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# Fabrication and evaluation of a carbon-based dual-electrode detector for poly(dimethylsiloxane) electrophoresis chips

The first carbon-based dual-electrode detector for microchip capillary electrophoresis (CE) is described. The poly(dimethylsiloxane) (PDMS)-based microchip CE devices were constructed by reversibly sealing a PDMS layer containing separation and injection channels to another PDMS layer containing carbon fiber working electrodes. End-channel amperometric detection was employed and the performance of the chip was evaluated using catechol. The response was found to be linear between 1 and 600 μM with an experimentally determined limit of detection (LOD) of 500 nM and a sensitivity of 30 pA/μM. Collection efficiencies for catechol ranged from 36.0 to 43.7% at field strengths of 260–615 V/cm. The selectivity that can be gained with these devices is demonstrated by the first CE-based dual-electrode detection of a Cu(II) peptide complex. These devices illustrate the potential for a rugged and easily constructed microchip CE system with an integrated carbon-based detector of similar scale.

**Keywords:** Microchip / Capillary electrophoresis / Electrochemical detection / Poly(dimethylsiloxane)
EL 4288

## 1 Introduction

Over the past ten years, the number of micrototal analysis systems (μ-TAS) that utilize electrophoresis for separations and fluid manipulation has increased dramatically. Advantages to working in a microchip capillary electrophoresis (CE) format are numerous and include fast analysis times, the use of high field strengths, minute consumption of solvents, and the possibility for disposable/portable devices. A thorough review of microchip CE systems is beyond the scope of this report, and readers are referred to a recent review on this topic [1].

The majority of microchip CE systems have been constructed from glass or quartz [1], although significant advances have been made using alternative materials such as plastics [2] and poly(dimethylsiloxane) (PDMS) [3]. The use of PDMS-fabricated devices for microchannel separations has attracted increased attention in recent years due to the ease with which these devices are constructed. Channels are formed in PDMS by curing the polymeric material over a master that contains a positive relief of the desired microchip design. The resulting PDMS chip is pliable, yet extremely durable, and can be sealed reversibly or irreversibly to a variety of materials [3]. Additionally, μm-scale resolution is possible, and devices can be constructed in laboratories with limited clean room capabilities [4]. Rapid prototyping can be accomplished using commercially available films as masks for photolithography procedures, greatly reducing the cost and time associated with producing chrome masks.

Detection in PDMS-based microfluidic systems has been accomplished primarily using laser-induced fluorescence (LIF), which has also been the most common detection mode used with all microchip CE devices [1, 3]. LIF offers high sensitivity and selectivity; however, only a small number of wavelengths can be used for excitation, and derivatization is often necessary. Mass spectrometry has also been used as a detection mode in PDMS devices [3, 5], although the instrumentation is costly and not inherently portable.

Our group and others have focused on the development of electrochemical (EC) detectors for microchip CE systems. These detectors offer a high degree of selectivity and sensitivity, and the electrodes and potentiostats are both amenable to miniaturization. Also, EC detectors at the μm scale are relatively inexpensive to fabricate in large numbers; therefore, their use in portable/disposable devices can be envisioned. Several examples of single-electrode EC detection in microchip CE devices in glass [6–10], plastics [11], and low-temperature cofired ceramics (LTCC) [12] have recently been reported.

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**Abbreviations: EC**, electrochemical; **5-HIAA**, 5-hydroxyindole-3-acetic acid; **PDMS**, poly(dimethylsiloxane)

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Previous work in our laboratory focused on the development of a PDMS-based microchip CE system consisting of a top layer of PDMS that contains the separation and injection channels and a bottom glass plate upon which multiple gold electrodes for EC detection were deposited [13]. The utility of these devices was demonstrated by the separation and detection of phenolic compounds including 5-hydroxyindole-3-acetic acid and tyrosine. Additionally, the selectivity that can be gained by operating in a dual-electrode format was demonstrated with compounds containing a reversible redox couple. One disadvantage of this approach is that the lifetime of the gold electrodes is dependent on the rate of grain boundary diffusion of the underlying chromium adhesion layer. Consequently, the electrodes have a tendency to detach from the glass plate after one day of continued use (approximately 150 separations). In addition, metal-based electrodes are not effective for direct amperometric detection of many compounds, including peptides, due to irreversible adsorption onto the metal surface [14].

