Anal. Chem. **1995, *67,* 3234-3245**

Electroosmotically Transported Baseline Perturbations in Capillary Electrophoresis

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Two anomalous capillary electrophoresis (CE) phenomena, referred to as the baseline shift and spontaneous-marker peak, are investigated. The baseline shift and spontaneous-marker peak have been observed in a simple CE system with no sample injection and no deliberately formed concentration boundaries, a sodium benzoate solution as the running electrolyte, and on-column UV absorbance detection. The baseline perturbations, which are believed to have physical origins at the capillary inlet, are transported along the capillary at the rate of electroosmotic flow. Baseline perturbations observed previously have been attributed to pH changes or temperature changes, and although the latter may influence our results somewhat, neither of these effects can explain the phenomena that we have observed. Instead, we believe these baseline shifts and spontaneous-marker peaks are attributable to changes in the actual concentration of the running electrolyte. Although the property of a capillary end which is responsible for the generation of baseline perturbations remains unknown, the transport of the concentration excursions and the origin of spontaneous-marker peaks are explained.

Capillary electrophoresis (CE) is now a well-established field with many unique features and valuable applications. Much effort has been devoted to the development of CE as a powerful separation tool, with attention focused on the role of migration in the transport of analyte species.[1-6] Recently, the role of electroosmosis in analyte transport in CE systems has become a topic of interest,[7-13] although for analytical purposes, electroosmosis is often intentionally controlled because of its variability[14-20] Our present interest, however, is predominantly in the study of electroosmosis and its implications for simple CE systems. In particular, we have investigated two anomalous CE phenomena which are believed to have physical origins and whose effects are transported along the capillary column at the rate of electroosmotic flow. These phenomena, which are interrelated perturbations in the baseline UV signal, representative of disturbances in the electrolyte concentration, are referred to as the "baseline shift" and 'spontaneous-marker signal". It is believed that these phenomena may exist in other CE studies but that the interests of those studies are such that the baseline perturbations discussed herein have been either discounted or obscured by other signals. Unlike most CE studies, the present work does not involve the injection of a sample onto the capillary column for subsequent analysis, nor does it concern the separation efficiency of a given CE system. Instead, the factors which affect the appearance and behavior of the baseline shift and spontaneous-marker signal are investigated.

Related work includes that of Vinther and co-workers,[21] who observed a positive shift in baseline UV absorbance occumng at the time of electroosmotic flow, which they attributed to a dependence of the absorbance on buffer pH. System peaks and baseline disturbances, occuring before the electroosmotic flow marker, were studied by Beckers[22] for a discontinous buffer system. Furthermore, temperature-induced fluctuations in the baseline *UV* absorbance have also been studied.[23,24] These other studies, although somewhat related to this work, are unable to provide an explanation of the origin of the baseline shift and spontaneous-marker signal described herein. Although such an explanation remains incomplete, we believe that a study of these features will provide us with a greater understanding of CE systems. Brief descriptions of these phenomena follow.

In order to provide a 'marker" of electroosmotic flow in CE, it is common practice to add a neutral species to injected analyte samples. On many occasions, however, we have observed spontaneous-marker signals in a simple system with on-column *UV* absorbance detection, a sodium benzoate solution as the running electrolyte, and no injection whatsoever. The spontaneous markers appear to originate at the instant of field application

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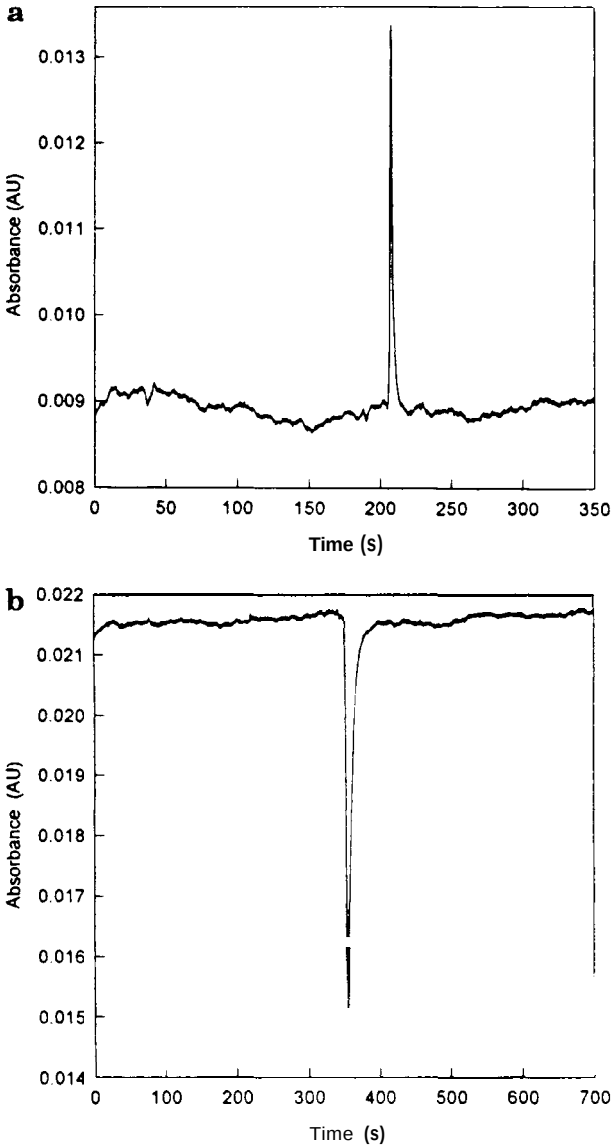

Figure 1. Capillary electropherograms showing typical spontaneous-marker peaks for a CE system with no injection, 20.0 mM NaBz original running electrolyte, 15.03 kV applied voltage, and detection at 225 nm. (a) A positive spontaneous-marker peak. Experimental conditions: column length, 57.30 cm; inlet-to-detector length, 31.20 cm; average measured current, 9.24 μA. (b) A negative spontaneous-marker peak. Experimental conditions: column length, 88.70 cm; inlet-to-detector length, 30.00 cm; average measured current, 6.39 μA.

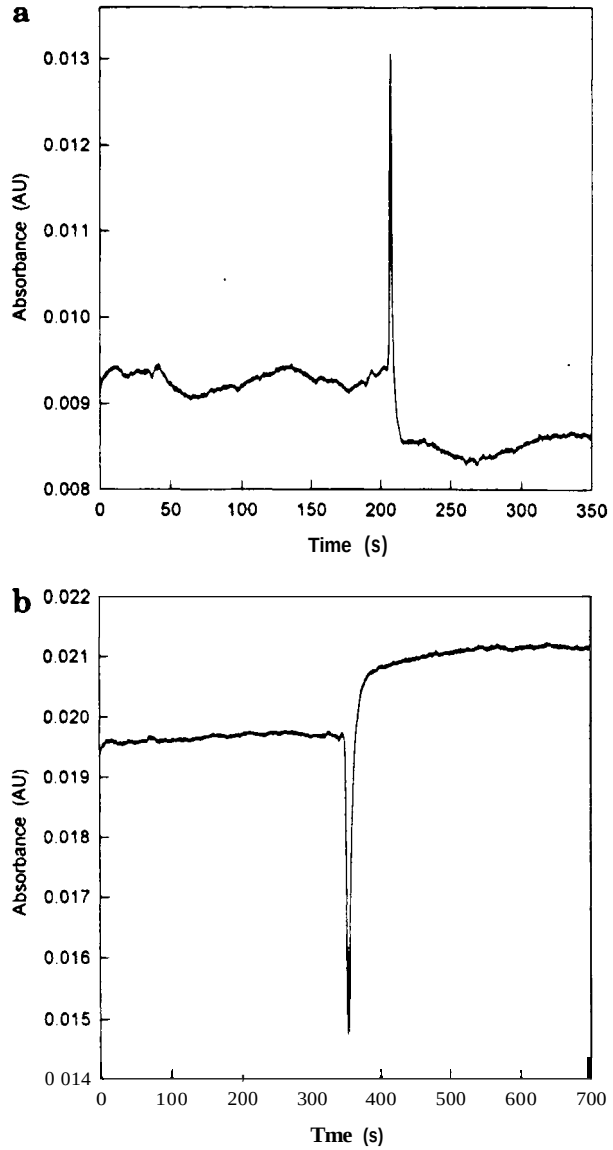

Figure 2. Capillary electropherograms showing typical spontaneous-marker peaks accompanied by baseline shifts resulting from changes in the applied field strength for a CE system with no injection, 20.0 mM NaBz original running electrolyte, 15.03 kV applied voltage, and detection at 225 nm. (a) A positive spontaneous marker accompanied by a negative baseline shift resulting from an increase (87.6 V cm $^{-1}$) in the applied field strength. Experimental conditions: column length, 57.30 cm; inlet-to-detector length, 31.20 cm; average measured current, 9.30 μA. (b) A negative spontaneous marker accompanied by a positive baseline shift resulting from an increase (56.7 V cm^{-1}) in the applied field strength. Experimental conditions: column length, 88.70 cm; inlet-to-detector length. 30.00 cm; average measured current, 6.36 μA.

at the column inlet, are peak-shaped, and may be either positive or negative of the baseline absorbance, depending on some presently unknown property of the capillary end. Examples of typical spontaneous-marker peaks are shown in Figure 1. We believe that spontaneous markers arise from diffusion-controlled processes at the interface between the solution in the column and that in the bulk electrolyte reservoir(s).

