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Microchip electrophoresis with electrochemical detection for the determination of analytes in the dopamine metabolic pathway

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Abstract

A method for the separation and detection of analytes in the dopamine metabolic pathway was developed using microchip electrophoresis with electrochemical detection. The microchip consisted of a 5 cm PDMS separation channel in a simple-t configuration. Analytes in the dopamine metabolic pathway were separated using a background electrolyte composed of 15 mM phosphate at pH 7.4, 15 mM SDS, and 2.5 mM boric acid. Two different microchip substrates using different electrode materials were compared for the analysis: a PDMS/PDMS device with a carbon fiber electrode and a PDMS/glass hybrid device with a pyrolyzed photoresist film carbon electrode. While the PDMS/PDMS device generated high separation efficiencies and good resolution, more reproducible migration times were obtained with the PDMS/glass hybrid device, making it a better choice for biological applications. Lastly, the optimized method was used to monitor L-DOPA metabolism in a rat brain slice.

Keywords

Brain slice; Dopamine; Electrochemical detection; L-DOPA; Microfluidics

1 Introduction

Dopamine is an important neurotransmitter [1,2] that has been implicated in reward, social behavior, movement, mood, and addiction, as well as neurological disorders such as Parkinson's and Huntington's diseases [3]. The most popular method for monitoring dopamine release *in vivo* is fast scan cyclic voltammetry [4,5]. However, it is not possible to measure dopamine and its metabolites simultaneously with this technique, making it impossible to investigate the effect of drugs or other treatments on the dopamine metabolic pathway (Fig. 1). Specifically, L-DOPA, the precursor to dopamine, has been the gold-standard treatment for Parkinson's disease for over half a century, and, therefore, much research has been performed regarding its role in neurotransmission and neuromodulation [6,7]. Methods for the simultaneous measurement of dopamine and L-DOPA along with

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their metabolites are important to understanding the roles of these compounds in biological processes and drug metabolism. Methods such as capillary electrophoresis with laser-induced fluorescence or electrochemical detection [8–11] and liquid chromatography with electrochemical detection [12] have been previously employed to analyze dopamine and/or related compounds in microdialysis samples and brain tissue.

Microchip electrophoresis (ME) is an excellent separation method for the analysis of biologically important molecules. Separations employing microchip electrophoresis are fast (sub-minute), highly efficient, and require low sample volumes (pL-to-nL) [13,14]. These qualities make microchip electrophoresis ideal for the analysis of time-sensitive, small volume, and precious biological samples. Additionally, microchip electrophoresis operates on a planar, chip-based platform, allowing integration of multiple steps (sampling, separation, detection) all onto a single device.

Many different substrate materials have been employed in microchip electrophoresis, including glass, PDMS, PMMA, and paper, among others [15]. For many applications, the material of choice is glass as it has the same properties as the fused silica used in traditional capillary electrophoresis—strong EOF, low analyte adsorption, and good optical clarity. PDMS is also widely used for microchip electrophoresis due to its low cost, ease of fabrication, and the fact that electrodes can be easily incorporated into the device. However, PDMS devices have several disadvantages, including low EOF, analyte adsorption, and inconsistent analyte migration times [16]. Glass/PDMS hybrid devices can be an effective compromise in an attempt to combine the consistencies of glass with the ease of fabrication of PDMS.

Electrochemical detection (EC), especially amperometry, has long been employed as a detection strategy in microchip electrophoresis, due, in part, to the ability to integrate electrodes directly into the microchip format [17–19]. Additionally, many important biological molecules are natively electroactive and do not require derivatization prior to their detection. Both metal and carbon-based electrodes have been incorporated directly in-chip for a variety of applications [18,19]. However, integrating electrodes for electrochemical detection into an all-glass microchip electrophoresis device can be difficult and has not been reported for carbon electrodes. The procedure for creating all-glass microchip electrophoresis devices with integrated electrodes involves high temperatures and pressures and requires a complete seal around the electrodes and channel, which can be challenging to accomplish [20,21]. For the detection of catecholamines and related compounds, various types of carbon electrodes integrated in polymer and plastic substrates have been employed, including carbon fiber [22], carbon paste [23], carbon ink [24], and pyrolyzed photoresist film [25,26], as they generate good responses for many biological, carbon-containing analytes.