It is well known that carbon-based electrodes are more desirable than metal-based electrodes for most organic analytes because of their lower overpotential and wider electrochemical range [15]. Carbon-based electrodes have been routinely used in CE-EC for the detection of electrochemically active peptides and amino acids [16]. More recently, carbon electrodes in the microchip CE format with single, thick-film screen-printed electrodes have been reported [10]. In this paper, we describe the design and fabrication of the first carbon-based dual-electrode electrochemical detector for microchip CE. This device employed two carbon fiber working electrodes configured in series. The detector was evaluated and characterized for both single- and dual-electrode detection and was applied to dual-electrode detection of a Cu(II) peptide complex.

## 2 Materials and methods

### 2.1 Reagents

The following chemicals and materials were used as received: SU-8 50 photoresist (MicroChem Corp., Newton, MA, USA); propylene glycol methyl ether acetate (Aldrich, Milwaukee, WI, USA); 75-mm silicon wafers (Silicon, Boise, ID, USA); 125-mm silicon wafers (MEMC Electronic Materials, St. Peters, MO, USA); Sylgard 184 (Fisher Scientific, Pittsburgh, PA, USA); carbon fibers (Avco Specialty Materials, Lowell, MA, USA); boric acid, anhydrous copper sulfate, sodium cyanide, tartaric acid, catechol, serotonin, epinephrine, and 5-hydroxyindole-3-acetic acid (5-HIAA) (Sigma, St. Louis, MO, USA). Des-Tyr leu-enkephalin was purchased from Bachem (Bruchs, Switzerland) and colloidal silver from Ted Pella (Redding, CA, USA).

### 2.2 Fabrication of PDMS chips

Fabrication of PDMS-based microfluidic devices has been well documented [4, 13] and was recently reviewed [3]. Briefly, masters were made on silicon wafers using an SU-8 photoresist and photolithographic procedures that utilized negative film masks designed in Freehand (PC Version 8.0; Macromedia Inc., San Francisco, CA, USA) and produced with a resolution of 2400 dpi (Lasergraphics, Lawrence, KS, USA). These masters were cast against a mixture of PDMS oligomer and cross-linking agent (Sylgard 184) and cured at 65°C for at least 1 h, after which time the PDMS was removed to yield a pattern of negative relief channels. In this work, two separate PDMS layers were fabricated (Fig. 1) – one containing the separation and injection channels and one containing two electrode channels for the carbon fibers. Two designs for the separation layer were used. One design incorporated a single T-injector; the separation channel was 3.3 cm long, 50 μm deep, and 25 μm wide. The second design contained a twin T-injector; the separation channel was 4.4 cm long, 50 μm deep, and 25 μm wide. Buffer access holes were made in the PDMS separation layer with a hole punch. The PDMS electrode layer was sealed to a glass plate for increased rigidity. The electrode channels were 35 μm wide and 35 μm deep with 5 μm spacing between the two channels. With the aid of a light microscope, 33 μm carbon fibers were placed in the electrode channels. When the fibers were secure in the channels, electrical contact was made to a copper wire with colloidal silver. The contact point was then covered with hot glue to insulate and secure the electrode. After electrode construction, the two layers were reversibly bonded together by aligning the first working electrode close to the end of the separation channel with the aid of a light microscope (Fig. 2).