The spontaneous-marker signal is sometimes accompanied by a fielddependent shift in baseline absorbance. In the case of positive marker signals, increasing the applied field results in a decrease in the baseline absorbance. Conversely, in the case of negative marker signals, increasing the applied field results in an increase in the baseline absorbance. The end of a column giving rise to the former case is referred to as a "depleting end", while that giving rise to the latter case is referred to as an "enriching end". Electropherograms generated at both types of "active" ends are shown in Figure 2. The baseline s h i i represent real changes in electrolyte concentration, as confirmed by independent measurements of absorbance, conductivity, and electroosmotic mobility. Thus, baseline shifts indicate the existence of electrolyte concentration boundaries, which move along the column and past the detector by way of electroosmosis only. The time at which a baseline shift occurs coincides with that at which a spontaneous marker appears, and both serve to "mark" the rate of electroosmotic flow.

It appears that the existence of fielddependent baseline shifts, coupled with spontaneous-marker signals, depends in an undetermined way on the physical geometry of the end of the capillary

column. Thus, altering the end of the column by cutting to form a 'new" end with a possibly different geometry, can result in the appearance or disappearance of these phenomena. Similarly, Cohen and Grushka[25] and Schwartz et al.[26] have shown that the capillary cut can influence separation efficiency and peak shape. Only a minority of column ends or 'cuts" successfully generated baseline shifts and spontaneous-marker peaks. Over the course of these studies, three capillaries were installed. The length of each of these was continually diminished throughout its lifetime by cutting small lengths of capillary from either end, thereby exposing new cross-sectional annuli of the capillary. Spontaneous-marker signals were observed for all of the columns. The majority of baseline shift studies were conducted on the second and third columns, over a period of about 4 months on each. On the second column, seven cuts were made, and only two of these (29% of all cuts) resulted in an 'active" column end which gave rise to baseline shifts. On the third column, 47 cuts were made, 10 of which (21% of all cuts) produced baseline shifts. The magnitudes of the baseline perturbations vary greatly depending on the cut of the column and are often small enough to go unnoticed under the conditions normally encountered in analytical electrophoresis. When large, however, baseline shifts and spontaneous markers can cause concentration enhancements or depletions significant enough to warrant consideration if quantitative analysis is to be undertaken.

EXPERIMENTAL SECTION

Apparatus. All experiments described herein were conducted with an Isco (Lincoln, NE) Model 3850 capillary electropherograph with on-column UV absorbance detection. Although rarely employed in these studies, sample injection was by syringe using a split-flow mechanism. The high-voltage power supply of this instrument was operated in constant voltage (0–30 kV) mode, with both positive and negative applied voltages being used. The capillary compartment of the electropherograph was not thermostated, but a fan maintained good ambient circulation. In order to record CE data with great accuracy, we used the analogue current, voltage, and absorbance outputs of the instrument These signals were monitored by a Hewlett-Packard Model HP-3497A data acquisition unit, which, in turn, was interfaced to a Hewlett-Packard Model HP-9000, series 200 computer. Computer programs, written in-house in HP Basic 3.0, enabled the computer to partially control the CE system and to collect, analyze, and store current and absorbance data.

Capillary Columns. In all experiments, we employed capillaries made of fused silica, coated with a thin layer of polyimide to improve their durability (Isco). Capillary dimensions were 50 μm i.d., 156.5 ym wall thickness, 16 μm coating thickness, and 54.95-98.75 cm length. The distance from the high-voltage end of the capillary to the detector ranged from 29.95 to 67.80 cm, while the distance from the detector to the grounded end ranged from 24.10 to 58.70 cm. Capillary columns were cut to length using a blunt, Isco ceramic capillary cutter. Cutting necessitated removal of the capillary ends from their fittings in the electropherograph. Cuts obtained in this way were not always "clean", as revealed by optical microscopy and scanning electron microscopy.

Since electroosmosis is very sensitive to the condition of the inside wall of the capillary, we subjected the column to a treatment which led to fairly reproducible electroosmotic flow rates (<1% relative standard deviation) over the course of several days. For a new capillary column, this treatment, referred to as 'conditioning", consisted of filling the column with 1.0 M NaOH (BDH, Toronto, Canada) for 1 h, followed by leaving it filled with 0.10 M NaOH overnight The column was then flushed sequentially with distilled, deionized water, 0.10 M HCI (Baxter/Canlab, Toronto, Canada), and again water. Even with this treatment, reproducible electroosmotic flow was not realized until the column had undergone about 1 month of regular use, with one column requiring 4 months of use before reproducibility was achieved. Electroosmosis increased throughout these periods of "conditioning", and soaking the column in water for extended periods of time (at least 2 days) when not in use appeared to be the most effective method of maintaining the electroosmotic flow rate at a stable value. Thus, we adopted a simple treatment which consisted of filling the capillary with water whenever it was idle, followed by refilling the column with fresh water for 1 h prior to daily experimentation. Column ends were submerged in water during storage to prevent evaporation from the column.

Solutions. Studies of the baseline shift and spontaneous marker signal were conducted mostly in simple, uni-univalent electrolyte systems. In most instances, a 20 mM sodium benzoate (NaBz) solution was used, prepared by dissolving reagent grade NaBz (Caledon, Georgetown, Canada) in deionized and distilled water. The measured pH of this solution was 6.98 at 23 °C. Although all experiments presented herein were conducted with NaBz solutions, we determined that the existence of baseline shifts and spontaneous markers was not unique to the NaBz system by conducting similar experiments with several other running electrolytes, all prepared from analytical grade reagents, including 20.0 mM potassium benzoate (KBz; Aldrich, Milwaukee, WI), 20.0 mM sodium salicylate (Nasal; BDH), and 15.0 mM trisodium citrate (Na_3Cit; BDH). The measured pH's of these solutions were 7.01 at 20 °C, 5.85 at 24 °C, and 8.22 at 25 °C, respectively. All solutions were passed through a cellulose acetate syringe filter (pore size 0.45 μm; Nalge Co., Rochester, NY) and were degassed under vacuum by a water aspirator for ~1 h prior to use.

Procedure. Some terminology should be defined at the outset to make sure that its usage is clear and consistent. The "inlet end" of the column is not necessarily that end of the column physically located near the injection port "Inlet" simply refers to the end through which electroosmotic flow carries solution into the capillary. Thus, during positive polarity experiments, the inlet end is indeed the high-voltage end of the capillary, located near the injection port, but during negative polarity experiments, the inlet is the low-voltage end of the capillary. Conversely, the "outlet" refers to the end through which solution exits the capillary. The solution reservoir in which the inlet end of the column is submerged is called the "supply reservoir", while the outlet end is submerged in the "receiving reservoir". The solution which fills the capillary column at the beginning of an experiment is called the "running electrolyte", and in most cases, the same solution is also used to fill the supply and receiving reservoirs. Reservoir volumes are about 10 mL. Care is taken to equalize

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the solution levels in the two reservoirs to inhibit hydrodynamic flow. The solution which enters the capillary from the supply reservoir as a result of electroosmotic flow during a CE experiment is called the "replacement electrolyte," and may or may not be the same as the running electrolyte.

The majority of experiments, referred to as 'uniform-electrolyte experiments", involved no sample injection and no deliberately formed concentration boundaries. The first such experiment conducted each day was referred to as the "equilibration run". After having first used a microliter syringe to fill the capillary with running electrolyte, the equilibration run served to flush the capillary by way of electroosmotic flow, to rid the capillary of any small bubbles which may have been present after conditioning, etc. Results from the equilibration run were disregarded. Uniform electrolyte experiments were then conducted to try to determine the effect of applied field, column end conditions, and other factors on the magnitude of both the baseline shift and the spontaneous marker signal. A single uniform-electrolyte experiment, or "run", consisted of applying a constant high voltage across the capillary for a certain length of time while recording absorbance and current data. At the end of the experimental run, the high voltage was turned off for a period of time. Typically, 2–5 min elapsed between runs with no field applied, and this period of time is referred to as the 'standard interlude" in what follows. However, the period of time between runs with no field applied was as short as 5 s in some cases. The next experimental run was commenced by applying a constant high voltage which was not necessarily the same as in the previous experiment. If, in fact, the applied voltage was not the same in two successive runs, then it is said that a change in applied field strength has occurred between those runs.

