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Microfabrication and Application of Recessed Gold Electrodes in Microchip Electrophoresis System

CHONGGANG FU
Department of Chemistry, Liaocheng University, 252059, Liaocheng, P.R. China

Abstract: Based on photolithographic technique, a simple and novel way to construct micro recessed gold electrodes (μRGE) using recordable compact disk was described in this paper. μRGE were characterized by a remarkable versatility, great availability, good reproducibility, insensitive response to the fluctuation of flow speed, and very low price. The applicability of μRGE in microchip electrophoresis system was demonstrated for the anodic detection of dopamine and catechol. A high sensitivity and low noise was obtained, consequently a lower detection limit (0.31 for dopamine and 0.62 μ mol/L for catechol) was achieved, which was far lower than that reported in the literature.

Key words: Microfabrication, Microchip, electrophoresis, electrochemical detection.

1. Introduction

Microfabricated capillary electrophoresis(CE) chip have received great interests in recent years.[1,2]. Such miniaturized devices have several advantages over the benchtop separation systems. e.g. higher throughput could be achieved while consuming only picoliters of sample volume. Currently, such electrophoresis chip rely primarily on Laser-induced Fluorescence (LIF) to obtain high detection sensitivities. Yet, LIF detection typically requires derivatization of the analytes with a fluorophore. Moreover, only a select number of wavelengths can be used for excitation. Recently, Electrochemistry (EC), an alternative detection technique has witnessed a great success in microchip CE system because of its high sensitivity, tunable selectivity, independence of path length, and inherent miniaturization. In the chip-based CE-EC system, the configuration and position of the working electrode play an key role for achieving high detection sensitivity

and separation efficiency. Woolley[3] first reported on capillary electrophoresis chip with integrated electrochemical detection, based on the photolithographic placement of the working electrode positioned outside the exit of the electrophoretic separation channel. Wang et al[4] described an chip-formatted eletrophoretic system with electrochemical detector based on sputtering the working electrode directly onto the channel outlet. The above two method for electrode preparation are both involved the access to the complex and expensive equipments. Recently, Wang et al[5] introduced easily-performed electroless deposition procedure for fabricating gold electrode just outside the exit of separation channel to serve as a working electrode. Although the chip-integrated electrode eliminates the need for a elaborate channel-electrode alignment, electrode cleaning and replacement due to severe surface poisoning and damage become hardly possible. For this reason, the stand-alone electrode seems promising. Wang et al[6] described a thick-film electrode detector for eletrophoretic chip. This coupling obviates the need for permanent attachment of the electrode, allows a convenient surface modification, a fast replacement of passivated electrode, or the comparison and use of different electrode materials. In this paper, we described a convenient way of construction of micro recessed gold electrode using recordable compact disks, investigated its reproducibility and stability, and examined primarily its utility as the detector of electrophoretic chips.

2. Experimental

2.1 Apparatus and Reagents

A home-built high-voltage power supply, with a adjustable voltage range between 0 and 2000V, was used for the electrophoretic separation. Amperometric detection was performed with an Electrochemical Analyzer 812 (CH Instruments, Austin, TX), which was connected to a Pentium 1.7G computer with 128M RAM. The glass microchannel chips, fabricated by combining photolithographic, wet-chemical etching and thermal bonding techniques, were made in the present laboratory using Au/Cr-coated glass slide.

All the solutions used were prepared with tri-distilled water, all the reagents were of analytical grade except the specially indicated. Dopamine, catechol and MES were obtained from Sigma, Potassium chloride, Potassium ferricynide was obtained from Beijing Chemicals Factory.

2.2 Preparation of μRGE

The electrodes used throughout this work were constructed with small ports of recordable compact disks (Kodak, bought from the local electric market).