### 2.3 Electrophoresis procedures

Stock solutions of catechol, epinephrine, serotonin, and 5-HIAA were prepared daily in water (1 mM). Appropriate dilutions were made with water prior to use. The electrophoresis buffer for the detection of catechol was 15 mM boric acid, pH 9.2. The separation of neurotransmitters (serotonin, epinephrine, and 5-HIAA) was obtained using a buffer of 100 mM boric acid, pH 9.2. A mixture of 50 mM boric acid, 3 mM tartaric acid, and 1 mM $CuSO_4$, buffered at pH 9.8, was used for the detection of the des-Tyr leu-enkephalin complex. A stock solution of peptide (1 mM in water) was diluted with run buffer to form the complex

prior to injection. In all cases, filtered and degassed buffer was introduced into the reservoirs and flushed through the channels *via* vacuum prior to use. Microchip CE separations were carried out in uncoated channels. Samples were injected by two different protocols, unpinched and pinched. Unpinched injections were applied to the single-T design and were performed as previously described by applying a high voltage to the sample reservoir (S) for 1 s with both the sample waste (SW) and detection reservoir grounded and the buffer reservoir (B) left floating (see Fig. 1) [9, 10, 13]. After the injection was complete, the separation voltage was applied to the buffer reservoir, and the separation was performed using a Spellman CZE 1000R high voltage power supply (Spellman High Voltage Electronics, Hauppauge, NY, USA) with the detection reservoir grounded. Pinched injections were performed on the twin-T design [17] and employed three 2-kV power supplies that were controlled through the USB port of a computer; these power supplies were constructed by the Instrumentation Design Laboratory at the University of Kansas. An injection sequence entailed filling the twin T by applying –1000 V to the sample waste reservoir for 20 s with the buffer, sample, and detection reservoirs grounded. After this fill time, the separation was initiated by applying 2000 V to the buffer reservoir and pushback voltages of 1200 V to the sample and sample waste reservoirs.

### 2.4 Electrochemical detection

Amperometric detection was performed in either a three-electrode (for single electrode detection) or four-electrode (for dual-electrode detection) format. The working electrodes were controlled by separate LC-4CE potentiostats (Bioanalytical Systems, West Lafayette, IN, USA). A platinum wire auxiliary electrode and a Ag/AgCl reference electrode (RE-6; Bioanalytical Systems) were placed in the detection reservoir to complete the cell. Data collection was performed with a DA-5 analog-digital converter (Bioanalytical Systems). The working electrodes were regenerated by applying a bipolar square wave of ± 1.5 V at 30 Hz for 30 s to $E_1$.

## 3 Results and discussion

### 3.1 Microchip fabrication

The general fabrication procedure for PDMS structures has been described previously [3]. The protocol for the construction of the PDMS-based microchip CE-EC system is shown in Fig. 1. The top PDMS layer contains the separation and injection channels. The bottom PDMS layer contains electrode channels into which carbon fibers are placed. The inherent hydrophobicity of the carbon fibers made them extremely compatible with the hydrophobic PDMS surface. The carbon fiber electrodes were surrounded on three sides by PDMS after they were placed in the electrode channel; this helped stabilize the carbon fibers. The fibers were easy to manipulate into the electrode channel, facilitating quick construction of the electrodes.

One concern that arises when using electrochemical detectors with CE is isolation of the working electrodes from the separation field. This is normally accomplished through end-column detection or the use of a decoupler [16]. In this application, end-column detection was employed. Once the bottom electrode layer was constructed, the carbon fiber electrodes were able to be reproducibly aligned 5 μm from the separation channel exit with the aid of a light microscope (Fig. 2). The two layers were then sealed in a reversible fashion. The PDMS layers were reusable, permitting reconstruction of electrodes, if necessary. The reversible seal between the PDMS layers allowed the disassembly, cleaning, and reconstruction of the devices when clogging or other problems occurred.

