ELSEVIER

Journal of Chromatography A, 716 (1995) 3–15

JOURNAL OF CHROMATOGRAPHY A

Emersion peaks in capillary electrophoresis

Christa L. Colyer, Keith B. Oldham*

Department of Chemistry, Trent University, Peterborough, Ontario K9J 7B8, Canada

Abstract

An anomalous phenomenon in capillary electrophoresis (CE), referred to as the 'emersion peak,' has been observed. The emersion peak is generated at the inlet end of the capillary whenever this end is temporarily removed from the 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 flow and is detected by on-column UV absorbance. Emersion peaks have been observed in a CE system with a uniform sodium benzoate electrolyte without sample injection or deliberately-formed concentration boundaries, and are attributed to the adsorption of benzoate at the air–solution interface formed upon emersion of the capillary inlet. Emersion peak size has been found to depend on the number of emersions, the duration of each emersion, the height to which the inlet is raised above the supply electrolyte reservoir during an emersion, the delay between completion of the emersion and application of the electric field, and the cut of the capillary forming the inlet end.

1. Introduction

The ability to inject and manipulate extremely small samples in capillary electrophoresis (CE) is, in part, responsible for the widespread use and application of this technique. For example, newly-developed methods of sample injection, such as the slide-type Nano-injector [1] and the Microdrop injector [2] permit reproducible small-volume injections in CE systems. Furthermore, it is now possible to use CE to analyze single biological cells [3–5], or to perform CE on a microchip [6–8]. When dealing with such small samples, the quantitative ability of CE may be compromised. Compensation for sampling biases, in order to improve quantitative precision in CE, has been addressed [9]. However, unintentional sample injection, by way of spontaneous [11] (also referred to as ubiquitous [12]) injection, may still interfere with small samples. Spontaneous injection is facilitated by simply bringing the capillary inlet into contact with (and subsequently withdrawing it from) the sample solution. Grushka and McCormick [12] were the first to investigate this phenomenon, which Dose and Guiochon [10,13] later attributed to diffusion into or out of the capillary inlet. Most recently, Fishman and co-workers [11] more fully characterized spontaneous injection. They proposed that an interfacial pressure difference, formed at the inlet of the capillary upon removal from the sample solution, was responsible for the unintentional injection of sample by way of spontaneous fluid displacement. The intentional use of spontaneous fluid displacement as a method of microcolumn sample injection has also been discussed [14].

However, before the capillary inlet is inserted into and subsequently removed from the sample solution, it is necessary for it to be removed from

* Corresponding author.

0021-9673/95/$09.50
SSDI 0021-9673(95)00312-6

the running electrolyte supply reservoir. We have found that absorbance signals, representing localized enhancements of the running electrolyte concentration and herein referred to as 'emersion peaks,' are generated at the inlet end of the capillary when this end is temporarily removed from the running electrolyte supply reservoir prior to application of the electric field necessary for electrophoresis. When the field is subsequently applied, the emersion peak, which represents a concentration excursion, is swept along the column and past the detector by way of electroosmosis alone in a two-ion electrolyte system. Both electrokinetic and hydrodynamic injections necessitate removal of the capillary inlet from its running electrolyte reservoir, and thus, involve emersion. As such, it is possible that an emersion peak is generated every time a sample is injected, and that this peak may introduce errors if the sample contains a neutral species, because the neutral signal and emersion signal will coincide.

The present work focuses on emersion peaks and does not involve the injection, intentional or otherwise, of a sample. Studies were conducted in a simple CE system with on-column UV absorbance detection and, in most instances, a sodium benzoate solution as the running electrolyte. The size of the emersion peak was found to be dependent on several factors, including: the cut of the capillary inlet, the height, duration and number of emersions, and the delay time between emersion and application of the electric field. During an emersion, an air–solution interface is created at the capillary inlet. We believe that the electrolyte may be positively adsorbed at this interface, and that this enriched interface may enter the capillary by spontaneous fluid displacement [15], thereby generating an emersion peak.

2. Experimental

2.1. Instrumentation

An Isco Model 3850 capillary electropherograph (Lincoln, NE, USA) with on-column UV absorbance detection was employed for all experiments described herein. The high-voltage power supply of this instrument was operated in constant voltage (15–30 kV) mode. Emersion peaks were generated as a result of removing either the capillary end from the running electrolyte reservoir housing the high-voltage electrode (prior to positive polarity experiments), or the capillary end from the electrolyte reservoir housing the grounded electrode (prior to negative polarity experiments). No intentional sample injection was conducted. The humidity and temperature inside the capillary compartment were not closely controlled. However, a fan inside the compartment maintained good ambient circulation, and several small, water-filled beakers in the compartment maintained an atmosphere saturated enough with water vapour to prevent significant evaporation from the running electrolyte reservoirs during any given day of experimentation. The analogue current, voltage and absorbance outputs of the instrument 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. Partial control of the CE system, along with data collection, analysis, and storage, was enabled by HP Basic 3.0 computer programs written in-house.

2.2. Capillary columns

Fused-silica capillaries with an external polyimide coating were employed in these studies (Polymicro Technologies, Phoenix, AZ, USA). Nominal capillary dimensions were 50 μm inside diameter, 156.5 μm wall thickness, 16 μm coating thickness, while measured dimensions ranged from 75.60 cm to 89.25 cm total length, and 28.70 cm to 59.25 cm working length (that is, length between inlet end and detector). Capillary columns were cut to length using a blunt, Isco ceramic capillary cutter. Optical and scanning electron microscopies were used to examine the cuts. Every effort was made to obtain 'clean' cuts (at 90° to the capillary axis, with no silica debris and no polyimide stripping), although each was inevitably unique and could therefore lead to

different air–solution interfaces when removed from the electrolyte reservoir.

