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Microfludic 플랫폼을 이용한 생체 분자의 voltammetric 분석

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Microfluidic platform for voltammetric analysis of biomolecules

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Abstract - A microfabricated chip with in-channel electrochemical cell using interdigitated gold electrode was fabricated for sensitive electrochemical analysis. The gold electrodes were fabricated on glass wafer using thermal evaporator and were covered using PDMS mold containing microchannel for analyte and electrolyte. The active area of each electrode was 250 μm × 200 μm with a gap of 200 μm between the electrodes. Microelectrodes results in maximum amplification of signal, since the signal enhancement effect due to cycling of the reduced and oxidized species strongly depends on the inter electrode distance. Analytes such as methylene blue and guanosine were characterized using the fabricated electrodes and their electrochemical characteristics were compared with conventional bulk electrodes. The device so developed shall find use as disposable electrochemical cell for rapid and sensitive analysis of electroactive species.

1. Introduction

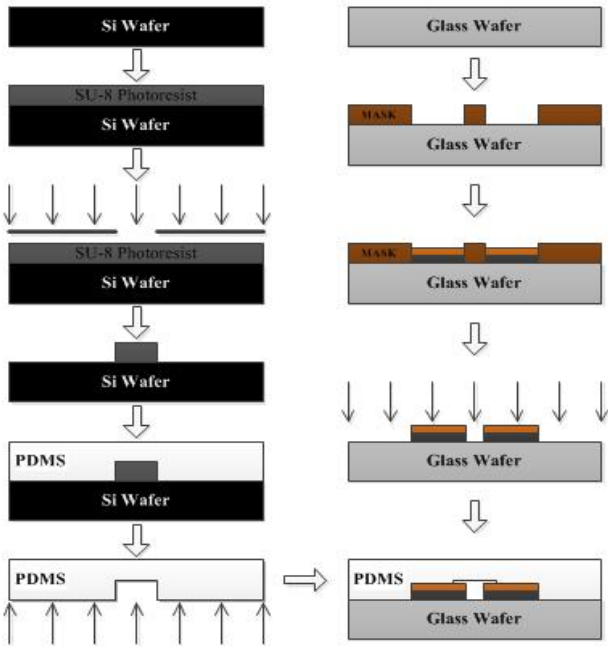
Electrochemical detection has widespread use in trace analysis of redox active organic and inorganic compounds, because it provides easy procedure for direct and selective detection [1]. Cyclic voltammetry in particular, determines the oxidation and reduction potential and electrochemical reaction rates of an analyte which can be used for their amperometric detection in processes such as biosensors and capillary electrophoresis [2]. In these regards, a microfluidic platform with microelectrodes can greatly facilitate sample handling, electrode cleaning and can enhance sensitivity by reducing interferences. The advent of microfabrication technology has widened the scope and applicability of microelectrodes. The behavior of microelectrodes differs from conventional sized electrodes in that nonlinear diffusion is the predominant mode of transport. This difference in mass transport from the bulk solution toward the electrode has several important implications that make microelectrodes very attractive in many areas of electroanalytical chemistry. These include reduced ohmic potential drop, a decreased time constant, a fast establishment of steady-state signals, and an increased signal-to-noise ratio [3,4].

For these reasons, in the present work, we developed an electrochemical cell on glass chip with sub millimeter sized gold microelectrodes using microfabrication technology. The microelectrode for electrochemical study i.e. working, reference and counter electrode were thermally evaporated at high vacuum through a shadow mask. The microchip contained negative molded PDMS layer containing a 1 cm long microchannel as a reservoirs for sample. The proposed device was tested to perform cyclic voltammetry using common analytes like methylene blue (MB) and guanosine and its performance was compared with conventional bulk electrodes.

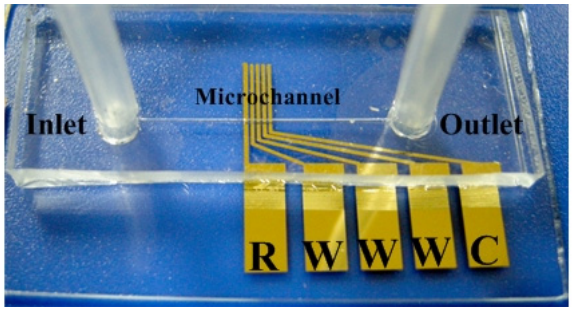
2. Experimental

Standard photolithographic techniques were followed to fabricate the device. The simple process flow for the fabrication of the Microfluidic device is shown in Fig. 1. Electrochemical detection was performed using an electrochemical analyzer (CHI 800B, CH Instruments, USA). For cyclic voltammetry; Ag/AgCl reference electrode, a Platinum wire counter electrode and a gold working electrode were used for bulk system while microfabricated gold microelectrodes were used for on-chip voltammetry.

CV of MB was performed in 200 mM potassium chloride as an electrolyte, while guanosine was analyzed in 200 mM sodium hydroxide at a rate of 100 mV/sec. For reference, control voltammetry of KCl and NaOH were also performed in a similar fashion as of the analytes. All the voltammetric measurements were performed at least 3 times for each condition (n=3) except otherwise stated.