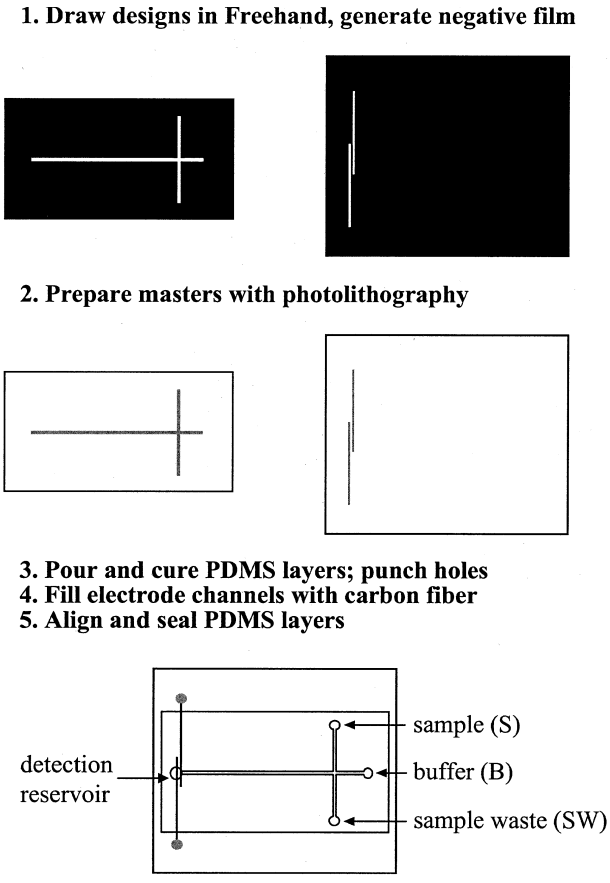



**Figure 1.** Schematic of the fabrication procedure used for the PDMS/carbon fiber microchip CE devices (not to scale).

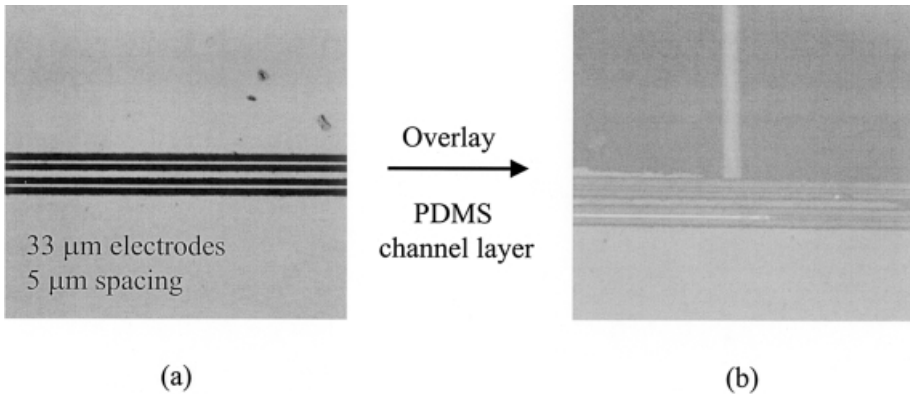



**Figure 2.** Photograph of (a) carbon fiber electrodes and (b) carbon fiber electrodes aligned at the end of the separation channel. Carbon fibers, 33 μm; electrode channel dimensions, 35 μm wide, 35 μm deep; electrode spacing, 5 μm. Separation channel, 35 μm wide, 50 μm deep.

### 3.2 Microchip evaluation

The response of the PDMS/carbon fiber device was evaluated using catechol as a test compound. The electrode response was reproducible and found to be linear between 1 and 600 μM ($r^2$ = 0.999) with a sensitivity of 30 pA/μM. The LOD was experimentally determined to be 500 nM. Additionally, these devices were evaluated using two different injection protocols, as described in Section 2.3. In general, it was found that utilizing the pinched injection protocol resulted in a more precise peak response for catechol under similar experimental conditions (pinched RSD of 3% *vs.* unpinched RSD of 8%; $n$ = 3), although the pinched injection methodology did not lead to a significant improvement in the separation efficiency for catechol. The overall efficiency for catechol ($N$) ranged between 2400 to 3000 theoretical plates (72 700–90 900 plates/m).

