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Development and optimization of an integrated PDMS based-microdialysis microchip electrophoresis device with on-chip derivatization for continuous monitoring of primary amines

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Abstract

An all-PDMS on-line microdialysis-microchip electrophoresis with on-chip derivatization and electrophoretic separation for near real-time monitoring of primary amine-containing analytes is described. Each part of the chip was optimized separately, and the effect of each of the components on temporal resolution, lag time, and separation efficiency of the device was determined. Aspartate and glutamate were employed as test analytes. Derivatization was accomplished with naphthalene-2,3,-dicarboxyaldehyde/cyanide (NDA/CN^-), and the separation was performed using a 15-cm serpentine channel. The analytes were detected using LIF detection.

Keywords

Aspartate; Cyanide; Glutamate; Microchip electrophoresis; Microdialysis; Naphthalene-2; 3-dicarboxaldehyde

1. Introduction

Microdialysis (MD) is a well-established sampling method for compounds present in the extracellular fluid of the brain as well as a number of other tissues [1–4]. The coupling of on-line MD to LC or electrophoresis yields a separation-based sensor that is capable of monitoring several analytes simultaneously in near real time [3,5–12]. An important consideration when using separation-based sensors to monitor biochemical events is the overall temporal resolution of the integrated system. In particular, many neurochemical processes occur on a very fast time scale. Temporal resolutions as high as 10 s have been reported by the Kennedy group, using MD coupled to CE [13]. However, faster measurements were not possible because the resolution was limited by the time it takes the analyte to diffuse across the probe membrane [14].

Microchip electrophoresis (MCE) is an attractive platform for on-line analysis of MD samples because of its minimal sample volume requirements (nL), small footprint, ability to

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perform fast, highly efficient separations, and easy integration of fluorescence detection [7, 15, 16]. Chips can be custom-designed for specific applications, and sample preparation steps (such as on-line derivatization for fluorescence detection) can be integrated into the chip [5, 7, 13, 17]. Our group and others have described MD-MCE systems with short linear separation channels as well as serpentine channels for monitoring amino acid neurotransmitters in in vivo brain microdialysate samples using a gated injection approach [5, 7, 18]. Alternatively, Martin et al. have employed a PDMS valving system for continuous monitoring of amino acids and catecholamines by MD sampling. [19–21] More recently, droplet-based microfluidics has become a popular approach for on-line analysis systems [8, 22, 23].

The present study is targeted toward the development of a totally integrated PDMS-based microchip device for the continuous monitoring of amino acid neurotransmitters. Although the approaches discussed above provide excellent temporal resolution for neurochemical studies, we have focused this paper on the development of a voltage-driven injection and separation system with the goal of ultimately using it in an on-animal separation-based sensor that can be controlled remotely via telemetry [24]. The components of the chip include (i) connections and interface to the MD sampling system; (ii) a prechannel mixer for on-line derivatization of the primary amines with NDA and CN^-; (iii) a flow-gated interface for injection of analyte into the electrophoresis channel [16,25]; and (iv) electrophoretic separation and analysis using a serpentine channel with fluorescence detection. The device incorporated standard methods for replenishing the buffer reservoirs (BRs), equilibration of the separation channel, and bubble removal without disturbing the MD flow. Each component of the system was separately optimized, and then evaluated for its effects on the overall temporal resolution of the device. As expected, the MD probe and associated tubing are the limiting factors for response time and temporal resolution.

2 Materials and methods

2.1 Chemicals

Boric acid, SDS, ACN, disodium fluorescein, amino acid standards, sodium cyanide (NaCN), sodium chloride, potassium chloride, calcium chloride, magnesium chloride, sodium bicarbonate, and disodium phosphate were all purchased from Sigma (St. Louis, MO, USA). Naphthalene-2,3-dicarboxaldehyde (NDA) was received from Molecular Probes (Eugene, OR, USA). All aqueous buffers and solutions were prepared using Milli-Q water and filtered using 0.22-μm Cameo teflon syringe cartridges from Osmonics (Minnetonka, MN, USA). Brain probes (CMA 12) were obtained from CMA (North Chelmsford, MA, USA). PDMS was obtained from Ellsworth Adhesive (Minneapolis, MN, USA).

