BASELINE PERTURBATIONS IN CAPILLARY ELECTROPHORESIS

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EMERSION PEAKS

INTRODUCTION

Absorbance signals, referred to as "emersion peaks," are generated at the inlet end of the capillary when this end is temporarily moved from solution prior to application of the electrophoretic field. This phenomenon, which is believed to have physical origins at the capillary inlet is transported along the capillary at the rate of electroosmotic(E0) flow and is detected by on-column UV absorbance. Emersion peaks have been observed in a CE system with a uniform sodium benzoate (NaBz) electrolyte without sample injection or delberately-formed concentration boundaries, and are attributed to the adsorption of benzoate at the air-solution interface formed upon emersion. Ernersion peak size has been found to depend on: (i) the number of emersions. (ii) the duration of each emersion, (iii) the height of emersion, (iv) the delay between completion of the emersion and application of the electric field, and (v) the cut of the capillary.

EXPERIMENTAL

Apparatus.

- Isco 3850 capillary electropherograph; on-column UV absorption detection at 225 nm
- constant voltage mode (15 - 30 kV); no sample injection

Capillary Columns.

- fused silica with polyimide coating (Polymicro Technol.): 50 µm i.d. x 395 µm o.d.
- total length ranging from 75.60 - 89.25 cm; inlet-to detector from 28.70 - 59.25 cm

Solutions.

- 20.0 mM sodium benzoate; degassed under water aspirator vacuum for 1 hr.; filtered through 0.45 µm cellulose acetate syringe filter

Procedure.

- emersion: lifting the capillary inlet above the solution to a certain height for a certain period of time before returning it to its original position; after emersion, two to five seconds ("standard delay") pass before application of the electric field

RESULTS

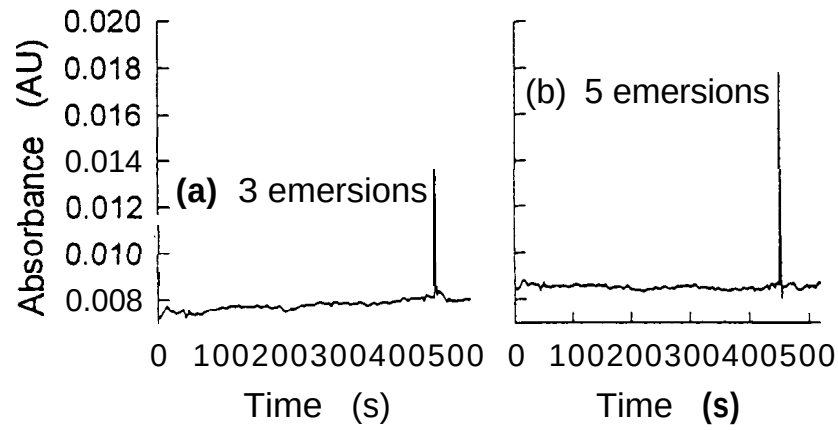

FIGURE 1. Capillary electropherograms showing typical emersion peaks resulting from (a) 3 emersions and (b) 5 emersions of the capillary inlet, each to a height of 17 mm above the level of solution in the supply reservoir for ≤ 2 s. Following the emersions, a 2 to 5 second ("standard") delay occurred prior to application of a constant voltage of 24.97 kV across the 8925 cm long capillary (inlet-to-detector length: 59.25 cm).

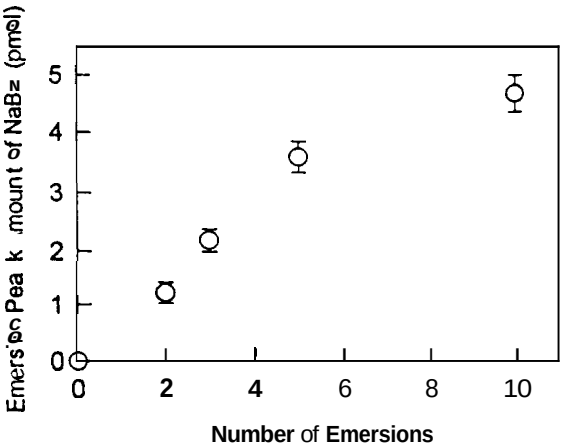

FIGURE 2. The amount of NaBz representative of an emersion peak as a function of the number of emersions of the capillary inlet. Error bars are based on propagated error in the calculated amounts of NaBz. Experimental conditions as described in Fig. 1.

