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Enzyme electrode formed by evaporative concentration and its performance characterization

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Abstract

A highly concentrated immobilized enzyme layer was formed on a small working electrode, and the behavior of the electrode as an amperometric sensor was examined. To this end, a super-hydrophobic layer was formed in an area other than the sensitive area by using polytetrafluoroethylene (PTFE) beads. A small droplet of an enzyme solution containing glucose oxidase (GOD) and bovine serum albumin (BSA) was placed on the sensitive area, concentrated by evaporation, and crosslinked with glutaraldehyde. With the same enzyme activity per unit area, the current density increased with smaller working electrodes. Also, the current density increased with higher enzyme loadings up to a limiting value. In addition, the linear range of the calibration plot was expanded to higher glucose concentrations. The enzyme electrode fabricated by the novel method was incorporated in a micro-flow channel. Compared with large enzyme electrodes with the same enzyme activity per unit area, smaller electrodes showed a significant increase in the current density and a decrease in the flow dependence. The conversion efficiency could be improved by narrowing the flow channel and increasing the number of electrodes, which was comparable with a large electrode placed in a shallow flow channel.

Keywords: Flow channel; Glucose; Polytetrafluoroethylene (PTFE); Super-hydrophobicity

1. Introduction

In the trend toward the miniaturization of analysis systems, electrochemical sensors have played a critical role and will be the focus of attention in the coming decade (Suzuki, 2000). This is because they are easily integrated in micro-flow channels with other components, such as pumps, valves, and circuits for signal processing. Since the current trend is directed toward a further reduction in the sample and reagent volumes, the highly sensitive and efficient detection of analytes in a very small amount of sample solution will surely be one of the next critical issues. In a previous study, we examined the behavior of an amperometric enzyme electrode placed in a micro-flow channel (Hashimoto et al., 2006). A conclusion to this study was that only a fraction of the analyte in the solution was consumed for detection, while most of the analyte was wasted without being effectively used. The tendency was enhanced with the increase in the flow rate. Although an option to achieve a highly sensitive efficient detection may be to place a wide enzyme layer on a large working electrode or in the upper stream of the flow channel (Hayashi et al., 2003), there are limitations to expecting the same effect when a narrow flow channel is used. Nevertheless, the requirement of highly sensitive efficient detection when using a working electrode with a limited area and an extremely small amount of sample solution in a narrow flow channel of limited length is becoming of particular importance for applications such as clinical analysis and the monitoring of cellular metabolites. The sensitivity of the sensor can be improved by increasing the enzyme loading on the working electrode (Alvarez-Icaza and Bilitewski, 1993; D'Urso and Coulet, 1993). In addition, the transport of substrate molecules to the immobilized enzyme layer and efficient collection of products on the working electrode are also effective to realize highly sensitive sensors.

As an electroanalytical technique, the application of a single microelectrode or a microelectrode array has already demonstrated its advantages (Štulík et al., 2000). The attractive properties are derived from the unique diffusional profile of the analyte. We considered if the same principle could be used for

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an electrode with an immobilized enzyme layer. In an analogy with microelectrodes, the transport of enzyme substrates should be improved by reducing the size of the sensitive area, including the immobilized enzyme layer and the working electrode. When the enzyme activity is low and the enzyme reaction is rate-limiting, the effect will be small. However, as the activity increases, the transport of the analyte becomes a major limiting factor, and the effect will be more significant. Several methods have been proposed for the immobilization of an enzyme on a small sensitive area mainly for the application to ion-sensitive field effect transistors (ISFET) (Kimura et al., 1988; Newman et al., 1992; Takatsu and Moriizumi, 1987; Nakamoto et al., 1988; Hanazato et al., 1989; Bidan, 1992; Bartlett and Cooper, 1993; Jiménez et al., 1995). However, a problem is that most of the precious enzymes used in the procedure are wasted accompanying denaturation, depending on the case. In addition, due to the limited amount of the immobilized enzyme, there seems to be a limitation in achieving a higher sensitivity in an amperometric enzyme electrode.

