Continuous Separations with Microfabricated Electrophoresis−Electrochemical Array Detection

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Abstract: A novel electrochemical detection scheme for channel electrophoresis has been developed. A microfabricated array of individually addressed microelectrodes has been used as a site-specific amperometric detector for continuous separations in a buffer-filled rectangular channel. Continuous separations of picomole quantities of dopamine and catechol by channel electrophoresis with this new detection scheme have been demonstrated. Dopamine, an electroactive neurotransmitter, has been continuously monitored for 60 min with the electrochemical array detector. The new detection technique should allow dynamic chemical events in volume-limited microenvironments (i.e. single cells) to be examined with channel electrophoresis.

Introduction

Capillary zone electrophoresis (CZE) has become a rapid, highly efficient analytical technique which can be used to separate a wide variety of chemical species.[1] Highly resolved separations and sensitive detection makes the technique attractive for the investigation of ultralow volume (nanoliter to femtoliter) microenvironments. Although CZE has been widely used to sample and separate species from complex biological microenvironments (e.g. single cells),[2−7] it is inherently a serial analysis; material is sampled for a brief period of time followed by electrophoretic separation. Several techniques to perform both rapid and repetitive chemical analysis with CE on short time scales have been developed.[8] These alternative approaches can provide sampling and extremely efficient separations on the millisecond time scale, which potentially allows many separations per second to be achieved. However, continuous sampling and separation from a real environment with these methods have yet to be demonstrated. Free flow electrophoresis on a chip has also been developed as a miniature sample pretreatment/separation method and also provides continuous sample introduction.[9] Analytical techniques which can continuously sample and electrophoretically separate material would be extremely useful for monitoring constantly changing microenvironments.

Channel electrophoresis, a dynamic separation technique that has originated from our laboratories, can provide continuous sampling and electrophoretic separations with the time resolution of a few seconds.[10−13] The channel electrophoresis technique consists of a sampling capillary coupled to an open rectangular channel. Uninterrupted application of a high potential across the capillary results in the continuous migration of material through the capillary and into the channel as the capillary is traversed across the channel width. Ionic material and buffer that has been delivered to the channel, migrate the channel length in straight paths under the influence of an applied potential field, and ionic analytes are separated as they migrate the channel length based on their electrophoretic mobility. At the channel exit, a site specific array detector is used to preserve the spatial integrity of the sample introduction process and provides a second time axis (sample introduction) for the continuous separation technique. Although fluorescence detection has been useful in our initial efforts[10−13] and in later work emanating from Sweedler's group,[14] relatively few compounds of biological relevance fluoresce with high efficiency. Therefore the technique is limited in its current stage of development by the detection schemes employed.

The major goal of the research reported here is to develop and demonstrate an electrochemical detection scheme for channel electrophoresis. Electrochemical detection is an attractive alternative to fluorescence detection owing to its high sensitivity (10^{-19} mol) and demonstrated success in single cell investigations by CZE.[15] In addition, many compounds of biochemical interest are easily oxidized, and hence problematic

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derivatization in nanoliter to picoliter environments can be avoided. A microlithographically fabricated array of platinum microelectrodes has been developed as an approach to electrochemical detection in channel electrophoresis. Microfabricated electrode arrays have been widely used in liquid chromatographic investigations;[16] however, their use in electrophoresis is less extensive. Thormann used an array of electrodes to monitor zone development in isotachophoresis,[17] and more recently, Slater described a microband array detector for capillary zone electrophoresis.[18] The electrode array detector described here consists of 100 individually addressed electrodes spaced across the channel exit and is used to provide a location-based amperometric response for species migrating across the exit of the narrow channel. The position of detection correlates to the time of sample introduction and thus the detector provides temporal information for the continuous separation technique.

Experimental Section

Chemicals. Dopamine (3-hydroxytyramine) hydrochloride, catechol, and MES 2-(*N*-morpholino)ethanesulfonic acid were purchased from Sigma and used as received. For the continuous separation experiments, stock solutions of dopamine and catechol were prepared in ∼25 mM MES buffer. Continuous migration experiments were performed with dopamine dissolved in 20 mM MES buffer. Buffer solutions were made in doubly distilled water and adjusted to the appropriate pH with solid sodium hydroxide. Buffers were filtered through 0.2 μm nylon filters (Supelco, Bellefonte, PA) prior to use.

