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Band-type microelectrodes for amperometric immunoassays

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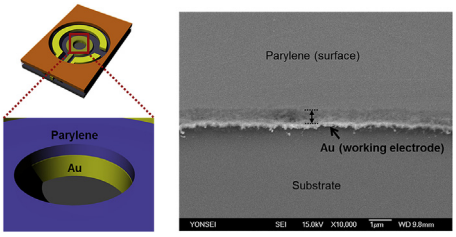
HIGHLIGHTS

- A band-type microelectrode was made using a parylene-N film as a passivation layer.
- The band-type microelectrode was 14-times more sensitive than circular-type electrode.
- The influence of geometry on microelectrode properties was simulated using COMSOL.
- The band-type electrode was applied to ELISA kits for hHBsAg and hHIV-antibodies.

GRAPHICAL ABSTRACT



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ABSTRACT

A band-type microelectrode was made using a parylene-N film as a passivation layer. A circular-type, mm-scale electrode with the same diameter as the band-type microelectrode was also made with an electrode area that was 5000 times larger than the band-type microelectrode. By comparing the amperometric signals of 3,5,3′,5′-tetramethylbenzidine (TMB) samples at different optical density (OD) values, the band-type microelectrode was determined to be 9 times more sensitive than the circular-type electrode. The properties of the circular-type and the band-type electrodes (e.g., the shape of their cyclic voltammograms, the type of diffusion layer used, and the diffusion layer thickness per unit electrode area) were characterized according to their electrode area using the COMSOL Multiphysics software. From these simulations, the band-type electrode was estimated to have the conventional microelectrode properties, even when the electrode area was 100 times larger than a conventional circular-type electrode. These results show that both the geometry and the area of an electrode can influence the properties of the electrode. Finally, amperometric analysis based on a band-type electrode was applied to commercial ELISA kits to analyze human hepatitis B surface antigen (hHBsAg) and human immunodeficiency virus (HIV) antibodies.

1. Introduction

Electrodes are classified as microelectrodes if one dimension of the electrode is on the order of micrometers. Microelectrodes have various advantages, such as: (1) an increased current caused by enhanced diffusion, (2) an increase in the signal-to-noise ratio, (3) fast establishment of a steady-state signal, and (4) a reduction in the influence of solution resistance. As the size of the electrode decreases, the double-layer capacitance also decreases and reduces the charging current, which is beneficial when conducting voltammetric analyses via the scanning electrode potential [1–5].

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Microelectrodes have been produced using various materials, including gold, platinum, and carbon nanotubes [6–8]. When a wide electrochemical window is required, diamond-like-carbon (DLC) and indium-tin (ITO) electrodes have been used [9,10]. These electrodes are known to have a relatively wide electrochemical window compared to conventional noble metal electrodes [8]. Microelectrodes have typically been made using the cross sectional area of metal wires with a high aspect ratio and UV-lithography [4,5,11,12]. However, the typical UV-lithography process has difficulties fabricating microelectrodes with dimensions below 1–2 μm. The fabrication of such microelectrodes also requires expensive processing steps with a stepper and focused ion beam (FIB) [13,14]. In this work, a band-type microelectrode is shown to have the following advantages: (1) Manufacturing of a band-type microelectrode is possible using standard UV-lithography technologies. A typical band-type microelectrode was prepared by a sequential deposition process and a series of etching processes [13]. From these processes, a band-type microelectrode with a thickness of 100 nm can be fabricated using standard UV-lithography technologies. (2) Sensitive amperometric measurement is possible using a band-type microelectrode due to the enhanced diffusion of analyte to the electrode. From the geometric effect of a band-type electrode, the properties of the microelectrode could be realized with a 100-fold larger electrode area compared with a conventional circular-shaped electrode. For the amperometric analysis, reference and counter electrodes were made via sputtering with metal masks, and the stability of an Ag/AgCl reference electrode was tested using the redox couple of ferricyanide. The sensitivity of the amperometric analysis based on the band-type electrode was demonstrated using 3,5,3′,5′-tetramethylbenzidine (TMB) samples at different optical density (OD) values and compared with a circular-type electrode with the same diameter.