2.2 Chip fabrication

All microchip devices were fabricated from PDMS utilizing standard photolithographic techniques that have been described previously [5]. Briefly, channel molds were prepared on silicon wafers (Telic Co., Valencia, CA, USA) using SU8–10 photoresist (Newton, MA, USA) and a high-resolution negative transparency (IGI, Minneapolis, MN, USA) containing the chip design. Approximately a 5-mm-thick layer of a mixture of PDMS and curing agent (10.5:1.5) was poured onto the channel mold followed by curing for 25–30 min at 90°C in a convection oven. Following this, the reservoir holes were produced using a biopsy punch (4-mm diameter), and inlets for hydrodynamic flow were produced by inserting 20-gauge luer stubs into the PDMS chip. Another thin PDMS layer (approximately 1 mm) was semicured in the oven for 15–18 min at 90°C until it was pliable to the touch. Then the channel layer was conformally contacted with the semicured layer and cured in the oven at 85°C overnight for semipermanent contact. The chip was interfaced with hydrodynamic flow using the 20-

gauge luer stubs that were embedded in the flow inlets. The other end of the luer stub was connected to the outlet of a syringe pump or that of an MD probe via fluorinated ethylene propylene tubing. The inlets for MD probe, NDA, and cyanide (CN^-) are labeled in Fig. 2 and are represented by the yellow, green, and blue dyes, respectively.

2.3 In vitro experiments

For these studies, a concentric MD probe (4-mm membrane length; CMA) was placed in the vial containing the amino acids of interest. The inlet of the probe was connected via fluorinated ethylene propylene tubing to a 1-mL syringe controlled by a 102 CMA MD syringe pump containing ultrapure water, and the outlet of the probe was connected to the microfluidic device.

2.4 Derivatization reactions

For off-line analysis, amino acid standards or microdialysates were derivatized with equal parts by volume of 7 mM NDA (dissolved in ACN) and 10 mM NaCN (dissolved in 50 mM boric acid buffer, pH 9.2). For on-line derivatization, 7 mM NDA (dissolved in 1:1 ACN:H_2O) and 10 mM NaCN (50 mM boric acid buffer, pH 9.2, and 5% ACN) were introduced independently into the mixing channel via a syringe pump. The flow rate for each inlet was optimized at 1 μL/min (total flow, 3 μL/min).

2.5 Chip operation

For off-line studies, a voltage of 2.7 kV was applied at the sample reservoir, −5 kV was applied at the sample waste (SW) with zero voltage at the BR using a four-channel high-voltage power supply (Ultravolt, Ronkonkoma, NY, USA). For separation, the same voltages were applied except that 5 kV was applied at BR (Fig. 1A). For on-line analysis, voltages of +5 and −5 kV were applied to the side channel and end of the separation channel, respectively. For sample injection, the separation voltage was floated during the period of injection (0.5 s). The separation buffer consisted of 20 mM borate, 10 mM SDS, 10% ACN, pH 9.2, unless otherwise noted.

2.6 LIF detection system

The detection system consisted of an epi-fluorescent microscope (Nikon Ti series). The chip device was placed on a glass microscope slide (Gold Seal cover glass #3334) and positioned on the microscope stage with clamps. The light source (mercury lamp) connected to the microscope by fiber optic cable was focused on a point of the separation channel approximately 0.2 cm from the waste reservoir through a 40× objective. Filter cubes housing the appropriate excitation/emission filters and dichroic mirrors were installed inside the carousel. Studies performed with fluorescein used FITC filter cubes obtained from Nikon. For analysis of NDA derivatives, a custom-built cube was purchased from Chroma (25-mm diameter z442/10× clean-up filter, 25-mm diameter 510 hq/50 m bandpass emission filter, and 25.5 × 36 mm laser dichroic filter and transmit 510 hq/50 m). A photomultiplier tube (Hamamatsu) was aligned to the side port of the microscope to collect the emission from the sample. Data were collected in a computer that was coupled to the PMT by means of a preamplifier (Stanford Research Systems, Sunnyvale, CA, USA) and data acquisition module (Chromgraph Interface). Electropherograms were analyzed using Origin software.

3 Results and discussion

This study focused on the development of a relatively simple voltage-driven microfluidic device for on-line derivatization and detection of primary amines. The ultimate goal is to integrate this into an on-animal separation-based sensor employing either amperometric or LIF detection. NDA derivatives are both fluorescent and electroactive [26]. An on-animal

system using electrochemical detection is currently under development in our group [24]. Although valving and droplet approaches provide excellent temporal resolution for on-line MD studies, they are much more difficult to miniaturize and control remotely than voltage-driven devices. The system described here could ultimately employ three battery-operated syringe pumps, a miniaturized HV power supply, and a potentiostat with telemetry for on-animal sensing of neurotransmitters. Therefore, we have focused on the optimization of the flow-gated approach for analysis in these studies.