Sample Injection. Fused silica capillaries, 145 μm o.d., 15 μm i.d, and 73.5−91 cm length (Polymicro Technologies, Phoenix, AZ), were used for continuous sample introduction. A helium-pressurized (∼80 psi) buffer reservoir was used to fill the sampling capillary with MES buffer. Application of a high voltage across the length of the capillary via a Spellman (0−30 kV) high-voltage power supply (Plainview, NY) was used to fill the capillary with analyte solution via electromigration. A portion of the capillary was attached to a precision translation stage (Newport, Fairport, NY) which was driven in 20.8 μm increments by a stepper motor. The exit of the capillary was positioned inside the beveled entrance of the channel, and during experiments, uninterrupted application of a high potential was used to continuously sample material on one end and deliver it to the channel at the other end.

Channel Structures. The rectangular channel consists of two quartz plates pressed together, separated by two thin strips of uniform glass microspheres (Duke Scientific, Palo Alto) dispersed in UV-cured adhesive.[11] Typical separation channels had usable widths of 1.5 cm and lengths of 4.8 cm with the channel internal height defined by the diameter of the uniform glass microspheres used. Channel structures were fabricated with spacers as small as 2 μm; however, for channel electrophoresis experiments, only 21 μm internal height channels were employed.

The sampling capillary and rectangular channel were both suspended across two buffer reservoirs. Two power supplies were used to provide a high voltage across the capillary and channel. Electrical connection to each electrode in the array was achieved via a dual row 50 pin surface mount header and a homemade current amplification device consisting of 100 hardware-addressed current amplifiers. All electrodes were held at the same potential, in a three electrode format, with the redox potential supplied by a BAS CV-1B (Bioanalytical Systems, West Lafayette, IN) potentiostat. A 1 cm^2 platinum electrode (Advanced Biosensor Technology, Yardley, PA) and a Ag/AgCl pellet electrode (World Precision Instruments, Sarasota, FL) were used as the counter and reference electrodes, respectively. A multifunction input/output AT-MIO-16 board with a bipolar (±10V), 12 bit A/D converter was interfaced to either a Gateway 486-DX33 or a Micron Millennia Pentium-133 computer for data collection.

Fabrication of an Electrode Array. The electrode array was fabricated in collaboration with the National Nanofabrication Facility at Cornell using standard photolithographic techniques. The array electrodes were directly formed across a 1 cm portion of a quartz plate which, upon attachment of another quartz plate, served as the bottom plate of the channel. Each platinum electrode was 95 μm wide and 0.12 μm high with a 5 μm space between adjacent electrodes. A 1 μm thick layer of SiO_2 was chemically deposited by plasma-enhanced chemical vapor deposition (PECVD) over the connection pathways as an insulation layer.

Data Collection and Analysis. Computer-controlled data acquisition and manipulation programs have been developed in-house using LabView for Windows 3.1 software. Spyglass Transform 3.0 (Spyglass Inc., Champaign, IL) was used for generation of two- and three-dimensional representations in Figures 3 and 4.

The apparent and electrophoretic mobility of dopamine and the electroosmotic flow coefficient were calculated using the typical equations.[19] Errors are reported as the standard error of the mean. The amount injected into the channel was calculated using a standard formula for electrokinetic injection,[19] with the potential applied across the capillary and the time duration per capillary step used to obtain the mass of analyte injected. The amount per capillary step was multiplied by a factor of 5 to approximate the amount injected per 95 μm wide electrode.

Results and Discussion

The concept of continuous separations in narrow channels has implications for single cell analysis, microdialysis, and two-dimensional separations through to large scale separations of arrays of biological macromolecules. The key elements in establishing a working system for ultralow-volume continuous electrophoretic separations are the sample introduction scheme, channel configuration, microfabrication method, and the detection scheme to preserve the spatial integrity. A depiction of the channel electrophoresis-electrochemical detection scheme is shown in Figure 1A. A three-dimensional representation of the channel and electrochemical array detector is shown in Figure 1B, with only five electrodes shown for clarity. This system allows continuous separations of nanoliter to picoliter volume samples with the sensitivity of electrochemical detection.

