

# On-Line Coupling of Microdialysis Sampling with Microchip-Based Capillary Electrophoresis

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**Microdialysis sampling is a technique that has been used for in vivo and in vitro monitoring of compounds of pharmaceutical, biomedical, and environmental interest. The coupling of a commercially available microdialysis probe to a microchip-based capillary electrophoresis (CE) system is described. A continuously flowing dialysate stream from a microdialysis probe was introduced into the microchip, and discrete injections were achieved using a valveless gating approach. The effect of the applied voltage and microdialysis flow rate on device performance was investigated. It was found that the peak area varied linearly with the applied voltage. Higher voltages led to lower peak response but faster separations. Perfusion flow rates of 0.8 and 1.0  $\mu\text{L}/\text{min}$  were found to provide optimal performance. The on-line microdialysis/microchip CE system was used to monitor the hydrolysis of fluorescein mono- $\beta$ -D-galactopyranoside (FMG) by  $\beta$ -D-galactosidase. A decrease of the FMG substrate with an increase in the fluorescein product was observed. The temporal resolution of the device, which is dependent on the CE separation time, was 30 s. To the best of our knowledge, this is the first reported coupling of a microdialysis sampling probe to a microchip capillary electrophoresis device.**

Microdialysis sampling is a technique that has been employed for continuous monitoring of compounds of biological and environmental interest both in vitro and in vivo.<sup>1</sup> As only analytes of low molecular weight can cross the semipermeable microdialysis membrane, recovered samples are free of large molecules such as proteins. Therefore, microdialysis can be used as both a sampling technique and a cleanup procedure.<sup>2–4</sup> Microdialysis samples are normally analyzed using methods such as liquid chromatography or capillary electrophoresis with electrochemical, fluorescence, or mass spectrometric detection. Analysis can be performed either on-line or off-line. With off-line analysis, collected fractions must fulfill the injection volume requirements of the

analysis system and contain enough liquid to physically manipulate the sample. Slow flow rates (0.1–2.0  $\mu\text{L}/\text{min}$ ) are generally preferred in microdialysis sampling, especially for trace analytes, due to higher analyte recoveries across the probe membrane.<sup>5</sup> When submicroliter perfusion flow rates are used, temporal resolution is limited by the lengthy collection times needed to obtain enough sample for analysis. In those cases, sample dilution is sometimes used to increase the sample volume for injection, which may affect detection sensitivity.

Capillary electrophoresis (CE) is an ideal separation method for the analysis of microdialysis samples.<sup>6–10</sup> At high field strengths, very fast, highly efficient CE separations are possible. As only nanoliters of sample are injected, the direct coupling of microdialysis to capillary electrophoresis permits the use of low perfusion flow rates. On-line coupling of microdialysis sampling with conventional capillary electrophoresis has been accomplished by our group<sup>11,12</sup> and others<sup>13–15</sup> with the best temporal resolution (12 s) achieved by Kennedy and co-workers.<sup>16</sup> For most on-line systems, temporal resolution has been limited by the separation time. In the case of very fast separations, equilibration of the microdialysis probe with the sample can be the limiting factor.<sup>16</sup> Both injection valves and flow-gated interfaces have been used to connect microdialysis to capillary electrophoresis.<sup>11,13</sup> In general, these systems are fairly large, which confines their application to benchtop analyses. In addition, tubing and connectors that can contribute to increased lag times and band broadening are required.

Coupling microdialysis to microchip systems is an attractive alternative to conventional analytical systems. Microchip platforms have characteristically small sample and reagent requirements

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with fast analysis times.<sup>17–20</sup> It is also possible to incorporate sample preparation and derivatization steps onto a planar chip format, minimizing the number of external connections required.<sup>21–24</sup> In addition, because lithographic methods are used to pattern the microchip, the same design can be mass produced. Microdialysis has been used as a sample introduction technique for flow injection analysis on-chip,<sup>25–29</sup> but to date, the coupling of a microdialysis probe to a separation-based miniaturized analysis system has not been reported.

An important requirement for on-line coupling of microdialysis sampling to microchip CE is that the device be able to accommodate the continuously flowing microdialysate. To accomplish this, the sample stream must enter and exit the device without disturbing the separation. Furthermore, the device must be able to discretely inject picoliter portions from this flowing sample stream into the separation channel for analysis. Harrison's group was the first to develop and evaluate intricate design manifolds to allow the introduction of large-volume samples into microfluidic devices.<sup>30</sup> Their design integrated a large sample introduction channel (SIC) with low flow resistance, allowing real-world samples to enter and flow through the chip. Channels of much smaller dimensions connected to the SIC were used for the electrophoresis separation. Their small dimensions made these channels highly resistant to fluid flow so that hydrodynamically pumped sample (at flow rates on the order of 1.0 mL/min) preferentially flowed through the SIC. A portion of this sample flow entered the smaller channels where a double T injector was used for sample injection into the separation channel. However, this approach is not compatible with microdialysis sampling, which typically uses flow rates in the 0.1–2  $\mu$ L/min range. A further limitation of the design was sample leakage from the injection channel to the separation channel. This was minimized by a series of flushing steps prior to injection and separation; however, this increased the overall analysis time, which would decrease temporal resolution. In addition, voltage control over more than one reservoir was needed.

