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Improving Microchip Capillary Electrophoresis with Electrochemical Detection Using a Bubble Cell and Sample Stacking Techniques

Qian Guan and Charles S. Henry

Department of Chemistry, Colorado State University, Fort Collins, CO, USA

Abstract

Two efforts to improve the sensitivity and limits of detection for MCE with electrochemical detection are presented here. One is the implementation of a capillary expansion (bubble cell) at the detection zone to increase the exposed working electrode surface area. Bubble cell widths were varied from 1× to 10× the separation channel width (50 μm) to investigate the effects of electrode surface area on detection sensitivity, LOD, and separation efficiency. Improved detection sensitivity and decreased detection limits were obtained with increased bubble cell width, and LODs of dopamine and catechol detected in a 5× bubble cell were 25 nM and 50 nM, respectively. Meanwhile, fluorescent imaging results demonstrated ~8% and ~12% loss in separation efficiency in 4× and 5× bubble cell, respectively. Another effort for reducing the LOD involves using field amplified sample injection (FASI) for gated injection and field amplified sample stacking (FASS) for hydrodynamic injection. Stacking effects are shown for both methods using amperometric detection and pulsed amperometric detection (PAD). The LODs of dopamine in a 4× bubble cell were 8 nM and 20 nM using FASI and FASS, respectively. However, improved LODs were not obtained for anionic analytes using either stacking technique.

Keywords

Bubble cell; Electrochemical detection; Field amplified sample injection; Field amplified sample stacking; Microchip capillary electrophoresis

1 Introduction

Recently, with the advent of microfluidic devices and lab-on-a-chip technology, interest in CE-based analyses has grown due to the high separation efficiencies afforded by CE as well as the potential to perform complex analyses in a point-of-care setting using miniaturized, portable devices [1]. Microchip CE (MCE) also has the advantages of decreased analysis time, integrated sample processing, high portability, high throughput, minimal reagent consumption, and low analysis cost [2,3]. However, one major limitation of CE and also MCE analyses is the poor concentration sensitivity caused by the limited volume of injected samples and the low absorption path-length if UV detection is used. To address these issues, z- or u-shaped optical path and multireflection cells have been employed in traditional CE and MCE formats [4–6]. Also bubble-shaped detection cells in which the optical path length was increased have been utilized to enhance absorbance detection sensitivity and LOD [7,8].

Correspondence: Dr. Charles S. Henry, Department of Chemistry, 1872 Campus Delivery, Fort Collins, CO 80523, USA
Chuck.Henry@ColoState.EDU **Fax:** +1-970-491-1801.

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Furthermore, LIF detection instead of UV absorbance provides lower detection limit in both CE and MCE [9].

An additional means of improving detection limits in CE and MCE is to employ sample preconcentration methods [10-14]. The most widely adapted approaches for on-line sample pretreatment include isotachopheresis (ITP) [15,16], sample stacking [17-20], solid phase extraction (SPE) [21-23], and sweeping techniques [24-27]. Field amplified sample injection (FASI) and field amplified sample stacking (FASS) are two widely used sample preconcentration techniques in traditional CE, and preconcentration factors from 10 to 1000 have been achieved [17-20]. For both methods, sample enrichment is based on the velocity change of the analytes between the sample and running buffers, but subtle differences exist in the sample introduction in which electrokinetic and hydrodynamic injection are employed in FASI and FAASS, respectively. Compared to ITP, which uses a binary buffer system to confine the sample between a leading and a terminating buffer, FASI and FAASS are more convenient techniques to incorporate with electrophoretic analysis because of the simple requirement of the manipulation of just two streams, the running and sample buffer. The first application of FASI in MCE was reported by Jacobson and Ramsey, resulting in no more than 10-fold detection enhancement due to the pressure-driven peak broadening effects [20]. To achieve further enrichment, one approach made use of the separation channel, or an additional branch channel, to load a large volume of low-conductivity sample solution, and then simultaneously pushed sample buffer out of the separation channel while stacking analytes [28,29]. In another approach an additional porous polymer structure was employed to stabilize the conductivity gradient boundaries to enhance detection sensitivity up to 1000-fold [30,31]. However, these sample stacking approaches were limited by more complicated MCE schemes, poorly controlled sample injection volumes and laborious procedures. Furthermore, most analytes enriched in stacking techniques were detected optically or with mass spectrometry [32-36]. Shim et al. reported on-chip electrochemical detection of trace DNA using microchip gel electrophoresis with FASI and FAASS [37]. An ~25,000-fold improvement in detection sensitivity was achieved when gold nanoparticles were added to the stacking and separation buffers containing a hydroxypropyl cellulose matrix as well as a conducting polymer-modified electrode.

