Use of an On-Column Fracture in Capillary Zone Electrophoresis for Sample Introduction

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INTRODUCTION

Capillary zone electrophoresis (CZE) is an analytical separation technique noted by its high resolution and small sample volume (*1*). A major problem in CZE is precise introduction of the small sample volume (~10 nL) into the capillary (*2*). Several different methods of injection have been developed for CZE. These techniques include electrokinetic and hydrodynamic methods. Electrokinetic injection works by applying a potential across the column, and sample is introduced by using electroosmotic flow and electromigration. Due to these properties, electrokinetic injection is biased in how it introduces differently charged analytes. Various hydrodynamic methods utilize a pressure gradient between the inlet and outlet ends of the capillary (*2–4*). Hydrodynamic methods require computer-controlled pressure or height adjustment systems to achieve adequate precision of injection. A simple alternative is to use the electroosmotic flow in the capillary as a micropump and pump the sample into the capillary. By using only the electroosmotic flow as a pump, no electromigration of analytes occurs and introduction of sample is no longer biased.

This method requires an electrical connection before the inlet end so that it is free of applied electrical field. Ewing (*5*) and Zare (*6*) have utilized porous glass and glass frits, respectively, to create an electrical connection on-column. These connections were made so that the outlet end of the capillary would be free for electrochemical detection or sample collection systems. We have made a similar electrical connection through a fracture in the column. However, instead of pushing the sample out the end of the column as Ewing and Zare have done, we apply the same principle to pull the sample into the capillary. The on-column fracture allows for ions and, therefore, current to pass but does not allow a significant quantity of sample to pass through. When a potential is applied between the fracture and the outlet end of the capillary, electroosmotic flow pulls sample into the capillary through the inlet. This approach is analogous to a syringe; the short segment of capillary acts as the needle and the long segment as the barrel. Thus, we have made an "electroosmotic syringe". The amount of sample introduced is proportional to the electroosmotic flow. Therefore, the magnitude of voltage applied for a fixed period of time will determine the quantity injected.

EXPERIMENTAL SECTION

Construction of On-Column Fracture. Three millimeters of the polyimide coating was burned off 5 cm from the end of a 55-cm-long fused silica capillary (Polymicro Technologies, Phoenix, AZ) having an inside diameter of 75 μm and outside diameter of 360 μm. Capillaries with thick walls (142.5 μm) were used because their strength made the fracture easier to construct. The capillary was glued to a 2-cm × 1-cm microscope slide with epoxy glue (SIG Mfg. Co., Montezuma, IA) so that the exposed fused silica was positioned 1 mm above the surface of the glass slide. By using a capillary glass cutter (Supelco, Bellefonte, PA), a small scratch is made on the top of the capillary. The capillary is then pushed up gently from the bottom, directly under the scratch, with a pointed stylist therby producing the fracture.

The fracture assembly is then placed in a 5-mL Teflon vial with a 1-mm hole drilled in the bottom. The short end of the capillary is pushed through a rubber septum that plugs the hole in the bottom of the vial. The capillary is cut so that the fracture is only 2–3 cm from the inlet end, and the total length of the capillary is 50 cm. The vial is filled with buffer solution, and a cap with a 1-mm hole is screwed on top. A 22-gauge platinum wire electrode is inserted through a small hole in the cap. This apparatus is shown in Figure 1.

Instrumentation. The CZE system was based on an in-house design. All high-voltage components of the system were contained in a Lucite cabinet fitted with a safety interlock that would interrupt the power supply when the cabinet door was opened. A Spellman RHR 30P 60/EI (Spellman High Voltage Electronics Corp., Plainsview, NY) power supply was used to apply the electric field across the capillary. The power supply was connected to 22-gauge platinum wire electrodes inserted in the appropriate 3-mL buffer reservoirs. On-column detection was performed at 254 nm with a modified BAS UV-8 detector (Bioanalytical Systems, West Lafayette, IN). Data acquisition was achieved by using either a PC, with Inject software, or a RYT recorder (Bioanalytical Systems, West Lafayette, IN). In the determination of possible leakage from the fracture, a Hewlett-Packard 8450 UV/vis spectrophotometer was used.