The lab-on-a-chip devices described here were fabricated completely in PDMS. Most previous reports of on-line MD-MCE have employed glass substrates [5, 7, 18]. However, the fabrication of glass microchips requires specialized equipment and clean room access that is not widely available, especially in academic laboratories. Therefore, PDMS can offer a quick, cheap, and reliable alternative substrate for many devices [27]. PDMS does suffer from some drawbacks compared to glass for electrophoresis separations with regard to the adsorption of hydrophobic analytes and slow deterioration of the EOF [28, 29]. The present study makes use of simple and standard fabrication steps to construct the PDMS devices. Each part of the chip was optimized separately, and then the fully integrated system was evaluated for on-line monitoring.

3.1 Optimization of gated injection in a serpentine chip (off-line analysis)

For the development of an MD-MCE system for high temporal resolution, rapid injection, and separation of samples on a chip device are essential. To maximize the peak capacity, a serpentine channel was employed in this device. Injection was accomplished using electrokinetic gating. This is the simplest approach to ultrafast injections that does not require intricate fabrication steps (e.g. soft lithographic valving) or the formation of droplets. The use of gated injection for electrophoresis in serpentine microchips has been reported previously [7, 30–32]. A 15-cm serpentine separation channel was employed for the separation (Fig. 1A). Analytes were injected using the procedure reported by Sandlin et al. [7]. The system was initially tested with fluorescein. The RSD for peak height for seven sequential injections of fluorescein was 4.2% ($n = 7$ injections). A solution containing glutamate, aspartate, and orthophosphoserine (OPS) (internal standard) that had been prederivatized with NDA/CN was then analyzed. In this case the separation lasted 10 s and the RSD values for peak heights for glutamate, aspartate, and OPS were 2.98, 3.61, and 5.90%, respectively ($n = 5$) (Fig. 1B).

3.2 Optimization of mixer for on-line derivatization

Once the MCE system described above was optimized for the three model analytes, the next goal was to develop an efficient precolumn mixer for the derivatization of the primary amine analytes prior to injection and separation on chip. In 2006, Huynh et al. reported the development of an on-line MD-MCE chip for the determination of amino acids and peptides that employed NDA and mercaptoethanol with in-channel derivatization [9]. Later, Nandi et al. described a prechannel derivatization approach for on-line analysis that employed an injection scheme in which the reaction mixture flowed into a large sample reservoir, was allowed to accumulate, and was then injected by applying the potential directly to this reservoir for the separation [5]. This approach led to a gap in data between injections, while the next sample was allowed to accumulate in the reservoir. In the device presented here, two different mixing channel designs were evaluated for on-line derivatization with NDA/CN. These designs are based on the dolomite micromixer chip from Dolomite Microfluidics and work by Ismagilov et al. [23].

First, the flow rates for analyte and reagent delivery into the chip were optimized for each design. Since efficient derivatization requires the delivery of all three reagents into the chip

at fixed flow rates, colored dyes were employed to investigate the efficiency of mixing for this design. For the chip shown in Fig. 2, uniform delivery and mixing was accomplished using a flow rate of 500 nL/min for all 3 dyes. Micrographs were taken at a few other areas (Fig. 2A and B) further down the reaction channel to show that mixing was occurring. Mixing of the three streams was accomplished by splitting and then reuniting the streams multiple times in the device. This design was then tested for precolumn derivatization with NDA/CN^- reaction by observing the actual fluorescence generated by the reaction. Bright fluorescence was observed using a 1 mM standard solution of aspartate, indicating that the on-line reaction with NDA/CN^- was occurring in the mixer (Fig. 2C and D).

3.3 Injection interface

Once the appropriate mixer and electrophoresis design had been determined, the method for injection of sample from the flowing stream into the chip needed to be optimized. Our approach was to use a flow-through gated injection design reported by the Chen et al. in 2001 that allows continuous injection and sample analysis from an uninterrupted flow stream [25]. The design needed to be modified for this application due to the integration of the mixer for derivatization, the much longer (serpentine) separation channel, and the use of hydrophobic PDMS as the chip substrate. Because the device was made of PDMS, the first challenge was to be able to reproducibly fill and condition the different channels in the chip with run buffer, perfusate, and reagents without having the different streams interfere with each other. In addition, the microdialysate and buffer solutions needed to be equilibrated within the PDMS microchannels for better resolution and peak height reproducibility.