RESULTS

Experiments that involved neither a deliberately formed concentration boundary nor an injected slug of analyte might be expected to produce no absorbance signals. Some such experiments, however, gave rise to absorbance signals, either positive or negative of the baseline. These spontaneous-marker signals appear to have their origin at the inlet end of the column, despite the fact that no sample was injected there or elsewhere. Accompanying these markers in some cases were fielddependent shifts in baseline absorbance. Both phenomena evidently represent concentration disturbances in the running electrolyte, and as such, are likely travelingalong the column and past the detector by way of electroosmotic flow alone. Thus, the typically peak-shaped spontaneous-marker signal, reminiscent of a small injected slug of an uncharged analyte, and the plateau-shaped baseline shift, reminiscent of a concentration boundary, serve as markers of the rate of electroosmotic flow in the CE system. The discovery of these phenomena is important not only in its own right, but also because both baseline shifts and spontaneous markers may be occurring at the same time as, and thus interfering with, the results of "typical" slug-type CE experiments. Uniform-electrolyte experiments were thus conducted to try to determine the effect of applied field, column end conditions, and other factors on the magnitude of both the baseline shii and the spontaneous-marker signal.

"Depleting ends" of the capillary somehow acted to deplete the concentration of electrolyte entering the column from the supply reservoir. For such ends, an increase in the applied electric field resulted in a decrease in baseline absorbance. Positive spontaneous-marker signals accompanied the baseline shift produced by depleting ends. In contrast, 'enriching ends" acted to enhance the concentration of electrolyte entering the column from the supply reservoir, and in these cases, an increase in the electric field resulted in an increase in baseline absorbance. Negative spontaneous markers accompanied the baseline shift produced by enriching ends. Although the two phen'omena—baseline shift and spontaneous marker - exist concurrently and presumably have a common cause, we will first attempt to characterize the baseline shift.

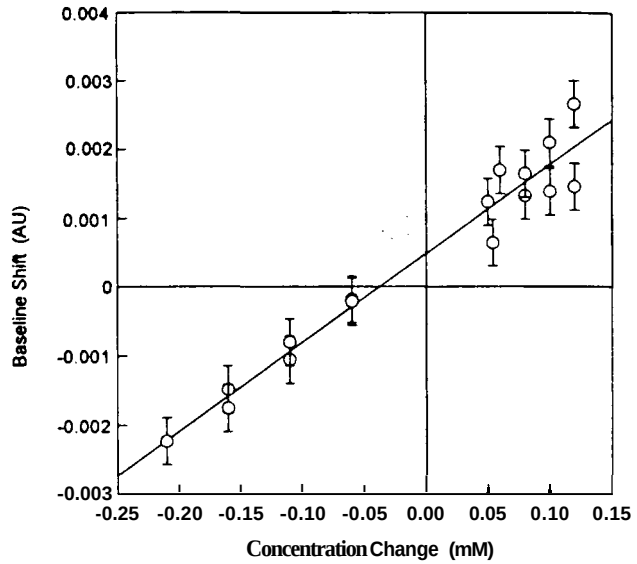

Figure 3. Calibration curve relating the magnitude of baseline shift (or change in absorbance) to the change in NaBz electrolyte concentration. Circles represent experimental data points, shown with error bars based on the standard error in the ordinate of the regression line (solid line). Slope of the regression line, 0.013 mM^{-1}; correlation coefficient, 0.95.

Baseline Shift. *Absorbance Studies.* In order to quantify baseline shifts, that is, to describe an observed change in UV absorbance in terms of the concentration difference between running and replacement electrolytes, we conducted a series of baseline shift calibration experiments. These experiments, conducted at a room temperature of 24.7 ± 0.6 °C, involved a series of successive "spikings" (beginning with the smallest and ending with the largest concentration increase) and a series of successive dilutions (beginning with the smallest and ending with the largest concentration decrease) of the 20 mM NaBz replacement electrolyte. Since most baseline shiis observed during uniform electrolyte experiments were fairly small, this calibration involved concentration increases of up to 0.12 mM and decreases of up to 0.21 mM. Figure 3 shows the resulting baseline shift calibration curve. By linear regression, the relationship between baseline shift (ΔA) and change in sodium benzoate concentration (Δc) was found to be

$$\Delta A\ (\mathrm{AU}) = (0.013 \pm 0.001)\Delta c\ (\mathrm{mM}) + (4.9 \pm 3.4) \times 10^{-4} \quad (1)$$

or equivalently

$$\Delta c\ (\mathrm{mM}) = (77 \pm 5)\Delta A\ (\mathrm{AU}) - (0.038 \pm 0.026) \quad (2)$$

with a correlation coefficient of 0.95. The fact that the regression

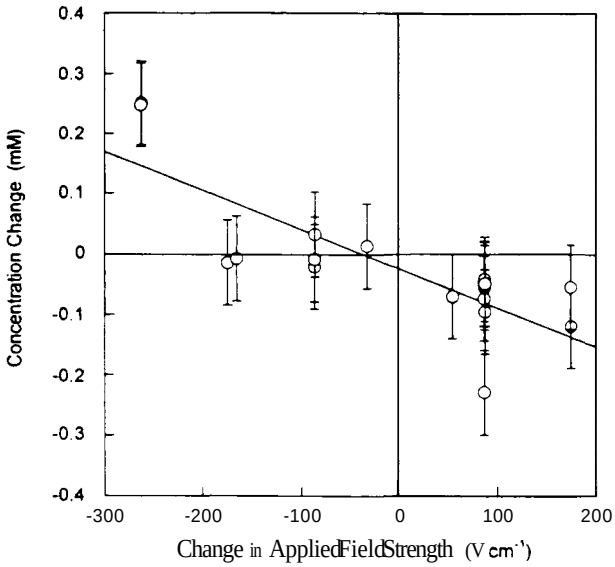

Figure 4. Effect of applied field on NaBz electrolyte concentration for cut (a). The solid line represents a linear least-squares fit of the data (slope, $-0.62\ \mu$M cm V^{-1}; correlation coefficient, 0.61). Error bars are based on the standard error in the ordinate of the least-squares fit.

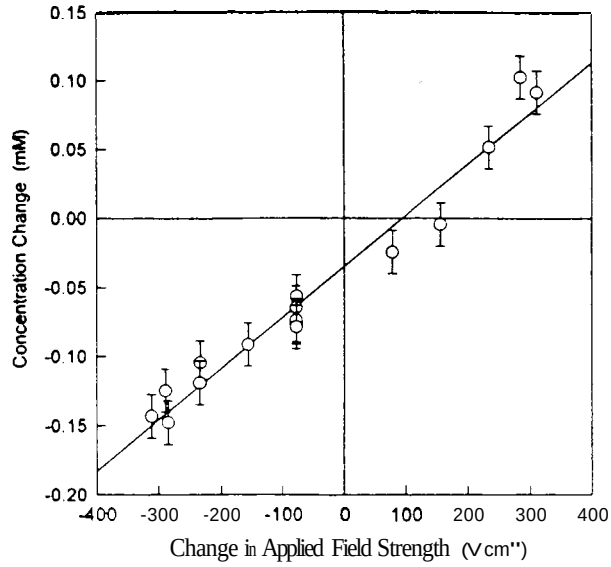

Figure 5. Effect of applied field on NaBz electrolyte concentration for cut **(b).** The solid line represents a linear least-squares fit of the data (slope, $0.37\ \mu$M cm V^{-1}; correlation coefficient, 0.96). Error bars are based on the standard error in the ordinate of the least-squares fit.

line does not pass through the origin, but instead has a positive intercept on the ΔA axis, indicates that a slight "drift" occurs in the baseline even when there has been no apparent change in electrolyte concentration. Possible reasons for this drift include changes in optical alignment or detector response over several hours of use or the gradual evaporation of solvent (water) from the supply reservoir, leading to a gradual increase in concentration of replacement electrolyte. Equation 2 was used to express the baseline shift resulting from an enriching or depleting column end in terms of concentration change.