Sample Introduction Procedures. During injections, the power supply was connected to the electrodes in the fracture assembly and the outlet reservoirs. The inlet end of the capillary was placed into the sample vial. Reproducibility and concentration studies were done with 3 kV (64 V/cm) applied for 30 s. Sample injections were timed by using a stopwatch. Inlet and outlet ends were placed at the same height to avoid siphoning. After injection, the positive electrode was transferred from the fracture reservoir to the inlet end buffer reservoir. During run conditions, the power supply was connected to the electrodes in the inlet and the outlet reservoirs, and 15 kV (300 V/cm) was applied across the entire capillary. The positive electrode was always either in the fracture assembly or inlet end buffer reservoir.

Reagents. Electrophoresis buffers were either 10 mM phosphate (Aldrich, Milwaukee, WI), pH 7.1, or 10 mM Trizma (Sigma, St. Louis, MO), pH 8.8. These were prepared by using in-house double distilled deionized water and were filtered by using 0.22-μm Nylon filters (Bioanalytical Systems, West Lafayette, IN). Mesityl oxide and adenosine monophosphate (AMP), both from Aldrich, were used for reproducibility and characterization studies.

RESULTS AND DISCUSSION

Characterization of Capillaries with On-Column Fractures. It was necessary to determine that the fracture in the capillary does not significantly affect the operation of the system. When electrophoresed from the inlet to the outlet, comparison of identical capillaries except for the presence or absence of a fracture showed no significant difference in column stability (electroosmotic flow), current, or efficiency (as seen by comparable peak width and shape). When the capillaries with the fracture are run at high voltages (20–30 kV) from the fracture to the outlet, there is a slight increase in current (<5%). This is attributed to lower resistance due to the shorter length of capillary. The electroosmotic flow was compared between identical capillaries with and without fractures. By using a weighing method developed by Altrich and Simpson (*7*), the electroosmotic flow was determined to be nearly identical (<3%). In this experiment, the column was arranged so the fracture allowed for the outlet to be free of applied electric field. The positive electrode was at the inlet end and the negative electrode was at the fracture reservoir, providing a 47-cm segment of capillary with applied potential and 3 cm of capillary at the end free of potential. This arrangement allowed for the buffer to be collected during electrophoresis. After 30 min, the collected buffer was weighed.

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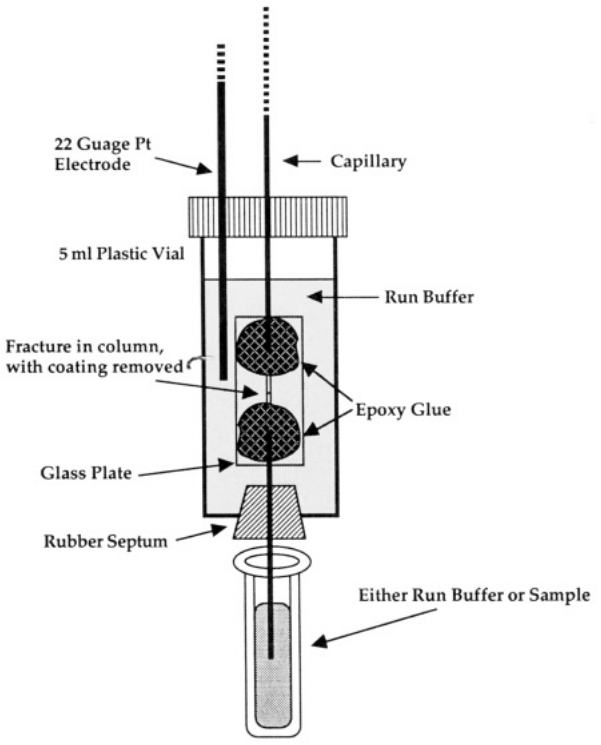

Figure 1. Fracture assembly and buffer reservoir.

In order to show that this method is valid, we needed to establish that the fracture did not leak. Both Ewing and Zare used porous glass or glass frits for their electrical connection, and no detrimental leaking problems were encountered. In this technique, the fracture created is very fine, and the two sections of capillary are never separated. The fracture allows for a charge to pass between the capillary and the buffer solution, but since the fracture is so small, minimal bulk flow through the fracture is expected. In order to determine if the fracture leaked, the capillary was arranged so that the outlet end would be free of applied electric field. Mesityl oxide (5%) in buffer was electrophoresed through for 1 h at a flow rate of 8 nL/s by applying a voltage of 300 V/cm across the capillary. The buffer–mesityl oxide was collected at the outlet end in a small vial. The sample collected was diluted to the same volume as the fracture reservoir. These two solutions were quantitated by UV absorbance. In addition, the capillary was switched so that the inlet was free of applied electric field, and the same 5% mesityl oxide–buffer was electrophoresed through for 1 h. The fracture reservoir was quantitated by UV absorbance and compared to the other two samples. These results are illustrated in Figure 2. These results encourage us to believe that the fracture does not leak to any significant amount and is an effective simple way of creating an electrical connection in a capillary.

