Rapid Determination of Metabolic Markers by Microchip CE-ECD

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The chemical make up of biological systems can be classified into three general categories, genomics, proteomics, and metabolomics. Genomics describes the chemical systems, mainly DNA and RNA that carry genetic information that codes for essential functions of the biological entity.[1-3] Proteomics is the set of proteins that are produced by the genetic code.[1-3] Metabolomics is the study of the small molecule metabolites that regulate and are regulated by genomic and proteomic elements of biological systems.[4-7] Analytical methods for genomics have been well developed over the last 20-30 years with gel electrophoresis (both slab and capillary leading the way) and microarrays adding new capabilities in recent years. Analytical methods for proteomics are developing along two general pathways, mass spectrometry-based methods for protein sequencing and microarrays for expression and interaction analysis. Analytical methods are not well developed for metabolomics for several reasons. First, the interest in metabolomics is more recent than genomics and proteomics and thus not as much time has been focused on this problem. Second, the chemistry of metabolomics is more complex. While genomics deals with nucleic acids (5 total) and proteomics deals with amino acids (20 total), metabolomics deals with many more compounds, including carbohydrates, lipids, amino acids, and many other small molecules. This complexity results in the need for multiple instrumental approaches for analysis of these complex systems. Third, metabolomic systems are in a constant state of flux and thus analytical methods must have reasonable high time resolution to be able to follow changes caused by external stress or disease.

It is the goal of our research to develop new methods for metabolomic analysis that make use of microfluidics and lab-on-a-chip technology. Lab-on-a-chip technology is particularly important in this arena of research because it provides fast analysis of small samples and can be adapted to the detection of a wide range of analyte classes. Specifically, we are attempting to adapt electrochemical detection methods to the analysis of complex biological samples using lab-on-a-chip devices. Electrochemical detection can provide a high level of sensitivity, often times bettering mass spectrometry. Furthermore, through the control of potential waveform and/or electrode material, specific classes of metabolites can be selected.

The development of a new lab-on-a-chip device with integrated electrochemical detection will be presented. Electrochemical

detection with microchip electrophoresis has traditionally been limited to easily oxidizable or reducable analytes present in micromolar concentrations.[8] Furthermore, electrodes were often times unstable, limiting the lifetime of the system. Our group has recently developed a new simple electrode design that incorporates microwire electrodes into poly(dimethyl siloxane) (PDMS) microchips. Using this design, we are able to incorporate multiple electrodes of different materials, which allows us to make two significant developments. First, we have been able to make use of pulsed amperometric detection (PAD) for class selective detection of alcohols (including carbohydrates), thiols, and amines. Second, we have been able to achieve the lowest reported detection limit for dopamine (5 nM or 1.25 zeptomoles). These developments and their application to metabolomic analysis will be presented here.

Experimental

Chip Fabrication. Microchips were fabricated from PDMS according to previously published methods in our group.[9-13] Briefly, molds were made using SU-8 2035 coated on a (100) Si wafer. Masks were produced using digital transparency technology to meet the pattern selected for the specific application. Once the mold was complete, commercially available PDMS (Sylgard 184) was mixed with a cross-linker (10:1 monomer:cross-linker) and degassed under vacuum. The degassed solution was poured over the mold and allowed to cure at 65°C for at least 2 hrs. After two hours, the cured PDMS was removed from the mold and reservoirs were created using a circular punch. Necessary electrodes were aligned in electrode alignment channels (Figure 1) on the chip and a second piece of un-patterned PDMS sealed on top to create the final chip. Electrical contact was made to the microwire and a 5 cm piece of Cu wire using Ag epoxy. Finally, the wire was held in place using hot glue.

Once the chip had been constructed, it was first wetted with methanol to fill the channels and remove any residual air bubbles. After rinsing with methanol, the channels were filled under vacuum with mobile phase buffer. Pt electrodes (1mm) were added to each reservoir to allow electrophoresis to be carried out. The electrochemical system consisted of a working electrode (Au, Pt, or Cu) and a counter electrode (1mm Pt). Electrophoresis experiments were performed with a custom-made high-voltage power supply described previously.[14]

Chemicals. SU-8 2035 photoresist and XP SU-8 developer were purchased from MicroChem Co. Sylgard 184 silicone elastomer and curing agent were obtained from Dow Corning. Aqueous solutions were prepared using analytical grade reagents and 18 MΩ resistance water (Milli-Q, Millipore). The running electrolytes were prepared by

weighing the desired amount of borate ($Na_2B_4O_7 \cdot 10H_2O$) (Fisher) and adjusting the pH with 2M NaOH (Fisher). Sample stock solutions (5mM) were prepared daily by dissolving the desired amount of each compound in 10mL of the running electrolyte. Dopamine, catechol, ascorbic acid, fructose (FRU), lactose (LAC), sucrose (SUC), maltose (MAL) and cysteine (Cys) were purchased from Sigma; arginine (Arg), histidine (His) and ampicillin (AMP) from Aldrich. Glucose (GLU) and penicillin G potassium salt (PEN) were purchased from Fisher and Fluka respectively.

