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Microfluidic Chip for High Efficiency Electrophoretic Analysis of Segmented Flow from a Microdialysis Probe and in Vivo Chemical Monitoring

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Abstract

An effective method for in vivo chemical monitoring is to couple sampling probes, such as microdialysis, to on-line analytical methods. A limitation of this approach is that in vivo chemical dynamics may be distorted by flow and diffusion broadening during transfer from sampling probe to analytical system. Converting a homogenous sample stream to segmented flow can prevent such broadening. We have developed a system for coupling segmented microdialysis flow with chip-based electrophoresis. In this system, the dialysis probe is integrated with a PDMS chip that merges dialysate with fluorogenic reagent and segments the flow into 8–10 nL plugs at 0.3–0.5 Hz separated by perfluorodecalin. The plugs flow to a glass chip where they are extracted to an aqueous stream and analyzed by electrophoresis with fluorescence detection. The novel extraction system connects the segmented flow to an electrophoresis sampling channel by a shallow and hydrophilic extraction bridge that removes the entire aqueous droplet from the oil stream. With this approach, temporal resolution was 35 s and independent of distance between sampling and analysis. Electrophoretic analysis produced separation with 223,000 ± 21,000 theoretical plates, 4.4% RSD in peak height, and detection limits of 90–180 nM for six amino acids. This performance was made possible by three key elements: 1) reliable transfer of plug flow to a glass chip; 2) efficient extraction of aqueous plugs from segmented flow; and 3) electrophoretic injection suitable for high efficiency separation with minimal dilution of sample. The system was used to detect rapid concentration changes evoked by infusing glutamate uptake inhibitor into the striatum of anesthetized rats. These results demonstrate the potential of incorporating segmented flow into separations-based sensing schemes for studying chemical dynamics in vivo with improved temporal resolution.

Keywords

Microdialysis; segmented flow; temporal resolution; electrophoresis; amino acids

INTRODUCTION

Microdialysis sampling coupled to analytical methods such as high performance liquid chromatography (HPLC) or capillary electrophoresis (CE) is a powerful approach for in vivo monitoring.[1,2] High temporal resolution is often an important goal in developing a microdialysis method.[3–7] For example, correlating neurotransmitter fluctuations in the brain with behavior requires temporal resolution on the time scale of behavior changes which is often in the seconds range.[8–10] An inherent limitation to obtaining high temporal resolution with

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microdialysis is broadening of sample zones due to Taylor dispersion that occurs between the sampling probe and the analytical platform.[11] Although temporal resolution of 3–30 s using microdialysis has been reported,[3,7,8,12–14] these studies used some or all of the following features: 1) high sampling flow rate (e.g. over 1 μL/min); 2) anesthetized subjects placed close to the detection system; or 3) short, small-bore connector capillaries for connecting the subject and analytical system to minimize dispersion during sample transfer. For many cases though, lower sampling flow rate (e.g. 50–300 nL/min) is useful to obtain higher relative recovery, better sensitivity, and quantitative sampling.[15] Longer connector tubing is often necessary for clinical applications[3,6] and for behavioral experiments. When using long connector tubing, larger bores are necessary to reduce back pressure exerted on microdialysis membrane and prevent leaks or ultrafiltration. Under conditions of long, large bore connecting tubing and low flow rates, Taylor dispersion becomes significant and obtaining high temporal resolution remains a challenge. In this work we describe a system that avoids these problems by coupling microdialysis with segmented flow to an electrophoresis chip for in vivo neurochemical monitoring.

Segmented flow analysis (also called continuous flow analysis[16]), in which sample plugs are carried by an immiscible fluid, is an excellent way to solve the problem because it prevents axial dispersion during transportation and storage. One approach to using segmented flow is to create a train of plugs on the inlet side of a sampling probe and pump them through the probe so that each plug collects sample at a point in time.[17] Although this approach achieves sub-second temporal resolution in sampling, coupling it with microdialysis membranes and in vivo sampling remains an unsolved problem. Previous work in our group has demonstrated that dialysate can be segmented immediately after sampling[11] to achieve temporal resolution as good as 15 s regardless of the downstream processing and connector tubing.

Previous work with microdialysis coupled to segmented flow has been limited to using enzyme assay for analysis of the plugs. To make microdialysis with segmented flow practically useful, it is necessary to couple segmented flow to more powerful, multi-analyte methods such as CE with laser-induced fluorescence (LIF).[7,18–21] Methods have been reported for electrophoretic analysis of plugs;[22,23] however, they have not yet been coupled to microdialysis nor do they have the analytical performance in terms of separation efficiency to be used for complex samples collected in vivo.

In this work we describe a system that segments dialysis flow, derivatizes the analyte stream with naphthalene-2,3-dicarboxaldehyde (NDA)/CN^-, transfers the samples to a glass microfluidic chip, and serially injects samples for electrophoretic analysis with >200,000 plates at 20 s intervals. This system required 3 technical innovations: 1) reliable transfer of plug flow to a glass chip; 2) efficient extraction of aqueous plugs from segmented flow; and 3) electrophoretic injection suitable for high efficiency separation with minimal dilution of sample. This microfluidic system was proven suitable for on-line in vivo chemical measurements of amino acids from the brain with ~30 s temporal resolution offering the combined advantages of maintained temporal resolution with segmented flow and high resolving power of electrophoresis.