Reproducibility. The peak areas of consecutive injections of a neutral marker (mesityl oxide) were achieved with 2.4% (n = 16) and 2.0% (n = 13) RSD at pH 7.1 and 8.8, respectively. Migration times were achieved with 0.3% (n = 16) RSD at pH 7.1. The capillary was conditioned for 5 min prior to each set of runs with 1 M NaOH, water, and run buffer. Additionally, the column was electrophoresed for 10 min to assure a steady electroosmotic flow. Since the quantity injected is proportional to the amount of electroosmotic flow, any slight change in electroosmotic flow would cause loss of precision. Uncoated fused silica capillaries, such as those used in these studies, do not have extremely stable or reproducible electroosmotic flow after running analytes that can adsorb to the surface. It has been proposed that cleaning the column with sodium hydroxide after each run will restore the capillary

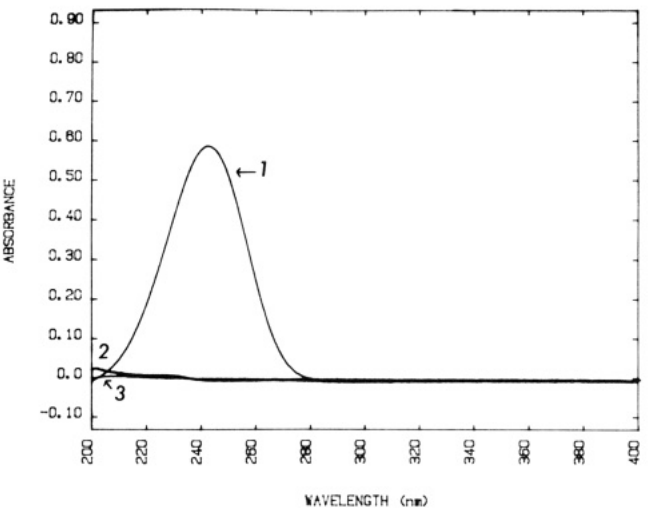

Figure 2. UV/vis absorbance of buffer reservoirs during leak test: (1) mesityl oxide that passed through column, (2) fracture reservoir when outlet is free of applied electric field, (3) fracture reservoir when inlet is free of applied electric field.

back to its original state (*8*). After repeatedly washing the column with 1 M NaOH, water, and buffer, the electroosmotic flow fluctuates and the column has difficulty returning to the original state. This effect has been described as the hysteresis of fused silica with acid and base (*9*). Therefore, separations producing slight changes in the characteristics of the capillary surface would require an internal standard to be used with this method. However, the other injection methods mentioned previously also require an internal standard to maximize precision.

We found a linear relationship (R^2 = 0.9977) between the amount of sample injected and length of time voltage was applied. Lower voltages for longer times produced better precision. Peak areas of triplicate injections, 0.24 mM AMP, at 3 kV were plotted against injection times ranging from 20 to 60 s. Different concentrations of AMP (0.12, 0.24, 0.35 mM) were injected for 30 s at 3 kV. A linear relationship of peak height versus concentration (R^2 = 0.9972), with the expected intercept at the origin, was obtained. Relative peak areas of samples with multiple components were identical within the limits of precision of the injection.

CONCLUSIONS

By fracturing the capillary and applying an electrical potential between the fracture and outlet, we have created an electroosmotic syringe. This technique can be used as an effective method for introducing small volumes of sample quantitatively into a capillary. It can be easily automated or manually controlled. In addition to uncoated fused silica capillaries, this method also works with bonded phases and surfactant coatings that still exhibit electroosmotic flow. A column coated with C-18/Brij 35 showed identical precision as uncoated capillaries. The simple fracture can also be used as a general method to separate the column into two segments. A capillary utilizing a fracture to separate the column and creating a segment of capillary with no applied electric field will allow for sample collection, electrochemical detection, or other detection methods. It appears that the glass frits or porous Vycor junctions used previously may be unnecessary.