Results and Discussion.

Electrochemical detection when coupled with lab-on-a-chip devices and particularly microchip electrophoresis, offers the powerful combination of low cost direct detection with instrumental simplicity and portability. There are many modern examples of portable electrochemical detectors, particularly in the blood glucose-monitoring field. The major limitations of existing electrochemical detectors interfaced with microchip electrophoresis include micromolar detection limits, poor electrode stability, and limited range of detectable analytes. Our group has recently developed a new, simple approach to the fabrication of electrochemical detectors for microchip electrophoresis that solves some of these problems.

Microchip characterization. We initially characterized our microchip to determine the stability and consistency of the design as well as the analytical performance using catecholamines as standards.[9] A schematic and photomicrograph of our design is shown in Figure 1. As can be seen, our electrode design uses an additional channel for electrode alignment that is perpendicular to and in the same plane as the separation capillary. Our approach allows use of a wide variety of electrode materials and sizes in a single microchip without the need for extensive microfabrication and photolithography.

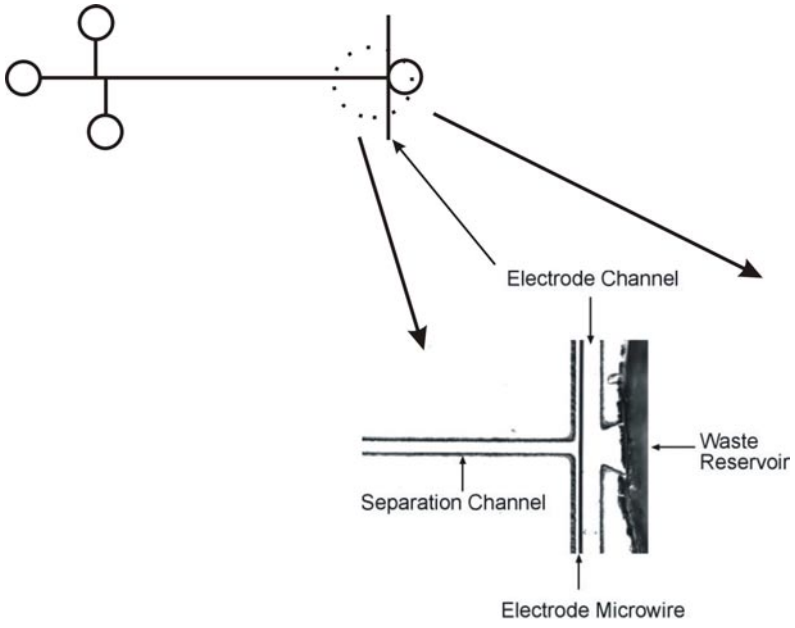

Figure 1: Top) Schematic of the microwire microchip design. Bottom) Photograph of a working electrode aligned in the electrode channel.

Figure 2 shows electropherograms for catechol obtained for three different electrode materials (Au, Pt, and Cu). Au and Pt showed the best response to catechol, as expected. The baseline for the Cu electrode had a significant drift. The drift is due to oxidation of the electrode at +0.8V. Cu electrodes are not normally used for amperometric detection because of this oxidation however we performed this experiment to show the versatility of the design. In addition to differences in electrode material, we were also able to evaluate differences in electrode size. Figure 3 shows the effects of electrode size on the peak intensity and separation efficiency during the separation of dopamine and catechol. The 25μm electrode gave better resolution but a lower peak height than the 50μm electrode. This is expected as the narrower electrode has less surface area and thus a lower collection efficiency. However, because the electrode has a smaller diameter it is able to resolve compounds better.

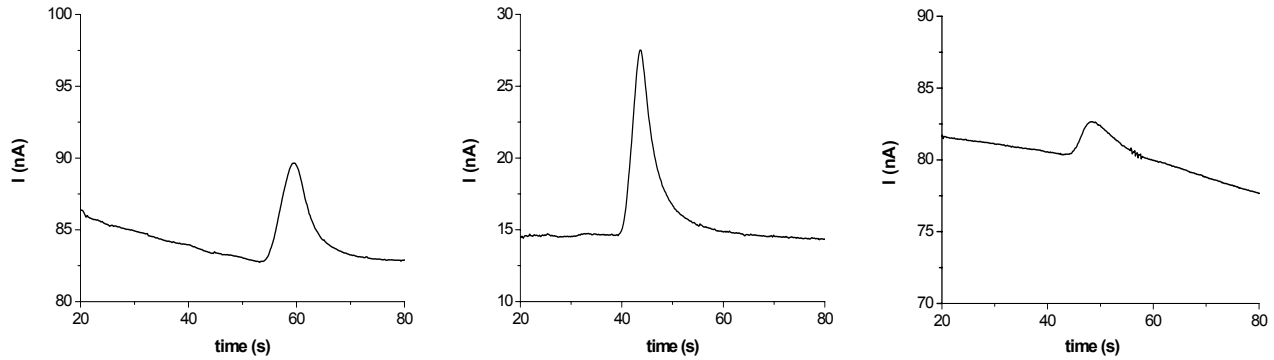

Figure 2: Electropherograms for detection of catechol at Left) Cu, Middle) Au, and Right) Pt electrodes.