EXPERIMENTAL SECTION

Chemicals

All chemicals were purchased from Sigma-Aldrich (St. Louis, MO) with the following exceptions. Salts for artificial cerebral spinal fluid (aCSF) were purchased from Fisher Scientific (Chicago, IL). NDA was purchased from Invitrogen (Eugene, OR). All aqueous solutions were prepared with water purified and deionized to18 MΩ resistivity using a Series 1090 E-pure system (Barnstead Thermolyne Cooperation, Dubuque, IA). Amino acid standards

of γ-aminobutyric acid (GABA), taurine (Tau), serine (Ser), glycine (Gly), glutamic acid (Glu) aspartic acid (Asp) were dissolved in aCSF. Octadecyletrichlorosilane (OTCS) was stored in a desiccator and was opened in dry N_2 atmosphere in an Aldrich Atmosbag (St. Louis, MO)

Microdialysis Probes

Side-by-side microdialysis probes with 18 kDa molecular weight cut-off membranes made from regenerated cellulose hollow-fibers were made in-house as described elsewhere.[24] Probes had 200 μm diameter, 2 mm sampling length and 40 μm i.d. × 100 μm o.d. fused silica capillaries for inlet and outlet tubing.

PDMS Chip Fabrication

The overall scheme of the system, which used two different chips, is illustrated in Figure 1. Fabrication of the PDMS chip with integrated microdialysis probe was previously described.[11] The segmented flow channel was 150 μm wide × 90 μm deep and the channels for NDA, dialysate, and KCN/EDTA were 75 μm wide × 90 μm deep. The 40 μm i.d. outlet capillary (3 cm total length) of the microdialysis probe was directly inserted into an access port on the PDMS chip. Other fluids were transferred to the chip through 50 μm i.d. × 360 μm o.d. capillaries. Segmented flow was pumped out of the chip into a 60 cm length of 150 μm i.d. × 360 μm o.d. high purity perfluoroalkoxy plus (HPFA+) tubing (Upchurch Scientific, Oak Harbor, OR) through a glass adaptor (Hampton Research, Aliso Viejo, CA).

Glass Chip Fabrication

Glass chips with the layout shown in Figure 2 were prepared using photolithography and wet-etching techniques.[25,26] Some surfaces were selectively modified with OTCS using laminar flow patterning similar to that described before.[23,27] Details of the fabrication procedure are provided in Supporting Information.

Device Operation

aCSF (145 mM NaCl, 2.68 mM KCl, 1.01 mM $MgSO_4$, 1.22 mM $CaCl_2$, 1.55 mM Na_2HPO_4, 0.45 Mm NaH_2PO_4, pH 7.4) was pumped through a microdialysis probe and into one channel of the PDMS chip at 300 nL/min flow rate. NDA in 50:50 mixture (v:v) of acetonitrile and 20 mM borate and cyanide with 0.5 M EDTA in 20 mM sodium borate at pH 9.5 were pumped through separate channels at 150 nL/min each (see Fig. 1).[28–30] The resulting solutions combined to form plugs at 0.3–0.5 Hz with 8–10 nL volume when perfluorodecalin was pumped at 1.5 μL/min through the cross-channel. The train of plugs flowed through 60 cm of 150 μm i.d. HPFA connector tubing to the glass chip for electrophoretic analysis.

All channels and reservoirs on the glass chip were filled with electrophoresis buffer (10 mM sodium tetraborate, 0.9 mM hydroxypropyl-β-cyclodextran (HPβCD), pH 9.7) prior to use. Voltage for performing electrophoresis was applied using a CZE1000R power supply (Spellman, Hauppague, NY). High-voltage relay for controlling electrokinetic-gated injection was from Kilovac (Santa Barbara, CA). Prior to use, channels of the glass chip were conditioned by using electroosmosis to pump 0.1 M NaOH for 15 min followed by deionized water for 15 min, and then electrophoresis buffer for 15 min through all channels. Detection on chip was accomplished using an in-house built confocal LIF detector.[31] Details of the detector and data analysis are provided in Supporting Information.

Testing of Temporal Resolution

The temporal response of the whole system was tested by equilibrating the microdialysis probe in a solution of amino acid standards dissolved in aCSF, which was constantly stirred at 37 °C. The concentration of the surrounding solution was then quickly altered by using pipettes to

remove and infuse solutions in the reservoir while monitoring the intensity of peaks in electropherograms. In continuous phase experiments, a Valco Cheminert cross (150 μm inner bore) was used to mix dialysate with derivatization reagents and to flow the mixture into a piece of 60 cm 150 μm i.d. × 360 μm o.d. fused silica capillary. Total fluorescence change was monitored near the outlet of the capillary.

Surgery and in Vivo Experiments

In vivo microdialysis experiments were performed on male Sprague-Dawley rats (Harlan, Indianapolis, IN) weighing 200–250 g. Rats were anesthetized with intraperitoneal (i.p.) injections of ketamine (65 mg/kg) and domitor (0.5 mg/kg) and mounted on a stereotaxic apparatus (Kopf Instruments, Tujunga, CA). Rats were maintained under anesthesia for the entire experiment by giving i.p. injections of ketamine (32.5 mg/kg) and domitor (0.25 mg/kg) as needed. The probe was inserted into the striatum at following coordinates: +1.0 mm anterior of bregma, +2.0 mm lateral of midline, and 8.0 mm deep from dura.[32] After inserting the microdialysis probe, the system was equilibrated by perfusing aCSF through the probe at 300 nL/min for 1 h before beginning measurements. In vitro calibration with standards was performed after in vivo measurements.

RESULTS AND DISCUSSION

NDA Reaction on PDMS Chip with Flow Segmentation

The dialysis probe integrated with PDMS chip allowed NDA/CN^- reagent to be added to a dialysate stream as it was segmented yielding 8–10 nL dialysate fractions that were derivatized as they flowed through the transfer tubing as illustrated in Figure 1. For this experiment, 10 μM amino acids were sampled and the fluorescent product detected 6 min later in the transfer tubing as a series of fluorescent spikes corresponding to individual plugs. The NDA reaction with amino acids generally takes 3 min or less to complete[30] and we were able to confirm that the 6 min transit time within plugs was sufficient for complete reaction (data not shown). Although all in vivo experiments reported here were performed on anesthetized animals, the 60 cm of tubing connecting probe and analysis chip is sufficiently long for many awake animal studies. We have previously used the PDMS chip shown in Figure 1 to add reagents for a glucose assay to dialysate as it was segmented.[11] This approach to reagent addition is also similar to that used for on-chip dilution of plugs as they are formed.[33] Figure 1 shows that the NDA/CN^- reaction could be performed in the same way.