In this study, the sensitivity and efficiency of detection of an amperometric biosensor were improved by forming a highly active immobilized enzyme layer only on a limited electrode area. In relation to this, we recently developed an evaporative method for concentration using a super-hydrophobic surface to increase the sensitivity of heavy metal detection by means of stripping analysis (Yanagimachi et al., 2005). In this technique, the entire area, excluding the working electrode, is covered with a super-hydrophobic layer, and a droplet of a solution containing analytes is concentrated to the sensitive area by evaporation. We believe that the same technique may be used for the concentrated immobilization of biomolecules. Spotting a precise amount of a very concentrated viscous solution on a very small working electrode is not easy even under a microscope. On the other hand, with this method, an appropriate amount of solution, which is easy to handle, is placed on the working electrode. At present, there is a technological challenge in reducing the size of the sensitive area surrounded by the super-hydrophobic layer to less than tens of micrometer. However, even with electrodes of sub-mm dimensions, an improvement of the sensitivity will be expected because the transport of molecules from the periphery of the electrode should be promoted. Here, GOD was used as a test enzyme because it is widely used and many applications are expected. In fact, the fabricated enzyme electrode showed a unique behavior, which was quite different from that of electrodes in which the immobilized enzyme layer was formed without such a concentrating procedure.

2. Experimental

2.1. Reagents and materials

The materials used for the fabrication and performance characterization of the device were obtained from the following commercial sources: a polyimide precursor solution (SP-341), from Toray Industries (Tokyo, Japan); PTFE beads (diameter: 1 μm), from Aldrich (Milwaukee, WI, USA); a thick-film photoresist (SU-8), from MicroChem (Newton, MA, USA); a dry-film photoresist (ME-1048 EA), from Hitachi Chemical Company (Tokyo, Japan); precursor solutions of polydimethylsiloxane (PDMS) (KE-1300T and CAT-1300), from Shin-Etsu Chemical (Tokyo, Japan); and GOD (165 units mg^{-1}), from Sigma-Aldrich (St. Louis, MO, USA). The other reagents were obtained from Wako Pure Chemical Industries (Osaka, Japan) and Kanto Kagaku (Tokyo, Japan).

2.2. Fabrication of the devices

Electrodes were formed on a glass substrate. For batch-style measurements, only a platinum working electrode was formed by sputter deposition with a chromium intermediate layer (Fig. 1(a)). The active area was circular (diameter: 300 μm to 2 mm) and was delineated with a polyimide layer. For experiments in a micro-flow channel, a three-electrode system was formed by a thin-film process (Fig. 1(b)–(d)). Platinum patterns were formed for the working and auxiliary electrodes and as a base layer for the reference electrode. A silver layer was additionally formed for the reference electrode on one part of the platinum pattern. The active areas for the electrodes and the pad areas were delineated with a polyimide layer. The active area for

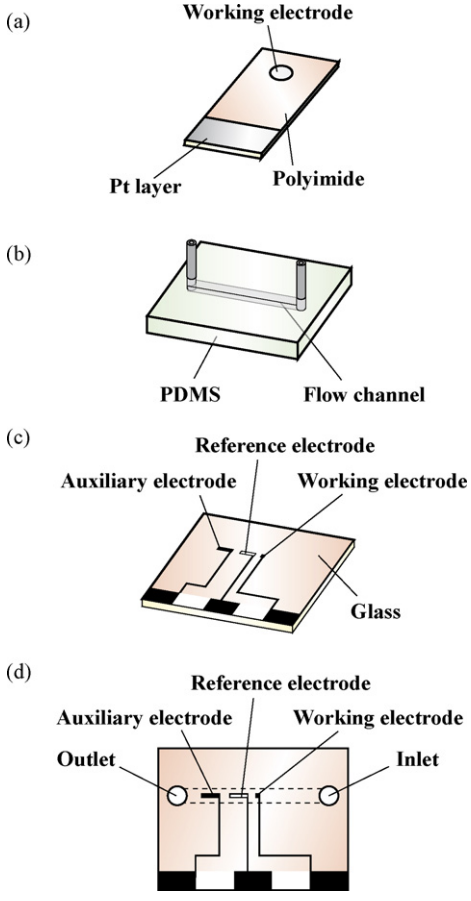

Fig. 1. Device used to characterize the electrode in a beaker and in a micro-flow channel. (a) Glass chip with only a single working electrode. (b) PDMS micro-flow channel with an inlet and an outlet. (c) Glass substrate with a three-electrode system. The dimensions of the chips are 15 mm × 20 mm. (d) Top view of the chip showing the relationship between the electrodes and the flow channel.