To overcome these challenges, an additional inlet was incorporated in the bottom of the chip adjacent to the buffer and SW reservoir (Fig. 2) that can be used to fill the chip with buffer using a syringe pump. This additional inlet could also be used to easily remove air bubbles from the separation channel and facilitate reconditioning of the separation channel. During the introduction of the MD perfusate containing the amino acid of interest and the reagents (NDA/CN^-) flow in the derivatization channels, air pockets can be created in the unfilled spaces within the PDMS channel. Over time, these bubbles were pushed into the separation channel and ultimately impeded sample injection and separation. Subsequent introduction of the separation buffer at a high flow rate (20–30 μL/min) through this additional inlet not only directed the bubbles exclusively toward the SW but also prevented the derivatized analytes from prematurely entering the separation channel. This ensures that all solutions are being maintained and directed through the appropriate channels for equilibration without inducing bubble generation. Once the chip was equilibrated using this procedure, subsequent injections and separations could be reproducibly carried out.

The system was first evaluated for continuous analysis of prederivatized cyanobenzisoindole (CBI) amino acids. A mixture of 5 μM CBI-amino acids was introduced at the probe and passed through the derivatization channel to be analyzed online on chip (Fig. 3). This experiment was performed to evaluate injection reproducibility from a continuous flow model using a probe and a derivatization channel. The peak height RSD values for serine, GABA, glutamate, aspartate, and OPS were calculated from the peaks displayed above the electropherogram to be 2.78, 3.26, 3.04, 2.89, and 3.17% (<5% for all amino acids), respectively. Although there appears to be more variation over the 20 min sampling period, the use of OPS as an internal standard can be used to correct for difference in injection efficiency over time. Also, a concentration change experiment was performed with the CBI derivatives of glycine, glutamate, and aspartate. Figure 4 shows the response obtained when concentration of the amino acids in the vial containing the MD probe was changed from 1 to 4 μM. The rise time in this case was calculated to be approximately 2 min, with a separation time of 35 s for each injection. A second concentration change from 4 to 8μM showed a 10-min lag time with a 2-min rise time.

3.4 On-line derivatization and analysis

To demonstrate the performance of the chip after initial design and flow optimization studies, on-line derivatization of amino acids followed by electrophoretic analysis was performed. First, the injection time was optimized for glutamate and aspartate (Fig. 5). An injection time of 0.1 s was chosen as optimum based on the separation efficiency. The peaks for the on-line derivatized amino acids (namely, glu and asp shown in the inset) exhibited RSD values of less than 5% ($n = 15$ injections). Subsequently, a concentration change experiment was performed in which the concentration of glutamate was increased from 3 to 10 μM (Fig. 6). The rise time in this case was much longer (5 min) compared to 2 min obtained for the prederivatized sample. This increase in rise time is most likely due to the kinetics of the reaction of the amino acids with NDA/CN that takes between 2 and 10 min, depending on the reaction conditions [33,34]. The fast flow rate (3 μL/min) due to the combined three flows through the mixing channel may not have allowed enough time for complete reaction. Figure 2 shows that it takes time for the three streams to mix before the reaction can begin. The use of lower flow rates for the MD and reagent introduction may improve the yield and, consequently, shorten the rise time.

4 Concluding remarks

The goal of this study was to develop an easily fabricated PDMS microfluidic device that can be coupled to MD in order to perform high-throughput sampling with prechannel on-chip derivatization. Each component was optimized and evaluated for its effect on temporal resolution, response time, and separation efficiency. It was found that the reaction kinetics of the derivatization reaction had the greatest effect on the response (rise) time of the separation-based sensor. Approximately half of the rise time was due to dispersion of the sample before being introduced into the separation channel. The lag time was consistent in both experiments at approximately 10 min. The time needed for the separation was not a limiting factor in the on-line MD-microchip system. The ultimate application of this device is near real-time in vivo monitoring of amino acid neurotransmitters with high temporal resolution for on-animal analysis.