The amperometric analysis based on a band-type electrode was applied to commercial ELISA kits to analyze hepatitis B surface antigens (hHBsAg) and the human immunodeficiency virus (HIV) antibody (HIV-ab). Recently, we demonstrated that electrodes consisting of a platinum wire, a sputtered gold electrode, and a DLC microdisk could be effectively used in the enzyme-linked immunosorbent assay (ELISA) as a detector [2,7,15]. Usually, the ELISA is used with a 96-well microplate, and microelectrodes can be effectively used to measure the assay results in each well. In the ELISA, analytes were specifically bound to the detection antibodies, which were immobilized on the microplate. Additionally, secondary antibodies (labeled with enzymes and fluorescent dyes) are added, and the quantification of the bound analytes is typically carried out by measuring the colorimetric, fluorescence, and luminescence signals. For commercial ELISA, the chromogenic reaction with horseradish peroxidase (HRP) and 3,5,3′,5′-tetramethylbenzidine (TMB) has been used most frequently. The chromogenic reaction is carried out by sequential oxidations of TMB and the assay results can be measured using amperometric methods: TMB → ox_1-TMB → ox_2-TMB [7,15,16]. The amperometric measurement for commercial ELISA kits were reported to have advantages, such as simple instrumentation, compared to optical systems and high sensitivity using microelectrodes, according to previous works [15,16]. Since the band-type electrode offers the above additional advantages, the influence of a band-type electrode on a commercial ELISA kit was demonstrated for the human hepatitis B virus surface antigen (HBsAg) test and the human immunodeficiency virus (HIV) antibody test. The amperometry of TMB was carried out using: (1) the current at the reduction potential of the oxidized TMBs and (2) the current at a convolution point of ox_1-TMB and ox_2-TMB.

2. Materials and methods

2.1. Materials

Ferricyanide, 3,5,3′,5′-tetramethylbenzidine (TMB), bovine serum albumin (BSA), horseradish peroxidase (HRP), and other analytical grade chemicals were purchased from Sigma-Aldrich Korea (Seoul, Korea). Human hepatitis B surface antigen ELISA kits were purchased from Elab Science Co., Ltd (Wuhan, China) and HIV ELISA kits were purchased from Bio-Rad Laboratories Corp. (Hercules, CA, USA). Polyetherimide (PEI) substrates were purchased from Goodfellow Co. (London, UK) and photoresists (AZ-GXR601) were purchased from Merck Co. (Darmstadt, Germany). Parylene-N dimers were purchased from Femto Science Co. (Korea). Polystyrene microplates were purchased from SPL Co. (Seoul, Korea).

2.2. Nanoelectrode fabrication

The band-type electrode was fabricated as described in Fig. 1(c). First, a working electrode with a diameter of 2 mm and a thickness of 100 nm was fabricated using UV-lithography. Then, a parylene film (parylene-N from Femto Science Co., Seoul, Korea) with a thickness of 500 nm was thermally deposited on the substrate [17–21]. After fabrication of the reference and counter electrodes using UV-lithography, an AZ GXR 601 layer was patterned on the electrode. Next, the parylene film was etched using a reactive ion etcher (RIE) and the gold electrode was wet-etched to expose the band-type electrode. Finally, the photoresist layer (AZ GXR 601) was removed and the Ag/AgCl paste was added as the reference electrode.

A circular-type, mm-scale electrode was fabricated as described in Fig. 1. The three electrodes were fabricated on a PEI substrate using UV-lithography with a photoresist (AZ GXR-601). First, the photoresist layer (AZ GXR-601) was spin-coated at 4000 rpm for 50 s. After soft-baking at 105 °C for 90 s, UV-exposure was carried out for 30 s using a film mask containing the shapes of the working, counter, and reference electrodes. The working electrode was fabricated to be circular in shape with a diameter of 2 mm. Then, a gold layer with a thickness of 100 nm was sputtered and lift-off was carried out with acetone using ultra-sonication. Additionally, the passivation layer was patterned using a photoresist (AZ GXR 601) and a UV-lithography. Ag/AgCl paste from Keti Co. (Korea) was added as the reference electrode.

2.3. Amperometric analysis

Amperometric measurements with a band-type microelectrode and circular, mm-scale electrodes were carried out using a commercial potentiostat from IVIUM Technologies (Netherlands). Cyclic voltammetry (CV) was performed in the potential range between −0.2 V and +0.6 V versus the Ag/AgCl reference electrode at a scanning rate of 50 mV/s. The stability of the reference electrode was estimated from the redox potentials after repeated CV analysis using 50 mM ferricyanide and 100 mM 3,5,3′,5′-tetramethylbenzidine (TMB) solutions. For the measurement of the amperometric signals from the ELISA kits, the electrode was inserted into a 96-well microplate. The amperometric signal was calculated as $\Delta I/I_0$ where I_0 is the initial current for the CV measurement and ΔI represents the difference between the reductive current at a reduction potential of 500 mV against the Ag/AgCl reference electrode and I_0.

2.4. Application of microelectrode to the commercial ELISA kit

The commercial ELISA tests were carried out according to the manufacturers' manuals. The human immunodeficiency virus (HIV)