Performing NDA derivatization using droplets was advantageous in at least two ways. NDA is hydrophobic and susceptible adsorbtion by native PDMS. Previous attempts to perform NDA derivatization of amino acids in continuous phases on a PDMS chip had to utilize channel surface treatment to increase its hydrophilicity.[34] With segmented flow, this is unnecessary since the reaction mixture was protected from contacting PDMS walls by a thin layer of perfluorinated oil. Another problem often encountered when using NDA/CN is precipitation.[29] Although this problem can be avoided with some capillary systems,[28,35,36] we found that on-line reaction in chips with continuous phases was frequently plagued with channel clogging due to precipitate formation. In this work, precipitation would occur where CN^- and saline solutions were brought into contact; however, these precipitates did not disrupt the formation of plugs, affect plug frequency or affect product yield for at least 2 h. We believe that the elastomeric property of PDMS and relatively high oil cross flow both contributed to the continuation of segmented sample flow even in the presence of precipitates. No precipitate was found within plugs so downstream CE analysis was not affected.

Transfer of Segmented Flow to Glass Chip

To achieve high-performance separation, it was desirable to use glass chips for electrophoretic analysis. This required developing a system for smooth transfer of segmented flows to glass chips. Commercially available glass chip connections are a convenient option for regular fluid access to microfluidic channels, but they are typically mounted on the chip surface and generate a 90 degree turn in the fluid path which causes plugs to coalesce producing irregular plug frequency. We elected therefore to use in-line connections similar to those used in microchip CE-electrospray ionization (CE-ESI) applications.[37,38] Such connections are prepared by etching a channel in the same plane as fluidic connections and gluing connecting capillaries in place (see Supporting Information for details). Unlike CE-ESI connection that tolerates little dead-volume, transport of segmented flow did not require rigorous seal at the capillary-channel interface. Images of plug transfer are provided in supporting information (Figure S2). This in-line configuration is a simple connection that assists the manipulation of segmented flow from outside tubing to glass-based microchips.

Desegmentation through Extraction Bridge

A key to electrophoretic analysis of segmented flow is to collect sample from aqueous plugs and transfer it to the electrophoresis channel. One approach is to transfer the plug directly to the electrophoresis channel.[22,23,39] This method has not yet yielded separations with sufficiently high efficiency to be used for complex dialysate samples. The second approach is to "desegment" the plug flow to create a continuous aqueous sample stream that is then injected using a conventional microfluidic injection.[23] Desegmentation is suitable for the dialysis application as long as the loss of temporal resolution due to recombining plugs is less than that lost due to sampling and rate of electrophoretic analysis. Thus far desegmenting injection has shown more promise for achieving high efficiency separations generating ~50k plates for an 18 s separation, compared to ~1k plates for direct injection of discrete plugs.[23]

In this work we developed an improved desegmenting and injection design for segmented flow electrophoresis shown in Figure 2A. In this method, aqueous plugs are removed from a segmented flow and transferred to a separate channel using an "extraction bridge". The extraction bridge is a shallow, hydrophilic channel that connects two deep channels, a hydrophobic one for incoming segmented flow and a hydrophilic one for extracted aqueous flow (Supporting Information, Figure S1B(i)). When aqueous plugs contact the bridge, they experience both capillary force from the shallow depth of the bridge and lower surface energy induced by hydrophilic channel walls, which guides the aqueous fraction preferentially through the bridge. Using this design, the entire aqueous plug is consistently transferred the extraction channel as illustrated in Figure 2B. Oil is prevented from entering the extraction bridge by the high flow resistance and high surface energy of the hydrophilic bridge area for fluorinated oil.

This phase separation method shares some similarity with previously reported work to separate either gas-liquid flow[40,41] or liquid-liquid flow.[42–44] However, it better met the need of carrying out subsequent high performance electrophoresis of the plug content by building the device on glass substrate instead of PDMS, Teflon or silicon.

High Efficiency CE Separation of Amino Acids

After phase separation, plugs fill the extraction channel (see Figure 2) which acts as a reservoir supplying sample to the sampling channel and cross-style CE injector. The volume of the extraction channel (9.5 nL) is such that each plug fills it and washes the previous plug past the sampling channel. The flow resistance of the sampling channel (6 μm deep × 42 μm wide × 4 mm long) is about 10^4 higher than the extraction channel (70 μm deep × 260 μm wide × 6.5 mm long) so that virtually no hydrodynamic flow occurs in the sampling channel.

Electrokinetic injection[45] was employed using the voltage scheme shown in Figure 2A. With a negative high voltage applied to the CE outlet and ground at the sample outlet reservoir, sample present in the extraction channel was drawn toward the injection cross by EOF through the sampling channel. This stream was gated by a cross-flowing buffer stream connected to positive high voltage through a relay. Changing the relay from closed to open (float) allowed sample to be injected onto the channel for separation. Figure 3 is an example of electropherograms of the separation of six amino acids dissolved in aCSF with 11.1 cm separation distance, 60 ms injection time and 720 V/cm applied to the separation channel. Injection was reproducible with an average of 4.4% RSD in peak height for 50 serial injections. Detection limits were 90–180 nM at the sampling probe for six amino acids.

Separation efficiency was over 200k theoretical plates even though samples contain high salts from the aCSF. This is a significant improvement over the 50k plates previously reported for a desegmenting injector.[23] It is also a significant improvement over previous chip-based electrophoretic analysis of microdialysate that used continuous phase flow.[13,31,46] Compared to previous methods of electrophoresis analysis of plugs, this device offered three major advantages. First, the plug was extracted without dilution, allowing shorter injections for improved separation efficiency without sacrificing LOD. Second, there was no hydrodynamic flow into the sampling channel or separation channel which could cause excess band broadening. Third, the chip layout allowed for application of higher electric field before bubbles formed, which also helped with higher separation efficiency.