An alternative approach to inject discrete samples from a continuously flowing stream was recently reported by Chen's group. A simple microchip CE design was employed that permitted flow-through sampling without perturbing the separation.<sup>31,32</sup> A plug of the hydrodynamically pumped sample flow was introduced into the separation channel using a gated injection scheme. Voltage was applied to a single reservoir and floated for 1–5 s to allow discrete sample injection prior to separation. Using this approach, better temporal resolution can be achieved over designs utilizing a double T injector as the time needed to fill the injection channel is eliminated. The microchip format has the added advantage that the flow-gated interface is built into the chip, eliminating some of the excess tubing and connectors needed for conventional systems. An in-depth overview of sample introduction techniques to microchip systems has recently been published.<sup>33</sup>

In this report, the direct coupling of a commercially available microdialysis probe to a microchip capillary electrophoresis device is described. Initial evaluation of the device employed fluorescein as a model analyte. The effect of microdialysis flow rate and applied voltages on performance of the device was examined. The ability of the device to sense a concentration change was evaluated. Finally, in vitro monitoring of the hydrolysis of fluorescein mono- $\beta$ -D-galactopyranoside (FMG) by  $\beta$ -D-galactosidase ( $\beta$ -gal) was accomplished using the microdialysis/microchip CE system. To the best of our knowledge, this is the first report coupling microdialysis sampling to microchip capillary electrophoresis for near-real-time monitoring of a biochemical process. Further integration of additional on-chip functions such as derivatization and detection could lead to portable separation-based sensors for pharmaceutical and biomedical applications.

## EXPERIMENTAL SECTION

**Chemicals and Reagents.** Disodium fluorescein 2-hydrate, sodium hydroxide,  $\beta$ -Gal, and FMG were purchased from Sigma (St. Louis, MO). Sodium phosphate (dibasic, heptahydrate), hydrochloric acid, sulfuric acid, hydrogen peroxide, and isopropyl alcohol were from Fisher Scientific (Fair Lawn, NJ). Nanopure water was obtained from a Labconco water filtration system (Kansas City, MO). All solutions were filtered using 0.22- $\mu$ m Cameo Teflon syringe cartridges from Osmonics, Inc. (Minnetonka, MN).

**Fabrication of the Microchip Device.** The microchip was fabricated in-house using standard photolithographic techniques. The microchip design was drawn using Microsoft Freehand 8.0 software, and a negative mask transparency was produced by Laser Graphics (Lawrence, KS). The mask was then placed on top of a piece of soda lime glass with predeposited layers of chrome and AZ1500 photoresist (Telic, Santa Monica, CA) and exposed to UV light for 15 s. The exposed glass was placed in AZ developer (Clariant Corp., Sommerville, NJ) for 3 min, rinsed with Nanopure water, and baked at 95 °C for 10 min before being placed in chrome etchant (CR-7S, Cyantek Corp., Fremont, CA) for 2 min to remove the exposed layer of chrome. The glass was then placed in a temperature-controlled, filtered circulating etch

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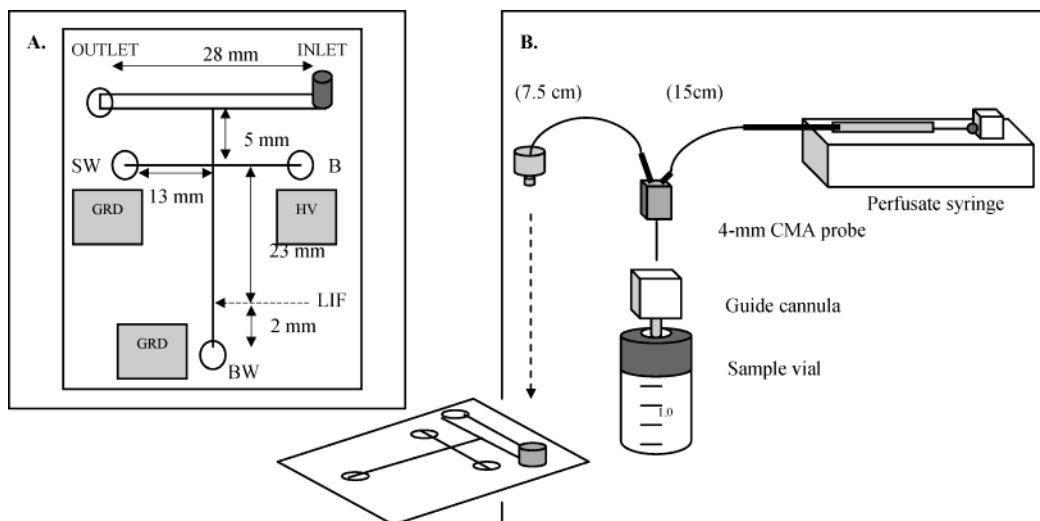


Figure 1. (A) Layout of the microdialysis/microchip CE device. The channel lengths are shown and the depths are described in the text. The voltage scheme is also represented. (B) Schematic of the microdialysis/microchip CE setup. Perfusate (20 mM sodium phosphate buffer, pH 7.4) was pumped into the 4-mm CMA microdialysis brain probe using a syringe pump. The probe was inserted into the guide cannula, which was pierced into the septum of the vial lid. Samples were continuously collected using the probe and were directly introduced into the chip. For the syringe sampling, a syringe filled with fluorescein disodium salt sample was directly connected to the chip device.

bath (Modutek, San Jose, CA) of buffered oxide etchant from Fox Scientific (Alvarado, TX) for 40 min. At each 10-min interval, the glass was taken out of the bath and dipped into a solution of 1 M hydrochloric acid, rinsed with water, and dried with a filtered stream of  $N_2$  gas to minimize the buildup of crystalline precipitates in the channels.<sup>34</sup> Once the etching of the channels was complete, the photoresist and chrome layers were removed using acetone and chrome stripper, respectively. The etch profiles were measured using a Tencor Alpha Step 200 profilometer (San Jose, CA). The separation channels were determined to be  $\sim 90 \mu\text{m}$  wide at the top of the channel and  $\sim 35 \mu\text{m}$  wide at the bottom, with a depth of  $\sim 20 \mu\text{m}$ . The sampling channel was 2 mm wide and  $\sim 20 \mu\text{m}$  deep. Channel dimensions are indicated in Figure 1A.