An important step in the development of electrochemical detection in channel electrophoresis has been the construction of ultrasmall (<25 μm) internal height channel structures. The small internal height is necessary to perform electrochemical detection without electrical isolation of the separation voltage.[20] The narrow internal height reduces the separation currents generated in the electrophoretic process, and due to the increased internal resistance of the structure, the majority of the separation voltage is dropped across the channel length, leaving a minimal potential field at the exit of the channel. Thus the working electrodes are not dramatically affected by the separation potential and hence further isolation is not necessary. Our initial attempts to use electrochemical array detection employed 48 μm internal height channels and were not successful. These experiments resulted in the deformation and/or removal of the electrode metal layers. The electrophoretic separations reported here have been performed in 21 μm internal height channels;

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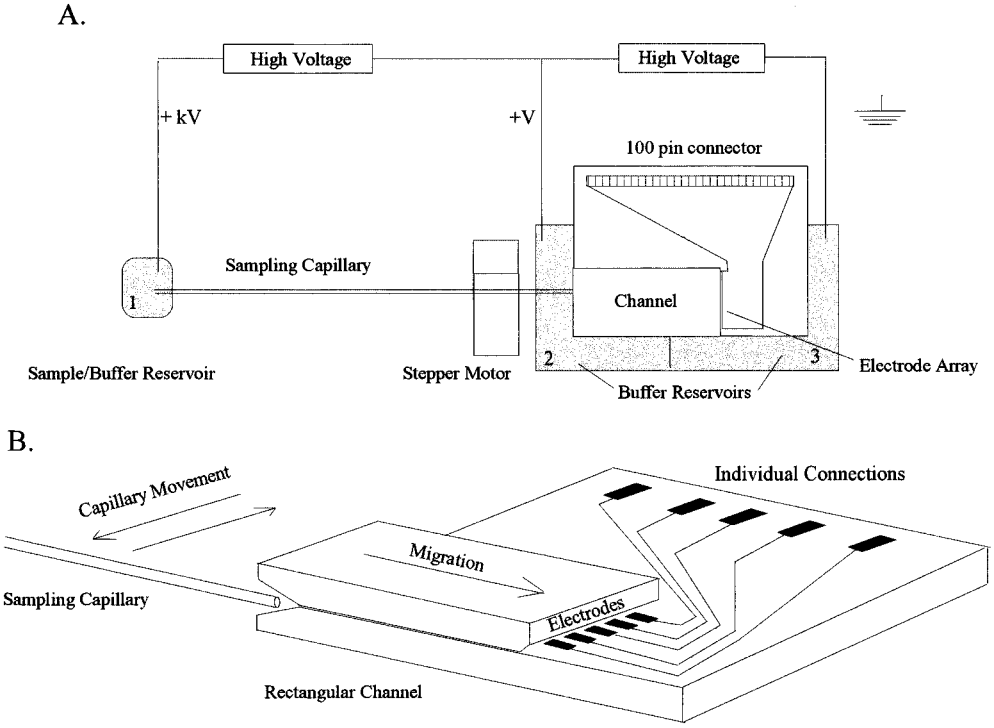

Figure 1. (A). A depiction of the channel electrophoresis technique. The sampling capillary is suspended across reservoirs 1 and 2, and the channel is suspended across buffer reservoirs 2 and 3. The stepper motor is used to move the capillary across the channel entrance. The counter and reference electrodes (not shown) are placed in reservoir 3. (B). A three-dimensional view of the channel electrophoresis scheme, shown with five electrodes, connections, and bond pads. The actual electrode array has 100 electrodes, connections, and bond pads directly fabricated onto the bottom plate.

however, even much narrower internal heights are possible. Figure 2A is an end-on SEM image of the entrance of a rectangular channel made using ~8 μm uniform glass microspheres to define the separation space. The smaller size channel structure was chosen since an image of a 21 μm internal height channel appears identical, except larger. It is important to realize that the spacers are applied only along the outer lengths of the quartz plates and do not interfere with the separation paths. The separation channel is in the space formed between the two plates as can be evidenced from the SEM in Figure 2B. A single 8 μm spacer covered by adhesive has been imaged in a region near the leftmost part of the channel entrance in Figure 2A and is shown in Figure 2C. Channel fabrication by the microsphere-adhesive technique[10,11] is both a simple and accurate way to define the separation space, with general applicability to a wide array of very small internal height separation structures.