To better understand the factors that limit separation efficiency in the current design, we examined the relationship of peak variance with both injection width and migration time along the channel length. GABA and Glu were chosen as model analytes because of their large difference in mobility. As shown in Figure 4A, peak variance decreased linearly with decreasing injection width until a minimum value of ~6×10^{-4} cm^2 was reached at 80 ms injection width suggesting that below this injection time the injector does not contribute significantly to peak width.

Plotting variance of zones against migration time (achieved by recording variance at different lengths along the channel) yields a linear trend with a slope of 10^{-5} cm^2/s (Figure 4B). This value is double the estimated diffusion coefficient of ~5×10^{-6} cm^2/s for a small molecule, which is expected if diffusion is the dominant source of band broadening.[47] The small y-intercept of 10^{-4} cm^2 indicated that extra-column effects contributed only 10–20% of the total bandwidth over 11.1 cm separation length. Based on these considerations, further improvement in efficiency would require higher fields; however, electric field higher than 720V/cm generated Joule heating. Therefore, future effort to improve efficiency should be directed towards improving heat dissipation.

Conservation of Temporal Resolution with CE Separation

In previous work, we found that temporal resolution with a 2 mm microdialysis probe using segmented flow was 30 s.[11] In such a case, temporal resolution is limited by broadening that occurs during sampling[48] and is fixed once segmented flow was formed. Because plugs were recombined prior to injection in this work, special care was required to not introduce extra mixing of contents of adjacent plugs that would further distort temporal distribution. To achieve this purpose, the sampling channel was placed as close as practical to the extraction bridge so that a de-segmented plug could be immediately sampled once it entered the extraction channel. The dead end of the extraction channel was also designed with a triangular shape to fit the flow trajectory of sample plug as it entered water extraction channel through extraction bridge facilitating rapid transfer with minimal unswept volume. With the above considerations, the final device proved to conserve temporal resolution as shown in Figure 5A. For this test, back-to-back injections were made every 7 s and separation length was 1.5 cm. A step change in

concentration at the dialysis probe was recorded as about a 35 s change in the electrophoresis traces, compared to 30 s before the extraction, suggesting minimal temporal distortion due to desegmenting. For more complex samples, it is desirable to use the full efficiency of the system. When we moved the detection point to 11.1 cm (Figure 5B), where the six amino acids could be fully resolved, injection could be made every 20 s. As expected, the concentration change finished in two electropherograms or 40 s. Temporal response was limited by the separation time in this situation. Further enhancement of the separation speed would be required to be able to analyze each droplet that is formed.

If no flow segmentation was employed but other conditions remained the same, temporal resolution worsened to 90 s for a step increase (Figure 5C) and was 130 s for a step decrease (Figure 5D). Segmented flow eliminated this bias allowing measurement of concentration dynamics in either direction with both good fidelity and high temporal resolution.

In Vivo Monitoring of Neurotransmitters

To test this system for in vivo chemical monitoring, we measured amino acids collected from rat brain during a pharmacological treatment. Figure 6A illustrates a typical electropherogram collected in vivo. Serine, glycine, glutamate and aspartate were resolved and basal concentrations were 34.1 ± 4.4, 6.0 ± 1.1, 1.8 ± 0.4, 0.7 ± 0.3 μM (mean ± SEM, n = 3), respectively. These values are in good agreement with previous reports.[49, 50] Interferences were found for GABA and taurine; however, optimization of the electrophoresis buffer is likely to allow these neuroactive amino acids to be resolved.

To demonstrate the capability of the system to detect rapid changes of neurotransmitter levels, 400 μM L-trans-pyrrolidine-2,4-dicarboxylic acid (PDC), a glutamate transport inhibitor[51] was infused into the striatum by reverse microdialysis. Figure 6B is an example of the chemical changes resulted from 5 min PDC stimulation. As expected, glutamate concentration in striatum was elevated to 206 ± 34% (mean ± SEM, n = 3) of basal upon PDC infusion. Aspartate also increased by 238 ± 19% (mean ± SEM n = 3).[52] No changes in serine and glycine levels were observed.[51] Re-perfusion of regular aCSF brought both neurotransmitter concentrations back to basal. The increase of glutamate and aspartate in response to PDC stimulation occurred at the limit of temporal resolution illustrating a rapid achievement of steady state concentrations after drug application. While the number of replicates is too low for a complete pharmacological study, the good reproducibility of the basal concentration and evoked changes show that the method has acceptable reproducibility for animal work.

A number of improvements could be made that might further enhance temporal resolution. Faster separation in the on-line system would be one approach. This would require a method to avoid the effects of Joule heating. Another approach would be to use this method in off-line mode in which case the connector tubing is used as a fraction collector. NDA products are stable for several hours[30] so in principle the sample plugs could be stored in tubing and analyzed later by pumping stored samples into the chip. In this approach samples could be pumped into the electrophoresis chip more slowly to allow more time for analysis while preserving the temporal resolution of sample collection; however, this would also require longer analysis times. Temporal resolution of sampling might be improved by better connections between dialysis probe and chip. Indeed, we have recently found that small changes in connections and droplet formation can improve temporal resolution to 10 s or better. Even more improvement might be obtained by using plugs created before the sampling probe[17] or by membrane-less sampling methods such as push-pull perfusion.[53]