In this study, the separation and detection of compounds in the L-DOPA metabolic pathway (Fig. 1) were optimized using microchip electrophoresis with electrochemical detection. As this method will be used in the future for on-line monitoring of brain microdialysis samples, maintaining biological and injection compatibility with the run buffer was paramount. Additionally, the performance of an all-PDMS device with a carbon fiber electrode was

compared to that of a PDMS/glass hybrid device with a pyrolyzed photoresist film carbon electrode. The optimized method was then employed *in vitro* to study the metabolism of L-DOPA by a brain slice. In the future, this method will be coupled on-line to microdialysis sampling for on-animal analysis of drug metabolism *in vivo*.

2 Materials and methods

2.1 Reagents

The following chemicals were used as received: AZ 1518 positive photoresist and AZ 300 MIF developer (AZ Electronic Materials, Sommerville, NJ, USA); SU-8 10 and SU-8 developer (Micro-Chem, Newton, MA, USA); L-tyrosine (L-Tyr), 3,4-dihydroxy-L-phenylalanine (L-DOPA), homovanillic acid (HVA), 3,4-dihydroxyphenylacetic acid (DOPAC), dopamine hydrochloride, 3-methoxytyramine hydrochloride (3-MT), sodium phosphate dibasic, sodium phosphate monobasic, and boric acid (Sigma-Aldrich, St. Louis, MO, USA); NaOH and 2-propanol (IPA) (Fisher Scientific, Fairlawn, NJ, USA); sodium dodecyl sulfate (SDS) (Thermo Scientific, Waltham, MA, USA); and PDMS and curing agent (Sylgard 184 silicon elastomer base and curing agent, Dow Corning Corp., Midland, MI, USA). Additionally, the following were also used: high temperature fused silica glass plates (4 in $\times$ 2.5 in $\times$ 0.085 in, Glass Fab, Rochester, NY, USA); 33 μ m diameter carbon fibers (Avco Specialty Materials, Lowell, MA, USA); copper wire (22 gage, Westlake Hardware, Lawrence, KS, USA); epoxy (J-B Weld, Sulphur Springs, TX, USA); colloidal silver liquid (Ted Pella, Inc., Redding, CA, USA); and 18.2 M Ω water (Millipore, Kansas City, MO, USA).

2.2 Fabrication of PDMS channels

The fabrication of PDMS microchips has been described elsewhere [27]. Briefly, a silicon master was created using SU8-10 negative photoresist spun onto a 4 in diameter silicon wafer to a thickness of 15 μ m using a Cee 100 spin coater (Brewer Science, Rolla, MO, USA). The wafer was then heated to 65°C for 2 min and ramped to 95°C for 5 min as the soft bake procedure on a programmable hotplate (Thermo Scientific, Waltham, MA, USA). After the soft bake, the coated wafer was covered with a negative transparency mask created using AutoCad (Autodesk, San Rafael, CA, USA) and printed onto transparencies (Infinite Graphics, Minneapolis, MN, USA) of the fluidic channels and exposed at 344 mJ/cm² using a UV flood source (ABM Inc., Scotts Valley, CA, USA). After exposure, the wafer was again transferred to a programmable hotplate for the post-bake at 65°C for 1 min, then 95°C for 2 min. The master was then developed in SU8 developer, rinsed with isopropyl alcohol, and dried with nitrogen. Finally, a hard bake was performed at 200°C for 2 h. This procedure produced a master with 15 μ m raised channels that were 40 μ m wide as measured by an Alpha Step-200 surface profiler (KLA-Tencor, Milpitas, CA). In this simple-t device, the separation channel was 5.0 cm and the side and top arms were 0.75 cm in length. To create the PDMS microchip from the silicon master, PDMS/curing agent was mixed at a 10:1 ratio and poured onto the master to form a thickness of about 2 mm. The PDMS was cured overnight at 70°C, after which the PDMS channels were peeled from the Si wafer. Reservoirs for buffer and pump waste were punched into the PDMS using a 4 mm biopsy punch (Harris Uni-Core, Ted Pella, Inc., Redding, CA, USA).