Electrode fouling is also a concern when utilizing EC detection. The fast separation times of microchip CE systems require a detector that can rapidly equilibrate in time for the next separation. If significant electrode fouling occurs between each run, the gain in speed of the separation is offset by the need to regenerate and equilibrate the electrode. The carbon fiber electrodes proved suitable for rapid analyses without significant fouling, as shown in Fig. 3. Forty-one consecutive injections of catechol were performed before the electrode was cleaned with a bipolar square wave. A 19% decrease in response is seen between the 1st and 41st injections; however, some of the variation in response can be attributed to the error introduced by the unpinched injection protocol that was used (8%), as discussed above. Upon application of a bipolar square wave, the electrode response was restored to 100%. Previous work in our laboratories with similar devices has shown that gold electrodes become substantially fouled after only 25 injections of catechol [13]. Figure 3 demonstrates the overall ruggedness and speed of microchip CEEC with carbon fiber electrode-based detectors. The entire experiment shown in Fig. 3 (46 runs) was completed in just over one hour.

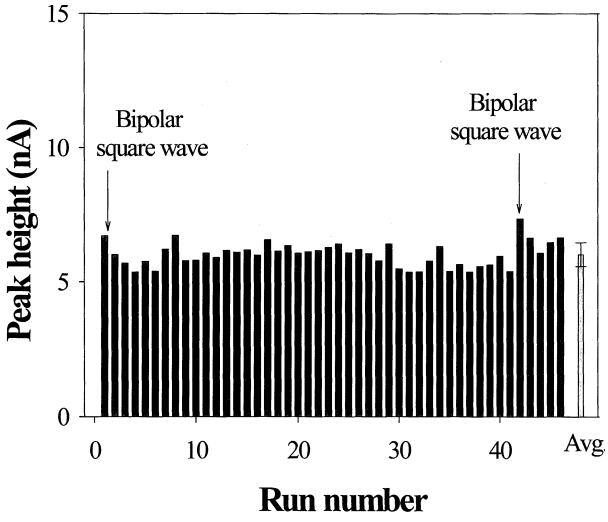



**Figure 3.** Response of 46 consecutive injections of 200 μM catechol (diluted in water). Prior to the 1st and 42nd injections a bipolar square ware (± 1.8 V at 30 Hz for 30 s) was applied to the working electrode. Separation conditions: 15 mM boric acid, pH 9.2; applied voltage, 915 V (267 V/cm); injection, 1 s (S to SW) at 915 V; $E_1$ = +900 mV *vs.* Ag/AgCl.

The applicability of these microchips to the determination of biologically important compounds is shown in Fig. 4. The neurotransmitters serotonin, epinephrine, and 5-HIAA were separated in under 90 s at a field strength of 310 V/cm. This separation was obtained using the pinched injection protocol. The separation of these three compounds was also possible with the unpinched injection protocol (data not shown); however, electrokinetic bias from the unpinched injection protocol was observed due to differences in the charge of the compounds. The peak tailing shown in Fig. 4 has been observed previously with PDMS-based devices and LIF detection [18]; the