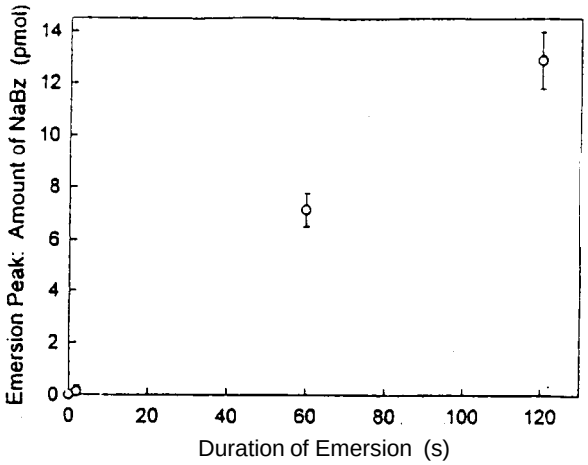

FIGURE 3. The amount of NaBz representative of an emersion peak as a function of the duration of the emersion. Error bars are based on propagated error in the calculated amounts of NaBz. Results are for single emersions to a height of 17 mm, with an applied voltage of -25.04 kV, and inlet-to-detector and total capillary lengths of 28.70 cm and 82.35 cm, respectively. Standard delay time was employed in all cases.

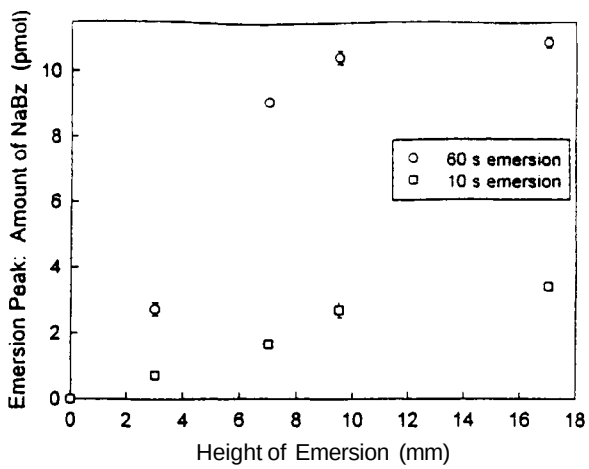

FIGURE 4. Emersion peak, represented as an amount of NaBz as a function of the height of emersion during single 10 s (□) and 60 s (○) emersions. Experimental conditions: 15.00 kV applied voltage, and inlet-to-detector and total capillary lengths of 48.75 *cm* and 75.80 cm, respectively. Standard delay time was employed in all experiments. Standard deviation of the mean associated with each data point is incorporated in the size of the data symbol or is shown as an error bar.