Next, we characterized the detection limit that could be achieved with the microwire design. Detection limits of 250nM and 100nM were achieved for 25 and 50μm Pt working electrodes respectively for dopamine. These detection limits are the lowest achieved for a non-decoupled design and are the result of extremely high collection efficiency. Typical collection efficiencies for microchip electrophoresis are on the order of 25-40%. The 25μm electrode had a collection efficiency of close to 50% while the 50μm electrode had a collection efficiency of 90%. This increase in collection efficiency permitted detection of extremely low levels of catecholamines.

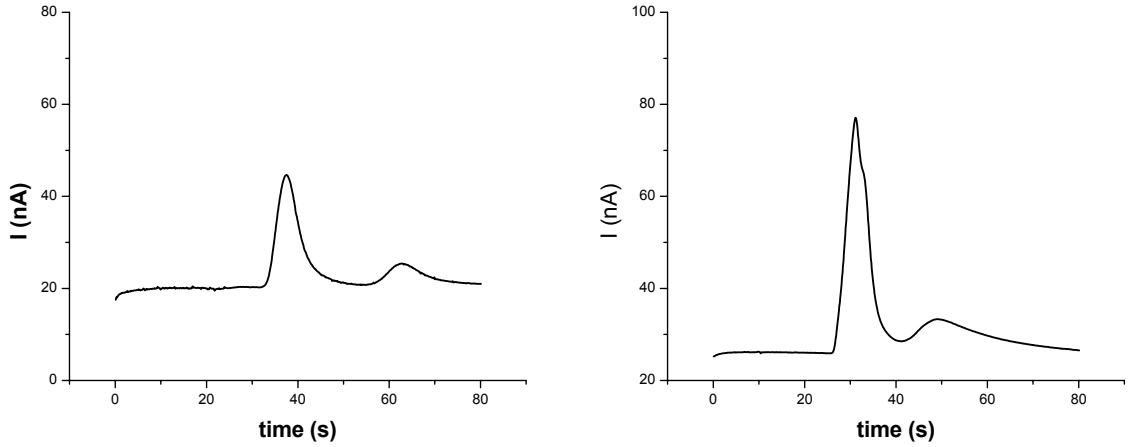

Figure 3: Electropherograms for dopamine and catechol obtained with 25μm (left) and 50μm (right) Pt working electrodes.

Current Decoupler. While we were able to achieve the lowest detection limits reported for microchip electrophoresis using our design, they were not low enough to profile catecholamines in biological systems. Detection limits in microchip electrophoresis/electrochemistry are typically limited by the need to measure nA-pA detection currents in the presence of a μA separation

current. One way to overcome this problem is to incorporate a current decoupler into the microchip.[15-19] A current decoupler serves as a grounding point in the separation channel prior to the working electrode that allows solution flow to continue past the electrode. Two general types of current decoupler exist, Pd electrode decouplers and joint decouplers. Using our microwire design, we have developed a simple and inexpensive decoupler that incorporates a Pd wire in the channel as a ground (Figure 3). Pd works well as a ground electrode because it can absorb H_2 up to 600 times its volume. Figure 3 also shows electropherograms for a mixture of dopamine, catechol, and ascorbic acid at different concentrations measured using the decoupled system. Using this system, we were able to achieve detection limits of 5nM for dopamine (1.25 zeptomoles) and 50 nM for catechol and ascorbic acid. These are the lowest detection limits reported to date for microchip electrophoresis/electrochemistry.