New capillaries were subjected to a conditioning procedure in order to develop reproducible electroosmotic flow. Conditioning consisted of filling the capillary with 1.0 *M* NaOH (BDH, Toronto, Canada) for one hour, followed by leaving it filled with 0.10 *M* NaOH overnight. The capillary was then flushed sequentially with distilled, deionized water, 0.10 *M* HCl (Baxter/Canlab, Toronto, Canada), and again with water. Despite this initial treatment, reproducible electroosmotic flow was not achieved until the capillary had undergone several weeks of regular use. The most effective method of maintaining the electroosmotic flow-rate at a stable value appeared to be filling the capillary with water for extended periods of time (at least two days) when not in use. After initial capillary conditioning, we therefore 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 one hour prior to daily experimentation. With the exception of intentional emersions, care was taken to keep the capillary ends wet at all times, by immersion in running electrolyte during experimentation or in water during storage.

2.3. Solutions

The vast majority of emersion peak experiments were conducted with a 20.0 m*M* sodium benzoate (NaBz) solution as the running electrolyte, 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. However, emersion peak experiments were also conducted with several other running electrolytes, all prepared from analytical grade reagents, including 20.0 m*M* potassium benzoate (KBz) (Aldrich, Milwaukee, WI, USA), 20.0 m*M* sodium salicylate (NaSal) (BDH), and 15.0 m*M* trisodium citrate (Na_3Cit) (BDH). The measured pH values of these solutions were 7.01 at 20°C, 5.85 at 24°C, and 8.22 at 25°C, respectively. Although the results are not presented here, the emersion of the capillary inlet from these other electrolytes also resulted in emersion peak formation. Prior to use, all solutions were degassed under vacuum by a water aspirator for approx. one hour after having been filtered through a cellulose acetate syringe filter (pore size 0.45 μm; Nalge Company, Rochester, NY, USA, and Lida, Kenosha, WI, USA).

2.4. Procedure

A 'typical' emersion experiment involved lifting the inlet end of the capillary above the running electrolyte solution in the supply reservoir for a certain period of time before returning it to its original position. The number, height and duration of emersions were sometimes varied. Upon completion of an emersion, two to five seconds passed (the 'standard delay') before application of the electric field. In some instances, this delay time was varied to determine its effect on the size of the emersion peak. Absorbance and current data were recorded while the run was in progress (that is, while the electric field was applied). Following an experiment, two to five minutes (the 'standard interlude') routinely elapsed with no field applied before commencement of the next experiment.

Since these experiments do not involve sample injection, it should be noted that the capillary 'inlet' does not necessarily refer to that end of the capillary physically located near the injection port. 'Inlet' simply refers to the end through which electroosmotic flow carries solution into the capillary. In all cases, emersion peaks resulted from emersion of the capillary inlet, but in some cases (specifically, positive polarity experiments) this involved emersion of the capillary end nearest to the high-voltage electrode and in other cases (specifically, negative polarity experiments) this involved the capillary end nearest to the grounded electrode. The solution reservoir in which the inlet end of the capillary is submerged is called the 'supply reservoir,' while the outlet end is submerged in the 'receiving reservoir'. Care was taken to equalize the solution levels in the two reservoirs, except when hydrodynamic flow was intentionally induced.

3. Results

The initial discovery of an emersion peak occurred by accident following a trimming of the inlet end of an installed capillary column in order to remove a suspected blockage. When the trimmed end was returned to its original position in the 20.0 m*M* NaBz supply electrolyte reservoir, and the electric field necessary for electrophoresis was applied, a prominent absorbance signal appeared in the capillary electropherogram after a time corresponding with that of a marker of electroosmotic flow. The field was then disabled and several minutes were allowed to pass before the field was reapplied. The inlet end of the capillary was not removed from solution in this second instance, and no absorbance signal was observed in the corresponding electropherogram. Thus, it appeared as if the removal of the column end from the supply reservoir, necessitated by the trimming procedure, was somehow responsible for the absorbance signal in the first run. To test the validity of this hypothesis, we conducted experiments employing intentional emersion manoeuvres, including variations of the number of emersions, the duration of the emersion, the 'delay time' (that is, the time elapsed between completion of an emersion and application of the high voltage), the height to which the capillary inlet was lifted during an emersion, the position of the capillary outlet relative to the elevated inlet, the capillary cut, and the concentration of the running electrolyte. Results from each variation of emersion experiment are presented here, and the possible causes of the emersion peak, as inferred from these results, are considered in the Discussion section of this paper.

3.1. Number of emersions

The size of the emersion peak was found to increase with the number of emersions of the capillary inlet. Multiple emersions were conducted by removing the capillary inlet from the supply electrolyte reservoir to some specified height for some specified duration, and then returning the capillary inlet to its original position. Then, within less than two seconds, the inlet was removed again to the same height for the same length of time. This procedure was repeated until the desired number of emersions had been conducted. Typical electropherograms generated after three and five emersions of the inlet end of the capillary are shown in Fig. 1. During each emersion, the capillary inlet was raised 17 mm above the level of solution in the supply reservoir for no more than two seconds. The amounts of NaBz representative of these emersion peaks, along with emersion peaks generated after two and ten emersions of a given capillary inlet, are shown in Fig. 2. For five or fewer emersions, the size of the emersion peak appears to depend linearly on the number of emersions. Peak size levels off, however, after ten emersions, perhaps indicating that some sort of saturation process occurs.