Electrochemical detection (ECD) is an attractive alternative to optical detection for microfluidic and lab-on-a-chip applications, because it not only offers detection limit comparable to fluorescence [38], but is also less expensive and complex. ECD systems can also be readily miniaturized, something which is hard to do with detection systems like LIF and MS [39,40]. Furthermore, based on the electroactivity difference of analytes, ECD has the advantages of specificity through redox chemistry, and selectivity through potential control [41,42]. By increasing the total number of working electrodes and thus the total number of applied detection potentials, MCE-ECD has the potential to meet the goal for increasing the amount of detected analytes in a single metabolic profiling analysis [43].

Most MCE-ECD systems use end-column detection where the electrode is placed at the end of the capillary [44]. A significant improvement in detection sensitivity has been achieved when the separation current was grounded by using microfabricated Pd or Pt electrodes as a decoupler before reaching the working electrode [45-47]. Our group has reported sensitive detection of a wide range of analytes by incorporation of a Pd microwire decoupler with Au or Pt working electrodes into a microchip CE-ECD device [43,48,49]. Recently, surface modification of working electrodes with nanomaterials, such as carbon nanotubes (CNT) [50-52], gold nanoparticles [37], and nanowires [53], has been shown to improve detection limits for some analytes. Significant improvements in the performance of MCE-ECD were observed using a carbon nanotube (CNT)-modified working electrode for the detection of several classes of hydrazine, phenol, purine, and amino acid compounds [54]. The broad and

significant catalytic activity exhibited by the CNT-modified electrodes has promise for a wide range of bioanalytical and environmental applications. In addition, detection sensitivity can be improved by increasing the surface area of the working electrode exposed to the fluid flow. Recently, our group presented the implementation of a capillary expansion at the detection zone, termed a bubble cell, to improve the compatibility and applicability of contact conductivity detection in MCE [55]. This design lowers the effective separation field in the conductivity cell and consequently reduces the voltage drop between the detection electrodes. Similar designs can also be employed to improve the detection sensitivity of our current MCE-ECD system since the expanded detection zone provides increased electrode surface area and lowers the analyte velocity in the detection zone.

In this paper, we report the use of a bubble cell to improve detection sensitivity and LOD for MCE-ECD. Bubble cell widths were varied from 1× to 10× of the separation channel width (50 μm) to investigate the effects of electrode surface area on detection sensitivity and LOD using amperometric detection. Improved detection sensitivity and decreased detection limits were obtained with increased bubble cell width, and LODs of dopamine and catechol detected in a 5× bubble cell were 25 nM and 50 nM, respectively. Fluorescent imaging results demonstrated ~8% and ~12% loss in separation efficiency in 4× and 5× bubble cell, respectively. Furthermore, preconcentration of the sample was performed using FASI and FASS individually on the MCE system with a 4× bubble cell in the detection zone to explore the applicability of these two stacking techniques with electrochemical detection. Stacking effects were shown for both methods characterized with amperometric detection and pulsed amperometric detection (PAD). LODs of 8 nM and 20 nM for cationic analyte dopamine were afforded using FASI and FASS, respectively. Sample enrichment was also obtained for anionic analytes using both stacking techniques but LODs were not improved due to a large baseline perturbation associated with the EOF.