CONCLUSION

This work demonstrates the feasibility of coupling segmented microdialysate flow with CE analysis on a chip for near real-time in vivo chemical monitoring. This method takes advantage of both conserved temporal resolution with segmented flow and high resolving power of CE. The system offers a significant improvement in separation efficiency over previous dialysis-chip CE combinations and previous systems for electrophoretic analysis of multiphase flow. Although this work used anesthetized rats, we envision that as the engineering challenge of securely mounting the plug generator to animals is overcome, the system can be applied to freely-moving rats for behavior, pharmacology and physiology investigations. With optimization of separation conditions more transmitters can be measured. Improvement in temporal resolution may be possible leading to systems that approach the temporal resolution of many sensors, yet offering greater versatility and capacity for chemical analysis.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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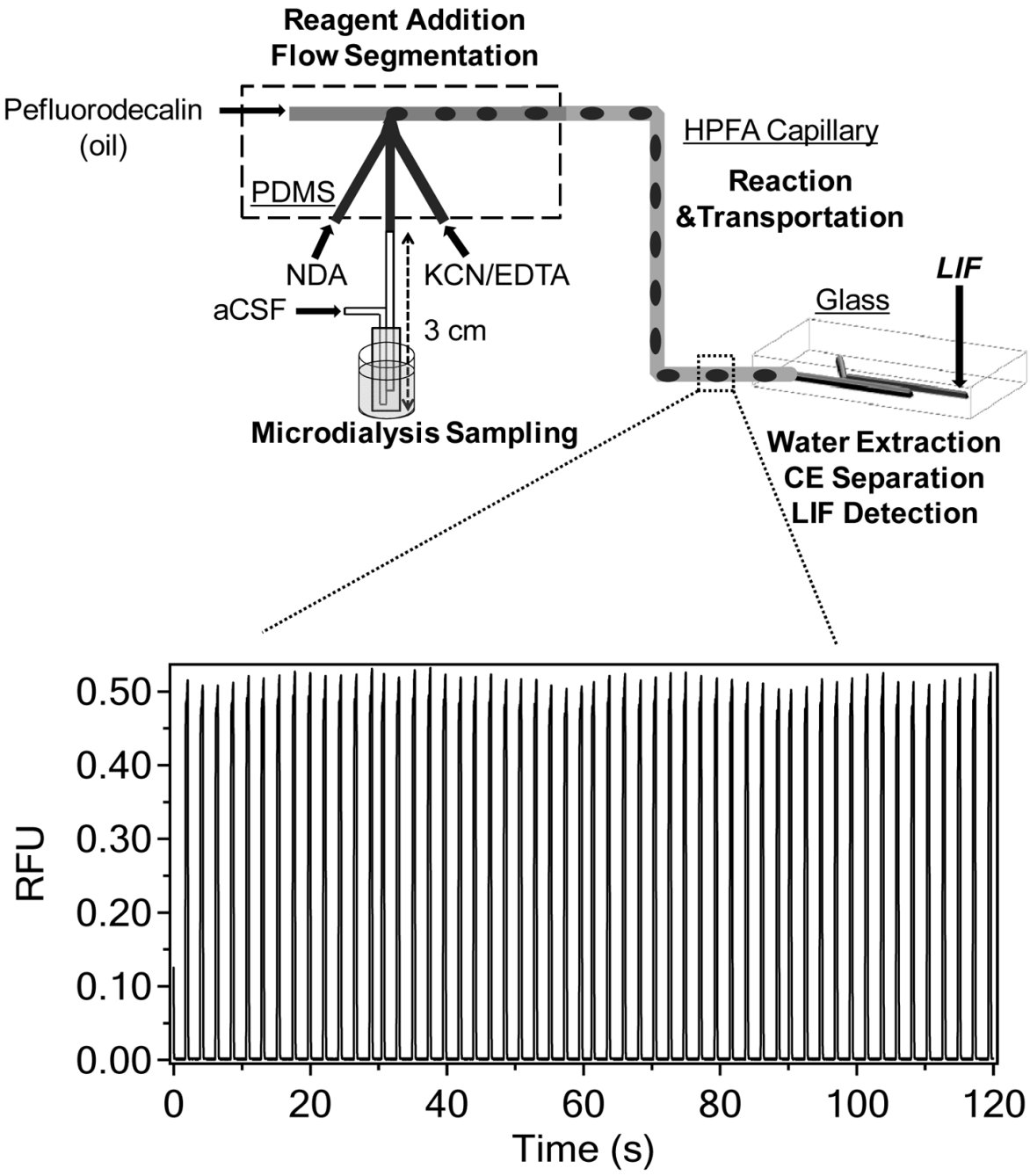

Figure 1.
Illustration of the dual-chip system used in this work. Inset graph shows reproducible total fluorescence intensity of plugs containing NDA derivatized amino acids as they pass through the HPFA connector tubing. RSD for peak height is 1.4%.