3.2. Duration of emersion

The time spent out of the supply reservoir by an elevated capillary inlet was varied in order to determine its effect on the size of the resulting emersion peak. Increasing the duration of the emersion from two seconds or less to two minutes resulted in a linear increase in emersion peak size, as shown in Fig. 3. These were single

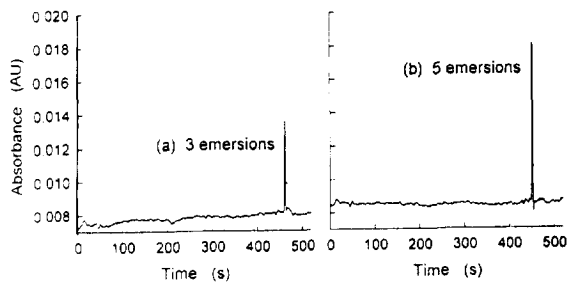

Fig. 1. Capillary electropherograms showing typical emersion peaks resulting from (a) 3 emersions and (b) 5 emersions of the capillary inlet, each to a height of 17 mm above the level of solution in the supply reservoir for ≤ 2 s. Following the emersions, a 2–5 s (standard) delay occurred prior to application of a constant voltage of 24.97 kV across the 89.25 cm long capillary (inlet-to-detector length: 59.25 cm). The original running electrolyte was 20.0 m*M* NaBz and detection was at 225 nm.

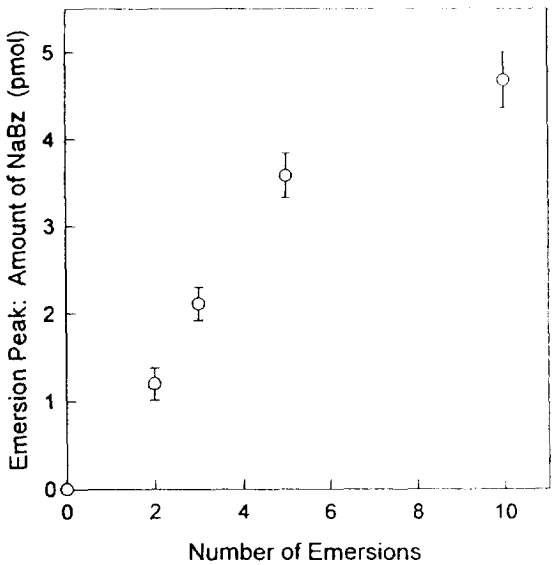

Fig. 2. The amount of NaBz representative of an emersion peak as a function of the number of emersions of the capillary inlet. Error bars are based on propagated error in the calculated amounts of NaBz. Experimental conditions as described in Fig. 1.

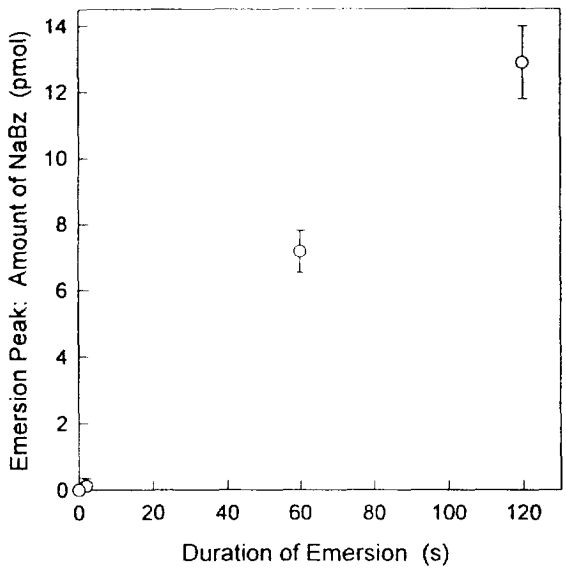

Fig. 3. The amount of NaBz representative of an emersion peak as a function of the period of time during which the capillary inlet was removed from solution during an emersion. Error bars are based on propagated error in the calculated amounts of NaBz. Results are for single emersions to a height of 17 mm, with 20.0 m*M* NaBz running electrolyte, an applied voltage of −25.04 kV, inlet-to-detector and total capillary lengths of 28.70 cm and 82.35 cm, respectively, and detection at 225 nm. Standard delay time was employed in all cases.

emersion experiments, with the capillary inlet raised 17 mm above the level of solution in the supply reservoir. Furthermore, these emersion peak experiments were conducted by emerging the end of the capillary located in the electrolyte reservoir housing the grounded electrode and subsequently applying a negative voltage. Thus, the capillary end located at the grounded side of the apparatus served as the 'inlet' for these experiments. Similar results were obtained when the high-voltage end served as inlet, indicating that the phenomenon of the emersion peak is in no way dependent on some feature of the high-voltage end of the apparatus.