2 Material and methods

2.1 Chemicals

Hydrochloric acid (37%), N-tris[hydroxymethyl]methyl-2-aminoethane-sulfonic acid (TES), sodium dodecyl sulfate (SDS), 3,4-dihydroxyphenethylamine (dopamine), catechol, ascorbic acid, propylene glycol methyl ether acetate, DL-homocysteine (Hcy), and reduced glutathione (GSH) were purchased from Sigma-Aldrich (St. Louis, MO). L-tyrosine (Tyr) and L-cysteine (Cys) were obtained from Fluka (Buchs, Switzerland). Sodium hydroxide and boric acid were purchased from Fisher (Pittsburgh, PA). Fluorescein was received from Eastman (Rochester NY, USA). Other reagents used for the fabrication of MCE-ECD include SU-8 2035 photoresist (Microchem, Newton, MA), Sylgard 184 elastomer and curing agent (PDMS) (Dow Corning, Midland, MI), 4 in. silicon wafers (University Wafer, South Boston, MA), and microwires made of 99.99% Pd (diameter 25 μm) and 99.99% Au (diameter 25 μm) (Goodfellow, Huntingdon, England).

2.2 Solution preparation

Solutions were prepared in 18.2 MΩ water from a Millipore Milli-Q purification system (Millipore Corp., Billerica, MA). 10 mM stock solutions of dopamine, catechol, ascorbic acid, Hcy, Cys and GSH were individually prepared weekly in 10 mM HCl, while 10 mM Tyr solution was prepared in 20 mM NaOH. All stock solutions were stored at 4°C. A 20 mM TES buffer containing 1mM SDS was adjusted to pH 7 with concentrated NaOH and used as background electrolyte (BGE) for the separation of dopamine, catechol and ascorbic acid analytes. In sample stacking experiments, running buffer was diluted to prepare each sample buffer for the appropriate stacking factor (SF): 20 mM TES, 1mM SDS (pH 7.0) for SF 1; 4 mM TES, 0.2 mM SDS (pH 7.0) for SF 5; 2 mM TES, 0.1mM SDS (pH 7.0) for SF

10; and 0.2 mM TES, 0.01mM SDS (pH 7.0) for SF 100. A 20 mM boric acid buffer was adjusted to pH 9.2 with concentrated NaOH and used as BGE for the separation of Tyr, Hcy, Cys and GSH. Again, sample buffers were prepared for stacking experiments by dilution of running buffers: 20 mM boric acid (pH 9.2) for SF 1; 10 mM boric acid (pH 9.2) for SF 2; 4 mM boric acid (pH 9.2) for SF 5; and 2 mM boric acid (pH 9.2) for SF 10.

2.3 PDMS microchip fabrication

The method used to fabricate PDMS microchips using incorporated microwires for detection has been published previously [48,56]. Fig. 1A and 1B show schematics of two typical PDMS microchips ($50\text{ }\mu\text{m} \times 50\text{ }\mu\text{m} \times 6\text{ cm}$) used in these experiments. Design A has a double T injector with a 625 pL volume [57,58] for pinched injection [59], while design B has a straight T injector suited for gated and hydrodynamic injection modes [60,61]. Both designs have a bubble cell in their electrochemical detection zones. A bright field image of the silicon mold (for design A) with a bubble cell width $5\times$ of the separation channel width ($50\text{ }\mu\text{m}$) is shown in Fig. 1C. Each electrode channel is $50\text{ }\mu\text{m}$ wide with $125\text{ }\mu\text{m}$ spacing between the channels. A $25\text{ }\mu\text{m}$ Pd decoupler and two $25\text{ }\mu\text{m}$ Au working electrode (WEs) are inserted to pass through the bubble cell and the electrode channel a, b and c on both sides, respectively. The rectangular tapers connecting the electrode channels and the bubble cell are $20\text{ }\mu\text{m} \times 56\text{ }\mu\text{m}$ and are shown in the red oval. A similar electrode assignment is used in design B with only one WE employed.