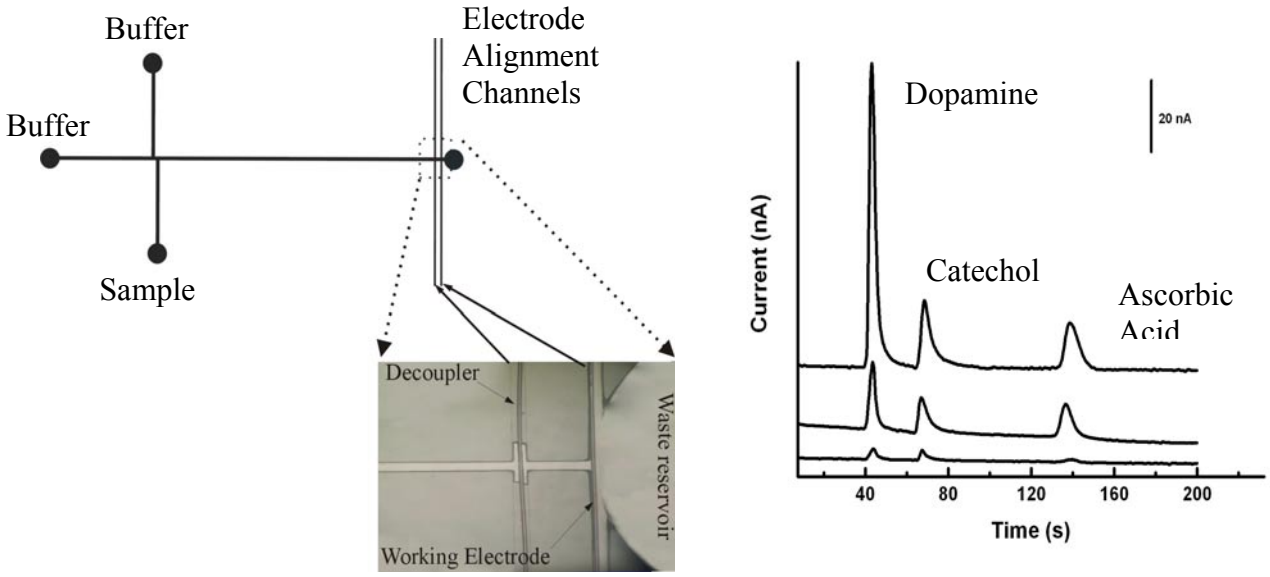

Figure 3: Left) schematic of the decoupled microchip and a photograph of the electrodes aligned in the channels. Right) Electropherograms for dopamine, catechol and ascorbic acid at three different concentrations using the decoupled microchip.

Detection of Metabolic Markers. Once we had finished characterizing our new electrochemical detection system, we began to evaluate the possibility of using the system to detect biological metabolites.[11] There are several common classes of metabolites that are studied in metabolomics, including carbohydrates, amino acids, and thiol compounds. Most of these compounds are not detectable using normal amperometry and thus we explore the use of pulsed amperometric detection (PAD). PAD uses a triple potential pulse to first clean the electrode and then regenerate the electrode before detecting the analyte. PAD is useful for compounds that are electrochemically active but also known to foul the electrode surface after oxidation. Common classes of compounds that can be detected by PAD include

alcohols (including carbohydrates), thiols and thioethers, and amines. Figure 4 shows electropherograms for all three classes of these compounds detecting using microchip CE-PAD.

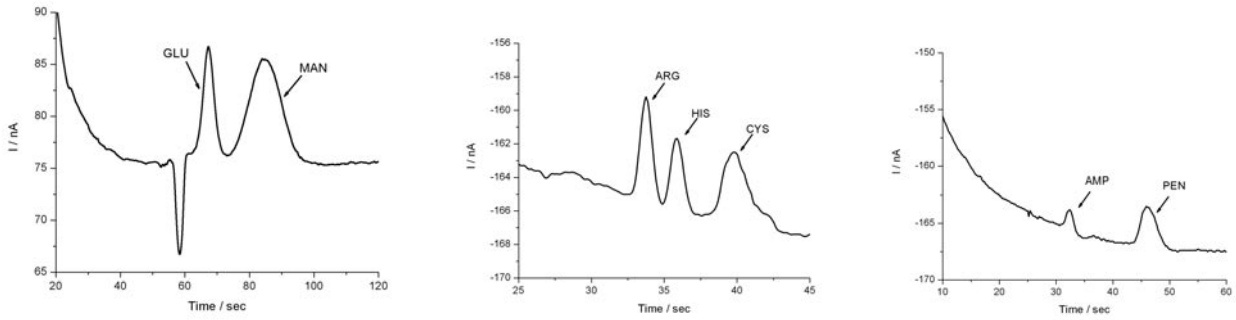

Figure 4: Electropherograms obtained using microchip CE-PAD. Left) Separation of Glucose (GLU) and Mannose (MAN). Middle) Separation of Arginine (ARG), Histidine (HIS), and Cysteine (CYS). Right) Separation of Ampicillin (AMP) and Penicillin (PEN).

Conclusions. We present here a new design for microchip capillary electrophoresis with electrochemical detection. Our design allows us to incorporate multiple stable electrodes into a single separation channel and achieve very high levels of sensitivity. The design also permits us to perform pulsed amperometric detection (PAD), which allows us to detect compounds that are not normally measured by electrochemical detection with electrophoresis. The use of this system for the profiling of metabolic markers of disease will be presented.

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