3.3. Delay time

Under normal experimental conditions, a two to five second delay (referred to as the 'standard delay') occurred between the time the capillary inlet was replaced in the electrolyte reservoir following an emersion and the subsequent application of high voltage. Since there is no electric field applied along the column during this delay, there will be no migration or electroosmosis taking place. However, transport of NaBz into or out of the column can occur by diffusion during the delay if a concentration gradient exists at the interface between the solution in the capillary and that in the bulk reservoir [10]. Furthermore, replacing the capillary inlet in the electrolyte reservoir may initiate convective mixing at the interface between the solution in the capillary and that in the bulk reservoir. A study was undertaken to determine the effect of delay time on the size of the emersion peak resulting from 10- and 60-s single emersions. Different capillary inlets were used for the 10- and 60-s emersion experiments. In all cases, the inlet was raised to a height of 17 mm above the level of solution in the supply reservoir during emersion. The amounts of NaBz representative of the resulting emersion peaks are shown as a function of the delay time in Fig. 4. The amount of NaBz

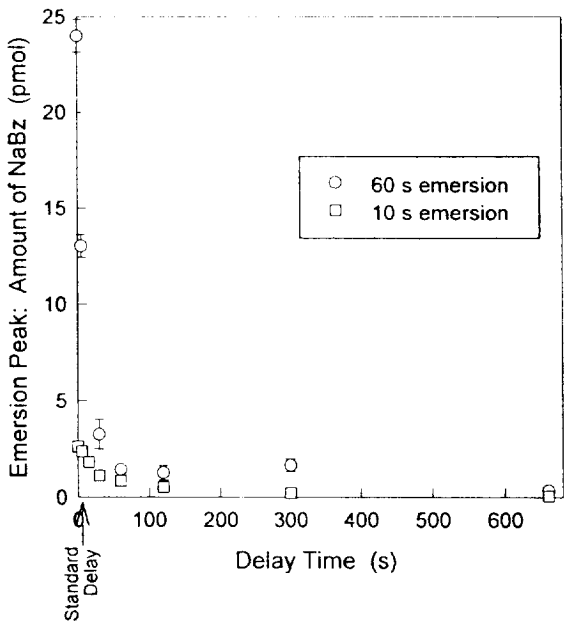

Fig. 4. Emersion peak, represented as an amount of NaBz, as a function of the delay time (that is, the time between replacing the capillary inlet in the electrolyte reservoir following an emersion and the subsequent application of high voltage). ○ = Results from single, 60 s emersions to a height of 17 mm; with 20.02 m*M* NaBz running electrolyte, 15.00 kV applied voltage, capillary length 87.30 cm, inlet-to-detector length 50.20 cm, and detection at 225 nm. □ = Results from single, 10 s emersions to a height of 17 mm; with 20.00 m*M* NaBz, 15.00 kV applied voltage, capillary length 85.50 cm, inlet-to-detector length 48.75 cm and detection at 225 nm. Standard deviation of the mean associated with each data point is less than the size of the data symbol or is shown as an error bar.

in the 10- and 60-s emersion peaks appears to decrease exponentially at short delay times. Regardless of delay time, emersion peaks generated by 60-s emersions are always larger than those generated by emersions of shorter duration (10 s).

3.4. Height of emersion

The capillary positioner of the electropherograph allows the capillary inlet to be lifted to a maximum height of 17 mm above the level of solution in the supply electrolyte reservoir. Thus, in a 'normal' emersion experiment, the inlet is raised 17 mm above the solution for some period of time, as discussed previously. It is possible, however, to emerge the capillary inlet to shorter heights, and it was found that the size of the emersion peak was dependent upon the height of the emersion. For 10- and 60-s single emersions with standard delay times, the effect of emersion height on the amount of NaBz in the emersion peak is shown in Fig. 5. As expected, the emersion peaks resulting from 60-s emersions are larger at every emersion height than those resulting from 10-s emersions. Both durations of emersion, however, gave rise to increasingly large emersion peaks as the height of the emersion increased. For emersion heights below 10 mm, the amount of NaBz in the emersion peak appears to increase linearly with height. Above 10 mm, however, the amount of NaBz in the emersion peak apparently begins to level off. This would indicate that some sort of saturation

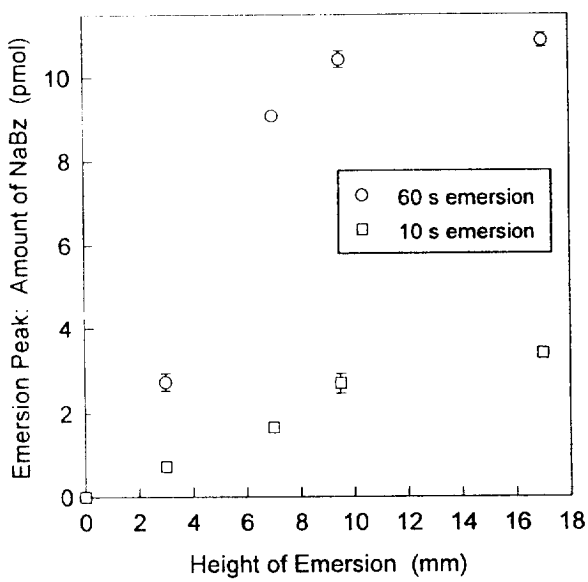

Fig. 5. Emersion peak, represented as an amount of NaBz, as a function of the height to which the capillary inlet was raised above the level of solution in the supply reservoir during single 10 s (□) and 60 s (○) emersions. Experimental conditions: 20.01 m*M* NaBz running electrolyte, 15.00 kV applied voltage, inlet-to-detector and total capillary lengths of 48.75 cm and 75.60 cm, respectively, and detection at 225 nm. Standard delay time was employed in all experiments. Standard deviation of the mean associated with each data point is less than the size of the data symbol or is shown as an error bar.

point or upper limit to the size of an emersion peak was being approached.

