INLINE ELECTROCHEMICAL DETECTION FOR CAPILLARY ELECTROPHORESIS

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ABSTRACT

This paper presents for the first time fabrication and initial measurements of an electrophoresis stage integrated with a novel inline electrochemical detection system. This detector system can be easily incorporated into plastic substrates and has less complex fabrication procedure compared to fluorescent detection systems. Potentiostatic and Open potential modes of detection are demonstrated. Techniques used to eliminate gas evolution at the detection electrodes and to reduce noise are discussed. Noise levels of 0.7 pA on a signal of 5 nA and drift in signal of 1.18 pA/s were measured for the potentiostatic mode of detection for the field and buffer concentration mentioned. Open potential mode had noise levels of 9.1 mV on a signal of 800 mV and a drift in signal of 0.22 mV/s. The potentiostatic mode was determined to be more stable based on the S/N ratios. Separation and detection of few biologically important compounds along with few electrochemical tags are demonstrated. Detection limits of 14.5 amol were measured for the potentiostatic mode of detection.

DEVICE STRUCTURE AND FABRICATION

Figure 1 shows the optical micrograph of the electrophoresis device with integrated electrochemical detection with 2 cm long separation channel.

INTRODUCTION

Capillary Electrophoresis (CE) and other analytical techniques have greatly benefited from micromachining. Microfabrication technology provides a means for implementation of complex CE devices with a high degree of functionality [1, 2] yielding fast and highly efficient separations. The majority of micromachined CE devices however have been fabricated using bonded, glass substrate technology with off-chip laser induced fluorescence detection. The advantages of miniaturization are reduced when large detection systems are used in microanalysis. The use of on chip detection techniques makes portable microanalysis systems practical and possible. Recently our group has demonstrated on chip fluorescence detection of DNA using photodiode on silicon [3], which is a step towards microfabricated detection system. Compatibility of silicon photodiode with plastic substrates, which are favored because of bio-compatibility and cost, is however difficult to achieve. The optical detection method also requires additional optical devices such as LEDs, lasers, and optical filters for efficient detection.

Alternatively, the signals can be detected electrochemically (EC) with just an appropriate set of electrodes, making it the simplest detection method to incorporate into the process flow of an existing microfabricated system. End column EC detection in micromachined CE systems has been demonstrated by a number of groups [4, 5]. Inline detection provides advantages of better resolution and possibly higher limit of detection. Inline Electrochemical (EC) detection techniques are commonly used in High Performance Liquid Chromatography (HPLC) systems [6], but they have not been employed in CE systems because of coupling of detection and separation fields and bubble generation in aqueous solution at the high CE fields. In this paper we present the implementation of an inline electrochemical detector that eliminates these two adverse effects.

DEVICE STRUCTURE AND FABRICATION

Figure 1 shows the optical micrograph of the electrophoresis device with inline electrochemical detection. The detection system consists of three electrodes, namely working, counter and reference electrodes, as seen in the micrograph.

Figure 1: Optical micrograph of Electrophoresis device with integrated electrochemical detection with 2 cm long separation channel

Figure 2 shows the simple process flow for the fabrication of the device. The electrophoresis channels are surface micromachined of polymer material (Parylene), using photo resist as a sacrificial layer [7]. Fabrication is a simple 4 mask process. The starting substrate can be of any material (silicon, glass, plastics), and provides only a mechanical platform on which the microsystem is built. Here we use silicon as the substrate material. A passivation layer of thermal oxide (0.2 μm) was next grown on the wafer under wet, trichloroethane
(TCA) conditions at 900 °C. A parylene base layer (5 μm) was deposited using chemical vapor deposition on the oxide which provides good adhesion. Next a 0.1 μm thick gold electrophoresis electrodes and detection electrodes are patterned. The sacrificial photoresist is then patterned using a 20 μm thick layer of AZ9260 (Clariant). The top layer of the channel is then formed by depositing a 5 μm thick parylene layer. Adhesion of this layer to the base parylene layer is assisted by a short oxygen plasma RIE at low power. The final parylene layer is etched in a O₂ plasma RIE using a thick photoresist mask. The channel reservoir openings as well as the electrode pad openings are etched at this stage. Photo-definable silicone rubber rings can be patterned around the reservoirs to increase the holding capacity.