2.3 Electrode fabrication

Carbon fiber electrode in PDMS—The placement of carbon fiber electrodes into PDMS has been described previously [28]. Briefly, a 5 in silicon master wafer containing a raised structure with the dimensions $35\ \mu\text{m} \times 40\ \mu\text{m}$ was created using the same procedure outlined in section 2.2. PDMS was poured over the master to create a trench with the same dimensions. Once hardened, the PDMS was placed on a glass plate to add structural stability, and a $33\ \mu\text{m}$ carbon fiber was placed into the trench. The carbon fiber was connected to detection electronics through silver colloidal liquid and copper wire.

Pyrolyzed photoresist film electrode on quartz glass—Pyrolyzed photoresist film electrode fabrication has been described previously [25,29]. Briefly, a 2.5×4 in plate of quartz glass was cleaned using acid and base piranha. After drying the substrate for 2 h at 200°C on a programmable hotplate, AZ-1518 positive photoresist was spun onto the substrate using a Cee 100 spin coater. Photoresist (4 mL) was dynamically deposited on the substrate while at 100 rpm for 10 s. The spin coater was then ramped at 500 R/s to 2,000 rpm and held for 20 s. The substrate was then heated to 100°C for 1.5 min on a programmable hotplate. Positive mask designs for the electrodes were created using AutoCad software and printed onto transparencies. The coated substrate was placed on a reflective surface, covered with the transparency mask, and exposed at $21.5\ \text{mW}/\text{cm}^2$ for 4 s using a UV floodsource. Post exposure, the substrate was developed in MIF 300 for about 10 s and rinsed with nanopure water.

Substrates with photoresist were then placed in a Linden-BlueM 3 Zone Tube furnace (Cole-Parmer, Vernon Hills, IL, USA), with a constant flow of nitrogen gas at about 5 psi throughout the pyrolysis procedure. The temperature program was ramped from room temperature to 925°C at $5.5^\circ\text{C}/\text{min}$ and held there for 1 h. The furnace was then allowed to cool to room temperature. Final electrode dimensions, as measured using a surface profiler, were $35\ \mu\text{m}$ wide and $2.3\ \mu\text{m}$ in height.

2.4 Electrophoresis procedure

Complete microchips for microchip electrophoresis were constructed by placing the PDMS channel layer in conformal contact with the electrode containing substrate (either PDMS with a carbon fiber electrode or glass with a PPF electrode), creating a reversible bond between the two. Care was taken to align the electrode at the very end of the separation channel (Fig. 2).

Prior to electrophoresis, the channels in the device were flushed with isopropyl alcohol, 0.1 M NaOH, and the separation buffer for about 5 min each. All electrophoresis procedures were accomplished using two Spellman CZE 1000R (Hauppauge, NY, USA) high voltage power supplies controlled using LabView (National Instruments, Austin, TX, USA) programs written in-house. A gated injection scheme was utilized for sample introduction and separation in the simple-t device. A gate was accomplished by applying 1900 V at the sample reservoir, 1600 V at the buffer reservoir, and holding the sample waste and detection reservoirs at ground (Fig. 2). All injections were accomplished by floating the buffer voltage for 1.0 s, then reapplying the voltages for the separation. Standard solutions (10 mM) were

prepared daily in 18.2 mΩ water and diluted into 15 mM phosphate buffer (pH 7.4) at the time of analysis. Unless otherwise noted, 100 μM standard solutions were employed.