2.4 MCE-ECD

Channels were first treated with 1 M NaOH for 30 min prior to running analyses. After rinsing with ultra-pure water, channels and reservoirs were then filled with BGE by applying pressure to a reservoir containing the solution. The buffer in the sample reservoir was replaced with sample solution prior to running analyses. The corresponding positions of sample (SR), sample waste (SW), buffer (BR) and buffer waste (BW) reservoirs for pinched, gated and hydrodynamic injection are shown in Fig. 1A and 1B, respectively. For both pinched and gated injections, equivalent volume of solutions were loaded in all reservoirs, while for hydrodynamic injection, the sample reservoir was filled with $80\text{ }\mu\text{L}$ of sample solution and the remaining reservoir were filled with $50\text{ }\mu\text{L}$ of buffer solution. Applied voltages were facilitated by a programmable high voltage power supply built in-house [62]. The Pd decoupler was always held at ground in all injection and separation phases to prevent exposing the detector electronics to high voltage. Pinched injection were performed by applying a high positive potential (450 V) to SR and BR, and a negative potential (-160 V) to SW. For its separation, a high positive potential (1200 V) was applied to BR while SR and SW were held at 450 V , allowing only buffer to pass through the separation channel. SW was held at ground for both gated and hydrodynamic injections as well as their corresponding separation phases. In gated mode, sample introduction was achieved by applying a high positive potential (1050 V) to SR and keeping floating in BR, while in hydrodynamic mode, both SR and BR were held at floating. Their following separation phases were performed by applying a proper high positive potential to BR while keeping voltage settings in all other reservoirs the same as their corresponding injection phases. Amperometric detection and pulsed amperometric detection (PAD) were employed (CHI 1010A Electrochemical Analyzer, CH Instruments, Austin, TX) in a two-electrode configuration [48]. The former was used for the detection of dopamine, catechol, and ascorbic acid, while the latter was used for the detection of Tyr, Hcy, Cys and GSH. A Pt wire (1 mm diameter) in the waste reservoir was acted as both auxiliary and pseudo-reference electrode [48]. Cleaning of Pd decoupler was done initially by running cyclic voltammetry (CV) from -1.0 V to 1.0 V at 0.1 V/s for 50 cycles. Two gold working electrodes were cleaned using CV by scanning from -0.5 V to 1.8 V at 0.5 V/s for 100 cycles while buffer was flowed over the electrodes.

3 Results and discussion

3.1 Bubble cell design

A bubble cell design (Fig. 1A and Fig. 1C for detection zone details) was tested with PDMS microchips using a dual working electrode detection configuration. All microchips had one downstream Au WE placed at the exit of the separation channel, with a Pd decoupler and an upstream Au WE placed in the channel of the bubble cell. Bubble cell widths changing from 1× to 10× of the separation channel width (50 μm) were chosen to investigate the effects of electrode surface area on detection sensitivity, LODs and separation efficiency at the upstream WE.

3.1.1 Characterization of bubble cell design with amperometric detection—

Dopamine and catechol were chosen as model analytes to characterize the new bubble cell design using amperometric detection. Significant increases in peak heights for both model analytes were observed at the upstream WE in the bubble cell as shown in Fig. 2. The peak heights of dopamine and catechol at the upstream WE increased approximately linearly from 1× to 5× bubble cell width ($R^2 = 0.9658$, $R^2 = 0.9648$, respectively), which can be attributed to the increase in the electrode surface area. Fig. 2 also shows a roughly linear decrease in the noise at the upstream WE from 12.99 ± 0.34 pA ($n = 4$) in a 1× bubble cell to 2.786 ± 0.098 pA ($n = 4$) in a 5× bubble cell ($R^2 = 0.9920$). The decrease in noise is a result of a decrease in the resistance of the solution in the bubble cell. Lower resistance leads to a decreased voltage drop in the bubble cell, which is the major source of noise with electrochemical detection. However, when increasing the bubble cell from 5× to 10×, increases in peak heights at the upstream WE were not as significant. These responses are probably caused by the increased band broadening produced in larger bubble cells. Furthermore, the noise in the bubble cell increased at 8× and 10× relative to 5× since two opposing phenomenon are at work. As the bubble cell width increases, this source of noise decreases. Meanwhile, more noise arising from double layer capacitance is noted in the bubble cell with larger electrode area. The 4× and 5× bubble cells appear to reach a minimum noise value at where these two phenomena are balanced. Approximate 4-fold improvements were obtained for both analytes as the detection sensitivities of dopamine and catechol increased from 0.121 nA/μM and 0.075 nA/μM in a 1× bubble cell to 0.448 nA/μM and 0.290 nA/μM in a 5× bubble cell, respectively. Meanwhile, the LODs of dopamine and catechol decrease from 0.40 ± 0.01 μM and 0.60 ± 0.03 μM in a 1× bubble cell to 0.025 ± 0.002 μM and 0.050 ± 0.004 μM in a 5× bubble cell, respectively, showing a factor of 16 and 12 decreases in the LODs for both analytes ($n = 4$ and $S/N = 3$). The decreased LODs are the results of increased peak currents and decreased noise in the 5× bubble cell. The detection linear ranges of dopamine and catechol also correspondingly expanded to 0.025–100 μM and 0.050–100 μM, respectively.