The effect of applied field on baseline shift was studied in detail for several "active", that is, enriching or depleting, capillary ends. In these studies, the applied field was held constant during each run, but was changed before commencing a new run. The "standard interlude" elapsed between runs. The correlation between baseline shift, expressed as a concentration change, and change in applied field strength (ΔE) for one particular depleting end, referred to as cut a, is shown in Figure **4.** Experiments were conducted at a room temperature of 22.6 ± 0.5 °C. Despite the scatter in the data, a decreasing linear relationship between concentration and applied field is evident. A regression line was calculated for these data and was found to have a slope of $-0.64 \pm 0.12\ \mu$M cm V^{-1} and an intercept on the Ac axis of -0.024 ± 0.070 mM. The negative slope is indicative of a depleting column end: as the magnitude of the applied electric field is increased, the concentration of solution entering the column from the supply reservoir under the influence of electroosmotic flow is decreased. That is, the column end somehow acts to deplete the concentration of NaBz entering the column from the supply reservoir, and this depleting ability is enhanced by the application of stronger electric fields. For example, if the applied field were to be increased by 150 V cm^{-1} (equivalent to increasing the voltage applied along the 57.3 cm length of the column by 8 6 **kV)** when conducting experiments with cut a as the inlet end, then the NaBz solution entering this column from the supply reservoir would be 0.12 ± 0.07 mM less concentrated than that already present in the column, even though the running and replacement electrolytes were initially identical 20 mM NaBz solutions.

To provide further evidence of "active" end behavior, we now characterize another capillary end, referred to as cut b. Cut b was also an "active" cut, but unlike cut a, cut b acted to enrich the concentration of solution entering the column. The correlation between baseline shift, expressed in terms of concentration change and change in applied field (ΔE) for cut b, is shown in Figure 5. Since the Δc versus ΔE data (collected at a room temperature of 24.7 ± 0.8 °C) appear to be linearly related, a linear regression of the data was carried out. The resulting slope was $+0.37 \pm 0.02\ \mu$M cm V^{-1}, while the intercept on the Δc axis was -0.035 ± 0.016 mM, and the correlation coefficient was 0.96. The positive slope is indicative of an enriching column end: as the magnitude of the applied electric field is increased, the concentration of solution entering the column from the supply reservoir under the influence of electroosmotic flow is also increased. This behavior is opposite to that of the depleting end discussed previously. Thus, it appears that cut b somehow acts to enrich the concentration of NaBz entering the column relative to that in the supply reservoir. For example, consider increasing the applied field by 150 V cm^{-1} (by increasing the applied voltage along the 64.30 cm long column by 9.6 **kV)** when conducting an experiment with cut b. The NaBz solution entering this column from the supply reservoir would be 0.02 ± 0.01 mM more concentrated than that already present in the column, even though the running and replacement electrolytes were initially identical 20 mM NaBz solutions.

Of even greater interest is the extreme enriching ability of a third cut, referred to as cut c. When an electric field of magnitude 280 V cm^{-1} was first applied so that solution would pass through cut c and replace the contents of the column by electroosmosis, a baseline shift of 0.097 AU occurred. Using the correlation between baseline shlft and concentration change given in eq 2, it was found that solution filling the column through cut c was 7.5 ± 0.4 mM more concentrated than the origmal running electrolyte. This large enrichment does represent a somewhat unique case,

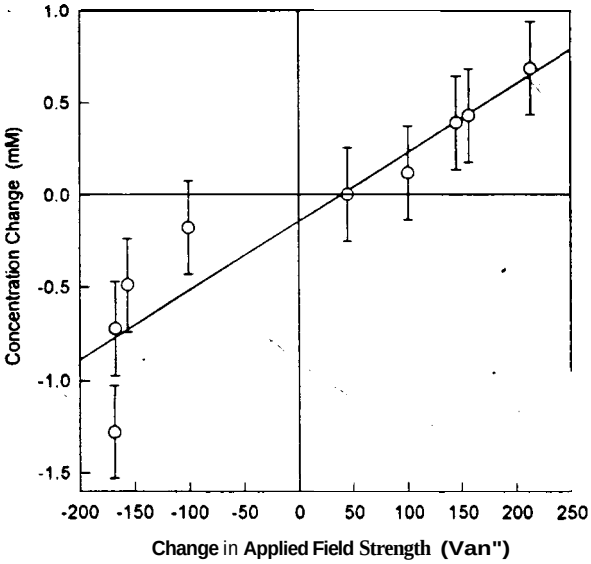

Figure 6. Effect of applied field on NaBz electrolyte concentration for cut c. The solid line represents a linear least-squares fit of the data (slope, 3.7 μM cm V^{-1}; correlation coefficient, 0.86). Error bars are based on the standard error in the ordinate of the least-squares fit.

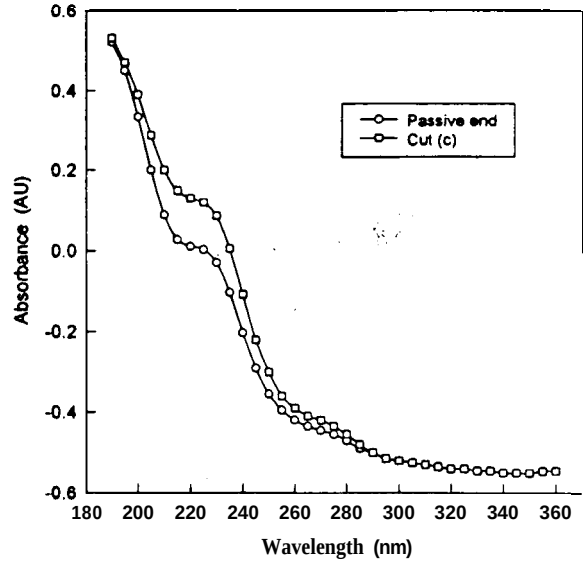

Figure 7. Absorbance spectra of NaBz solutions: solution entering the column by way of electroosmosis through the passive column end opposite cut c is indicated by circles; solution entering through cut c is indicated by squares. The zero of absorbance was set at 225 nm when the column was filled with the original 20.0 mM NaBz running electrolyte.

in that it occurred only during the first experiment with solution entering the capillary through the end which had previously been functioning as the outlet Thus, a change in solution flow direction and field polarity and strength had occurred. The effect of subsequently changing the applied field on concentration of electrolyte entering this particular end, at a room temperature of 22.3 ± 0.4 °C, is shown in Figure 6. Again, a linear relationship was observed, and regression of the data gave a line with slope +3.7 ± 0.6 μM cm V^{-1}, intercept −0.14 ± 0.25 mM, and correlation coefficient 0.86. This positive slope, which is 10 times greater than that for cut b, indicates that the enriching (or depleting) ability of all cuts is not equal and that cut c was extremely effective at enriching the concentration of NaBz entering the column. In fact, the concentration of NaBz entering the column through cut c would be 0.42 ± 0.26 mM greater than that already present in the column if the applied field 'were to be increased, as in the previous examples, by 150 V cm^{-1} (achieved by increasing the applied voltage along the 89.25 cm length of column by 13.4 kV).

To conlirm that cut c was functioning as an enriching end and that the significant absorbance changes observed during these experiments were, indeed, due to real changes in the concentration of NaBz entering the column through this cut, we recorded the absorbance spectra of first the original .NaBz running electrolyte, which entered into the column by way of electroosmotic flow through the 'passive" column end opposite cut c, and then the solution which had entered the column by way of electroosmosis through cut c. These spectra, recorded after the electric fields which had been used to fill the column were disabled, are shown in Figure 7. Moreover, it should be noted that the absorbance spectrum of 20.0 mM NaBz was found to be independent of applied field strength during experiments with a passive column end, and so any change in the absorbance spectrum cannot be attributed to some effect of the electric field on the optics of the CE instrument. The similarity in shape between the two spectra in Figure 7 indicates that the electrolyte in the column is pure NaBz in each case, although the greater absorbance demonstrated by the solution entering through cut c indicates that this cut did, indeed, enrich the concentration of NaBz relative to the supply and receiving reservoirs.

During the experiments investigating the effect of applied field change on electrolyte concentration, cut c was positioned in the capillary electropherograph such that it was submerged in the reservoir housing the grounded rather than the high-voltage platinum electrode, and large negative voltages were applied along the length of the capillary. If, in fact, some physical feature of cut c was responsible for the enrichment of solution during these experiments, then its enriching behavior should be observable regardless of its position in the electropherograph. To verify this, the column was reversed, so that cut c was submerged in the reservoir housing the high-voltage electrode, and then large positive voltages were applied along its length at a room temperature of 25.5 ± 0.1 °C. Indeed, in its reversed position, cut c continued to act as an enriching end, as shown in Figure 8, although the effect of changing the applied electric field on the concentration of electrolyte entering the column was somewhat less dramatic than it had been in its original position. In its new position, the rate of change in concentration as a function of applied field for cut c was found to be (by way of linear regression of the data in F i r e 8) +2.3 ± 0.1 μM cm V^{-1}. This means that increasing the applied field by 150 V cm^{-1} would result in an increase in concentration of NaBz entering the column through cut c of 0.33 ± 0.06 mM, as opposed to an increase of 0.42 ± 0.26 mM in its original position. The intercept on the Ac axis and the correlation coefficient for Figure 8 were −0.021 ± 0.056 mM and 0.98, respectively. It should be noted that reversal of the column subjects the exposed cut to some physical stress, in that it must be removed from and refitted through tight ferrules. This may, in some way, alter the condition of the cut, and so we cannot be sure that cut c in its reversed position was identical to cut c in its original position. Nevertheless, the degree to which a column end is able to deplete or enrich the concentration of electrolyte entering the column under the influence of electroosmotic flow appears to depend on the very nature of the end itself.