2.5 Electrochemical detection and data analysis

Electrochemical detection was accomplished using a two electrode system (carbon working, Ag/AgCl reference (BASi, West Lafayette, IN, USA)) with an electrically isolated potentiostat (Pinnacle Technology, Inc., Lawrence, KS, USA). The sampling rate for this device was 10 Hz, and data acquisition was performed through wireless transmission and visualized with Pinnacle Acquisition Laboratory (PAL 8400) software. This potentiostat has been used previously in our group for in-channel amperometric detection [30]. For all separation optimization and characterization experiments, the working electrode (carbon fiber or PPF) potential was held at 0.8 V (vs. Ag/AgCl). For brain slice experiments, the working electrode potential was held at 1.0 V (vs. Ag/AgCl), as that potential is further along the current limiting plateau. The electrode was placed at the channel outlet (Fig. 2). This electrode alignment has been employed previously in our lab and yields higher efficiency separations than other detection configurations with minimal interference between the separation and detection voltages [31]. All data were analyzed using Origin 8.6 software (OriginLab, Northampton, MA, USA) after baseline subtraction. In calculating performance parameters for the PDMS/PDMS device, three different microchips were employed and the migration times, resolution values, and number of theoretical plates/meter for up to 25 total injections (across the three chips) were averaged. However, due to occasional co-migration between both dopamine and DOPAC and dopamine and HVA in this device (section 3.2.1), theoretical plate values for those analytes were based on only 23 total injections. With the PDMS/glass device, performance parameters were calculated based on a total of 30 injections across three different microchips (10 injections per chip). Resolution values for both devices were calculated between the stated analyte and the following peak/analyte, as shown in Table 1.

2.6 Brain slice

A brain from a male Wistar rat weighing 385 g was obtained postmortem and used in this experiment. The rat was previously used for a seizure trial [32], and 50 mg/kg 3-mercaptopropionic acid was administered to the rat i.p. over 48 h prior to its death and the experiments described herein. All experiments were performed in accordance with regulations of the Institutional Animal Care and Use Committee (IACUC) at the University of Kansas, which operates with accreditation from the Association for Assessment and Accreditation of Laboratory Animal Care (AAALAC). The day of the experiment, the brain was removed postmortem and immediately placed in a specimen container surrounded by liquid nitrogen. Prior to the microchip electrophoresis experiments, the brain was partially thawed in ice-cold aCSF, and sagittal slices (~ 3 mm thick) were prepared by hand using a razor blade. The brain slices were then allowed to thaw completely in aCSF. An individual brain slice was then placed in 515 μL of 15 mM phosphate (pH 7.4) in a 2 mL centrifuge tube. Using a water bath, the temperature was maintained at 37°C for the entire experiment. An initial baseline aliquot (25 μL) was removed immediately and 10 μL of a 10 mM stock solution of L-DOPA was added to the liquid surrounding the slice so that the brain slice was

surrounded by 200 μ M L-DOPA in 15 mM phosphate (pH 7.4). Subsequent aliquots (25 μ L) were removed from the supernatant and analyzed at indicated time points.

3 Results and discussion

3.1 Separation optimization (PDMS/PDMS device)

3.1.1 Background electrolyte—The initial separation optimization of five compounds in the dopamine metabolic pathway was performed using an all-PDMS 5 cm simple-t device (Fig. 2). In these studies, the working electrode was a 33 μ m carbon fiber electrode. The ultimate goal of this separation is on-line analysis of brain microdialysis samples on-animal, where artificial cerebrospinal fluid will be employed as the perfusate. Therefore, a 15 mM sodium phosphate buffer at pH 7.4 was chosen as the background electrolyte to maintain injection compatibility. Additionally, better electrochemical responses were observed in this system with sodium phosphate acting as the background electrolyte when compared to boric acid (data not shown). To establish a strong, consistent gate and sample injection, separation voltages and injection time were optimized using the 15 mM sodium phosphate buffer (pH 7.4). These parameters are key to both inhibiting sample leakage into the separation channel during separation and injecting an adequate amount of sample to detect. Voltages of 1900 V and 1600 V applied to the sample and buffer reservoirs, respectively, produced a stable gate with a separation field strength of $\sim$ 220 V/cm, as calculated using Kirchhoff's laws [33]. These voltages enabled the highest field strength possible, while still maintaining a prolonged chip lifetime when using phosphate as the background electrolyte. These voltages, combined with an injection time of 1.0 s, allowed adequate sample introduction into the channel. While sodium phosphate concentrations higher than 15 mM were also investigated, the high ionic strength lead to excessive Joule heating, resulting in rapid deterioration of the PDMS and a reduction in electroosmotic flow (data not shown). A concentration of 15 mM sodium phosphate allowed for a prolonged chip life while still maintaining a good buffering capacity.