A cover plate was prepared by drilling access holes into another piece of soda lime glass using a 1.5-mm-diameter diamond drill bit (Jules Borel, Kansas City, MO) and a Dremel tool (Ace Hardware, Lawrence, KS). Prior to bonding, the cover and channel plates were placed in a piranha bath consisting of 7 parts sulfuric acid and 3 parts hydrogen peroxide for 30 min. The two halves were then placed in a second bath composed of water, hydrogen peroxide, and ammonium hydroxide (5:2:2) for another 30 min. The two plates were then rinsed thoroughly with water and dried with  $N_2$  gas. The substrate surfaces were then brought in contact with a few drops of water, and the assembled glass plates were placed in a Fisher Scientific Isotemp programmable oven for bonding. The following program was used for bonding: (1) ramp to 500 °C at 10 °C/min, hold for 30 min; (2) ramp to 580 °C at 2 °C/min, hold for 1 h; (3) ramp to 650 °C at 2 °C/min and hold for 4 h; (4) ramp down to 450 °C at 2 °C/min; (5) ramp to 25 °C at 10 °C/min.

Once the chip was bonded, the surface was cleaned with isopropyl alcohol. A 2-mm-thick layer of cured poly(dimethylsiloxane) (PDMS; Ellsworth Adhesives, Germantown, WI) containing prepunched access holes with a diameter of  $\sim 3.5$  mm was

irreversibly sealed to the surface of the glass chip by plasma oxidation (PDC-32G plasma cleaner/oxidizer, Harrick Scientific, Ossining, NY). A low-dead-volume Microtight union (P-720) from Upchurch Scientific (Oak Harbor, WA) was cut in half using a Dremel tool, and one of the halves was glued to the PDMS layer of the microchip device using Loctite Plastix adhesive (Ace Hardware). A male fitting (F-125, Upchurch Scientific) containing polyetheretherketone (PEEK) tubing (0.65-mm o.d. and 0.12-mm i.d. (Bioanalytical Systems, West Lafayette, IN) could be threaded into the halved Microtight union piece and served as a physical interface between the microdialysis tubing and microchip. The layer of PDMS served to increase the reservoir volume as well as provide a layer to which the halved Microtight union was adhered. The combined dead volume of the interface connector, which includes the 1.5-mm-diameter access hole drilled into the 1.5-mm-thick glass, was  $\sim 2.1 \mu\text{L}$ . PEEK and fluorinated ethylene propylene (FEP) (0.65-mm o.d. and 0.12-mm i.d.) microdialysis tubing and tubing connectors (MD-1510) were purchased from Bioanalytical Systems.

**Microdialysis Sampling Setup.** Two sampling configurations were used in our experiments: (1) direct connection to a syringe (no probe in the system) and (2) a 4-mm CMA/12 microdialysis brain probe (CMA Microdialysis, North Chelmsford, MA) submerged in an amber 2-mL vial (Fisher Scientific) containing sample (Figure 1B). For the microdialysis/microchip CE device studies, the brain probe was inserted into a guide cannula that had been placed into a 4-mm piece of FEP tubing. The FEP tubing was used to prevent pinching of the cannula and probe. This encased probe was then pushed through the septum of the vial lid, and Plastix glue was applied around the opening to hold the fixture in place. The sample vial containing the probe was placed as close as possible to the microchip device. A syringe pump (MD-1001, Bioanalytical Systems) operating at flow rates between 0.2 and 1.0  $\mu\text{L}/\text{min}$  was used to pump the perfusate solution (20 mM sodium phosphate buffer, pH 7.4) through the probe. Fifteen centimeters of FEP tubing was used to connect the syringe pump

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to the probe. The total length of tubing between the probe and the chip interface was 5 cm; 5.0 cm of FEP was interfaced with 2.5 cm of PEEK tubing with tubing connectors. The 2.5-cm piece of PEEK tubing was inserted into the Microtight male fitting to provide fluid transfer into the microchip device. The rigid PEEK tubing could be easily manipulated into the Microtight fitting, and a few drops of Plastix glue were used to keep the tubing in place. In the direct syringe sampling mode, the microdialysis probe was replaced by the syringe sampling system.