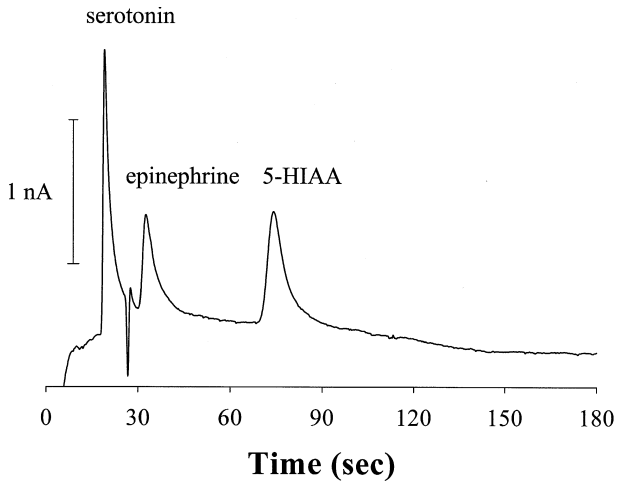



**Figure 4.** Separation and detection of three neurotransmitters at a single carbon fiber electrode. All compounds are 300 μM diluted in water. Separation conditions: 100 mM boric acid, pH 9.2; applied voltage, 2000 V to B with pushbacks of 1200 V to S and SW; field strength, 310 V/cm; injection, 20 s fill of 250 μm twin T with –1000 V applied to SW and B, S, and cell grounded; $E_1$ = +900 mV *vs.* Ag/AgCl.

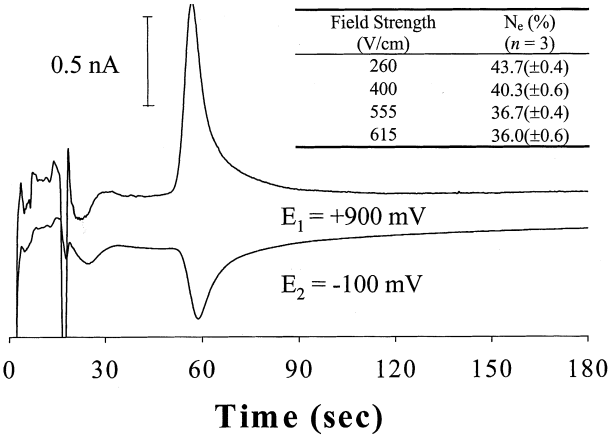
end-column detection scheme that was employed in these studies could be contributing to this phenomenon as well.

### 3.3 Dual-electrode detection

EC detection is inherently selective due to its tunable nature. Dual-electrode detection in a serial mode offers additional selectivity due to the ability to monitor reversible redox reactions. The utilization of serially configured dual-electrode detectors with conventional CE has been shown to be useful for the selective detection of many compounds, including phenolic acids, catecholamines, thiols, and disulfides [19–21]. One of the reasons dual-electrode detection has not been more widely utilized in conventional CE is the difficulty in aligning two electrodes at the end of the capillary. Alignment of two electrodes at the exit of the separation channel in these microchip devices was easy to achieve with the reversible sealing procedure. New, complete systems could be made reproducibly in only a few hours, with a significant portion of this time allocated to curing of the PDMS. As compared to the similarly configured and constructed dual-electrode microchip CE devices utilizing gold electrodes previously reported by our group [13], these carbon fiber devices exhibit longer lifetimes. The issue of grain boundary diffusion, which is encountered when utilizing lithographically produced metal electrodes [22], is not a concern with the carbon fiber detector. In this study, devices were used continuously for up to five days. If problems with the chip arose, the devices could be disassembled and cleaned, the carbon fiber electrode replaced, and the two layers realigned in under 30 min. The same PDMS layers could be used multiple times.

Catechol exhibits a chemically reversible redox reaction and is frequently employed as a model compound for the evaluation of dual-electrode detectors. At the first electrode, an anodic signal is obtained for the oxidation of catechol to *ortho*-benzoquinone; a cathodic signal is seen at the second electrode due to the reduction of *ortho*-benzoquinone back to catechol. Collection efficiencies are defined as the ratio of the cathodic and anodic signals. The collection efficiencies ($N_e$) for catechol (Fig. 5) ranged from 36.0 to 43.7%, depending on the field strength. Lower field strengths resulted in slightly greater collection efficiencies due to slower mass transport across the electrodes. These collection efficiencies are greater than those previously reported for similarly configured dual-electrode EC detectors in conventional (25–35%) [21] and microchip CE (26–29%) [13].