3.1.2 Impact of bubble cell widths on separation efficiency—Next, separation efficiencies were measured as a function of bubble cell width using fluorescein as a model analyte. Fig. 1C shows the three positions chosen to measure separation efficiencies in the separation channel and the bubble cell detection zone, respectively. Position 1 was located in the separation channel before the bubble cell, while positions 2 and 3 were selected between the decoupler and upstream WE and the upstream and downstream WE, respectively. The separation efficiencies at positions 2 and 3 relative to position 1 were monitored to determine the effect of bubble cell width on separation efficiency. Separation efficiencies were measured using the decoupler and applying 1 V on both upstream and downstream WEs to replicate electrochemical experiments.

Fig. 3 shows that separation efficiencies at positions 2 and 3 relative to position 1 decrease with increasing bubble cell width. Also, the separation efficiency at position 2 was higher than at position 3 for the same bubble cell width. As the bubble cell width increases, the velocity of fluid flow at the same position in the bubble cell decreases due to the larger channel volume. The decrease in fluid velocity causes an increase in the residence time of the analyte, resulting in band broadening of the analyte peak and a decrease in separation efficiency. In addition, separation efficiency decreases with increasing distance from the decoupler as fluid flow in this region is predominantly hydrodynamic. Compared to the 1× bubble cell, the loss in separation efficiencies at positions 2 and 3 relative to position 1 are 8.15% (position 2), 12.5% (position 3) in a 4× bubble cell, and 11.4% (position 2), 14.3% (position 3) in a 5× bubble cell, respectively. Therefore, as a compromise between the loss in separation efficiency and improved detection sensitivity as well as detection limit in a large bubble cell, microchips with a 4× bubble cell in the detection zone were selected for further experiments performed with sample stacking techniques.

3.2 Sample stacking techniques: FASI and FASS

For both FASI and FASS techniques, a long plug of sample, prepared in a low concentration buffer, is injected into the separation channel by either electrokinetic forces or hydrodynamic flow [20]. The analytes become stacked at the boundary between the sample and running buffers due to the higher electric field strength resulting in faster migration of the analytes in the sample buffer relative to the running buffer. The stacking mechanism occurs for ionic analytes, with the positively and negatively charged analytes stacking up in front of and back of the sample plug, respectively [63]. The neutral compounds are left in the sample plug and coelute [63,64]. Theoretically, the amount of sample being stacked is simply proportional to the resistivities between the sample buffer and the running buffer. Since the ratio of resistivities is simply the inverse of the ratio of concentrations, stacking factor (SF) was defined as the ratio between the running and sample buffer concentrations. SFs of 1, 2, 5, 10 and 100, respectively, were chosen to investigate sample stacking effects in MCE-ECD.