![Channel Fabrication Diagram](image)

**Figure 2: Fabrication process flow**

In the device tested, this step was avoided out of convenience and the rings were applied by hand. The wafer was diced using a diamond tip saw. The devices were then released in acetone for 20 hours followed by an IPA rinse and a N₂ dry.

**EXPERIMENTAL**

**Separation conditions.** Separations were performed in these devices using a sieving matrix consisting of 0.5 %(w/v) hydroxyethylcellulose (HEC) (Polysciences, Inc., Warrington, PA; MW 90000-105000) and 0.1% Tris/Boric Acid/ EDTA buffer (Sigma Chemical Co., St. Louis, MO). Approximately 5 μl of the buffer solution is loaded in one reservoir and allowed to fill the entire channel by capillary action. The remaining reservoirs are also filled with approximately 5 μl of buffer solution. The channel was pre-electrophoresed at 150 V/cm for 5 minutes. This was found to concentrate the HEC in the separation column to more than 0.5 % resulting in a higher resolving power of the gel. A 0.2 μg/μl sample of probe solutions was loaded into the injection reservoir. The sample was cross injected using a pinched injection scheme.

**Instrumentation.** A stereo microscope (Olympus SZX12) equipped with a fluorescence illuminator (100W Mercury lamp illuminator) was used to observe the separations. A high sensitive potentiostat specially designed (Gamry instruments, PA) was used to measure the output of the detection electrodes and set different modes of operation of the electrodes.

**Detection system design.** EC detection system consists of three electrodes, the working, counter and reference electrodes. The working electrode (WE) being the electrode at which the transfer of a chemical signal into an electrical signal takes place is made of 100 nm thick Au, 10 μm in width and 160 μm in length. Au was chosen as the working electrode as it provides a high hydrogen over potential which aids in the prevention of bubble generation over a greater range of potential differences. The working electrode should be an ideally polarizable electrode, meaning that its potential should not change with the current. The reference electrode (RE) maintains its potential with respect to the solution and hence should ideally be non-polarizable. Typically Ag/AgCl is used as the RE because of its near ideal non-polarizable behavior. In micro EC cells, since the amount of current handled is very small, metals can be used for the RE if its surface area is made much larger the WE area. A large area metal RE is acceptable because the effect of current variation on potential is weak. In our device, all the electrodes are made with Au, to simplify the fabrication process.

**Figure 3: Current voltage characteristics of bubble generation at electrode**

When an electrode is floating it takes up the potential of the solution. In a CE channel there is a gradient of potential equal to the electric field applied. Thus if the distance between the WE and CE electrodes is sufficiently large, then the potential difference between them will be greater than 1.2V, the theoretical potential for gas evolution. Under this condition if the two electrodes are connected, gas evolution...
will occur. The effect of potential difference on the gas evolution is shown in Figure 3. In order to avoid bubble generation the entire detection system was designed to be less than 80 μm in width. The RE was also designed to envelope the WE as shown in Figure 4(i), so that the region around the WE is maintained at a stable potential. This helps in reducing noise in the detection signal as then the entire electrochemical cell floats with a single solution potential. A typical potentiostat-electrochemical cell system is shown in Figure 4(ii).

RESULTS AND DISCUSSION

Electrochemical detection can be achieved by two methods. In potentiostatic detection, the potential of the WE with respect to RE is maintained at a fixed value, and the current through the electrochemical cell is monitored. The potential of the WE determines the reaction taking place at the electrode. Thus by maintaining the potential, only the desired reaction is allowed to take place. The peak in the current then indicates the occurrence of the desired reaction. In open circuit potential (OCP) mode, the potential of the electrode is allowed to float and is measured periodically. The reaction occurring at the surface of the electrode modifies the OCP of the electrode and this can be monitored. Potentiostatic mode utilizes three electrode configuration and hence has lesser noise compared to OCP mode as seen from Figure 5.