Continuous electrophoretic separations have been used to demonstrate the new electrochemical detection scheme. Since the sampling capillary is moved across the channel width at a precise rate, the channel width dimension serves as a time axis for sample introduction. A mixture of dopamine and catechol is continuously deposited over a 5 min time period across the channel entrance by moving the sampling capillary along its width in 0.6 s time increments. A three-dimensional electropherogram of the results of this experiment are shown in Figure 3. Dopamine, a cation at pH = 5.8, migrated the channel length at a higher velocity than the neutral species, catechol, and was detected first. The average migration times for dopamine and catechol, as observed at 10 arbitrarily chosen electrodes spanning the channel width, are 133 ± 2.6 and 374 ± 12 s, respectively. For this data, the electroosmotic flow coefficient, calculated from the migration time of the neutral species catechol, has a value of $1.13 \pm 0.03 \times 10^{-4}$ cm^2 V^{-1} s^{-1}. The apparent and electrophoretic mobilities for dopamine have been determined to be $3.15 \pm 0.06 \times 10^{-4}$ cm^2 V^{-1} s^{-1} and $2.02 \pm 0.05 \times 10^{-4}$ cm^2 V^{-1} s^{-1}, respectively. The peak heights for dopamine and catechol appear to vary as a function of location across the width of the channel. The variation in peak height is not due to a change in sample concentration during the experiment but rather to different amperometric responses obtained at each of the electrodes in the array. Future work will involve normalization of electrode responses as a function of position across the channel exit.

The three-dimensional electropherogram shown in Figure 3 demonstrates that the detection scheme preserves the spatial and hence temporal resolution of the sampling process. Since the capillary was moved from right to left (from electrode 100 toward electrode 1), material deposited early in the experiment is detected by electrodes at a location that corresponds to the right side of the channel. Figure 3 shows that dopamine has been detected near electrode 100 earlier in time than at electrode 1. Although some zone broadening is apparent, it should be noted that no attempt has been made to optimize the separation parameters for the current data. The observed broadening is most likely due to the high concentration of sample relative to the separation buffer distorting the electric field in the sample zone as described by Jorgenson.[19] Despite this, the analytes are clearly and continuously resolved. The amount of dopamine injected per step was ~700 fmol, which corresponds to 3.5 pmol of dopamine injected across each 95 μm wide electrode. The mass detection limit calculated for dopamine at electrode 50 is ~25 fmol and holds promise for single cell experiments where a typical nerve or hormonal cell contains femtomole levels of messenger.[21] Considerable improvement in mass detection limits is expected as the system is reduced to smaller channel internal heights, as the coulometric efficiency of the detector is

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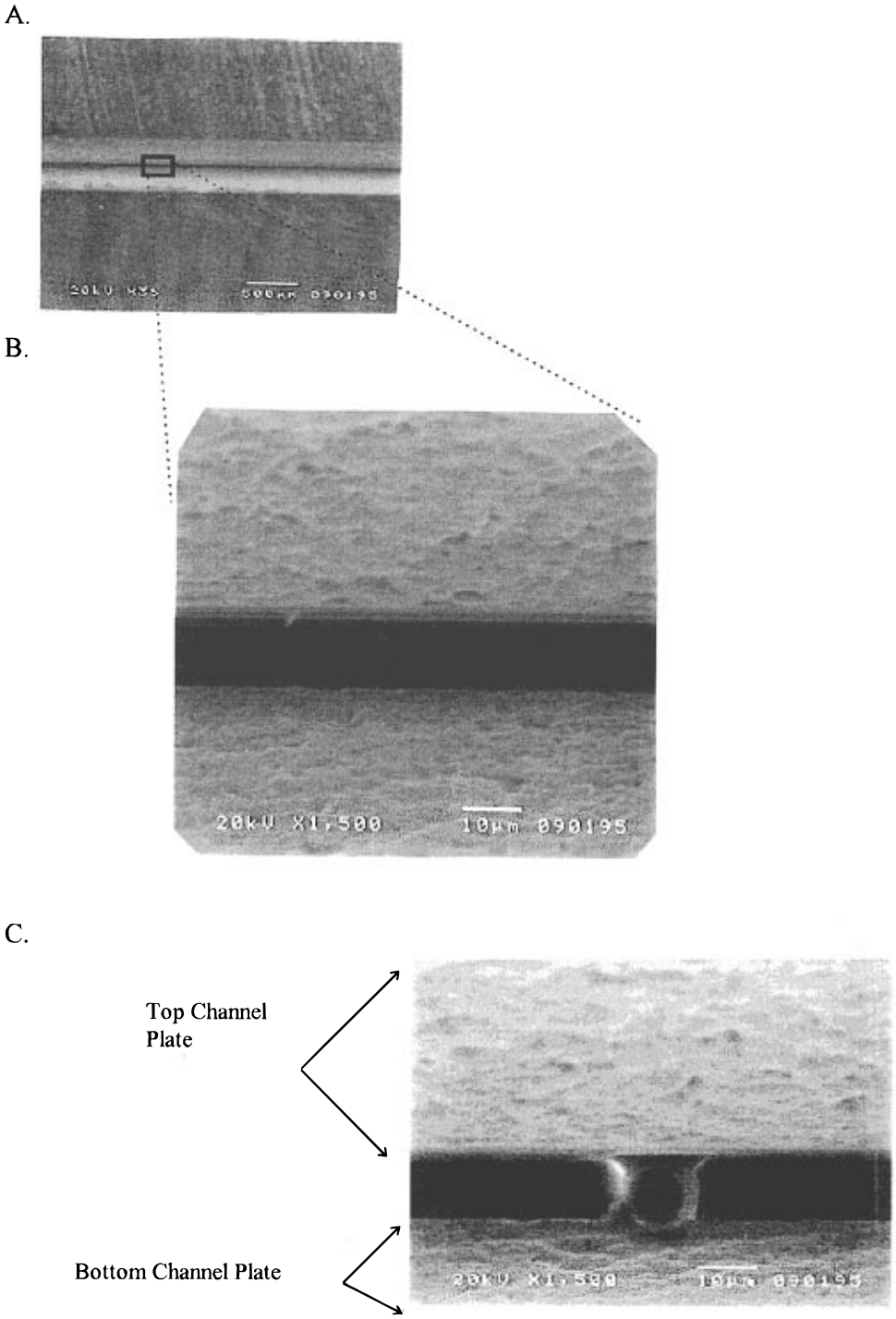