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Abbreviations

BR	buffer reservoir
CBI	cyanobenzisoindole
MCE	microchip electrophoresis
MD	microdialysis
OPS	ortho-phosphoserine
SW	sample waste

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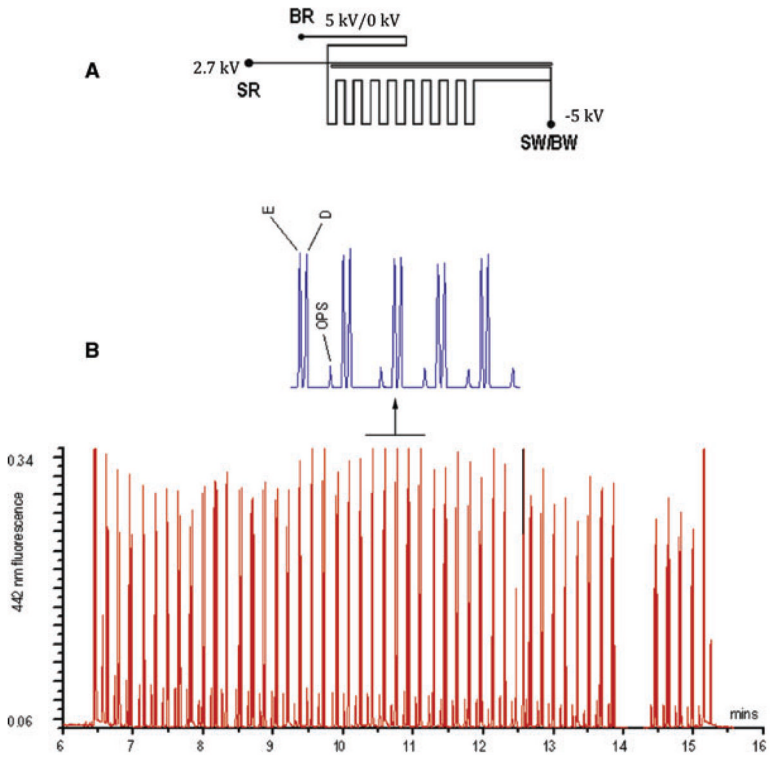

Figure 1.

(A) Chip design and injection for off-line simulated flow-through studies.[7] Sample was injected from sample reservoir (SR) electrokinetically with SR at 2.7 kV, buffer reservoir (BR) at 0 kV, and sample waste (SW)/BW at −5 kV. Separation was performed with SR at 2.7 kV, BR at 5 kV, and SW/BW at −5 kV. (B) Separation of 7 μM glutamate (E), aspartate (D), and orthophosphoserine (OPS), an internal standard) with temporal resolution of 15 s. The continuous set of electropherograms in the bottom panel shows a set of 53 continuous injections and separations. Separation buffer: 20 mM borate, 10 mM SDS, 10% ACN, pH 9.2.

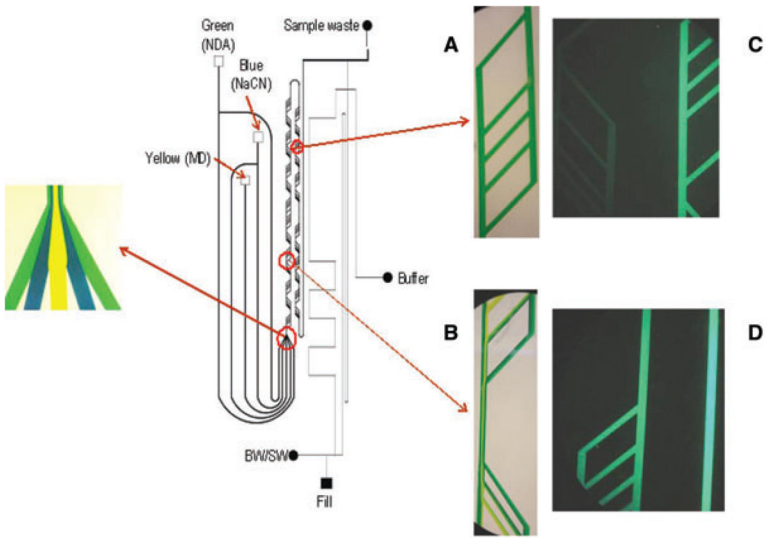

Figure 2.