Figure 5: (i) Potentiostatic detection of FeCA (ii) Open circuit potential measurement for CE of FeCA

Figure 5(i) shows the potentiostatic detection of a single electrochemically active compound. The compound chosen was Ferrocene di carboxylic acid (FeCA). It has a reduction potential of 0.5V, as measured from CV measurements. The potential difference between the WE and RE was maintained at 0.8V in all the experiments. The response of the system is affected by the switching of potentials in the electrophoresis channel from the injection to separation mode in pinched type injection scheme. Therefore we see a jump in the current at t=60 sec. when the potentials are switched.

Figure 6: (i) Potentiostatic detection of CE separation of (A) FeCA, (B) Histamine. (ii) Potentiostatic detection of CE separation of (A) FeCA, (B) Histamine and (C) Epinephrine.

Figure 5(ii) shows the OCP mode detection of the same compound. The signal is affected by variation of the electric field along the channel. Thus the noise level is much higher. The decay time of the signal is also higher. In the case of potentiostatic detection the system is brought back to equilibrium by imposition of a feedback in potential to the WE. However in OCP detection, no external input is used to restore the equilibrium after the influence of the electroactive compound. This accounts for the higher decay time.

The noise levels in the two different modes of operation were estimated by performing the experiments without any analyte present. The separation field was 150 V/cm. The buffer and gel concentration were the same as in other experiments. In case of the potentiostatic mode, the average rms noise level was 0.7 pA on a signal level of 5 nA. There was
a drift in the signal of 1.18 pA/s. In the OCP mode of operation the noise level was 9.1 mV on a signal level of 800 mV and the drift was 0.22 mV/s. The S/N ratio for the potentiostatic mode was higher than for OCP mode due to the potential feedback mechanism and the three electrode configuration. The minimum detectable signal (assuming S/N = 2) was 14.5 amol.

Figure 6(i),(ii) shows the detection of two and three electroactive compounds. In these plots the peak heights are approximately in the ratio of their concentration.

Figure 7(i) shows detection of four electroactive compounds. The reaction of serotonin is a reduction reaction leading to a dip in the current observed. Thus the type of reaction can also be deduced from this data. Figure 7(ii) shows the separation of compounds which can be used as electrochemical tags to DNA. These compounds are believed to attach themselves to the minor groove of the double stranded DNA [8]. Replacing the metal atoms in the compounds and utilizing the properties of potential discrimination of chemical species, color coding of DNA can be achieved.

Figure 7: (i) Potentiostatic detection of (A) FeAC, (B) Histamine, (C) Epinephrine and (D) Serotonin. (ii) Detection of (A) Fe(bipyridine), (B) Ru(bipyridine), (C) Fe(phenanthroline) and (D) Ru(phenanthroline)

Figure 8: (i) Detection of (A) Fe(II), (B) Fe(III) and (C) FeCA. (ii) Variation of the transit time of the band with electrophoresis field

Figure 8(i) shows the oxidation and reduction reaction occurring at the WE and CE. The analyte consists of ferrocyanide (Fe(CN)_6^{2-}) and FeCA. The ferrocyanide undergoes oxidation at the WE at a potential of 0.2V, and it is transformed into ferricyanide. This ferricyanide is then reversibly converted into ferrocyanide at the CE which is downstream to the WE. Figure 8(ii) shows the variation in electrophoresis mobility of FeCA with the variation in the electric field. The result is consistent with that observed for gel electrophoresis in [9].

SUMMARY

In this paper we presented the implementation of an inline electrochemical detection system for capillary electrophoresis. Two electrochemical detection schemes were used, and biologically important neuro-transmitters were separated and detected using these techniques. The important issues of potential coupling and bubble generation in electrochemical detectors for CE are discussed, and effective solutions were implemented. The detection limit was 14.5 amol for the specific gel, buffer and electrochemical conditions. The versatility of electrochemical methods in discriminating compounds according to the type of reaction occurring at the electrode surface was demonstrated.

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References