**Detector Setup.** The epifluorescence LIF detection setup has been described elsewhere.<sup>35</sup> Briefly, three mirrors were used to direct a beam from a 25-mW argon ion laser (Omnichrome, Chino, CA) into an optics cage that contained a dichroic mirror (505DRLP, Omega Optical, Brattleboro, VT). Irises were used to align the incoming beam into the optics cage apparatus. The dichroic mirror directed the beam 90° toward an X–Y translatable objective. A long working distance objective (NA = 0.6, 40× LWD Plan Fluorite PCPLFL, Olympus America, Melville, NY) focused the laser beam to a spot size of ~30 μm on the separation channel. The microchip was positioned on a platform secured to an X–Y–Z translator (Thor Labs, Newton, NJ). Fluorescence was collected by the same microscope objective, passed through the dichroic, and redirected 90° with a mirror into a photomultiplier tube (R1527, Hamamatsu USA, Bridgewater, NJ). Spectral filtering and polarizing film were used before the beam entered the optics cage and before the collected fluorescence reached the PMT. The analog signal was electronically filtered with a Stanford Systems preamplifier (Sunnyvale, CA) and converted using a data acquisition system that interfaced with a PC and Chrom Perfect Spirit software from Justice Systems (Melbourne, FL).

**Microchip CE Conditions.** A 10 mM stock solution of fluorescein disodium salt was prepared in water and diluted with buffer to the appropriate concentration for the experiments. Sodium phosphate buffer (20 mM) was prepared and adjusted to a pH of 7.4 with 1 N NaOH. All buffer and sample solutions were sonicated and filtered with 0.22-μm syringe filters prior to use. At the beginning of each day, the chip was rinsed with 0.1 N NaOH for 15 min, followed by a buffer rinse for 15 min, and then electrophoresed for ~10 min at an applied voltage of 4000 V. Microdialysis probes were stored in vials of water and perfused with water overnight at a flow rate of 0.5 μL/min prior to connection to the microchip device.

Platinum electrodes were placed into the buffer, buffer waste, and sample waste reservoirs of the microchip to serve as electrical contacts to the high-voltage power supplies. The voltage scheme employed for introducing sample into the device is shown in Figure 1A. The injection method for operation of the chip device was based on a flow-through gated scheme described elsewhere.<sup>31</sup> Briefly, fluid sampled through the probe (or directly from the syringe) entered the wide sampling channel through connective fittings (Figure 1B). The sampling channel is designed to direct the majority of flow to the outlet while introducing a fraction of this stream to the injector T. As the sample stream enters the microchip injection T, buffer pumped electroosmotically from the buffer reservoir directs the sample flow away from the separation channel to the sample waste reservoir. To introduce discrete

sample plugs into the separation channel, the voltage applied at the buffer reservoir was floated for 1 s. The voltage was then resumed to allow simultaneous separation and sample gating.

A positive-polarity eight-channel high-voltage power supply (Jenway, Essex, England) controlled by a Toshiba laptop computer was used to apply voltage. Voltages between 3 and 5 kV were applied to the buffer reservoir only. As described by Seiler et al., Kirchhoff's rules were used to calculate field strengths of the separation channel.<sup>36</sup> The values were calculated to be 313, 366, 418, 470, and 522 V/cm for the applied voltages of 3000, 3500, 4000, 4500, and 5000 V, respectively.

**Optimization of Device Parameters.** The microdialysis sampling system was equilibrated for at least 15 min at the desired flow rate before connecting to the microchip. Voltage and flow rate experiments were carried out to determine the optimal conditions for the operation of the chip device. The applied voltage was systematically varied from 3000 to 4500 V at 500-V intervals while keeping the flow rate constant at 0.5 μL/min. This experiment was carried out for both microdialysis sampling and direct sampling from the syringe pump. In both cases, the sample consisted of 100 μM disodium fluorescein prepared in phosphate buffer. To determine the effect of flow rate on the response of the device, the syringe pump flow rate was varied from 0.2 to 1.0 μL/min at a constant voltage of 4000 V. Electrophoresis run times for optimization of flow rate and voltage experiments were 40 s. Stirring was used for optimization studies with microdialysis probe sampling to aid in analyte mass transport across the probe but was found to be unnecessary for subsequent experiments.

**Fluorescence Microscope Imaging of Flow Profiles at the Microchip Injector.** To visually inspect the effect of voltage on the flow profile at the injector T, fluorescence microscope images were taken. A solution of 200 μM fluorescein in a syringe was continuously pumped at a constant flow rate of 0.8 μL/min through a 45-cm piece of FEP tubing. This tubing was connected to a 1.5-cm piece of PEEK tubing, inserted into the male fitting, and threaded into the union interface of the microchip. The chip was placed on the stage of the fluorescence microscope and flow profiles at the injector T at various voltages were then imaged. Fluorescence images of flow profiles were taken using an Axiolab fluorescence microscope (Zeiss USA, Thornwood, NY) connected to a RGB AutomatiCam CCD camera from MicroImage (Boyer-town, PA). The data from the CCD camera were then processed using Scion Image software (Scion Corp., Frederick, MD) on a PC. The 5× microscope objective was used for images taken, resulting in an overall magnification of 50×. The buffer used in these experiments was 20 mM boric acid, pH 9.2.

**Evaluation of Device Response and Lag Time.** The response and lag time of the microdialysis/CE microchip were evaluated by moving the microdialysis probe from a vial of 50 μM to a vial of 100 μM disodium fluorescein solution in phosphate buffer. The 50 μM fluorescein sample injections were equilibrated after 10 min of the experiment, and at ~18 min, the vial was replaced with the 100 μM solution. A 20 mM sodium phosphate solution, pH 7.4, was used as the run buffer and perfusate; the microdialysis flow rate was 1.0 μL/min, and the applied voltage was 4000 V.