(A) Design and characterization of dolomite micromixer-based chip. The micrographs with colored dyes show the flow and mixing pattern in various locations of the derivatization channel. Panel (B) shows segregated flow streams, whereas (A) demonstrates a uniform color pattern indicating mixing. The micrographs (C, D) demonstrate the fluorescence in the derivatization channels with 1 mM Asp, 7 mM NDA in ACN, and 10 mM NaCN in 50 mM borate buffer at pH 9.2.

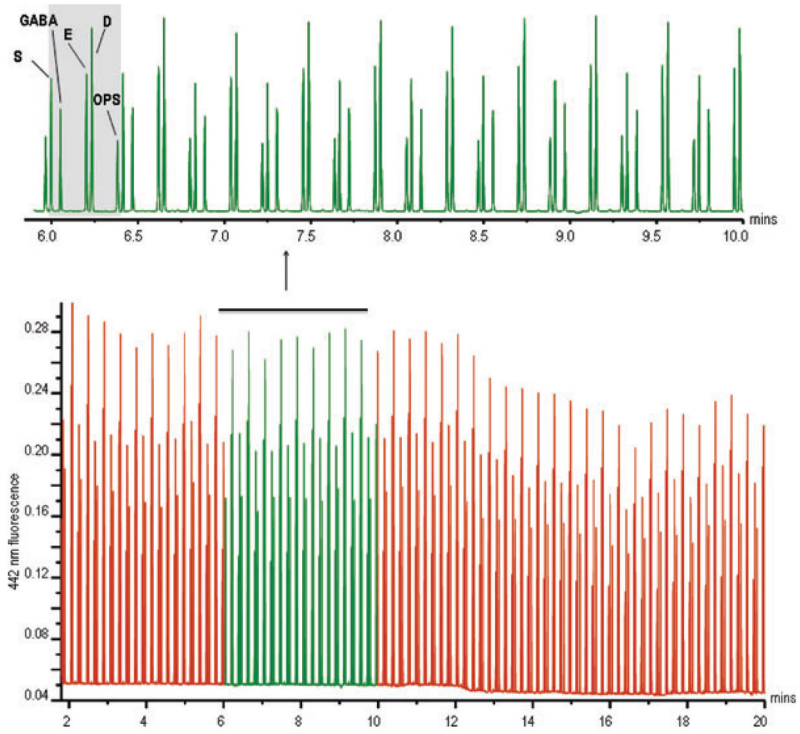

Figure 3.

(A) On-line flow through sampling analysis with prederivatized amino acids 5 mM serine (S), GABA, glutamate (E), aspartate (D), and ortho-phosphoserine (OPS), internal standard) with temporal resolution of 25 s. Separation buffer: 20 mM borate, 10 mM SDS, pH 9.2, injection time 0.5 s, 500 V/cm separation voltage. The mi-cromixer chip was used with 1 inlet for prederivatized amino acids.

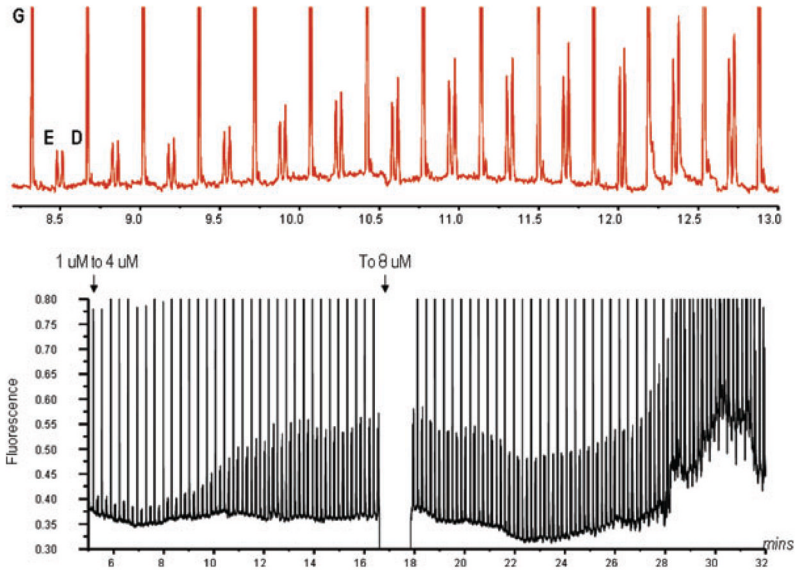

Figure 4.