3.2.1 Stacking characterization with dc amperometric detection—Dopamine, catechol and ascorbic acid were chosen as model analytes to characterize the stacking effects of FASS and FASI with amperometric detection. Fig. 4 compares electropherograms of 50 μ M analytes obtained with a straight T injector using FASI and FASS, respectively. Electropherograms for each stacking condition have been offset for clarity. First, significant sample stacking was achieved for positively charged dopamine when using a 5-s gated injection in FASI as evidenced by a significant peak height increase and peak width decrease with increasing SF. A similar trend was also observed for dopamine preconcentrated by using a 60-s hydrodynamic injection in FASS compared to a 25-s hydrodynamic injection under the nonstacking condition. The peak height enhancement of dopamine at SF 5, 10, and 100 relative to SF 1 are 2.84, 3.63, and 4.28-fold in FASI, and 1.71, 2.92, and 2.21-fold in FASS, respectively. Both FASI and FASS exhibited diminishing sample enhancement above a threshold SF. A reason for this behavior can be attributed to a laminar back flow inside the capillary generated by the difference in the EOF rate between sample and running buffers as noted by others [63,65]. The laminar flow disturbs the original plug profile, reducing the effectiveness of the stacking process. From these experiments, the lowest detection limit for dopamine of 8.02 ± 1.51 nM and 20.0 ± 3.5 nM ($n = 4$) were achieved with SF 100 in FASI and SF 10 in FASS, respectively. As expected, catechol, as a neutral analyte, did not stack in either FASI or FASS modes. Here the broader peak and smaller peak height of catechol obtained from the stacking conditions relative to the nonstacking condition were the result of bandbroadening caused by a mobility mismatch between sample and running buffers. Furthermore, negatively charged ascorbic acid also did

not achieve any obvious stacking. The much smaller observed mobility of this analyte may result in a much smaller sample plug volume being injected and stacked than the positively charged analyte dopamine with a higher observed mobility.

3.2.2 Stacking characterization with pulsed amperometric detection—Several reports have shown the ability to stack negatively charged analytes using FASI and FASS [27,66,67]. This was not observed in the above results with ascorbic acid. Therefore, three negatively charged amino acids associated with oxidative stress in human disease, Tyr, Hcy and Cys, were chosen as model analytes to further investigate the stacking effects of FASI and FASS coupled to PAD. Integrating a self-cleaning cycle prior to the measurement, PAD has proven to be effective in the detection of a large number biomolecules with $-\text{OH}$, $-\text{NH}_2$, and $-\text{SH}$ functional groups [68–73]. Example electropherograms of three analytes and the stacking effects by the comparison of their peak heights and half peak widths (HPWs) using a 5-s FASI with different SF conditions are shown in Fig. 5A and 5B, respectively. Of the three analytes, Tyr has the highest observed mobility, inducing the longest sample plug injected into the separation channel. Therefore, Tyr was easily stacked by FASI, showing an increase in its peak height with SF 2 and reaching the largest increase with SF 5. There was no further increase in peak height when using SF higher than 5, as a result of the mobility mismatch between sample and running buffers. The increased HPW of Tyr obtained from SF 5 to 10 also confirmed the existence of bandbroadening. Hcy, having a lower apparent mobility, showed significant increase in peak height with SF greater than 2. For Cys with the lowest apparent mobility, SF 2 produced decreased peak heights compared to the nonstacking condition (SF 1), however, higher SFs showed improved peak heights. Since stacking and broadening functioned against each other, the optimal stacking effect on peak height showed analyte dependency for different SFs. In addition, all three analytes produced their smallest HPWs with SF 5. One more thing to note in Fig. 5A is that an increasingly large fluctuation in the baseline appeared around 160 seconds where neutral analytes would elute when higher SFs were employed in FASI.

Also, comparisons of nonstacking, FASI (SF 5) using a 5-s gated injection, and FASS (SF 5) using a 25-s hydrodynamic injection were performed. Both FASS and FASI showed sample stacking for all four analytes Tyr, Hcy, Cys and GSH. (Please see supporting information Fig. 1A and 1B for example electropherograms, their comparisons on stacking effects, and the corresponding data discussion). Although significant enhancement in peak heights were seen when detecting analytes at their relative high concentrations, improved detection limits were not achieved using FASI and FASS for these four analytes when compared to their LODs under nonstacking conditions. The exact reason for this is not known at this time but may be a limitation of the increased baseline noise associated with the use of the PAD waveform. The methods do, however, increase the sensitivity of the analysis relative to nonstacking conditions.