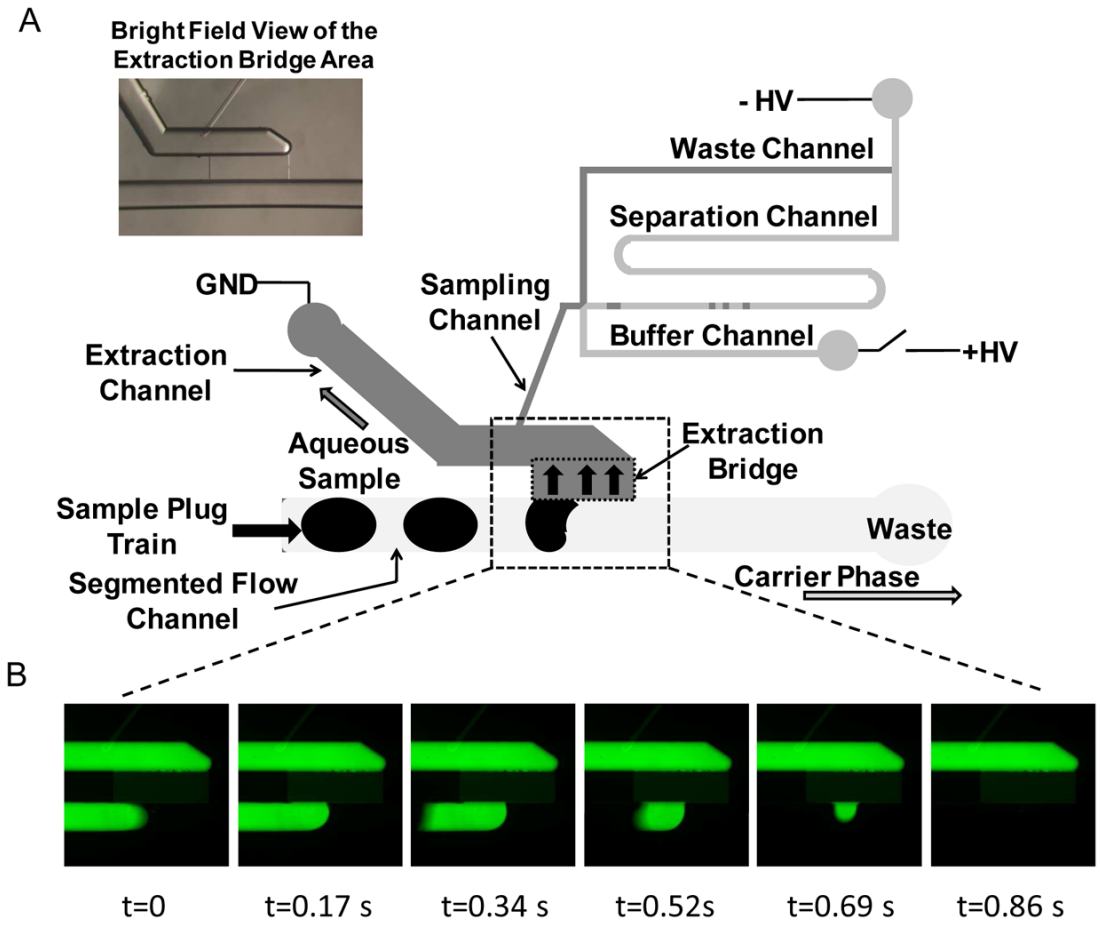

Figure 2.

(A) Illustration of the operational scheme of the CE chip. Incoming sample plugs reach the extraction bridge interface and are separated from carrier phase. By applying negative high voltage to the CE outlet and grounding the sample outlet reservoir, sample is drawn to the injection cross by electroosmotic flow and is gated by a cross-flowing buffer stream that is connected to positive high voltage through a relay. As the relay position is changed from closed to open for 60–200 ms second, injection is made and electrophoresis separation is carried out. Inset is the bright field picture of the extraction bridge interface. All the sampling and electrophoresis channels are 42μm wide by 6 μm deep. (B) Series of micrographs illustrating the approach and extraction of plugs through extraction bridge. Black region in the segmented flow channel represents the continuously flowing carrier phase.

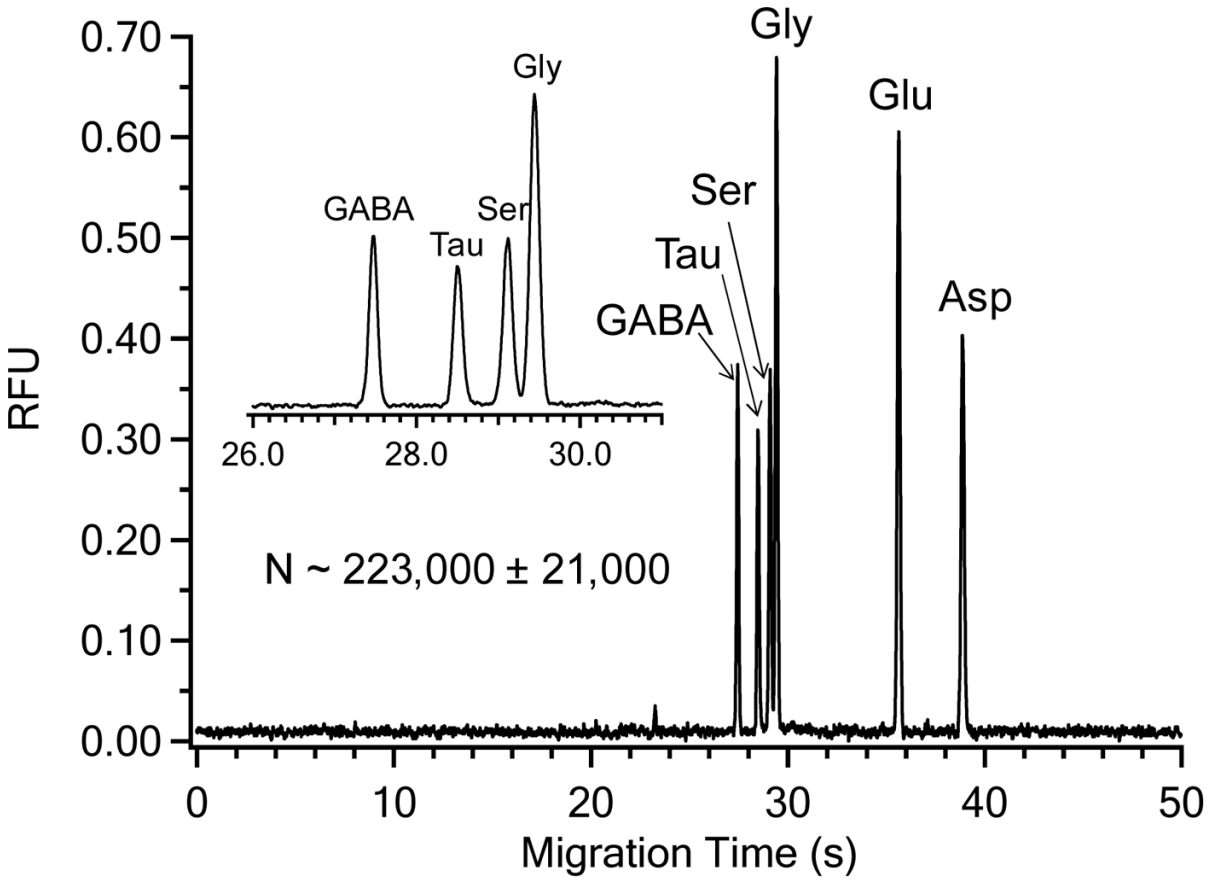

Figure 3.