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**On-Line Monitoring of an Enzyme Assay.** The time course of the hydrolysis of fluorescein mono- $\beta$ -D-galactopyranoside by  $\beta$ -D-galactosidase<sup>37</sup> was monitored using the on-line microdialysis/microchip CE system. In these studies, a solution containing 440  $\mu$ M FMG substrate dissolved in 20 mM phosphate buffer, pH 7.4, was initially sampled for over 10 min. Serial injections were conducted until a baseline response was achieved. After  $\sim$ 650 s, the vial lid was removed and 41 units of  $\beta$ -Gal enzyme was added to the vial and mixed using an Eppendorf pipet. The cap was then replaced and the serial injections resumed. A 1.0  $\mu$ L/min flow rate, 5000 V applied voltage, and 30 s of analysis time were used in these experiments. Both the electrophoresis run buffer and perfusate consisted of 20 mM sodium phosphate, pH 7.4.

## RESULTS AND DISCUSSION

Microdialysis was successfully coupled to microchip capillary electrophoresis using the device shown in Figure 1. The chip used in this work was based on initial reports by Chen and co-workers,<sup>31,32</sup> who used a similar design for the study of protein affinity interactions but did not use a microdialysis probe. Their experiments were performed using low field strengths (100–250 V/cm, total applied voltages between 500 and 2000 V) and relatively high flow rates (2–7  $\mu$ L/min). The higher flow rates employed by Chen could produce ultrafiltration through the probe.<sup>38,39</sup> To employ this design for microdialysis sampling, the application of higher field strengths and lower flow rates was investigated. A step change was then conducted to evaluate system response. Last, the on-line microdialysis/microchip CE device was used to monitor an enzyme reaction.

**Effect of Voltage on Sample Injection and Device Performance.** It is important for the CE separation to be as fast as possible to maximize temporal resolution. At higher field strengths, both analysis speed and separation efficiencies increase. For this reason, the effect of the separation voltage on peak height and the separation efficiency was investigated. These experiments employed disodium fluorescein as the model compound. To determine the effect of the microdialysis probe on the system performance, the response obtained for fluorescein sampled through a microdialysis probe and pumped directly from a syringe was monitored separately.

Sample was injected using the sequence shown in Figure 1 and described in the Experimental Section. For initial experiments, a flow rate of 0.5  $\mu$ L/min and a separation voltage of 4000 V (418 V/cm) were used. Figure 2 shows sequential injections of 100  $\mu$ M fluorescein dissolved in phosphate buffer sampled through the microdialysis probe. The reproducibility was good with an RSD of 3.3% ( $n = 5$  injections).

At a constant sample introduction flow rate, the effect of separation voltage on the peak height of fluorescein was then investigated (Figure 3). As the voltage was increased from 3000 to 4500 V, the peak height decreased by almost 40% (see Table 1). The RSDs for the peak height ranged from 3.3 to 5.6% ( $n = 5$ ) depending on the voltage. Although the overall peak height decreased with higher voltages, peak efficiencies and sampling rates (and therefore temporal resolution) were improved, as evidenced by the decreasing peak widths and retention times

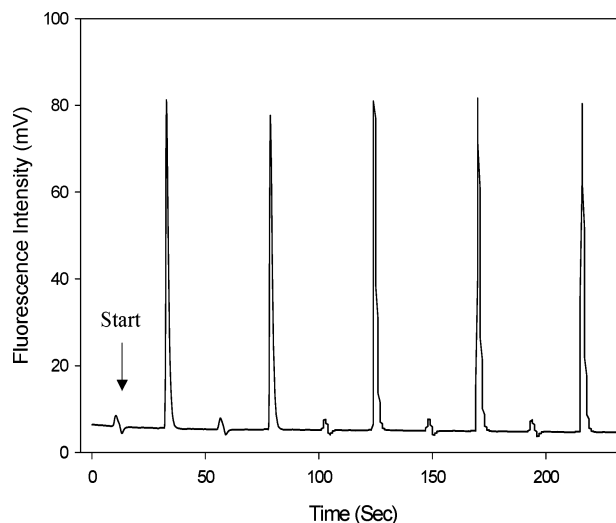


Figure 2. Sequential injections of 100  $\mu$ M disodium fluorescein sampled from a microdialysis probe. The applied voltage was 4000 V (418 V/cm) at a flow rate of 0.5  $\mu$ L/min.

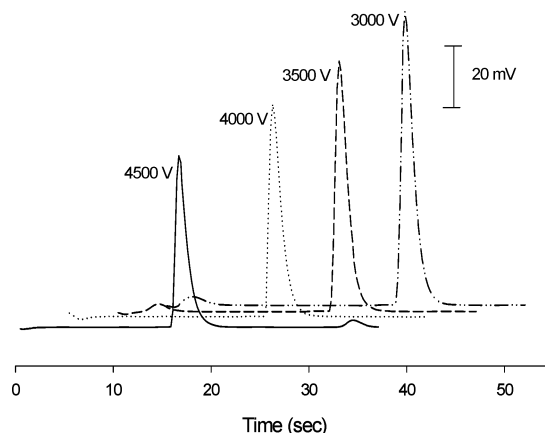


Figure 3. Comparison of 100  $\mu$ M disodium fluorescein peak injections at 4500, 4000, 3500, and 3000 V with probe sampling. The flow rate used was 0.5  $\mu$ L/min. 4000, 3500, and 3000 V traces are offset 5, 10, and 15 s, respectively.