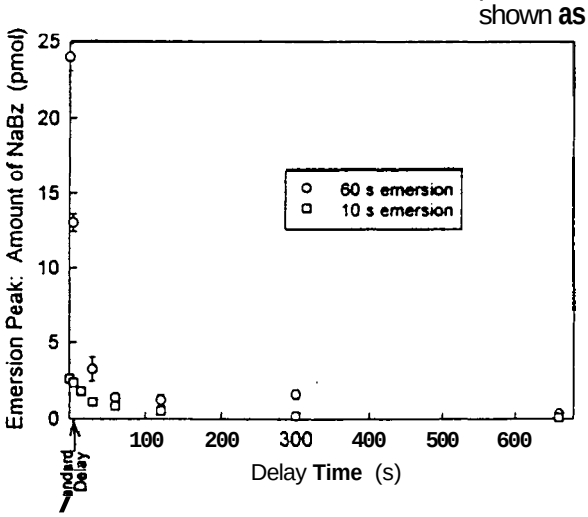

FIGURE 5. Emersion peak, represented as an amount of NaBz as a function of the delay time (that is, the time between replacing the capillary inlet in the electrolyte reservoir following an emersion and the subsequent application of high voltage). ○'s represent results from single, 60 s emersions to a height of 17 mm, with 15.00 kV applied voltage, capillary length 87.30 cm, and inlet-to-detector length 50.20 cm. □'s represent results from single, 10 s emersions to a height of 17 mm, with 15.00 kV applied voltage, capillary length 85.50 cm, and inlet-to-detector length 48.75 cm. Standard deviation of the mean associated with each data point is incorporated in the size of the data symbol or is shown as an error bar.

TABLE I.
SIZE OF EMERSION PEAK AS A FUNCTION OF CAPILLARY CUT

Capillary Cut[a]	Capillary Length (cm)	Inletto-Detector Length (cm)	Emersion Peak: Amount of NaBz[b] (pmol)
a	87.30	50.20	132 ± 0.6 (*N*=13)
b	86.45	49.70	2.2 ± 0.3 (*N*=8)
c	85.50	48.75	9.7 ± 0.5 (*N*=10)

[a] Electrophoresis experiments conducted with cuts a to c of this capillary involved a single emersion of the inlet to a height of 17 mm for 60 s with an applied voltage of 15.00 kV. In all cases, the running electrolyte was 20.0 mM NaBz, the standard delay time was empioyed, and detection was at 225 nm.

[b] Amounts of NaBz represent averages of the number N of replicate experiments indicated in parentheses, expressed ± standard deviation of the mean.

DISCUSSION

It is evident from these results that a narrow zone of enriched electrolyte is somehow being formed at the capillary inlet during emersion, and that this zone is then swept along the column and past the detector by electroosmosis. The result is the appearance of an emersion peak a small but well-defined peak in the electropherogram corresponding to the time of electroosmotic flow, appearing even in the absence of any intentionally (or unintentionalty) loaded sample. Based on our experimental results, the most feasible explanation for the emersion peak phenomenon b the adsorption of benzoate ions at the air-solution interface created at the elevated capillary inlet during emersion.

Increasing the number of emersions, or increasing the duration of any given emersion would provide a greater opportunity for the adsorption of benzoate at the air-solution interface, and for the subsequent transport of the enriched interface into the capillary column by way of spontaneous fluid displacement. Consequently, one would expect the size of the resulting emersion peak to increase with the number and duration of emersions, as illustrated in Figs. 2 & 3. Increasing the emersion height would provide an increased opportunity for fluid displacement by hydrodynamic flow, allowing more of the adsorption-enriched interface to enter the capillary inlet. Hence, the resulting emersion peak size would increase with increasing emersion height, as in Fig. 4. The "standard delay" between the time the capiilary inlet was replaced in the electrolyte reservoir following an emersion and the subsequent application of high voltage was 2 to 5 s. Increasing the delay time increases the opportunity for NaBz to diffuse from the cappillary inlet, thereby decreasing the size of the resulting emersion peak, as seen in Fig. 5. Another factor that appears to affect emersion peak size is the actual inlet itself (its shape, size, surface, properties, etc.). Table I shows that similar emersion experiments conducted with different capillary inlets result in d i r e n t emersion peaks. Different inlets would result in d i r e n t air-solution interfaces and hence, d i i r e n t amounts of adsorbed NaBz.

CONCLUSIONS

Since it is necessary to emerse the capillary inlet prior to any sort of sample injection, an emersion peak could be generated in addition to any sample peaks. Thus, care must be taken to minimize the size of the emersion peak so that it will not lead to quantitative error in is capillary electrophoresis. Emersion peak size could be diminished by reducing the amount of electrolyte adsorbed at the air-solution interface or by limiting the transport of the adsohance-enriched solution into the capillary inlet. At the very least care should be taken to ensure that emersion of the capillary inlet is reproducible (same height, duration, etc.) and that the same delay time exists between emersion and initiation of an electrophoretic separation. In this way, It should be possible to minimize variability in the emersion peak size and, therefore, minimize the impact of emersion peaks on capillary electrophoresis experiments.