The whole procedure is as follows: Firstly, a whole CD was immersed in concentrated nitric acid for 2 min, then took out and thoroughly rinsed with tap water to remove the plastic protective film from its surface. Secondly, the disk was cut into as many as rectangular slices (1.5×0.5 cm) as possible with a large scissors. Each slice was sequentially rinsed with isobutnol and de-ionized water and dried in oven at 110 ˚C for 30 min. Thirdly, a layer of negative photoresist is spin-coated on the upper and side face of a slice to completely cover the gold layer, then dried at 90 ˚C for 30 min, after that the photoresist in two circular regions was exposed for 80s under UV lamp. The exposed regions were defined by the printed pattern on a photomask made from a transparent plastic film. The smaller region (40μm in diameter) served for working electrode; the larger (2mm in diameter) for electrical contact. After development in petroleum ether, the slice was dried at 90 ˚C for 30 min. Electrical contact was performed with a copper wire using conductive silver paint. Thus a μRGE was obtained with 5μm recession.

3. Results and Discussion

3.1 Electrochemical Properties of μRGE

Potassium ferricynide as a model compound was used for examining the electrochemical properties of a μRGE. Figure 7.1 shows a cyclic voltammogram of a μRGE in 5 mmol/L $K_4Fe(CN)_6$ and 0.5mol/L KCl. This diagram is of zigzag, which is typical property of a microelectrode. The dominating process

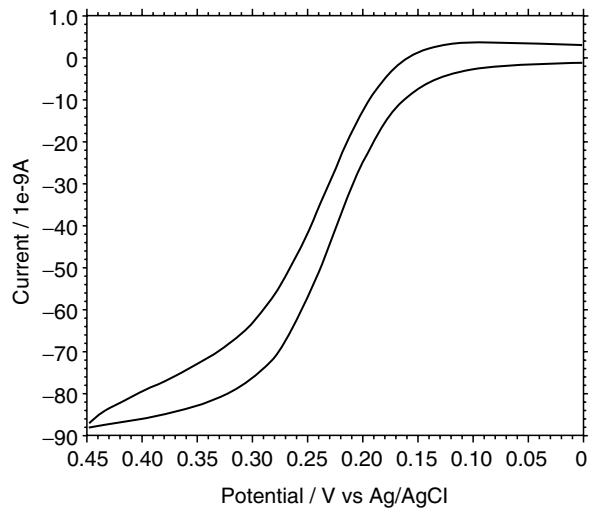

FIGURE 7.1. Cyclic voltammogram of a μRGE in 5 mmol/L $K_4Fe(CN)_6$ and 0.5mol/L KCl.

of mass transport in static solution is radial diffusion for microelectrode, whereas linear diffusion for macroelectrode. Generally, the rate of radial diffusion is far greater than that of linear diffusion. Therefore, the microelectrode is more prone to attain a steady state of mass transport; a larger limiting current density could be obtained. Moreover it is hardly affected by convection. So the microelectrode is particular suitable for the microfluidic system.

The effect of the potential scan rate on the electrode response was examined in the range of 10-500 mV/s. As shown in Figure 7.2, the limiting current doesn't significantly vary with increasing scan rate up to 100 mV/s. After that the limiting current rapidly increase with the scan rate, but the relationship doesn't obey Randles equation. This phenomenon could be explained as following: In case of lower scan rate, the diffusion layer is relatively thick, so the radial diffusion plays an important role. In this case, the limiting current i_1 should obey the modified Bond[7] equation. That is:

$$i_1 = \frac{AnFcD}{l + r} \tag{1}$$

where n represents number of electrons transferred, F the Faradic constant, D the diffusion coefficient, C the concentration of the electroactive species in the bulk of the solution. r and A the radius and surface area of the working electrode, l the depth of the recession. As shown in Equation(1), i_l is independent of the scan rate. When the scan rate further increase, the diffusion

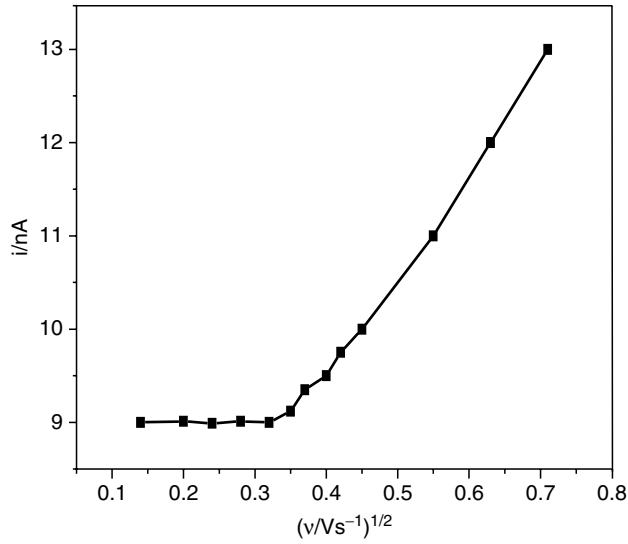

FIGURE 7.2. Effect of the potential scan rate on the electrode response.