<Fig. 1> Schematics of on-chip electrode fabrication



<Fig. 2> Image of on-chip CV system

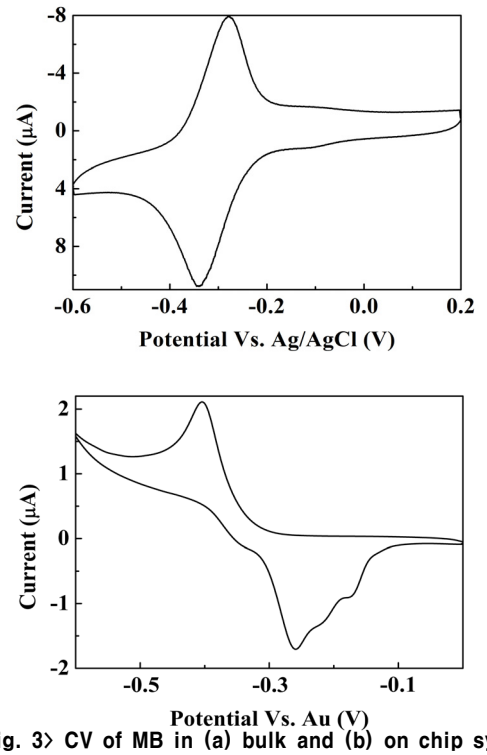
3. Results and Discussion

3.1 Device characterization

The image of our microfabricated electrochemical cell depicting all the microelectrodes for voltammetric study has been shown in Fig 2. The width of electrode was 200 µm and the distance between two electrodes was 200µm. As the dimension of microchannel were 250 µm (width) × 200 µm (height) × 1 cm (length), the effective active electrode area used for voltammetric study was 250 µm × 200 µm with a sample requirement of just 1 µL inside the microchannel.

3.2 Electrochemistry of Methylene blue (MB)

MB is a redox active dye which is used in various biochemi cal studies. The cyclic voltammograms of 100 µM MB at diffe rent electrodes are shown in Figure 3. The cyclic voltammogr am of 100 µM MB shows a cathodic process (EpC) at -0.28 V (conversion of MB to leucomethylene blue, a two-electron p rocess) using conventional electrodes [fig.3a]. The correspondin g anodic process appeared at -0.34 V (release of a proton). Co mpared with conventional electrode, microelectrodes showed a cathodic process (EpC) at -0.27 V and the anodic process app eared at -0.39 V [fig.3b]. A control experiment with 200 mM electrolyte without any analyte produced no peak.



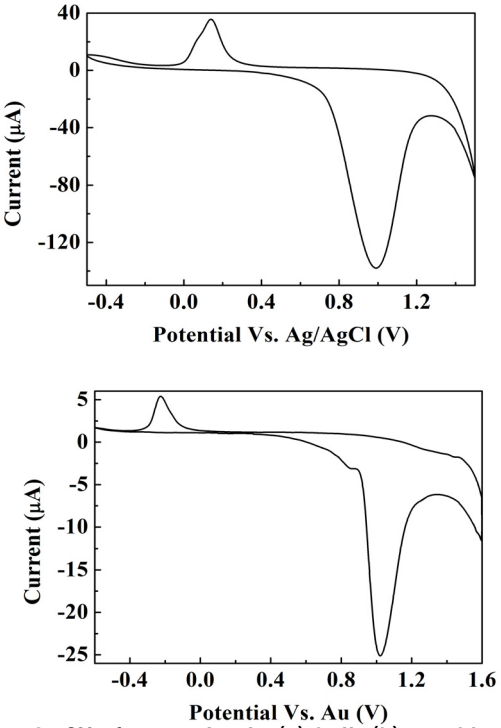
<Fig. 3> CV of MB in (a) bulk and (b) on chip system

3.3 Electrochemistry of Nucleosides

In this study, guanosine was used as a bio molecular analytes. Guanosine is a part of DNA molecules and hence its detection is useful in detecting DNA. Cyclic voltammetry of 1 mM guanosine using conventional technique produced oxidation peak at 0.99 V [fig. 4a]. On-chip voltammetry showed oxidation potential of guanosine at 1.02 V [fig. 4a]. The potential obtained using on-chip voltammetry is similar with that of bulk voltammetry but the current level is much higher than conventional technique. The pronounced electrochemical response at microelectrode was strong evidence that our system is at par with the conventional system.

4. Conclusions

In the present work, fabrication of a simple microfluidic electrochemical cell on glass chip using lithographic techniques was demonstrated. The cell consisted of interdigitated goldmicroelectrode of 200 μm in thickness. The reservoir was made using PDMS microchannel and bonded to glass. The functionality of device was checked by performing on-chip cyclic voltammetry and comparing it with conventional voltammetric analysis. Analysis of methylene blue and guanosine were successfully performed using the proposed device and the voltammograms obtained showed good correlation with conventional bulk electrode system while having low background current and sharper detection peaks. We propose that such devices shall facilitate low-noise electrochemical measurements in microenvironments without deviating from bulk electrode chemistry.



<Fig. 4> CV of guanosine in (a) bulk (b) on chip system

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