The selectivity that can be achieved with dual-electrode detection is especially useful when dealing with complex biological samples. Sensitive LC-based methods using dual-electrode electrochemical detection have been reported for both electroactive and nonelectroactive peptides using postcolumn copper complexation [23, 24]. In this approach, copper reacts with the peptide amide backbone to form a Cu(II) peptide complex. The Cu(II) complex is oxidized at the first electrode; the resulting Cu(III) complex can be reduced back to a Cu(II) complex at the




| Field Strength (V/cm) | $N_e$ (%) ($n$ = 3) |
|---|---|
| 260 | 43.7(±0.4) |
| 400 | 40.3(±0.6) |
| 555 | 36.7(±0.4) |
| 615 | 36.0(±0.6) |

**Figure 5.** Dual-electrode detection of catechol (200 μM) with carbon fiber electrodes in a serial configuration. Separation conditions: 15 mM boric acid, pH 9.2; applied voltage, 1800 V (555 V/cm); injection, 1 s (S to SW) at 915 V; $E_1$ = +900 mV *vs.* Ag/AgCl; $E_2$ = –100 mV *vs.* Ag/AgCl. Inset table shows collection efficiency *vs.* field strength for 200 μM catechol.

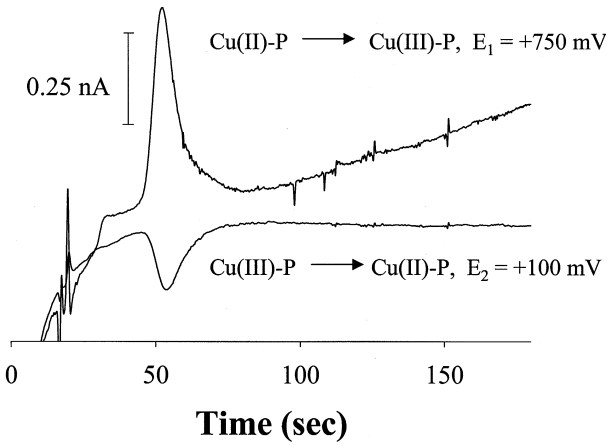



**Figure 6.** Dual-electrode detection of a des-Tyr leu-enkephalin copper complex with carbon fiber electrodes (complexed prior to injection, 215 μM). Separation conditions: 50 mM boric acid, 3 mM tartaric acid, 1 mM copper sulfate, pH 9.8; applied voltage, 1800 V (555 V/cm), injection, 1 s (S to SW) at 915 V; $E_1$ = +750 mV *vs.* Ag/AgCl; $E_2$ = +100 mV *vs.* Ag/AgCl.

second electrode. The reduction of the complex at the second electrode takes place at a mild reduction potential (+100 to +200 mV), which results in a better S/N ratio and fewer possible interferences. Compounds not exhibiting stable Cu(III) complexes, such as most amino acids, will not produce a response at the second electrode.

Our laboratory has pursued the development of a methodology for the detection of peptides in biological matrices using copper complexation and CE with single-electrode electrochemical detection [25]. In this study, des-Tyr leu-enkephalin was chosen as a model peptide to demonstrate the applicability of dual-electrode microchip CE-EC for the detection of copper-peptide complexes. Des-Tyr leu-enkephalin is not inherently electroactive, and cannot be detected without Cu complexation. Figure 6 shows a dual-electrode electropherogram of the des-Tyr leu-enkephalin copper complex. The complex exhibits a chemically reversible couple with a collection efficiency of 31%. The noise is also considerably less at the second electrode. Both the oxidation of the Cu(II) complex and the reduction of the Cu(III) complex can be monitored under these conditions.

## 4 Concluding remarks

A complete PDMS-based microchip CE device with an integrated carbon fiber dual-electrode detector has been developed. Reversible sealing of the separate PDMS layers provided a reusable device that could be made with minimal effort. The device has proven useful for the separation and detection of biologically important neurotransmitters. In addition, dual-electrode electrochemical detection of a Cu(II) peptide complex using electrophoresis (microchip or capillary) was accomplished for the first time. Future work will address optimization of the detector and development of an on-chip decoupler to minimize interferences from the separation current.

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