3.1.2 Addition of SDS—A strong, stable EOF is necessary in microchip electrophoresis to establish a consistent gate and reproducible sample injection and to enable all analytes to reach the detector. Unfortunately, PDMS does not possess a high negative surface charge as does glass, so SDS was added to the background electrolyte in the PDMS-based device to generate a negative charge at the channel walls [34]. With the low SDS concentrations in this system, dopamine co-migrated with other analytes of interest. However, if the SDS concentration was increased to concentrations above the critical micellar concentration [35], the positively charged dopamine interacted electrostatically with the negatively charged SDS micelles, causing it to migrate later (Fig. 3A). The other analytes, which are not positively charged at pH 7.4, were unaffected by the negative micelles and their migration times remained unchanged. At an SDS concentration of 15 mM, the migration time of dopamine was substantially increased, permitting it to be resolved from the other analytes of interest.

3.1.3 Addition of boric acid—Due to the similarity in the chemical structures and charges of L-Tyr and L-DOPA as well as that of HVA and DOPAC, their separation was initially challenging. Both L-Tyr and L-DOPA possess amine and carboxylic acid functional

groups, and the only difference in their structures is that L-DOPA contains a catechol moiety while L-Tyr does not. The same is true for HVA and DOPAC. In order to resolve these pairs of analytes, an additional separation strategy was employed. Borate has long been used as a background electrolyte in microchip electrophoresis, and it is well known that it complexes with catechol moieties [36]. Because L-DOPA and DOPAC are catechols, while L-Tyr and HVA are not, boric acid was added to the separation buffer as a complexation reagent to resolve the compounds (Fig. 3B). A concentration of 2.5 mM boric acid was sufficient to provide good resolution for five analytes. Therefore, the optimal separation buffer was determined to be 15 mM phosphate, 15 mM SDS, and 2.5 mM boric acid at pH 7.4. These conditions resulted in separation efficiencies between 60,000 and 290,000 theoretical plates/meter for the range of analytes, resolutions of 1.2 or better, and a complete separation in under 60 s (Table 1).

3.2 Microchip material optimization

3.2.1 PDMS/PDMS microchip with carbon fiber electrode—For the separation optimization described in the previous section (section 3.1), an all-PDMS device was employed. However, it quickly became apparent that reproducibility of analyte migration times injection-to-injection and chip-to-chip was problematic in this device. Previous researchers have reported that PDMS-based devices suffer from migration time irreproducibility due to inconsistencies in the EOF over time due to a changing channel surface [37]. As can be seen in Table 1, this was also the case for the separation of the analytes in this study. When using the PDMS/PDMS device, the migration times deviated by 10–15% intra-day (data not shown) and 14–21% overall (Table 1). Dopamine was especially problematic, with a migration time of 56 ± 9 s for all experiments. Additionally, in some experiments the dopamine peak would slowly decrease in migration time over multiple injections on the same microchip and begin to co-migrate with the other peaks (data not shown). While the resolution between the other analytes remained relatively constant or increased, the resolution between dopamine and the other analytes deteriorated. This is evident from the high standard deviation in resolution number for DOPAC and dopamine (1.7 ± 2.4). It is believed that this inconsistency in the migration of dopamine was due to the high SDS concentration in the run buffer, as dopamine was the only analyte affected by the SDS micelles. This effect may have been caused by the dynamic equilibria between the PDMS and SDS, as well as dopamine and SDS changing over time. There is also the possibility that this effect is due to continuous SDS adsorption by PDMS. Because dopamine is an important analyte in this system, reducing its migration time variability was key in these studies.