Concentration change experiment with pre-derivatized glycine (G), glutamate (E), and aspartate (D). The top panel shows a snapshot of the rise time from 4 to 8 μM (from 9 to 12 min). The bottom panel demonstrates the continuous injections and separations with concentration changes at three points (1 μM–4 μM and then to 8 μM indicated by the arrows). Buffer conditions: 20 mM borate, 10 mM SDS at pH 9.2. The amino acids were derivatized with 7 mM NDA in ACN, 10 mM NaCN in 50 mM borate buffer at pH 9.2. Injection time is 0.5 s with a separation potential of 500 V/cm.

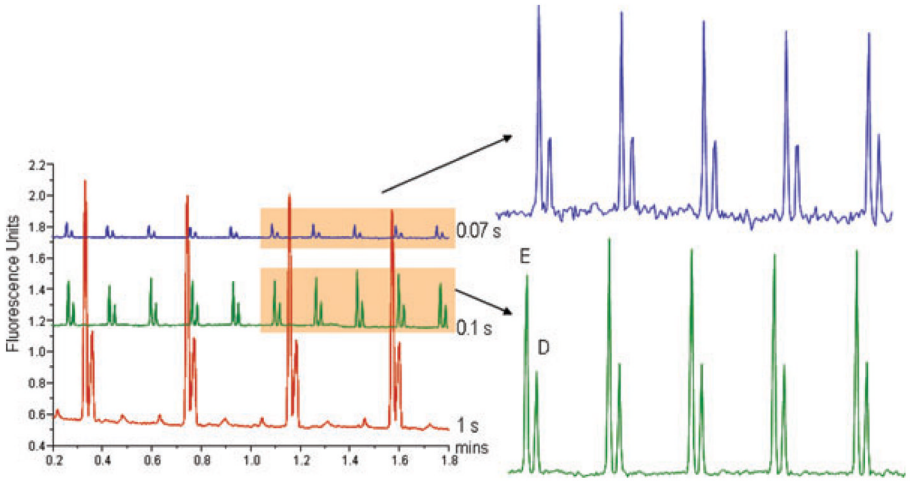

Figure 5.

On-line derivatization of 10 μM of glutamate (E) and aspartate (D). Injection times (1, 0.1, and 0.07 s) were optimized for baseline separation of the analytes. Separation buffer: 20 mM borate, 10 mM SDS, pH 9.2. On-line derivatization was performed with 7 mM NDA in ACN, 10 mM NaCN in 50 mM borate buffer at pH 9.2. The separation potential was 500 V/cm.

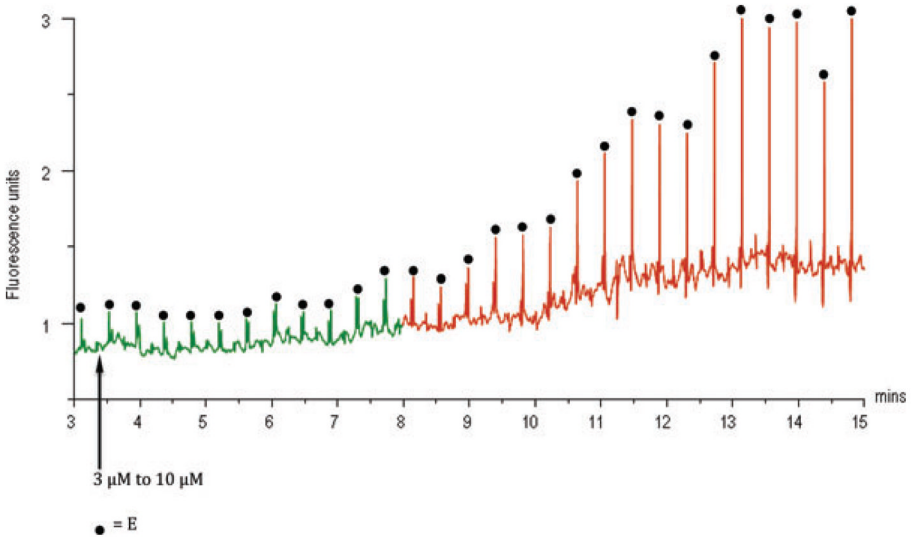

Figure 6.

On-line derivatization with concentration change of glutamate (E) demonstrating the rise time (8–13 min) of approximately 5 min. Separation buffer: 20 mM borate, 10 mM SDS, pH 9.2; separation potential 500 V/cm. Derivatization was performed with 7 mM NDA in ACN, 10 mM NaCN in 50 mM borate buffer at pH 9.2.