4 Conclusions

Here, a simple implementation of a bubble cell detector for MCE-ECD was described. The surface area of WE exposed to the fluid flow entering into the detection zone increases with increasing bubble cell width. This ability affords improved detection sensitivity and lower LODs for model analytes with an acceptable loss of separation efficiency. The lowest LODs of dopamine and catechol detected in a 5 $\times$ bubble cell were 25 nM and 50 nM, showing a 16-fold and 12-fold decrease compared to the straight channel design, respectively. We have also demonstrated that FASS and FASI allow sample stacking on a MCE-ECD system with increased peak height and decreased HPW. Using stacking in conjunction with a 4 $\times$ bubble cell, we obtained LODs of 8 nM and 20 nM for dopamine by using FASI and FASS, respectively. However, these stacking techniques did not significantly improve LODs for

anionic analytes. Further optimization of our current MCE-ECD design may be necessary to enhance the stacking impact and improve LOD. This may include using either narrow channel, inversion of the applied electric field, or negative pressure for the introduction of large volume of low-conductivity sample solution. With use of a bubble cell and sample stacking techniques, the improvement obtained on detection sensitivity in MCE-ECD has the potential to reach nM detection limits for redox active biological molecules.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Abbreviations

Cys	L-cysteine
ECD	electrochemical detection
FASI	field amplified sample injection
FASS	field amplified sample stacking
GSH	reduced glutathione
Hcy	DL-homocysteine
PAD	pulsed amperometric detection
TES	N-tris[hydroxymethyl]methyl-2-aminoethane-sulfonic acid
Tyr	L-tyrosine

5 References

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Figure 1.

Schematic of PDMS microchips (50 μm $\times$ 50 μm $\times$ 6 cm from buffer to waste reservoir) with a double T injector (250 μm , 625 pL) in design (A) for pinched injection and a straight T injector in design (B) for gated and hydrodynamic injections, respectively. The sample, buffer and sample waste side channels are all 1 cm in length. All reservoirs are 5 mm in diameter. (C) Bright field image of silicon mold with a bubble cell width 5 $\times$ of the separation channel width (50 μm). The gold color seen in Fig. 1C is the native color of the SU-8 photoresist when photographed. Electrode channels a, b and c are 50 μm wide with 125 μm spacing between the channels for passing a 25 μm Pd decoupler and two 25 μm Au working electrode (WEs) through the bubble cell and the electrode channel a, b and c on both sides, respectively. 1, 2 and 3 are three positions chosen to measure separation efficiencies in the separation channel and the bubble cell detection zone, respectively.

Figure 2.

Changes in peak heights (left Y axis label) of 100 μM dopamine and catechol and noise level (right Y axis label) at the upstream WE in 1× – 10× bubble cells. Experimental conditions: field strengths: 200 V/cm; pinched injection time: 10 s; BGE: 20 mM TES, 1mM SDS (pH 7.0); E_{Det} : 1.4 V.

Figure 3.

Separation efficiency comparisons among 1 $\times$ – 5 $\times$ bubble cells. Insert picture shows three positions chosen in a bubble cell to collect electropherograms of 20 μ M fluorescein on each PDMS microchip. Experimental conditions: field strengths: 200 V/cm; pinched injection time: 7 s; BGE: 20 mM boric acid (pH 9.2).

Figure 4.

Example electropherograms for 50 μM dopamine, catechol and ascorbic acid detected on PDMS microchips with 4 $\times$ bubble cell using FASI (A) and FASS (B) sample pre-concentration techniques. Experimental conditions: $V(\text{SR}) = 1050 \text{ V}$, $V(\text{BR}) = 900 \text{ V}$ in FASI; $V(\text{SR}) = 885 \text{ V}$, $V(\text{BR}) = 900 \text{ V}$ in FASS; running buffer: 20 mM TES, 1mM SDS (pH 7.0); sample buffer: diluted running buffer with SF 1, 5, 10, and 100, respectively; E_{Det} : 1 V.

Figure 5.

(A) Example electropherograms for 250 μ M Tyr, Hcy and Cys detected on a PDMS microchip with 4 $\times$ bubble cell using FASI. Experimental conditions: V (SR) = 1050 V, V (BR) = 900 V; gated injection time: 5 s; running buffer: 20 mM boric acid (pH 9.2); sample buffer: diluted running buffer with SF 1, 2, 5, and 10, respectively; E_{Det} : 1.6 V. (B) Comparisons of peaking heights (left y axis) and HPWs (right y axis) of analytes Tyr, Hcy and Cys using different stacking factors in FASI.