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Determination of Halocarbons in Drinking Water by Direct Aqueous Injection Gas Chromatography

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INTRODUCTION

Recently, the determination of halocarbons in drinking water has become an important item in environmental monitoring. For the analysis of halocarbons, a common method requires the use of a gas chromatograph equipped with an electron capture detector (ECD). Other techniques that have been in use are purge and trap (P&T) (*1*), liquid–liquid extraction (LLE) (*2*, *3*), headspace (*4–6*), and direct aqueous injection (*7*, *8*). These methods have different advantages and shortcomings. For example, purge and trap is sensitive and accurate enough to detect organic compounds, but the device is complicated and analysis time is longer. Liquid–liquid extraction is simpler, but the extract is easily contaminated and background may affect accuracy. Headspace has avoided some shortcomings in the two aforementioned methods, but in water samples of different chemical composition and salt content, the distribution coefficients are varied and will affect the accuracy (*9*).

This paper describes the characteristics of direct aqueous injection (DAI) and its actual application. This method is easy to perform. On the one hand, it has sufficient accuracy, good precision, and high sensitivity for 0.4–1-μL injections. On the other hand, its operation does not affect the ECD. With direct aqueous injection on column, the separation of water from halocarbons requires a special column. By using a porous polymer (GDX-103) as the support, 1% SE-30 is coated on the GDX-103 polymer beads. The column is much better than an OV-101 column (Chromosorb W HP DMCS as support) and provides satisfactory results.

EXPERIMENTAL SECTION

Chemicals and Apparatus. The following compounds were analyzed: (1) methanol, (2) chloroform, (3) tetrachloromethanol, and (4) $Na_2S_2O_3 \cdot 5H_2O$. Bromodichloromethane, chlorodibromomethane, and bromoform were of chromatography grade. The pure water used in this study did not contain halocarbons.

The gas chromatograph was a Hewlett-Packard 5890A fitted with an electron capture detector. Chromatograms were recorded with a Hewlett-Packard 3392A reporting integrator. High-purity nitrogen was used as the carrier gas, and the gas line was equipped with moisture and oxygen traps.

Procedures. A standard mixture containing five halocarbons (see Table I) was prepared: Methanol was used as the solvent for the stock solution, while pure water was used to prepare the dilute test solution.

Sampling and Analysis. First, about 10 mg of sodium thiosulfate was added to the sample to remove residual chlorine in the tap water. Second, the tap was opened and run until a steady stream was achieved, and the sample container was then filled to a point short of overflowing. The tube was capped and shaken until the thiosulfate was dissolved. The water sample was then analyzed by the indicated GC conditions. Injection volume was 0.4–1 μL. Quantitative computation was based on peak areas. Samples were compared with standards run under the same conditions by using the HP 3392A integrator.

Table I. Standard Mixture of Halocarbons in Water[a]

amount, μg/L				
$CHCl_3$	CCl_4	$CHBrCl_2$	$CHBr_2Cl$	$CHBr_3$
12	2	16	4	20

[a] Each component concentration in the standard mixture of five carbons was close to that in the drinking water samples.

Table II. Accuracy of the Method (% Recovery)

	% recovery				
method	$CHCl_3$	CCl_4	$CHBrCl_2$	$CHBr_2Cl$	$CHBr_3$
DAI	105	99	102	110	99
LLE (EPA)	110		125	94	114
P&T (EPA)	102	81	101	98	89

Table III. Precision of the Method (Relative Standard Deviation (%))

	rel std dev, %				
method	$CHCl_3$	CCl_4	$CHBrCl_2$	$CHBr_2Cl$	$CHBr_3$
DAI (0.4 μL)	5.3	6.2	4.1	10.8	2.1
DAI (1.0 μL)	2.4	7.4	2.7	3.1	9.9
LLE (EPA)	11		1.4	9.9	1.2
P&T (EPA)	0.6	25.6	5	6.5	9

Table IV. Detection Limits[a]

limit, μg/L				
$CHCl_3$	CCl_4	$CHBrCl_2$	$CHBr_2Cl$	$CHBr_3$
1.2	0.4	1.0	0.9	2.6

[a] Range 16.

RESULTS AND DISCUSSION

Selection of the Column. In DAI, a key problem was rapid elution of water. This problem would affect the separation of water from trihalomethanes and the analytical accuracy. In headspace and other methods, the stationary phases that have been used in separation of halocarbons are SE-30, OV-101, squalane, SP-1000, SP-2100, OV-11, etc., and the support is either Chromosorb or Carbopack. A column of OV-101 on Chromosorb W HP DMCS can separate water from trihalomethanes, but the effect is not ideal (see Figure 1). The column packing used for this work was SE-30 on

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