Figure 2. Scanning electron micrograph of the beveled entrance of an 8 μm internal height rectangular channel structure. (A) A low magnification (35×), end-on image of a 3.5 mm portion of a narrow channel. (B) A high-magnification image (1500×) of an ~85 μm long portion of the channel structure. The channel internal height, the black region between the two plates, is between 8 and 9 μm, which is consistent with the size of the glass microspheres used (7.9 ± 0.8 μm, 17% CV). (C) A single glass microsphere, shown between the quartz plates.

expected to increase. As discussed above, the technology is available to construct channels with internal heights as small as 2 μm.

Another unique feature of the channel electrophoresis technique is its ability to continuously sample from a microenvironment over time periods from minutes to hours. Long duration sampling has been achieved by moving the capillary back and forth across the channel entrance while continuously sampling material by electrokinetic injection. Figure 4 is a top view graph of the results of such an experiment where a solution of dopamine has been continuously sampled for 60 min. From the data in Figure 4, one pass of the sampling capillary corresponds to 300 s of sample introduction. This is a dramatic extension of the time of sample introduction as compared to conventional CE analyses which typically employ short injection times (1−10 s). This aspect of the technique makes continuous, long-term electrophoretic analyses possible, a feature which cannot be achieved with a repetitive, discrete sample introduction technique. The data also reveal that over the time course of the experiment, uniform migration of analyte occurs inside the rectangular channel. Thus, throughout the 60 min of continuous sample introduction, the microvolume structure exhibits little fluctuation in migration across its width and, hence, the position of detection can be used to