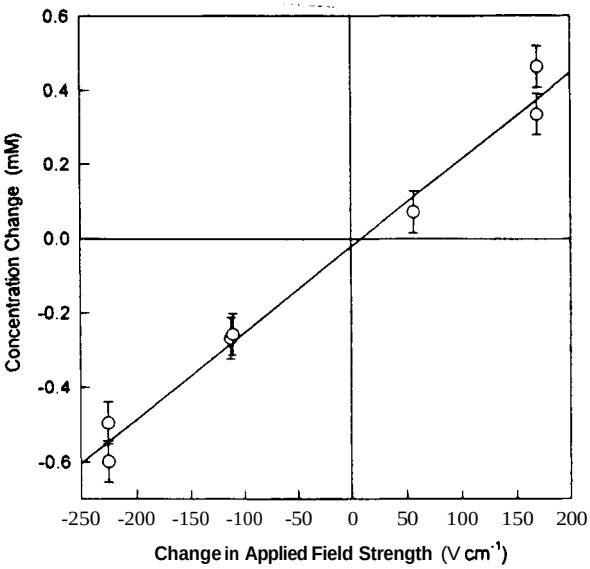

Figure 8. Effect of applied field on NaBz electrolyte concentration for cut c in its reversed position. The solid line represents a linear least-squares fit of the data (slope, 2.3 μM cm V^{-1}; correlation coefficient. 0.98). Error bars are based on the standard error in the ordinate of the least squares fit.

In an effort to determine if enriching or depleting behavior was caused by some physical or geometrical feature of the cut, scanning electron micrograph (SEM) images of several cuts, both passive and active, were recorded using a Cambridge S90 stereoscan scanning electron microscope. Samples were not conductively coated before imaging with an accelerating voltage of 25 kV. Unfortunately, there was no visible feature present in (or absent from) the images that could distinguish between an active cut and a passive cut. The exposed surface of the capillary resulting from all cuts appeared to be nonuniform, with features such as chips out of the fused silica, fused silica 'overhangs", debris remaining on the cut surface, or removal of small pieces of polyimide coating (which may have occurred either during cutting or perhaps during imaging). It is unclear from the SEM images if any of these features are responsible for the enriching or depleting ability of some column ends.

Baseline Shift. ***Conductivity Studies.*** The changes in absorbance used to characterize the "active" behavior of some column ends are supported by conductivity (current) and electroosmotic flow rate measurements. For an enriching end, an increase in applied field strength leads to an increase in absorbance. If an increase in electrolyte concentration is indeed responsible for this increase in absorbance, then the conductivity of the solution should also increase. This increase in conductivity was observed whenever a column end appeared to be enriching the solution entering the column and so provided an additional piece of evidence for this phenomenon.

The equation relating solution conductivity κ to the concentration c of a uni-univalent electrolyte is

$$\kappa = Fc(\mu^+ + \mu^-) \qquad (3)$$

where F is the faraday constant and μ^+ and μ^- are the electrophoretic mobilities of the cation and anion, respectively. This equation can be combined with that relating conductivity to current I,

$$\kappa = IL/VA \qquad (4)$$

where L is the length of the column, V is the constant applied voltage, and A is the cross-sectional area of the column. An equation is thus developed which enables us to calculate the concentration c of our uni-univalent electrolyte from the measured conductivity (or current) and applied voltage:

$$c = \frac{\kappa}{F(\mu^+ + \mu^-)} = \frac{IL}{FVA(\mu^+ + \mu^-)} \qquad (5)$$

This equation was used to calculate the concentrations of solutions filling the column during experiments conducted on a column-into which solution flowed first from the end opposite cut c and then through cut c itself. Initially, a positive voltage (+25.03 kV) was applied, and because cut c was positioned in the grounded reservoir, solution entered the column from the supply reservoir containing 20.0 mM NaBz by way of electroosmosis through the end opposite to cut c (that is, through the high voltage end). The concentration of this replacement electrolyte, calculated from eq 5 and based on a conductivity of 0.18 ± 0.01 Ω^{-1} m^{-1} (average measured current, 10.1 ± 0.1 μA) and mobilities of 51.9 x 10^{-9} and 33.5 x 10^{-9} m^2 s^{-1} V^{-1} for Na+ and Bz$^-$ at 25 °C, respectively,[27] was 22 ± 2 mM. In view of factors that could have a bearing on the magnitudes of the mobilities and uncertainty in the exact value of A, this can be considered good agreement with the concentration of the running electrolyte.

The polarity was then reversed by applying −24.96 kV, and since the magnitude of the resulting field was approximately the same as in the positive polarity experiment, the Joule heating would be comparable. Under the influence of this field, solution entered the column through cut c by way of electroosmosis. The calculated concentration of this replacement electrolyte, based on a conductivity of 0.28 ± 0.02 Ω^{-1} m^{-1} (final measured current, 15.2 ± 0.2 μA), was 34 ± 2 mM. Notice that the increase in conductivity resulting from filling the capillary with sodium benzoate solution through enriching end c by electroosmosis was *>50%.* The enrichment of the running electrolyte predicted by these conductivity measurements (14 ± 2 mM), conducted at a room temperature of 22.6 ± 0.1 °C, was greater than that predicted by the absorbance measurements (7.5 ± 0.4 mM) discussed previously. The discrepancy between these results may, in part, be attributed to the use of mobility values at infinite dilution and 25 °C in the calculation of concentration by way of eq 5. In addition, the calibration curve relating change in absorbance to change in concentration (Figure 3) covered a range of <0.4 mM, and as such, may not have been valid for quantifying significantly larger changes in concentration, as in these examples. It is clear, however, from both absorbance and conductivity measurements that the concentration of solution entering the column through cut c was enriched.

A subsequent experiment was conducted by applying −29.00 kV, and this led to a calculated concentration of 38 ± 3 mM, based on a conductivity of 0.31 ± 0.02 Ω^{-1} m^{-1} (final measured current, 19.8 ± 0.2 μA). Thus, the conductivities measured during this

(27) Oldham. K B.: Myland. J. C. ***Fundamentals of Electrochemical Science:*** Academic Press. Inc.: San Diego. 1994 p 12

and previous experiments indicated that the concentration of 'electrolyteentering the column through cut c was greatly enriched compared to the original running electrolyte and that the extent of enrichment appeared to depend on the magnitude of the applied field.

Baseline Shift, *Electroosmotic Mobility Studies.* In addition to using absorbance and conductivity measurements to support the concept of an enriching column end, it is also possible to use measurements of electroosmotic mobility. In this work, the electroosmotic flow velocity was confirmed by injection of a small slug of electrolyte with the same composition as but at a slightly different concentration than the running electrolyte. The rate of mobilization (or electroosmotic velocity ν^{eo}) of the baseline shift is proportional to the electroosmotic mobility μ^{eo}, which itself depends on the ζ potential of the column, according to the equation[28]

$$\nu^{eo} = \mu^{eo}E = (E\zeta/\eta)\epsilon \qquad (6)$$

where E is the applied electric field, η is the solution viscosity, and ϵ is the permittivity of the medium. The ζ potential, in turn, depends on the surface charge density a and double-layer thickness β:[29]

$$\zeta = \sigma\beta/\epsilon \qquad (7)$$

while the double-layer thickness depends on the concentrations and charge numbers of all solute ions:[30]

$$\beta = \left(\frac{RT\epsilon}{F^2\sum_i z_i^2 c_i}\right)^{1/2} \qquad (8)$$

Here, R represents the gas constant, T is the temperature of the solution, and all other symbols are as previously defined. Thus, the electroosmotic mobility is dependent on the concentration '(and the temperature) of the electrolyte. Since the baseline shift is believed to represent a concentration disturbance, it should act to alter the rate of electroosmotic flow somewhat.