3.5. Position of capillary outlet relative to emersed inlet

By emerging the capillary inlet, a gravitational head is created, thereby generating a hydrodynamic flow of solution in the capillary toward the outlet end. The extent of this flow is limited by the surface tension of the solution in the capillary, which prevents solution from receding into the column and leaving behind a gap or slug of air. The size of the gravitational head can be altered not only by altering the height of the emersion, but also by changing the elevation of the capillary outlet, together with the receiving reservoir, during an emersion. Such outlet elevation can 'compensate' for some or all of the inlet elevation, thus reducing the effective height of the emersion. The difference between the elevation of the capillary inlet and the capillary outlet/receiving reservoir is referred to as the 'inlet head'. When the outlet is raised above the inlet, the inlet head is negative. The effect of inlet head on emersion peak size was determined by conducting a set of experiments in which the capillary outlet, together with the receiving reservoir, was raised to varying heights just before the inlet was emersed for 60 s to its maximum height (17 mm) above the level of solution in the supply reservoir. At the end of each emersion, the capillary inlet and outlet were respectively returned to their original positions, and the high voltage applied after the standard delay time had elapsed. The results of these experiments are shown in Fig. 6. For positive inlet heads, the amount of NaBz representative of the emersion peak increases with increasing head, just as it did with increasing emersion height in Fig. 5. The size of an emersion peak for any given positive inlet head in Fig. 6, however, differs from that in Fig. 5 for a comparable emersion height. This apparent discrepancy may be attributed to the fact that the two sets of experiments were conducted one month apart and so some property of the capillary inlet may well have changed due to conditioning, wear, etc. (see section

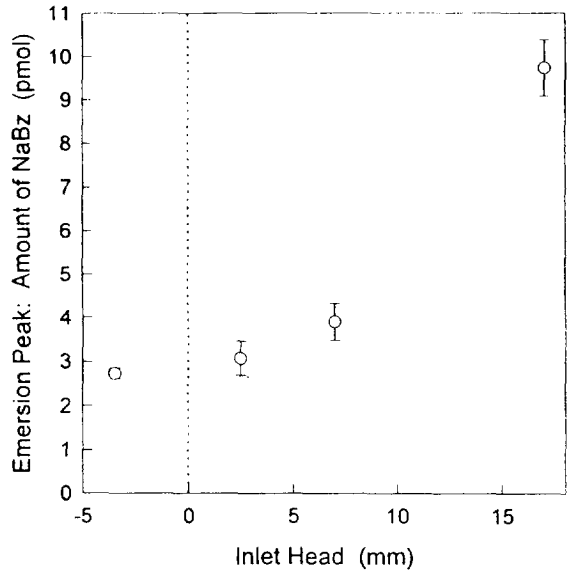

Fig. 6. The amount of NaBz representative of an emersion peak as a function of the inlet head (that is, the difference between the elevation of the capillary inlet and outlet). Error bars are based on the standard deviation of the mean associated with each point. Results are for single, 60 s emersions with inlet height equal to 17 mm in all cases. Capillary length: 85.50 cm and inlet-to-detector length: 48.75 cm. Other experimental conditions are as described in Fig. 5.

entitled Capillary cut). The emersion peak does not disappear when the inlet head is negative; it is simply diminished in size relative to peaks generated from positive inlet heads during emersion. This is in agreement with the findings of Fishman et al. [11] and Grushka and McCormick [12] who found that spontaneous sample injection could occur even when hydrostatic flow is towards the inlet end of the capillary.

3.6. Capillary cut

As mentioned above, the cut of the capillary forming the inlet end can have a significant effect on the size of emersion peaks generated at that end. Optical and scanning electron microscopies were used to determine that the cuts appeared clean, although there was no way to ensure that the wall thickness at all points along the capillary was equal, or that the angle of the cut relative to the capillary axis was exactly right. Hence, each

Table 1
The effect of capillary cut on the amount of NaBz in emersion peaks

Capillary cut[a]	Capillary length (cm)	Inlet-to-detector length (cm)	Emersion peak: amount of NaBz[b] (pmol)
a	87.30	50.20	13.2 ± 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 m*M* NaBz, the standard delay time was employed, 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.

cut exposed a new and different annulus of the capillary to the solution. These different cuts or annuli resulted in different sizes of emersion peaks, as documented for one particular capillary in Table 1. For this capillary, the average amount of NaBz in the peak generated by one, 60 s emersion to a height of 17 mm, ranged from 2.2 (± 0.3) pmol to 13.2 (± 0.6) pmol, depending upon the cut. Even though the height of emersion, the number of emersions, and the duration of emersion were not varied, the size of the resulting emersion peaks in these experiments differed significantly, therefore indicating the importance of the capillary cut.