An electropherogram showing high efficiency separation of GABA, taurine (Tau), serine (Ser), glycine (Gly), glutamic acid (Glu), and aspartic acid (Asp) standards dissolved in aCSF using 11.1 cm separation length. Amino acid concentrations were 50 μM. Separation buffer was 10 mM sodium tetraborate, 0.9 mM HPβCD at a pH of 9.7. Electric filed strength was 720 V/cm and injection width was 60 ms. Inset is the enlargement of early migrating peaks.

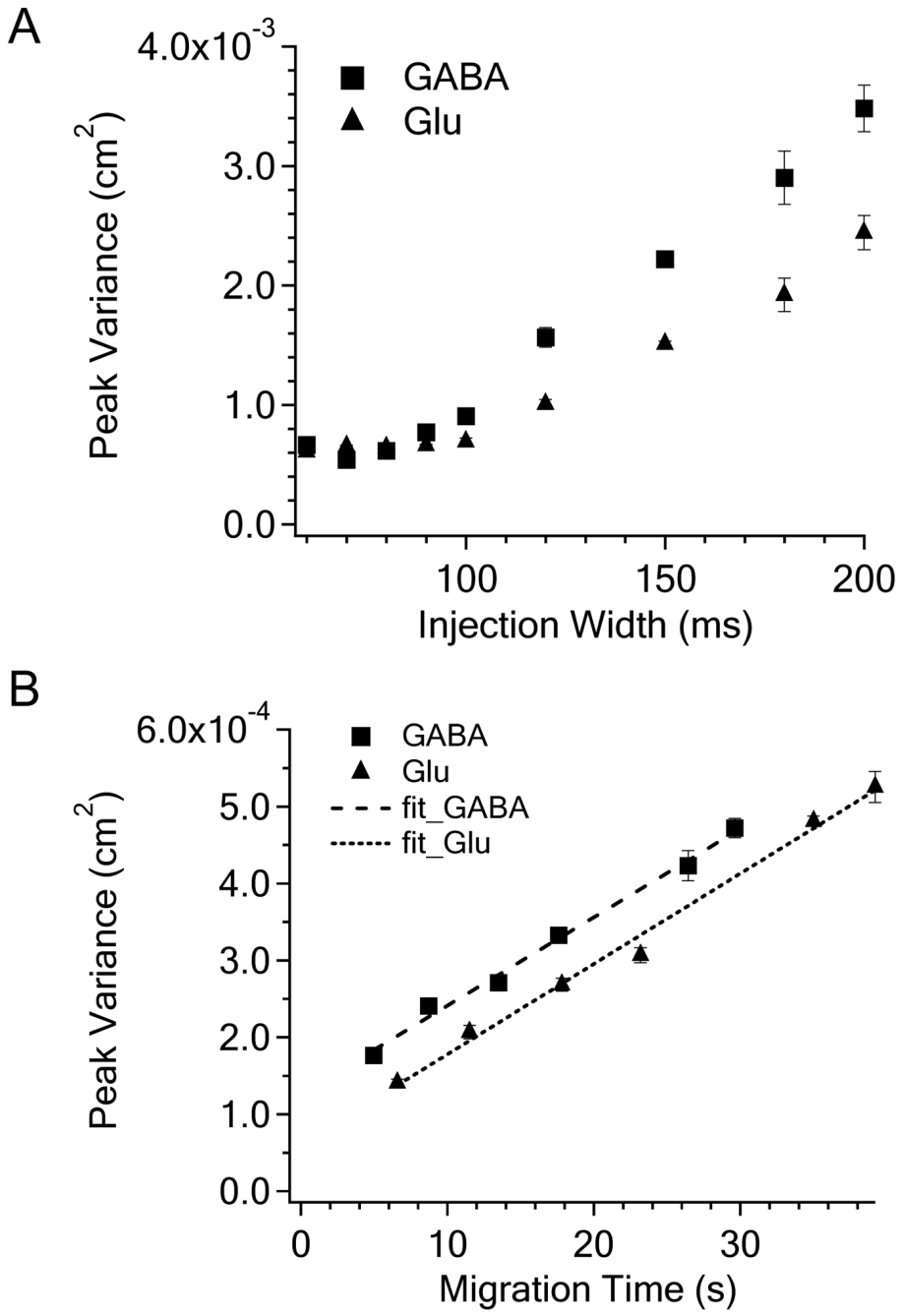

Figure 4.

Effect of injection width (A) and migration time (B) on peak variance for glutamate (Glu) and GABA using chip shown in Figure 3. Data in (A) were obtained at 11.1 cm migration distance, while injection time was altered. Data in (B) were obtained with 80 ms injection width, while migration distance was altered and migration times were recorded. The field strength was kept at 720 V/cm. Separation buffer was the same as in Figure 3.