From eq 8 it can be seen that if the concentration of the running electrolyte is increased, the double-layer thickness will decrease. This, in turn, will decrease the ζ potential according to eq 7, and finally will decrease the electroosmotic mobility, as predicted by eq 6. Since cut c has been shown to function as an enriching end, it should, by virtue of passing more concentrated electrolyte through the column, reduce the ζ potential and hence the electroosmotic velocity. Because cut c was originally positioned in the grounded reservoir, the initial application of +25.03 kV, at a room temperature of 22.6 ± 0.1 °C, filled the column with solution through the 'passive" end opposite to cut c by way of electroosmosis with $\mu^{eo} = (4.71 \pm 0.04) \times 10^{-4}$ cm^2 V^{-1} s^{-1}. Upon applying a constant voltage of –24.96 kV, the magnitude of the electric field was approximately the same as in the previous experiment but in a direction such that solution would enter the column through cut c by way of electroosmosis. The electroosmotic mobility in this case was $(4.00 \pm 0.04) \times 10^{-4}$ cm^2 V^{-1} s^{-1}, indicating that the ζ potential had, indeed, been decreased. Such a decrease could be attributed to an increase in electrolyte concentration. Of course, a change in concentration would lead to a subsequent change in the amount of Joule heating and thus in the temperature. Because of the dependence of temperature on concentration, and the dependence of electroosmotic mobility on both of these variables, the effect of increasing the concentration on the electroosmotic mobility is quite complex.

When a constant voltage of **–29.00** kV was subsequently applied to allow flow through cut c, a further decrease in electroosmotic mobility was observed ($\mu^{eo} = (3.88 \pm 0.03) \times 10^{-4}$ cm^2 V^{-1} s^{-1}), indicating a further increase in concentration. Thus, measurement of electroosmotic mobility provided evidence of the enriching behavior of cut c, as did the measurement of current and absorbance.

Spontaneous-Marker Peak. Since the baseline shift did not occur in isolation, we will now discuss the concomitant phenomenon of the spontaneous-marker signal. This signal appeared only when the column end gave rise to baseline shifts. Enriching capillary ends gave rise to negative spontaneous-marker peaks, while depleting ends gave rise to positive spontaneous markers. Often, the marker peaks were skewed rather than Gaussian in shape. Whereas baseline shifts occurred only when the applied field was changed, spontaneous markers appeared whenever there was a period of time with no field applied and when the inlet end of the capillary was enriching or depleting, regardless of change in field strength. Thus, when the field strength remained unchanged between runs, the only feature in the electropherogram was a spontaneous marker, as seen in Figure 9a, but when the field was altered, a spontaneous marker was superimposed onto a baseline shift, as seen in Figure 9b. The size of these markers appeared to depend primarily on the extent of enrichment or depletion and on the length of time elapsed with no field applied between experimental runs.

When no electric field is applied along the column, as is the case between CE runs, transport of NaBz through the column by either migration or electroosmosis will cease. Thus, diffusion will become the only mechanism of ion movement Diffusion of NaBz into or out of the column will occur if a concentration gradient exists at the interface between the solution in the column and that in the bulk reservoir.[31] Thus, it seems reasonable to assume that spontaneous-marker peaks could be formed as a result of diffusion of NaBz into or out of the column between runs, given that the observed baseline shifts represent concentration boundaries formed at the capillary end. To investigate this possibility, the effect of no-field time on spontaneous marker size was studied for cut c in its original and reversed positions. The results are presented in Figure 10. Because cut c is an enriching end, it produces negative spontaneous markers, and so the marker peaks are negative of the baseline. Using eq 2 along with peak widths at half-height, these 'negative" peaks were transformed into amounts of NaBz representative of the markers. As expected, the spontaneous markers generated by cut c in its original position were larger than those generated in its reversed position at all times, due to the greater enriching ability of cut c before reversal. In both cases, however, spontaneous markers approached a maximum decrease in the amount of NaBz as the standard

(28) Laidler. K J.; Meiser. J. H. ***Physical Chemistry,*** Benjamin/Cummings Publishing Co., Inc.: Menlo Park. *1982:* p *841.*

(29) Reference *28.* p 794.

(30) Grossman. P. D. Factors Affecting the Performance of Capillary Electrophoresis Separations: Joule Heating. Electroosmosis, and Zone Dispersion. In ***Capillary Electrophoresis.*** *"Theoryand Practice;* Grossman. P. D . Colburn. J C.. Eds.: Academic Press. Inc.: San Diego. *1992;* p *18.*

(31) Dose. *E V.*. Guiochon. G. *Anal. Chem.* 1992. *64. 123–128*

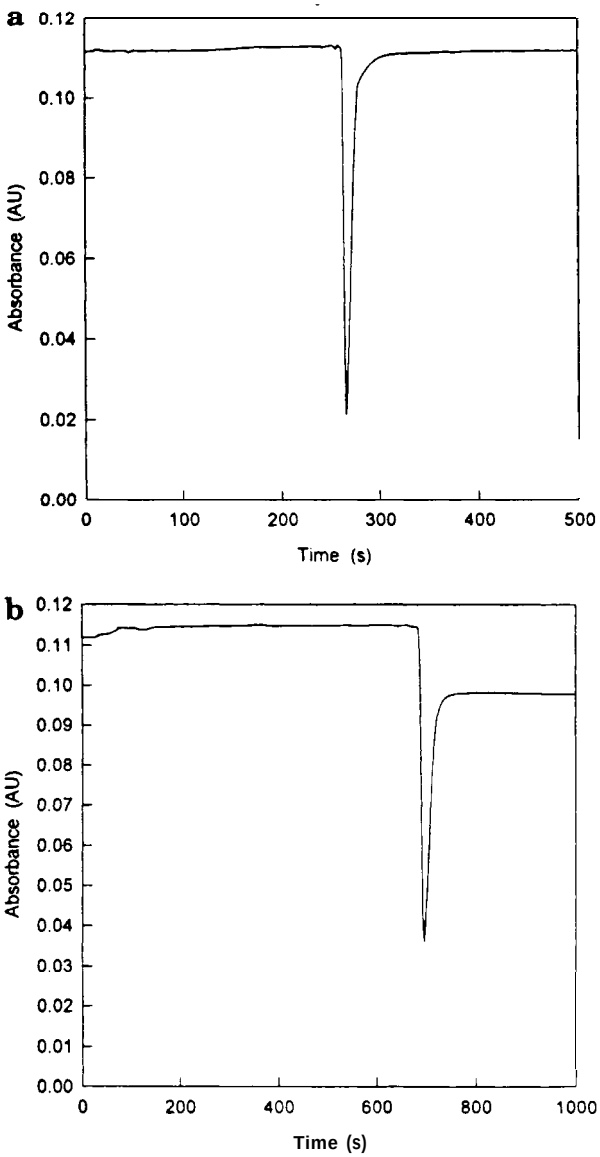

Figure 9. Capillary electropherograms showing negative spontaneous-marker peaks generated at enriching capillary end c with no injection; 20.0 mM NaBz as the original running electrolyte; column length, 88.70 cm; inlet-to-detector length, 30.00 cm; and detection at 225 nm. (a) A negative spontaneous marker with no accompanying baseline shift. Experimental conditions: applied voltage in this and in previous run, −24.99 kV; average measured current, 15.54 μA. (b) A negative spontaneous marker accompanied by a negative baseline shift. Experimental conditions: applied voltage in previous run, −24.99 kV; applied voltage in this run. −10.04 kV; average measured current, 5.87 μA.

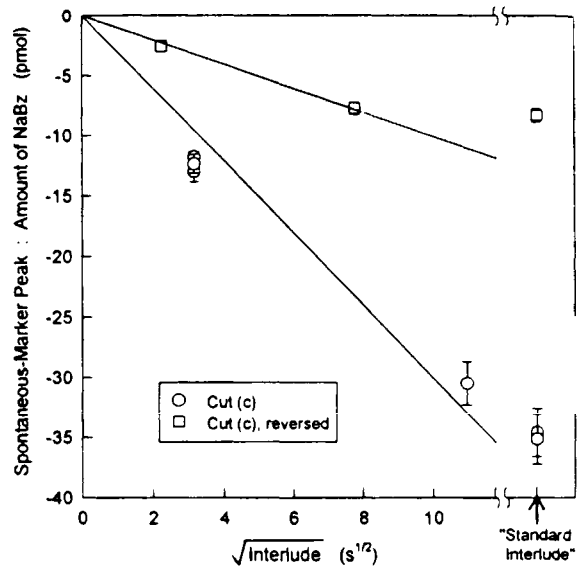

Figure 10. Spontaneous-marker peak size, represented as an amount of NaBz, as a function of the square root of the interlude (where the interlude represents the time elapsed between runs with no field applied). Solid lines represent least-squares fits of the data (excluding standard interlude data) for cut c in its original (circles) and reversed (squares) position. Propagated error in the calculated amounts of NaBz are less than the size of the data symbol or is shown as an error bar.

interlude was approached. The amounts of NaBz represented by the markers approached zero as the time between runs approached zero. This implies that if the applied field were never disabled, spontaneous-marker peaks would not be generated. Experimentally, this was observed. Recall that baseline shifts occur only when the applied field is changed, while spontaneous-marker peaks occur regardless of applied field strength changes, provided there is some no-field time between runs. Thus, if the applied field is changed in the middle of a uniform electrolyte experiment, one would expect to see a baseline shift without an accompanying spontaneous-marker peak. This was indeed the case during an experiment with cut c in its reversed position, when the applied field was decreased from 282 to 110 V cm^{-1} in the middle of a run, generating a baseline shift of -3.6×10^{-3} AU (equivalent to a decrease in concentration of 0.32 ± 0.03 mM) but not generating a spontaneous marker. Thus, it appears that some period of time with the field disabled is essential to the occurrence of the spontaneous-marker peak.