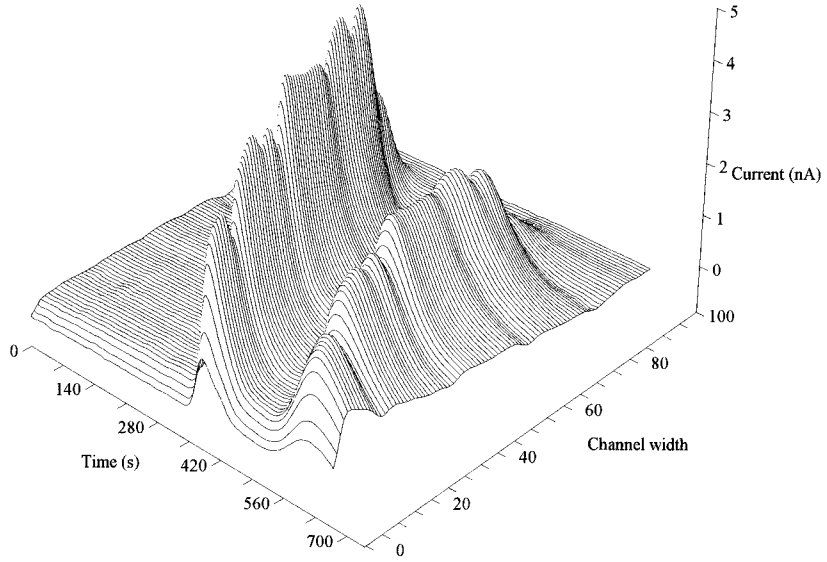

Figure 3. Continuous separation of dopamine and catechol in 21 μm internal height channels with electrochemical detection. A 15 μm i.d. fused silica capillary was electrokinetically filled with a solution containing dopamine (7.43 mM) and catechol (11.9 mM) in MES buffer (24.89 mM, pH = 5.8). Conditions: channel separation, 551 V and 4.8 cm length; injection potential, 10 kV and 73.5 cm length; capillary movement, 0.6 s/20.8 μm step for a total of 500 steps. The electrode array was positioned in the end-column detection format, 400−500 μm inside the beveled exit, and held at 0.7 V vs Ag/AgCl. The data acquisition rate was 0.4 Hz per 100 electrode scan. The first scan was background subtracted and the data was four-point moving window averaged by software developed in-house.

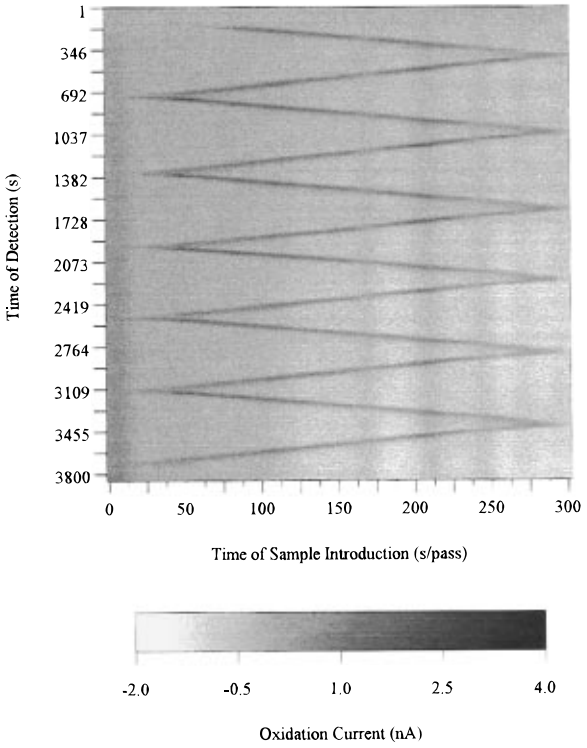

Figure 4. Continuous migration of a 7.2 mM solution of dopamine with electrochemical array detection. The channel was 4.8 cm long and had an internal height of 21 μm. The separation buffer was MES (20.0 mM, pH = 5.7). The injection capillary was 91 cm long with an internal diameter of 15 μm. A voltage of 6 kV and 700 V was applied across the capillary and channel reservoirs, respectively. The injection capillary was stepped at a rate of 0.6 s/step back and forth across the channel six times. The amount of material injected per 95 μm electrode in this experiment was 1.4 pmol. The data acquisition rate was 2 Hz per 100 electrode scan.

accurately preserve the time resolution of the dynamic sampling process.

The demonstration of long-term process monitoring combined with a detection scheme that can be used for biologically relevant analytes allows numerous avenues of research to be envisioned. In addition to our long-term goals, the methodology should provide a new approach for coupling microdialysis sampling to electrophoretic separations. Also, metabolism in living biological systems might be investigated with channel electrophoresis. The temporal resolution of sampling could be used to determine kinetic parameters from processes occurring in volume-limited environments, and the chemical selectivity of electrophoretic separation could be used to identify and quantitate metabolic intermediates.

Conclusions

An electrochemical detection scheme for channel electrophoresis has been developed. Our long-term goal for the development of channel electrophoresis has been its application to the study of dynamic chemical events that occur at single cells. The new detection scheme represents a major step toward our overall goal by increasing the number of analytes which can be studied and by making the overall technique more suitable for the analysis of biological microenvironments. Two facets of channel electrophoresis with the new detection scheme have been demonstrated: long-term, continuous monitoring of a single chemical species and the continuous separation of multiple analytes. Further refinement of the electrochemical detection scheme, along with its application to biological systems, is in progress in our laboratories.

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