layer is relatively thin, both radial and linear diffusion process determine the attitude of limiting current, representing a quasi-stable state of mass transport. Expectedly, As enough high scan rate attained, the electrode response would follow Randles equation[8]. i.e.

$$\boldsymbol{i}_l = 2.69 \times 10^5 \, \boldsymbol{n}^{3/2} \, \boldsymbol{AD}^{1/2} \, \upsilon^{1/2} \, \boldsymbol{c} \tag{2}$$

where ν is the scan rate. But it is a pity that experiments using higher scan rate than 500mV/s were not able to be performed because of limitation of the instrumentation used (allowable maximum scan rate 500 mV/s).

3.2 Stability and Reproducibility of μRGE

μRGEs fabricated using photolithographic technique and versatile recordable gold CDs possess the advantages of great availability, very low price and easy-to-fabrication. The reproducibility of eleven parallelly made μRGEs were examined using cyclic Voltammetry. It was found that the variance coefficients of limiting current were within 5%, indicating that the fabrication method is highly reproducible.

The robustness and stability of a μRGE was studied by periodically determining the area-normalized capacitance (C) over several successive days. If there is no Faradic current, the value of C could be obtained according to the following equation:

$$\boldsymbol{C} = \frac{\boldsymbol{I}_c}{\upsilon \boldsymbol{A}} \tag{3}$$

where A is the apparent area of working electrode. If the seal of a μRGE is perfect, that is the insulation layer of photoresist around the recession has no deficiency, the value of C would always remain constant. Experimental results show that the capacitance of the studied μRGE didn't significantly vary during seven successive days of immerse in 0.5 mol/L KCl solution. Longer time immerse results irreproducibility probably due to the damage of the insulation layer. Better results could be obtained if the μRGE was stored in dryness.

3.3 Application of μRGE in Electrophoresis Chip System

Figure 7.3 displays a electropherogram for an equimolar mixture of dopamine and catechol (each at 1×10^{-4} mol/L) using a μRGE as the detection electrode. The primary results show that the μRGE display well-defined concentration dependence. The calibration curve was linear with sensitivities of 0.15 and 0.08 nA/μM for dopamine and catechol. Based on three-time signal-to-noise ratio, detection limits of 0.31 and 0.62μM was obtained for dopamine and catechol respectively. Such values were lower than those reported in the literature[3]. So low detection limits are attributed to the insensitiveness

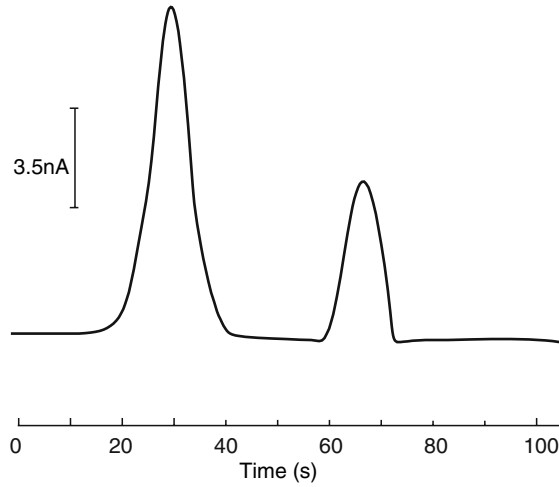

FIGURE 7.3. Electropherogram for an equimolar mixture of dopamine and catechol. Condition: 25mM MES buffer (pH6.8), separation voltage, 1800V; amperometric detection at 0.70V vs. Ag/AgCl.

to hydromechanical conditions for μRGEs. More extensive applications of μRGEs.in electrophoresis chip are in progress in our laboratory.

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