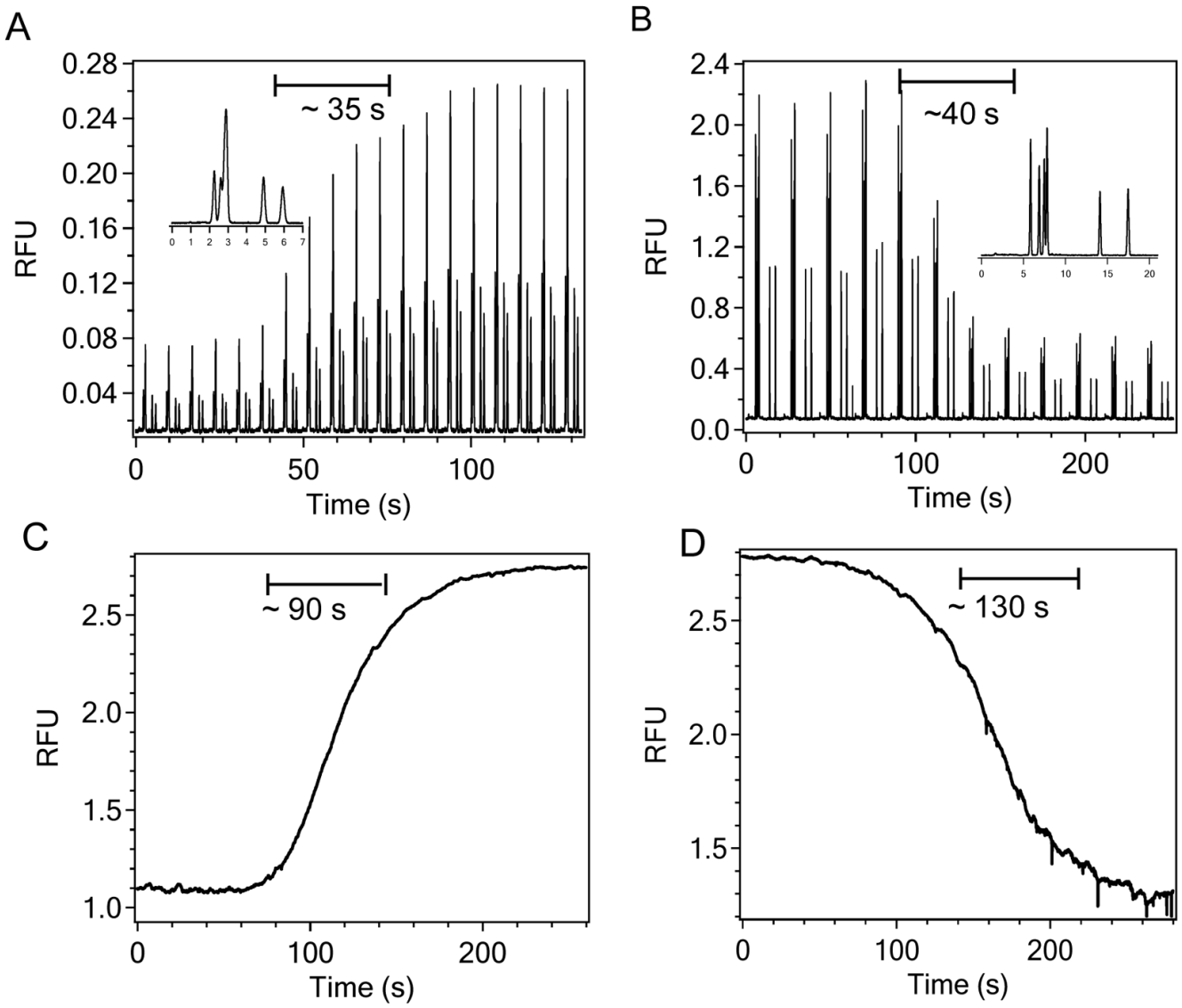

Figure 5.

(A) and (B) Temporal responses obtained with the system using segmented flow. Electropherograms in (A) were collected every 7 s at 1.5 cm. Electropherograms in (B) were collected every 20 s at 11.1 cm. Electric field and separation buffer were the same as described in Fig. 4. (C) and (D) Temporal responses obtained at the end of 60 cm 150 μm i.d. capillary after microdialysate were mixed with NDA derivatization reagents at PEEK cross. Step changes of concentration were made between 10 μM and 50 μM amino acid standard solutions following the protocol describe in Experimental section. Temporal resolutions are denoted on the graphs.

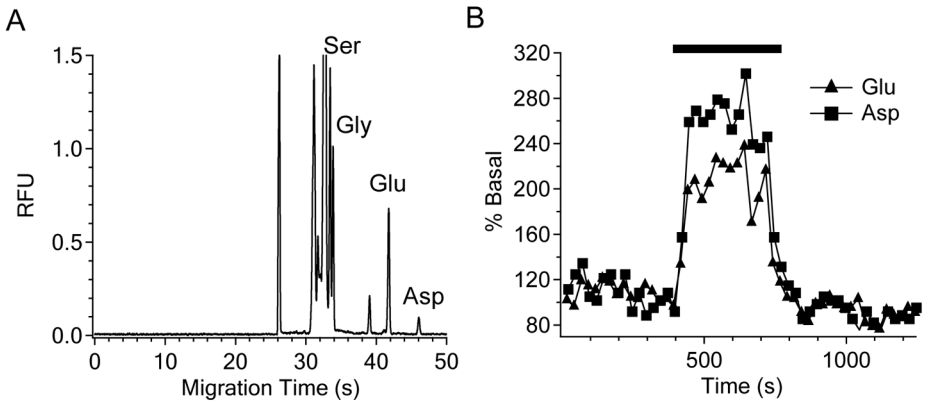

Figure 6.

(A) Typical electropherogram obtained in vivo from the rat striatum with on-line derivatization using the set-up. Peaks for serine (Ser), glycine (Gly), glutamate (Glu) and aspartate (Asp) are labeled in the graph. Electric field was 647 V/cm. Injection width was 80 ms. (B) Effect of PDC infusion on glutamate and aspartate basal levels. The black bar denotes infusion of PDC corrected for dead time (~6.5 min). Each point represents peak height of glutamate or aspartate from one electropherogram collected every 25 s.