3.2.2 PDMS/glass microchip with pyrolyzed photoresist film electrode—In contrast to devices made from PDMS, glass devices exhibit a strong, reproducible EOF and, therefore, more reproducible migration times. However, carbon electrodes are not currently compatible with the bonding procedures used to make all-glass devices, so in this study a PDMS/glass hybrid device was employed. This device consisted of three walls of PDMS (channel substrate) and one wall of glass (electrode substrate). The use of a PDMS/glass hybrid device made it possible to substitute a pyrolyzed photoresist film (PPF) carbon electrode for the carbon fiber electrode. PPF electrodes have been shown to display higher

sensitivities and lower LODs than carbon fiber electrodes, due to a decrease in background noise [29]. In the PDMS/glass hybrid device, the migration time of all analytes increased relative to that in the PDMS/PDMS device. While the EOF values for PDMS/glass devices have been shown to be faster than that in PDMS/PDMS devices [37], these previous studies were performed without the use of a surfactant in the run buffer. The addition of high amounts of surfactants in PDMS microchips have been shown to generate a much faster EOF [34]. We believe that the faster migration times in the PDMS/PDMS device are due to this effect. With regards to the separation reproducibility, the migration time reproducibility was dramatically improved (e.g., 65 ± 5 s for dopamine) due to the stabilizing presence of just one wall of glass when using the hybrid microchip. The RSD for migration times in the PDMS/glass device ranged from 4–6% intra-day (data not shown) and 7–9% overall (Table 1). However, the separation efficiencies and resolution were decreased compared to those in the all-PDMS device (Table 1). This trend is in agreement with previous studies by our group using laser-induced fluorescence detection to compare PDMS, glass, hybrid PDMS/glass, and polyester toner devices [37]. Using the PDMS/glass hybrid device, efficiencies between 30,000 and 180,000 theoretical plates/meter and resolutions of 1.1 or better in under 65 s were still achieved. Although there was decreased efficiency and resolution with the hybrid device, it was used for all further studies due to the dramatic improvement in migration time reproducibility. Prior to the brain slice studies, the migration time of another possible dopamine metabolite, 3-MT, was investigated. 3-MT was completely resolved from other analytes under the separation conditions (Fig. 3C).

3.3 Analysis of L-DOPA metabolism in brain slice

To investigate the conversion of L-DOPA into dopamine *in vitro*, a brain slice was incubated in 15 mM phosphate (pH 7.4) buffer at 37°C. The solution surrounding the brain slice was then spiked with L-DOPA at $t = 0$, and the total solution volume surrounding the brain slice at this time was 500 μ L. Every 10 min, a 25 μ L aliquot of the brain slice solution was removed and analyzed by microchip electrophoresis with electrochemical detection. Following the addition of L-DOPA to the brain slice, several additional peaks appeared in the electropherogram, as can be seen in Figure 4A. At the 10 min mark, a peak appeared that could correspond to either HVA or DOPAC. Unfortunately, the definitive identity of this peak could not be elucidated based solely on the migration times. Later, at $t = 30$ min, another peak appeared, corresponding to dopamine; its appearance over time can be seen in Figure 4B. After its appearance, the dopamine peak continued to increase over time, reaching a maximum at 50 minutes. The appearance of the dopamine peak at a later time than the HVA/DOPAC metabolite peak was expected, as the concentration of these metabolites is higher than that of dopamine due to the fast reuptake and metabolism of dopamine in the brain [38]. In the future, dual-series electrodes will be incorporated into the device to further confirm analyte identification through voltammetric characterization [22,28].