3.7. Running electrolyte concentration

Although the vast majority of our experiments were conducted using 20.0 m*M* NaBz as the running electrolyte, some involved other electrolyte concentrations. Table 2 shows the effect of running electrolyte concentration on emersion peak size for single emersions of capillary cut 'c' to a height of 17 mm above the level of solution in the supply reservoir. The enhancement, equal to the amount of NaBz calculated from emersion peak size, is an absolute quantity. That is, it is not calculated relative to the running electrolyte concentration, but rather, it represents an absolute increase in the amount of NaBz present. No trend towards increasing or decreasing peak size with increasing electrolyte concentration is evident from the results in Table 2. In fact, within the ranges of uncertainty given by the standard deviations associated with each amount of NaBz, the enhancement represented by the emersion peaks appears to be constant, regardless of running electrolyte concentration.

Table 2
The effect of running electrolyte concentration on the amount of NaBz in emersion peaks resulting from single emersions (10 and 60 s) of capillary cut c to a height of 17 mm

NaBz concentration (m*M*)	Amount of NaBz representative of emersion peaks[a] (pmol)	
	10 s Emersion peak	60 s Emersion peak
10.00	1.9 ± 0.2	7.3 ± 0.2
20.00	2.4 ± 0.2	9.7 ± 0.5
29.99	1.9 ± 0.4	8.6 ± 0.1

Other experimental conditions: 15.00 kV applied voltage, 85.50 cm capillary length, 48.75 cm inlet-to-detector length, standard delay time, and detection at 225 nm.
[a] Amounts of NaBz represent averages of 2 to 10 replicate runs, and are expressed with average deviations or standard deviations of the mean.

4. 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 unintentionally) loaded sample. Based on our experimental results, the most feasible explanation for the emersion peak phenomenon is the adsorption of benzoate ions at the air–solution interface created at the elevated capillary inlet during emersion. It may be thermodynamically favourable [16] for benzoate ions (or other organic anions) to align themselves at the air–solution interface, with sodium counterions (or other cations) close by in the solution, thereby creating a region of 'enriched' sodium benzoate solution. This region would then be 'loaded' into the column by way of spontaneous fluid displacement [15], forming a concentration excursion which would later be swept past the detector by electroosmotic flow, and which would appear as an emersion peak.

It is useful to compare the amount of NaBz representative of a 'typical' emersion peak to the amount which could be adsorbed at a 'typical' air–solution interface in order to determine the feasibility of this mechanism of peak formation. If we assume that a monolayer of benzoate is adsorbed at the air–solution interface, and that each benzoate ion occupies 60 $Å^2$ [17], then the surface concentration of benzoate would be about $3 \cdot 10^{-6}$ mol m^{-2}. If the interface formed is hemispherical (concave inside the capillary), the amount of adsorbed benzoate would be only 0.01 pmol, whereas at a pendant hemispherical interface (extending to the outer capillary wall), the amount of adsorbed benzoate would be 0.6 pmol. Often, the droplet remaining on the capillary inlet as a result of emersion extends beyond the annulus of the capillary [18]. At such a pendant 'extended' hemispherical interface (extending from the annulus along the capillary wall to a distance equal to the previous immersion), the amount of adsorbed benzoate would be 19 pmol. The last two of these proposed geometries for the air–solution interface would allow for sufficient adsorption of benzoate to produce an enhancement 'typical' of an emersion peak, and so adsorption may be a possible explanation for this phenomenon. Furthermore, the last two geometries would require the subsequent action of spontaneous fluid displacement to transport the enhanced electrolyte region inside the capillary inlet. Without spontaneous fluid displacement, the enriched region of solution in the pendant droplet would simply be 'washed away' upon reimmersion of the inlet in the supply electrolyte reservoir, and so no emersion peak would be observed.

According to this proposed mechanism of emersion peak formation, there are three main factors which could affect peak size: the size/shape of the initial air–solution interface; the time during which adsorption can occur at the interface; and the extent to which the enriched interface is transported into the capillary. These three factors could, in turn, be affected by variation of the number, duration, and height of emersions, as well as by variation of the capillary cut and running electrolyte concentration.

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. With repeated emersions, new air–solution interfaces are being generated, and benzoate adsorption may occur at each new interface. Consequently, one would expect the size of the resulting emersion peak to increase with the number of emersions, as illustrated in Fig. 2. However, when more than one emersion is conducted, the duration of each is often brief (<2 s). This brief emersion duration does not provide much time for the adsorbance-enriched electrolyte region to be transported into the capillary by spontaneous fluid displacement. Increasing the duration of any given emersion should provide additional

time for adsorption and/or spontaneous fluid displacement, thus producing a larger emersion peak. Such results were, indeed, observed (see Fig. 3) lending support to the interfacial adsorption hypothesis for emersion peak formation.

Regardless of the duration of emersion, increasing the emersion height would provide a greater driving force for fluid displacement by way of hydrodynamic flow. Such an increased opportunity for fluid displacement would allow more of the adsorption-enriched solution to enter the capillary inlet, and hence, the resulting emersion peak would be larger. Fig. 5 illustrates such an increase in emersion peak size with increasing emersion height.

It has been postulated [11] that increasing the emersion height may also increase evaporation from the capillary inlet, due to removal of the inlet from the humidified region directly above the supply electrolyte reservoir. If, indeed, evaporation from the capillary inlet was occurring, this may be construed as a possible contributor to the emersion peak. At first glance, the evaporation of solvent (water) from the running electrolyte on or just inside the elevated capillary inlet may be thought to leave behind a locally more concentrated region of electrolyte which would be seen as an emersion peak. However, Fishman et al. [11] found that evaporation from the capillary inlet did not simply concentrate the solution present at the inlet, but rather, resulted in solution loss from the inlet. In the present work, such solution loss would be expected to reduce the size of the emersion peak, since the adsorbance-enriched solution surface would be lost to evaporation. Emersion peaks, however, were seen to increase in size as the inlet was removed to greater heights (and supposedly less humid atmospheres) and thus, evaporation does not appear to significantly affect our results.