Table 1. Effect of Voltage on Retention Time, Peak Width, and Peak Height of 100  $\mu$ M Fluorescein Sampled through the Microdialysis Probe<sup>a</sup>

voltage (V)	retention time (s)	peak width at 10% (s)	peak height (mV)
4500	16.02 $\pm$ 0.06	1.274 $\pm$ 0.005	61.6 $\pm$ 2.8
4000	19.56 $\pm$ 0.23	1.298 $\pm$ 0.008	74.9 $\pm$ 2.5
3500	21.69 $\pm$ 0.65	1.328 $\pm$ 0.011	90.9 $\pm$ 5.1
3000	23.57 $\pm$ 0.91	1.370 $\pm$ 0.007	104.1 $\pm$ 3.5

<sup>a</sup> The sample introduction flow rate used was 0.5  $\mu$ L/min.

shown in Table 1. It appears that as the voltage applied to the buffer reservoir is increased, the volume of sample at the injection T is effectively reduced. Thus, a smaller amount of sample is injected into the separation channel, leading to the decreased peak heights. This concept is illustrated in a separate experiment where images of the injection T were taken using a fluorescence microscope. At a constant flow rate of 0.8  $\mu$ L/min, the flow profile was monitored at 300, 1500, 3000, and 4000 V (Figure 4). With increasing voltage, the hydrodynamically pumped sample stream

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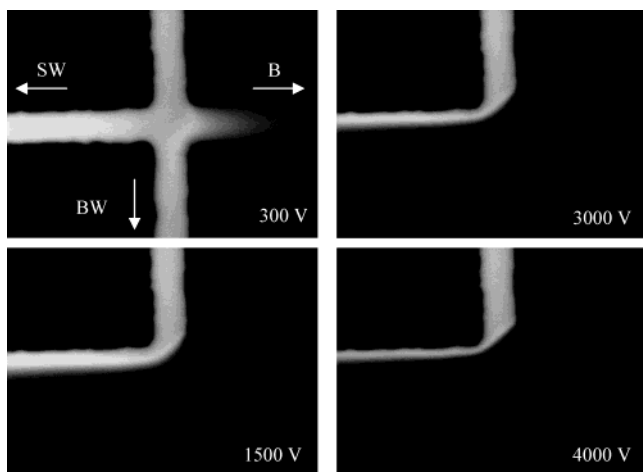


Figure 4. Fluorescence microscope images of flow profiles at the microchip injector T. In these images, a syringe of 200  $\mu\text{M}$  fluorescein pumped at a flow rate of 0.8  $\mu\text{L}/\text{min}$ . The buffer used for these images was 20 mM boric acid, pH 9.2. Buffer reservoirs are indicated by the following abbreviations: SW, sample waste; B, buffer; and BW, buffer waste. Arrows indicate the corresponding direction of each of the chip reservoirs.

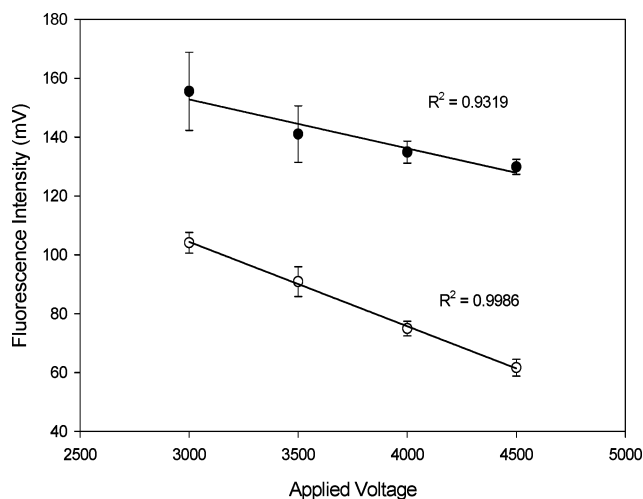


Figure 5. Comparison of 100  $\mu\text{M}$  disodium fluorescein peak injections ( $n = 5$ ) at 4500, 4000, 3500, and 3000 V. (●) denotes injections sampled from the syringe and (○) from the probe. The flow rate was kept constant at 0.5  $\mu\text{L}/\text{min}$ .

is reduced at the injector. This results in smaller sample plugs when the voltage is floated to facilitate injection.

The peak heights for the injections of 100  $\mu\text{M}$  disodium fluorescein sampled through the microdialysis probe were then plotted as a function of voltage. The response was linear with a correlation coefficient of 0.9986. A similar experiment was carried out with sampling directly from a syringe containing 100  $\mu\text{M}$  disodium fluorescein. This also gave a linear dependence of peak height versus voltage ( $r^2 = 0.9319$ ) although the correlation coefficient was not as good. Both lines are plotted in Figure 5. Since sample analyte recoveries through the microdialysis probe are typically less than 100% for moderate to high flow rates, a higher detector response for the direct syringe sampling was expected.<sup>40,41</sup> Using the relative peak height values at 4000 V, the recovery for fluorescein was calculated to be 55.5%.