BASELINE SHIFT AND SPONTANEOUS MARKER PEAK

INTRODUCTION

Two anomalous capillary electrophoresis (CE) phenomena, referred to as 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, sodium benzoate running electrolyte, and on-column VV absorbance detection. The baseline perturbations, believed to have physical origins at the capillary inlet, are transported along the capillary at the rate of electroosmotic flow.

Spontaneous markers appear to originate at the instant of field application 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. 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 can be accompanied by a field-dependent shift in baseline absorbance. In the case of positive marker signals, increasing the applied field results in a decresse 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 capillary 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." The baseline shifts represent real changes in electrolyte concentration, as confirmed by independent measurements of absorbance, conductivity, and electroosmotic mobility. Baseline shifts and spontaneous markers can cause concentration enhancements or depletions significant enough to warrant consideration if quantitative analysis is to be undertaken.

EXPERIMENTAL

Apparatus, Capillary Columns & Solutions.
(as for " Emersion Peak" experiments)
Procedure.

- no sample injection and no deliberably-formed concentration boundaries
- "Uniform-electrolyte experiment*: application of a constant high voltage across the capillary (initially filled with the same electrotyte solution filling the inlet/outlet reservoirs) for a certain length of time while recording absorbance and current data.
- typically, 2 to 5 minutes (the "standard interlude") elapse with no field applied between experiments.

RESULTS

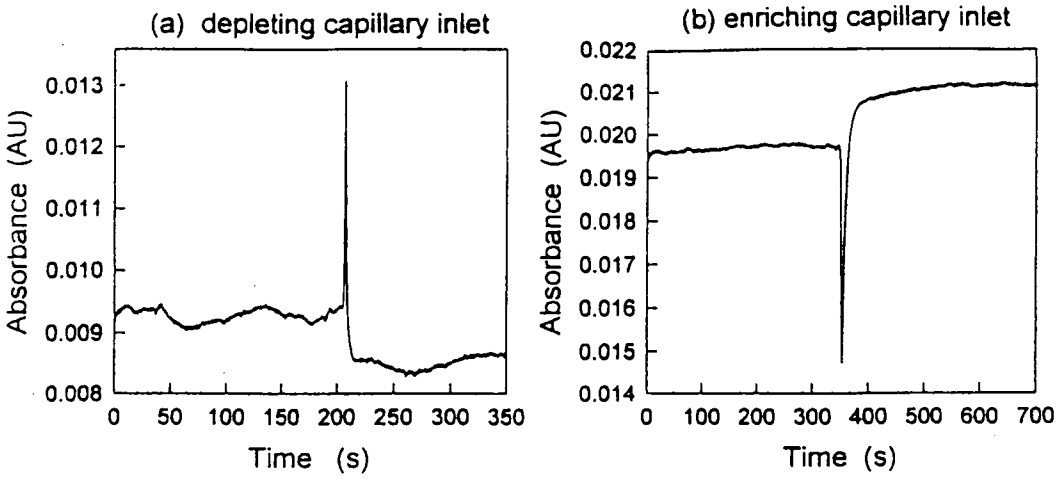

FIGURE 6. Capillary electropherograms showing typical spontaneous marker peaks accompanied by baseline shifts resulting from an increase in the applied field strength for a CE system with no injection, 20.0 mM NaBz original running electrolyte, and 15.03 kV applied voltage (a) A positive spontaneous marker accompanied by a negative baseline shift generated at a depleting inlet by increasing the applied field strength by 87.6 V cm^{-1}. Capillary length: 57.30 cm; inlet-to-detector length: 31.20 cm. (b) A negative spontaneous marker and positive baseline shift generated at an enriching inlet by increasing the applied field strength by 56.7 V cm^{-1}. Capillary length: 88.70 cm; inlet-to-detector length: 30.00 cm.