Furthermore, our study of the position of the capillary outlet relative to the emersed inlet does not indicate any significant variation in the humidity in the capillary compartment (which housed both the capillary inlet and outlet). During these experiments, the capillary inlet was always lifted to the same position (17 mm) above the supply electrolyte reservoir, thereby minimizing any possible variation in humidity. The capillary outlet, however, together with the receiving electrolyte reservoir, was lifted to various heights. This resulted in a change in the 'inlet head,' that is, the difference between the elevation of the capillary inlet and the capillary outlet. Such a change in the inlet head would be expected to change the driving force for hydrodynamic flow and, thus, the extent of transport of the adsorption-enriched interface into the capillary inlet. If evaporation was solely responsible for the formation of the emersion peak, we would expect to see no variation in peak size during these experiments. However, the emersion peak size increased with increasing inlet head (see Fig. 6), suggesting that the transport of the enriched air–solution interfacial region into the capillary inlet is critical to the formation of an emersion peak. Perhaps surprisingly, the emersion peak existed even for negative inlet heads (that is, when hydrodynamic flow would be towards the capillary inlet). Others [11,12] have similarly found that spontaneous sample injection occurs even when there is solution flow towards the capillary inlet. It is reasonable to expect that adsorption of benzoate at the air–solution interface would occur regardless of the direction of solution flow, although the adsorption-enriched region would not be transported into the capillary as readily when solution flow is towards the inlet. The fact that emersion peak size is greatly reduced for negative inlet heads (see Fig. 6) indicates that less of the adsorption-enriched region is finding its way into the capillary. Perhaps some diffusive process or convective mixing is ensuring that some small amount of the enriched solution makes its way into the capillary even without the assistance of hydrodynamic flow or spontaneous fluid displacement.

To further investigate the effects of capillary compartment humidity on emersion peak formation, we intentionally generated a 'dry' atmosphere inside the capillary compartment. This was achieved by replacing the small water-filled beakers normally present in the compartment with dishes of Drierite (BDH): a hygroscopic

$CaSO_4$ compound coated with $CoCl_2$ indicator. The average amount of NaBz representative of emersion peaks was 11.5 (± 2.1) pmol for the 'dry' capillary compartment under the following conditions: single, 60 s emersions to 17 mm above supply electrolyte reservoir; capillary length: 87.30 cm; inlet-to-detector length: 50.20 cm; applied voltage: 15.00 kV. When the Drierite was removed and the small water-filled beakers replaced in the capillary compartment, the average amount of NaBz representative of the subsequently generated emersion peaks was 13.3 (± 1.1) pmol. Although the 'dry' atmosphere resulted in nominally smaller emersion peaks, there is no significant difference between the effects of 'dry' and 'wet' atmospheres on emersion peaks when the standard deviations of the means are considered.

To intentionally cause evaporation from the capillary inlet during some experiments, a stream of dry argon gas (Praxair, Mississauga, Canada) was directed at the capillary just above the inlet during emersion. All other conditions were the same as for the emersion experiments described above with water-filled beakers in the capillary compartment. The average size of emersion peaks generated with Ar flowing (two experiments) was only 7.4 (± 0.2) pmol, compared to 13.3 (± 1.1) pmol without Ar. This significant difference appears to support the idea of solution loss (as opposed to solution concentration) due to evaporation, thereby resulting in the diminution of emersion peak size. Of course, typical variations in humidity that may occur in our capillary compartment would never be as extreme as the effect of a direct stream of dry Ar gas, and so we may consider the effects of 'normal' evaporation on our emersion peaks to be minimal.

Another factor that appears to affect emersion peak size is the actual inlet itself (its shape, size, surface properties, etc.). This effect is confirmed by Cohen and Grushka [19], who have discussed the influence of capillary cut on separation efficiency and peak shape. Table 1 shows that similar emersion experiments conducted with different capillary inlets result in different emersion peaks. Factors such as surface morphology, wettability, outside diameter, and angle of cut can modify the air–solution interface at the capillary inlet [11]. If adsorption of benzoate at the air–solution interface is indeed responsible for the formation of the emersion peak, then it is reasonable to expect to see variation in emersion peak size with variation, however subtle, of the capillary inlet. Optical and scanning electron microscopies have confirmed that each cut of the capillary is unique and may consequently lead to unique air–solution interfaces upon emersion.

Finally, our study of the effect of running electrolyte concentration on emersion peak size provides further support for interfacial adsorption as the mechanism of emersion peak formation. Virtually identical emersion peaks were obtained for emersion experiments conducted with sodium benzoate solutions ranging from 10.0 to 30.0 m*M* (see Table 2). Under moderate conditions, the size and shape of the air–solution interface formed at the capillary inlet should be almost independent of running electrolyte concentration. Thus, the amount of benzoate which can be adsorbed at that interface should be independent of running electrolyte concentration: different electrolyte concentrations simply represent different 'pools' from which the same maximum amount of surface-adsorbed benzoate is supplied. If the extent of surface adsorption is the same, then the resulting emersion peaks will be the same despite different running electrolyte concentrations, as was observed experimentally. These results support the idea of surface adsorption as a possible mechanism of emersion peak formation.