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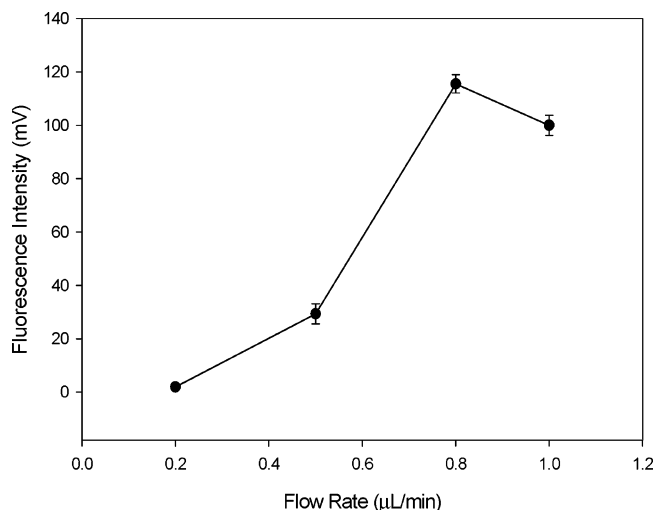


Figure 6. Effect of sample introduction flow rate on peak height. Flow rates of 0.2, 0.5, 0.8, and 1.0  $\mu\text{L}/\text{min}$  were used. The sample was a solution of 100  $\mu\text{M}$  disodium fluorescein, and the voltage was kept constant at 4000 V.

#### Effect of Flow Rate on Sample Injection and Device Performance.

Flow rate is an important parameter in microdialysis sampling, influencing analyte recovery through the probe. Recovery increases with decreasing flow rate, leading to higher analyte concentrations. Recoveries close to 100% have been reported at flow rates of 100 nL/min or less, obviating the need to correct sample concentrations for recovery.<sup>5</sup> The effect of flow rate on the microdialysis/microchip CE system was investigated using a 100  $\mu\text{M}$  disodium fluorescein solution in phosphate buffer. The solution was sampled through the microdialysis probe, and the response for fluorescein was determined at flow rates of 0.2, 0.5, 0.8, and 1.0  $\mu\text{L}/\text{min}$ . For this experiment, the applied voltage was kept constant at 4000 V.

It was found that the slower flow rates led to decreased peak heights (see Figure 6). This observation can be explained in terms of the chip design. For gated injections employing a simple T design, two high-voltage power supplies are typically needed to establish two separate electroosmotically pumped streams.<sup>42</sup> In the flow-through design that was used for this work, one stream is pumped hydrodynamically, and therefore, only one power supply is needed. The extent of hydrodynamic pumping, which is controlled by the flow rate, influences the amount of sample available to enter the T. At a constant voltage, reducing the flow rate results in less sample being pumped into the injection T. As can be seen in Figure 6, flow rates between 0.8 and 1.0  $\mu\text{L}/\text{min}$  resulted in the best detector response for fluorescein injections. Although lower flow rates typically lead to greater recoveries for most microdialysis investigations, it was found that in this case it contributed to a reduced detector response.

**On-Line Microdialysis/Microchip Capillary Electrophoresis.** The direct coupling of microdialysis sampling and microchip CE yields a separation-based sensor that can be used for on-line monitoring of enzyme reactions and in vivo release of neurotransmitters. The ultimate goal of these on-line devices is to provide

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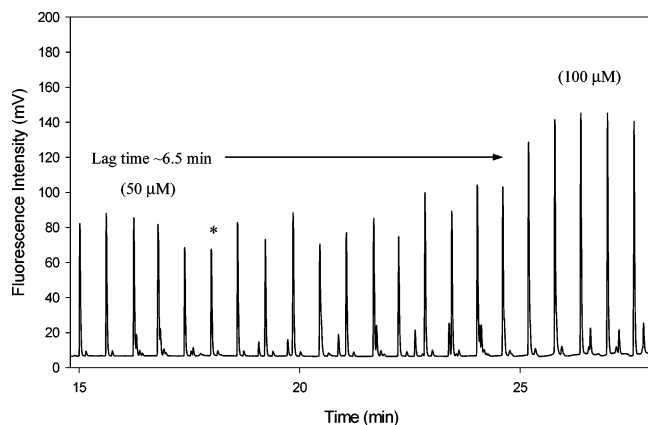


Figure 7. System response evaluation of microdialysis/microchip CE device. A step change experiment with disodium fluorescein was conducted. The concentration of fluorescein was changed from 50 to 100  $\mu\text{M}$ ; the asterisk represents the time at which the concentration increase was initiated. The flow rate used was 1.0  $\mu\text{L}/\text{min}$ , the applied voltage was 4000 V, and an analysis time of 35 s was used.

near-real-time monitoring with excellent temporal resolution. The actual temporal resolution needed is dependent on the type of experimental system that is being monitored. For example, pharmacokinetic studies can take several hours while in vivo release of neurotransmitters can occur on a second-to-subsecond time scale. The fast separations that can be achieved using microchip CE can accommodate studies requiring subminute resolution. Using the developed on-line system described, a temporal resolution on the order of 30 s was achieved.

The ability of this microdialysis/microchip CE device to sense a change in concentration was also evaluated. To study this, the microdialysis probe was initially inserted into a vial of 50  $\mu\text{M}$  fluorescein. Figure 7 shows sequential electropherograms that monitored the step change when the fluorescein concentration was increased from 50 to 100  $\mu\text{M}$ . Equilibrated injections of 50  $\mu\text{M}$  fluorescein sampled through the probe gave an average peak height of 73.2 mV, and injections of 100  $\mu\text{M}$  fluorescein resulted in an average of 136.3 mV. The RSDs were 15 ( $n = 25$ ) and 2.5% ( $n = 4$ ), respectively.