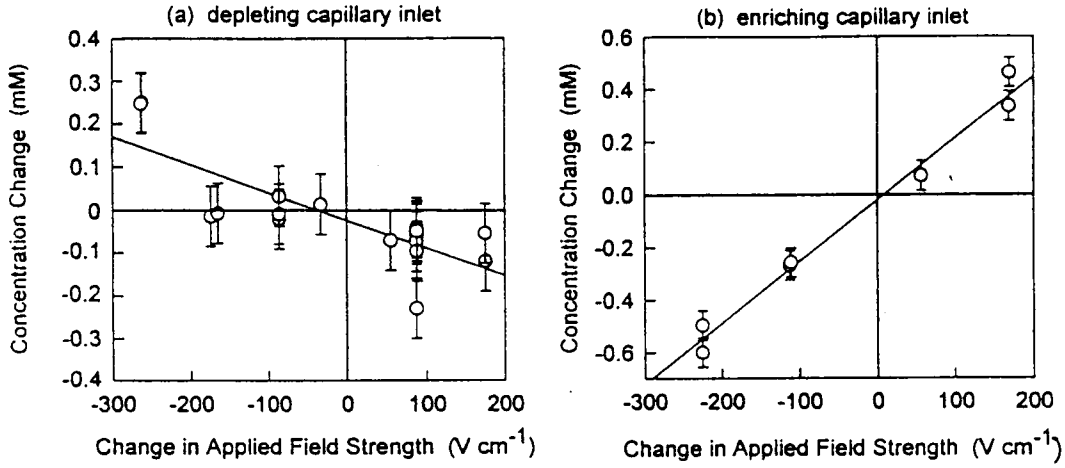

FIGURE 7. The effect of change in applied field on NaBz electrolyte concentration for the (a) depleting capillary inlet, and (b) enriching capillary inlet of Fig. 6. In each case, the solid line represents a linear least squares fit of the data. Error bars are based on the standard error in the ordinate of the least squares fit. Experimental conditions as in Fig. 6.

DISCUSSION & CONCLUSIONS

Depleting capillary inlets somehow acted to deplete the concentration of electrolyte entering the capillary 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 a depleting end, as illustrated in Fig. 6(a). The correlation between baseline shift, expressed as a concentration change, and change in applied field strength for one particular depleting end is shown in Fig. 7(a). The negative slope is indicative of a depleting inlet: as the magnitude of the applied electric field is increased, the concentration of solution entering the capillary from the supply reservoir by electroosmosis is decreased by some as yet unknown mechanism.

In contrast, enriching inlets acted to enhance the concentration of electrolyte entering the capillary, and so 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, as seen in Fig. 6(b). The correlationbetween baseline shift, expressed as a concentration change, and change in applied field strength for one particular enriching end is shown in Fig. 7(b). The positive slope is indicative of an enriching column end: as the magnitude of the applied field is increased, the concentration of solution entering the capillary also increased.

Whereas baseline shifts occurred only when the applied field was changed between runs, spontaneous markers appeared whenever there was a period of time with no field applied (i.e., an "interlude"). The size of spontaneous-marker peaks appeared to depend primarily on the extent of enrichment or depletion and on the length of the no-field interlude. During the interlude, "enriched" electrolyte can diffuse out of an enriching capillary inlet, or the original electrolyte can diffuse into a depleting capillary inlet, thereby generating a negative or positive spontaneous marker, respectively.

In summary, a change in electric field is necessary to generate a baseline shift at an "active" (i.e., enriching or depleting) capillary inlet, while an interlude with no-field applied is necessary to generate a spontaneous-marker peak at an active inlet. Baseline shifts appear to give rise to marker peaks by a diffusive mechanism, but the mechanism by which baseline shifts arise remains unknown.