DISCUSSION

Baseline shifts have been observed previously. Vinther et al.[21] observed shifts attributable to pH changes, but we do not expect significant pH changes to occur in our system. The electrolysis of water would, indeed, result in the production of hydrogen ion in the supply electrolyte reservoir, thus establishing a buffer system (benzoate/benzoic acid) which would prevent significant changes in pH. Consider, for example, a series of 10 experiments conducted in 1 day, each lasting 10 min with a typical current of 10 μA flowing. The amount of hydrogen ion produced in the supply electrolyte reservoir as a result of these experiments was calculated to be about 6×10^{-7} mol, but after taking into consideration the HBz/Bz buffer system formed, this would result in only a 0.3 decrease in pH relative to the running electrolyte's original pH. This was confirmed experimentally when, after 100 min of operation with an applied voltage of 20.01 kV ($I \approx 10$ μA), the measured pH of the 20.02 mM NaBz solution in the inlet reservoir had changed from 6.75 to 6.43 (at 22 °C).

Moreover, if a significant pH change were somehow to occur, it would be a progressive effect, and so one might expect to observe a progressive change in absorbance. Experimental changes in absorbance were not progressive: they occurred in both positive and negative directions, depending upon the capillary end and the change in applied field. Furthermore, measured absorbances of sodium benzoate and benzoic acid solutions were found to be indistinguishable at 225 nm, and so a change in pH (or a change in the relative amounts of benzoate and benzoic acid present) would not result in any observed change in absorbance. Finally, if baseline shifts were pH induced, one would expect to

see such features during every experiment, with every capillary and with any change in field strength, since the electrolysis of water would proceed regardless of these experimental variables. Such constancy in baseline perturbations was not observed. Baseline shifts have also been linked[23,24] to temperature changes in the running solution caused by Joule heating. While such thermal effects are undoubtedly present in our experiments, they alone cannot explain the phenomena that we have observed.

We attribute the baseline shift and spontaneous marker signals to changes in the actual concentration of the sodium benzoate solution in the capillary observed at the site of spectrophotometric analysis. We did consider a number of other possible explanations of the changed absorbance, such as its being due to temperature induced changes in the refractive index of the solution or some other artifact, induced in the detector by changes in temperature or field strength rather than by concentration changes. However, such possibilities are ruled out by the persistence of the signal even when the applied voltage is turned off during a run, which interrupts the flow, as well as both heat generation and the electric field. Moreover, the several independent pieces of experimental evidence that we report above all point to concentration excursions being responsible for both phenomena: the permanent baseline shift and the transient spontaneous-marker peaks.

Flow rate through the capillary is not a quantity that is open to direct measurement in CE as usually conducted. However, subsidiary experiments employing short slugs of analyte provided access to this quantity and demonstrate that flow through our apparatus occurred at a rate consistent with electroosmosis driven by a zeta potential of about −100 mV. Knowing the flow rate, one can calculate the delay before a concentration perturbation, initiating at the capillary inlet, would reach the spectrophotometer. In all cases, this calculated delay closely matched the experimental residence time in the column prior to the signal—baseline shift and/or spontaneous-marker peak—being observed. We regard this as very strong evidence that these phenomena, when they exist, have their origins at the mouth of the column and are propagated down the column at a velocity close to that of electroosmotic flow.

These effects are not trivial. Permanent concentration enrichments of 37% were observed in one extreme case, with more typical activity generating 1 or 2% enrichment or depletion. Nevertheless, in 75% of the cases studied, there was either no effect whatsoever, or it was at too low a level to be experimentally significant Whether or not the concentration excursion occurred and, if it did occur, its magnitude and sign depended entirely on some still-unidentified aspect of the end of the capillary tube. Paring the end of the capillary can destroy or create activity, though we have yet to learn how to do this in other than a random fashion.

There are four important questions still to be answered: First, what property of an end causes it to be active? Second, how does that property induce and maintain a concentration excursion in the solution entering the capillary? Third, how does the concentration step move along the column, ultimately making its existence manifest as a baseline shift at the spectrophotometer? And fourth, what is the origin of marker peaks and why are they inversely related to the baseline shift? We have no convincing answer to the first question. We have some qualitative guesses as putative answers to the second question. We believe that we can satisfactorily answer the third and fourth questions.

Though it may not be the logical place to start, let us first examine the circumstances surrounding the third question: How does a concentration step propagate? Imagine a concentration junction $c_2|c_1$ of a uni-univalent electrolyte to exist between two fixed points x_2 and x_1 in the column. These points are in homogeneous regions, but the concentration is not uniform between them. In general, there will not be a sharp concentration discontinuity, but we may nevertheless associate "the boundary" between the two regions with a specific point x_b along the column, such that

$$(x_1 - x_b)c_1 + (x_b - x_2)c_2 = n/A \tag{9}$$

where A is the cross-sectional area of the capillary and n is the total amount of electrolyte between x_1 and x_2. Let us inquire about the speed with which electrophoresis causes this boundary to move.

A current I flows from left to right across the boundary, carried in part by cations moving across the boundary from left to right and in part by anions moving in the opposite direction. In a time interval Δt, the increase in the amount of cations in the boundary zone $x_2 < x < x_1$ is

$$\Delta n^+ = (E_2\mu_2^+c_2 - E_1\mu_1^+c_1)A\Delta t \tag{10}$$

where each E denotes the field strength and each μ^+ is the cation mobility ($m^2\ s^{-1}\ V^{-1}$). The corresponding increase in anion content is

$$\Delta n^- = (E_1\mu_1^-c_1 - E_2\mu_2^-c_2)A\Delta t \tag{11}$$

but, of course, electroneutrality requires that these two Δn expressions be equal. This equality is also evident from the standard expressions

$$I/AF = E_1(\mu_1^+ + \mu_1^-)c_1 = E_2(\mu_2^+ + \mu_2^-)c_2 \tag{12}$$

relating current to field strength. Combining eqs 10 and 12 leads to

$$\Delta n/\Delta t = \frac{I}{F}\left[\frac{\mu_2^+}{(\mu_2^+ + \mu_2^-)} - \frac{\mu_1^+}{(\mu_1^+ + \mu_1^-)}\right] = \frac{I}{F}(t_2^+ - t_1^+) \tag{13}$$

where, in the final step, the cationic transport number t^+ is introduced.

Now, all terms in eq 9 except n and x_b are constants, and therefore, $\Delta n/A = (c_2 - c_1)\Delta x_b$. Combining this result with eq 13 leads to

$$\Delta x_b/\Delta t = \frac{I}{AF}\left(\frac{t_2^+ - t_1^+}{c_2 - c_1}\right) \tag{14}$$

This expression gives the electrophoretic velocity v^{ep} of the boundary. Stockrnayer[32] derived this result, and it was used by Gaš[24] to estimate the speed with which disturbances propagate.

(32) Stockmayer. W. H. Trans. N Y Acad Sci 1951. 13. 266-269

The electrophoretic velocity will be zero if, as it would be reasonable to assume, the transport numbers in the two regions are equal. There are (at least) three effects that could lead to the cation having a different mobility in the two regions. First, there is the direct effect of concentration itself. Ionic mobilities are known to decrease from their infinite dilution values as the concentration increases, for reasons that are incorporated into the Onsager theory.[33,34] Second, the electric field will be stronger in the more dilute region, and this is known to increase ion mobilities (the Wien effect[35]). Third, because the electrical conductivity will be less in the more dilute region, heat generation will be greater there, leading to a higher temperature and consequently higher mobilities. Though the third effect is likely the most important, all three of these effects conspire to make ionic mobility greater in the more dilute region. However, all three effects operate on both the cation and the anion, and we are unable to predict whether the sodium ions or the benzoate ions would be more affected. A proportionate increase, i.e., $\mu_2^+/\mu_1^+ = \mu_2^-/\mu_1^-$, would leave the transport numbers unchanged. Certainly it is hard to imagine that t_2^+ would differ from t_1^+ by more than 1% for a 1 mM difference between c_2 and c_1, and the discrepancy in transport number would likely be much less than this. For a current of 8 μA, which was typical of our experiments, we then estimate from eq 14 that $|v^{ep}| < 0.4$ mm s^{-1}.