5. Conclusions

The existence of absorbance signals, representing localized enhancements of the running electrolyte concentration and referred to as 'emersion peaks,' has been documented. Emersion peaks are generated at the capillary inlet provided the inlet is removed from solution for some brief period of time before commencing the electrophoretic run. Emersion peaks repre-

sent concentration excursions which travel electroosmotically from the inlet and past the detector. The mechanism of emersion peak formation is believed to be the adsorption of the electrolyte anion (benzoate in these experiments) at the air–solution interface generated at the capillary inlet during emersion. Prior to application of the electric field for electrophoresis, the surface-enriched region of solution is transported into the capillary by way of spontaneous fluid displacement or hydrodynamic flow. Factors which affect the properties of the air–solution interface were shown to affect the emersion peak. These include the number of emersions, the duration of each emersion, the height to which the capillary inlet is lifted above the supply electrolyte reservoir during an emersion, and the capillary cut. In addition, emersion peak size was found to depend on the delay time between replacement of the capillary inlet in the supply electrolyte reservoir following an emersion and the subsequent application of the electric field. Evaporation which may occur under typical ambient conditions did not significantly affect emersion peak size.

Since it is necessary to emerge the capillary inlet prior to any sort of sample injection, an emersion peak would be generated in addition to any sample peaks in routine capillary electrophoresis. The emersion peak would coincide with the absorbance signal of any neutral sample component. Even in the absence of any neutrals, the emersion peak would appear at a time corresponding to electroosmotic flow and thus, could be mistaken as a bona fide neutral component of the sample. Thus, care must be taken to minimize the size of the emersion peak so that it will not lead to quantitative errors in capillary electrophoresis. Emersion peaks could be eliminated by designing strategies for sample injection that do not require emersion, or could be diminished by reducing the amount of electrolyte adsorbed at the air–solution interface or by limiting the transport of the adsorbance-enriched solution into the capillary inlet. Reducing the amount of adsorbed electrolyte could be achieved by minimizing either the duration or number of emersions, or by minimizing the size of the air–solution interface (for example, by using capillaries with smaller outside diameters). Limiting the transport of the adsorbance-enriched solution into the capillary could be achieved by minimizing either the duration of the emersion or the height of the emersion (to minimize the contribution of forward hydrodynamic flow to the transport of the enriched solution). Also, countering spontaneous fluid displacement with hydrodynamic flow in the opposite direction (by creating, for example, a negative inlet head) would limit transport of the enriched solution into the capillary. At the very least, care should be taken to ensure that emersion of the capillary inlet is reproducible (same height, duration, etc.), that the inlet itself remains unchanged, 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.

Acknowledgements

We thank Dr. Artjom Sokirko of the Trent University Chemistry Department for many helpful discussions regarding this work. Also, we would like to acknowledge the generous financial support of the I.O.D.E., the Trent-Queen's Graduate Program in Chemistry, and the Natural Sciences and Engineering Research Council of Canada (NSERC).

References

[1] T. Hanai and J. Tsuruta, Instrum. Sci. Technol., 22 (1994) 151.
[2] D. Sziele, O. Brüggemann, M. Döring, R. Freitag and K. Schügerl, J. Chromatogr. A, 669 (1994) 254.
[3] T.M. Olefirowicz and A.G. Ewing, Chimia, 45 (1991) 106.
[4] A.G. Ewing, J. Neurosci. Meth., 48 (1993) 215.
[5] H.K. Kristensen, Y.Y. Lau and A.G. Ewing, J. Neurosci. Meth., 51 (1994) 183.

[6] D.J. Harrison, K. Fluri, K. Seiler, Z. Fan, C.S. Effenhauser and A. Manz, Science, 261 (1993) 895.
[7] K. Seller, Z.H. Fan, K. Fluri and D.J. Harrison, Anal. Chem., 66 (1994) 3485.
[8] A. Manz, E. Verpoorte, C.S. Effenhauser, N. Burggraf, D.E. Raymond and H.M. Widmer, Fresenius J. Anal. Chem., 348 (1994) 567.
[9] T.T. Lee and E.S. Yeung, Anal. Chem., 64 (1992) 1226.
[10] E.V. Dose and G. Guiochon, Anal. Chem., 64 (1992) 123.
[11] H.A. Fishman, N.M. Amudi, T.T. Lee, R.H. Scheller and R.N. Zare, Anal. Chem., 66 (1994) 2318.
[12] E. Grushka and R.M. McCormick, J. Chromatogr., 471 (1989) 421.
[13] E.V. Dose and G.A. Guiochon, Anal. Chem., 63 (1991) 1063.
[14] H.A. Fishman, R.H. Scheller and R.N. Zare, J. Chromatogr. A, 680 (1994) 99.
[15] A. Marmur, in M.E. Schrader and G.I. Loeb (Editors), Modern Approaches to Wettability: Theory and Applications, Plenum Press, New York, 1992, Ch. 12.
[16] N.K. Adam, The Physics and Chemistry of Surfaces, Oxford University Press, London, 3rd ed., 1941, Ch. III.
[17] E.G. Lewars, Optimized geometry calculation by Spartan SGI Version 3.1.3GL.
[18] H.A. Fishman, personal communication, Dec. 1994.
[19] N. Cohen and E. Grushka, J. Chromatogr. A, 684 (1994) 323.