There are a few factors that may have contributed to the peak height variability in the step change experiment, including buffer depletion and a decrease in the magnitude of electroosmotic flow over the time course. However, it is thought that the primary cause may be the syringe pump. Slight flow rate variation can occur when operating at low flow rates ( $<1 \mu\text{L}/\text{min}$ ), resulting in response fluctuations that could be detected by the analytical system. Future studies will investigate this issue.

The system lag time, defined as the time it takes the analyte to be transferred from the probe to the injection T on the device prior to separation, was 6.5 min. The amount of tubing used to connect the microdialysis probe to the chip was 7.5 cm. The corresponding volume associated with that amount of tubing was found to be 0.9  $\mu\text{L}$ .<sup>43</sup> Since the flow rate used in this experiment was 1.0  $\mu\text{L}/\text{min}$ , the lag time attributed to the microdialysis tubing is less than 1 min. Therefore, the device dimensions prior to the injection T were the major contributors to the lag time of the system. Last, the device response time was examined. The time

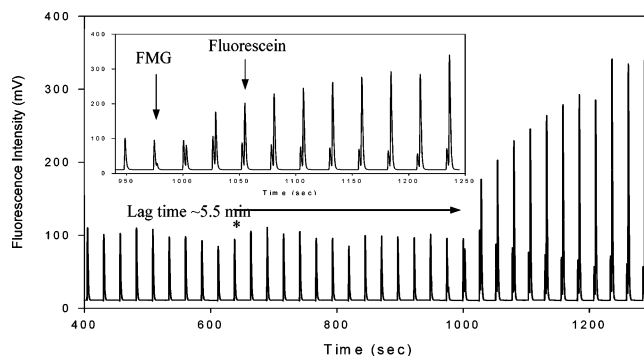


Figure 8. Hydrolysis of fluorescein mono- $\beta$ -D-galactopyranoside by  $\beta$ -D-galactosidase continuously monitored on-chip. The asterisk represents the time at which the enzyme was added into the vial. The flow rate was 1.0  $\mu\text{L}/\text{min}$ , the applied voltage was 5000 V, and the analysis time was 30 s.

for the probe to achieve  $\sim 90\%$  of the 100  $\mu\text{M}$  signal was found to span two sampling intervals of 35 s.

**Monitoring of an Enzyme Assay Using On-Line Microdialysis/Microchip CE.** To demonstrate a real-world application of the on-line microdialysis CE microchip system, an enzyme assay was monitored. Fluorescein mono- $\beta$ -D-galactopyranoside is a substrate that is hydrolyzed by  $\beta$ -D-galactosidase enzyme to form fluorescein and galactose. Both the substrate and the product are fluorescent, and the reaction typically takes less than 10 min at pH 7–7.5.<sup>44,45</sup> This enzyme reaction has been monitored on microchip devices previously but without the use of a microdialysis probe.<sup>37,46</sup> Figure 8 shows the results of the experiment; the inset is an expansion of the overall electropherogram during the time from 925 to 1250 s. Using this device, it was possible to monitor both the disappearance of the substrate and formation of the product. The intensity of the FMG peak started to decrease just before 1000 s with a concurrent increase in the fluorescein peak. The lag time in this case was 5.5 min. Again, some variation in peak height is seen prior to 1000 s (RSD = 7.9%). The variation seems to follow a pattern, suggesting syringe pump pulsing of the sampling system. Nonetheless, this work clearly demonstrates the potential of this system for near-real-time monitoring of a dynamic biological process.

## CONCLUSIONS

The first direct coupling of microdialysis sampling to a microchip-based capillary electrophoresis system has been described. Advantages of coupling microdialysis to microchip CE include fast analysis times, small injection volumes that facilitate the use of low perfusion flow rates, and the potential for integration of additional functions. Sequential injections of disodium fluorescein were used to optimize the system parameters, and the on-line microdialysis/microchip CE device was used to monitor the hydrolysis of fluorescein mono- $\beta$ -D-galactopyranoside by  $\beta$ -D-galactosidase. Using a modest flow rate and high separation field strengths, a temporal resolution of 30 s was achieved with this system. The simple interface eliminated issues typically encountered when coupling microdialysis to conventional CE systems,

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making this approach a viable option for researchers outside of the analytical community. Further characterization is necessary for quantification and assessment of response linearity and will be carried out in subsequent work. In the future, we will focus on decreasing the system lag time and integrating derivatization and detection components on-chip to create a portable separation-based sensor for on-line in vivo monitoring applications.

#### ACKNOWLEDGMENT

The authors thank Dr. James Landers and his research group at the University of Virginia for help with the LIF system and Dr. Steven Soper from Louisiana State University for his suggestions on detector alignment. Also advice from Dr. Elisabeth Verpoorte and Dr. Christopher Culbertson regarding procedures for high-

temperature glass bonding was greatly appreciated. We acknowledge the editorial assistance of Nancy Harmony in the preparation of the manuscript as well as Pradyot Nandi, Sara Logan, and Kathy Heppert for their technical expertise. This research was supported by the National Science Foundation (CHE-0111618) and the National Institutes of Health (5R01-NS42929). Additional funding for B.H. from the National Institutes of Health Biotechnology Training Grant (GM-08359) for a predoctoral fellowship is gratefully acknowledged.

Received for review April 29, 2004. Accepted August 17, 2004.

AC049365I