This estimate of a ceiling to the electrophoretic velocity of the boundary is to be compared with the value $v^{eo} = 1.5$ mm s^{-1} which is typical of the electroosmotic velocities in our experiments. Thus, while we cannot rule out the possibility of an electrophoretic contribution (additive or subtractive), it would appear from this analysis that electroosmosis is the major factor in transporting concentration excursions from the inlet to the spectrophotometer, in agreement with the experimental evidence.

Could the factors that have been discussed above be responsible not only for the propagation of the concentration excursion but also for its creation? There is no doubt that thermal effects due to Joule heating are of paramount importance in electrophoresis and must be considered in any thorough analysis of conditions during CE. We estimate that the local temperature elevation may be as high as 30 K in some of our experiments. In the literature, the greatest attention has been directed toward an understanding of the radial distribution of temperature, because this has a bearing on the resolution of electrophoretic peaks and thereby directly affects the success of chemical analysis by CE. In our case, however, it is to the axial dimension that we must look for the most likely explanation of our effects. While recognizing that this is possibly an oversimplication, we therefore ignore radial distribution of the variables in what follows.

The axial heat flux density (W m^{-2}) is given by the sum of two terms, one representing conduction and the other convection:

$$j_h = -k\left(\frac{\partial T}{\partial x}\right) + \varrho C v T \qquad (15)$$

Here k denotes the thermal conductivity (W m^{-1} K^{-1}) of the solution, ϱ its density, and C its heat capacity (J kg^{-1} K^{-1}). T is the local temperature, and v is the (largely electroosmotic) axial velocity which, if we ignore the thermal expansion of the liquid and the capillary, we can regard as a constant

The change in the axial heat flux density along the capillary is one of three factors that leads to the possibility of a change in the local temperature with time. Another is Joule heating, which is proportional to the square of the current density, and the third is conductive heat loss through the capillary wall. These three factors lead to the three right-hand terms in the equation

$$\varrho C\left(\frac{\partial T}{\partial t}\right) = \left(\frac{\partial j_h}{\partial x}\right) + \frac{(I/A)^2}{\kappa} - \frac{2h}{r}(T - T_0) \qquad (16)$$

In this equation, κ is the local electrical conductivity (Ω^{-1} m^{-1}) equal to $Fc(\mu^+ + \mu^-)$, r is the radius of the capillary, and A ($= \pi r^2$) is its cross-sectional area. T_0 represents the ambient temperature, and h is an overall heat transfer coefficient (W m^{-2} K^{-1}) of the capillary wall related[36,37] to the dimensions and thermal conductivities of the silica capillary and its polyimide coating. A detailed derivation of the previous two equations is described by Gaš[24] who, however, omitted the final term in eq15 because he excluded electroosmosis from consideration. The inclusion of convection could have important ramifications beyond the introduction of this term, making Gaš's conclusions inapplicable to our system.

The equations incorporated into expression 12 apply to regions in which the properties are uniform; they require augmentation when applied to regions in which electrolyte concentration c, electric field strength E, and temperature T are all functions of the axial dimension x. In a nonuniform region, we have

$$I/AF = E(\mu^+ + \mu^-)c - D^+\left[\left(\frac{\partial c}{\partial x}\right) + \sigma^+ c\left(\frac{\partial T}{\partial x}\right)\right] + D^-\left[\left(\frac{\partial c}{\partial x}\right) + \sigma^- c\left(\frac{\partial T}{\partial x}\right)\right] \qquad (17)$$

where D^+ and D^- are the diffusivities of the cation and anion, the σ^+ and σ^- terms being the corresponding Soret coefficient[38,39]

The mathematical problem presented by eqs 15–17 is strongly nonlinear, as noted by Gaš[24] and an analytic solution is out of the question. In principle, computer modeling of the system is possible, but the main impediment is the paucity of information about the values of the various parameters-diffusivities, Soret coefficients, and mobilities—and how they depend on concentration, temperature, and field. Doubtless by choosing appropriate concentration, field, and temperature dependences of these parameters for the sodium and benzoate ions, one could model a wide variety of behaviors. One might even obtain simulations that match some of our experimental observations, but in this eventuality we would hesitate to claim that we had then 'explained" the effects.

Our main reason, though, for not pursuing these approaches is the inescapable experimental evidence that the baseline shift originates in a concentration disturbance at the capillary inlet,

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where the equations are inapplicable. Moreover, the magnitude and sign of the concentration excursion evidently depend on some feature of the capillary end that can be changed by recutting but which is sufficiently intimate that is it not revealed by optical or electron microscopy. Some ends are 'active", some are not This brings us to the first two of the four questions that were enumerated at the beginning of this section: What property of the capillary end makes it active, and how does that property cause activity?

Frankly, we have no idea what the special property of an end is that makes it active nor how it comes about that some active ends reproducibly induce a positive concentration excursion, while others, with equal reproducibility, cause a negative excursion. We imagine that the important feature could be a geometrical anomaly a small notch, crack, or flute, for example. The feature is resistant to the normal, careful handling of day-to-day usage; it endures rinsing with a jet of water, for example. There is some evidence, however, that more stressful actions, such as forcing the end into a tight-fitting ferrule, do impair the activity of an active end. The positioning of each end of the column in the electrolyte reservoirs may play a role in activity, although our experiments have not shown this to be so.

Recall that the inlet is simply the cut end of the capillary dipping vertically into a small beaker of electrolyte solution. The distinctive feature of the inlet is the rapid, convergence-induced change in the values of the salient properties as one proceeds from the bulk solution toward and through the aperture. The properties that change in this unique way are the flow velocity, the field strength, and presumptively the temperature. If changes in any of these properties lead to changes in the transport number of either the cation or anion, then a change in concentration can arise. Unfortunately, knowledge of the dependence of transport numbers on such properties is very limited, and so it is difficult to predict what sort of concentration changes could be expected in different regions of the capillary. We speculate that there may be some angular dependence of the properties, arising perhaps from whatever anomaly is responsible for activity, that enhances the magnitude of the concentration excursion compared with the symmetric conditions that apply when that anomaly is absent. Possibly hydrodynamic factors are involved, but this is still speculation.

Though the origins of the baseline shii remain conjectural, once it is accepted that they represent real concentration changes developed at the capillary inlet, spontaneous-marker peaks can be explained rather simply. Recall that these peaks develop if, but only if, the field is interrupted between runs. Consider that we have an "enriching end" at the column inlet Then, during the interlude between runs, the capillary, filled with enriched solution, is sitting in the supply reservoir which contains unenriched solution. There will be a slow diffusion of electrolyte from the capillary into the reservoir. Then, when the field is reapplied and electroosmosis resumes, there will be a renewal of the enrichment process at the inlet But now there will be a small slug of partially depleted solution, trapped between the 'old" enriched solution and the 'new" enriched solution. This slug will travel at the usual electroosmotic velocity to the spectrophotometer, where it will cause a temporary negative excursion in absorbance. This is exactly what is observed with enriching ends: a negative spontaneous-marker peak observed after a delay corresponding to the electroosmotic journey from the inlet Conversely, positive peaks are observed with depleting ends, and no peak whatsoever is found with inactive ends. If the field is changed, an active end will display both a baseline shift and a spontaneous-marker peak, but without an inter-run interlude, the baseline shift is not accompanied by a marker peak.

In summary, we believe that we understand what baseline shifts and spontaneous-marker peaks are. We can explain how baseline shifts give rise to marker peaks, but we do not understand the mechanism by which baseline shifts arise.

ACKNOWLEDGMENT

We thank Professors R Cassidy (University of Saskatchewan) and B. Gaš (Charles University) for their very helpful comments on this work. The authors gratefully acknowledge support from the Natural Sciences and Engineering Research Council (NSERC) of Canada (C.LC. for a 1967 Science and Engineering Scholarship; KB.0. for a Research Grant; and A.V.S. for a NATO Science Fellowship). C.LC, also acknowledges the Trent-Queen's Graduate Program and a doctoral scholarship from the I.O.D.E.

Received for review February 22, 1995. Accepted June 20, 1995.®

AC9501912

® Abstact published in *Advance* ACS *Abstracts.* August 1. 1995.