4. Concluding remarks

The separation and detection of six analytes in the dopamine metabolic pathway was accomplished in under 65 s with microchip electrophoresis and electrochemical detection

using a separation buffer consisting of 15 mM phosphate, 15 mM SDS, and 2.5 mM boric acid at pH 7.4. This separation was characterized with both all-PDMS and PDMS/glass hybrid devices and, while the all-PDMS devices did generate a more efficient separation, the PDMS/glass hybrid device exhibited much better migration time reproducibility. This method was then used to monitor L-DOPA metabolism in a rat brain slice over time using microchip electrophoresis with electrochemical detection. In the future, this method will be coupled to microdialysis sampling and employed on-animal to monitor catecholamines in a freely roaming animal (sheep).

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Abbreviations

ME	microchip electrophoresis
EC	electrochemical detection
PDMS	polydimethylsiloxane
PPF	pyrolyzed photoresist film
EOF	electroosmotic flow
DA	dopamine
HVA	homovanillic acid
DOPAC	3,4-dihydroxyphenylacetic acid
3-MT	3-methoxytyramine
L-Tyr	L-tyrosine
L-DOPA	L-3,4-dihydroxyphenylalanine

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Figure 1. Compounds in the dopamine metabolic pathway. Enzymes are: TH (tyrosine hydroxylase), AADC (aromatic L-amino acid decarboxylase), MAO (monoamine oxidase) and COMT (catechol-o-methyltransferase).

Figure 2. Simple-t microchip electrophoresis with electrochemical detection device. (A) Applied voltages and channel dimensions. (B) Electrode alignment at the end of the separation channel with a 35 μm PPF carbon electrode.

Figure 3.

Separation optimization of analytes in dopamine metabolic pathway. (A) Effect of SDS concentration on analyte migration times. Run buffer is indicated SDS concentration and 15 mM phosphate (pH 7.4). Each point corresponds to three sequential injections. (B) Effect of boric acid concentration on analyte migration times. Run buffer is indicated boric acid concentration and 15 mM phosphate (pH 7.4) and 15 mM SDS. (C) Optimized separation of analytes in the dopamine metabolic pathway. Analyte identities are indicated in the figure. Separation optimization in (A) and (B) was performed on an all-PDMS device with a carbon

fiber working electrode at 0.8 V (vs. Ag/AgCl) while (C) was performed on a PDMS/glass hybrid device with a PPF working electrode at 1.0 V (vs. Ag/AgCl).

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Figure 4. Microchip electrophoresis with electrochemical detection analysis of L-DOPA metabolism by a rat brain slice. (A) Electropherograms of standards and L-DOPA metabolism at 50 min aligned at L-DOPA peak. Peak identities are: (1) L-Tyr, (2) L-DOPA, (3) HVA, (4) DOPAC, (5) dopamine, and (6) 5-MT. (B) Appearance of dopamine after L-DOPA administration, monitored over time. Each point corresponds to three sequential injections of the same sample.

Table 1

Separation performance parameters

Analyte	PDMS/PDMS microchip ^a			PDMS/glass microchip ^b		
	t _m (s)	R	N·10 ³ /meter	t _m (s)	R	N·10 ³ /meter
L-Tyr	36 (± 5)	1.2 (± 0.4)	100 (± 26)	38 (± 3)	1.1 (± 0.2)	99 (± 20)
L-DOPA	39 (± 6)	4.4 (± 1.0)	60 (± 30)	41 (± 3)	3.3 (± 0.6)	30 (± 12)
HVA	50 (± 10)	1.8 (± 0.4)	290 (± 90)	52 (± 4)	1.6 (± 0.3)	180 (± 50)
DOPAC	53 (± 11)	1.7 (± 2.4)	230 (± 50)	56 (± 5)	2.4 (± 0.4)	130 (± 30)
Dopamine	56 (± 9)	-	190 (± 100)	65 (± 5)	-	70 (± 30)

^aPDMS/PDMS values were calculated using 3 different microchips and n > 23 total injections^bPDMS/glass values were calculated using 3 different microchips and n = 30 total injections