Anal. Chem. **2000,** *72,* 1925−1929

Technical Notes

A Disposable Amperometric Sensor Screen Printed on a Nitrocellulose Strip: A Glucose Biosensor Employing Lead Oxide as an Interference-Removing Agent

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A new type of disposable amperometric sensor is devised by screen printing thick-film electrodes directly on a porous nitrocellulose (NC) strip. The chromatographic NC strip is then utilized to introduce various sample pretreatment layers. As a preliminary application, a glucose biosensor based on hydrogen peroxide detection is constructed by immobilizing glucose oxidase (GOx) on the NC electrode strip and by formulating a strong oxidation layer (i.e., PbO_2) at the sample loading area, placed below the GOx reaction band. The screen-printed PbO_2 paste serves as a sample pretreatment layer that removes interference by its strong oxidizing ability. Samples applied are carried chromatographically, via the PbO_2 paste, to the GOx layer, and glucose is catalyzed to liberate hydrogen peroxide, which is then detected at the electrode surface. The proposed NC/PbO_2 strip sensor is shown to be virtually insusceptible to interfering species such as acetaminophen and ascorbic and uric acids and to exhibit good performance, in terms of the sensor-to-sensor reproducibility (standard deviation, $\pm 0.026-\pm 0.086$ μA), the sensitivity (slope, -0.183 μA/mM), and the linearity (correlation coefficient, 0.994 in the range of 0−10 mM).

Various types of disposable sensing strips are used routinely for the determination of biomolecules in physiological fluids. These strips may employ either an electrochemical[1−11] or a colorimetric[12−18] detection scheme. Many researchers have investigated the use of thick-film amperometric electrode strips in the fabrication of disposable biosensors.[1−11] Such sensors are usually made by screen printing electrodes with suitable conducting pastes (e.g., carbon, Ag/AgCl, etc.) on a substrate and then immobilizing a bioreagent layer on the electrode surface.

Porous membrane-type substrates such as nitrocellulose (NC) membranes typically are used to construct diagnostic strips (e.g., pregnancy test strips) based on immunochromatographic principles.[14−18] Samples are carried chromatographically from the sample loading area to the detection zone through various types of sample pretreatment zones formed on a porous substrate: e.g., labeled or immobilized antibody, detergent, and enzyme. These strip assay systems utilize enzymes, fluorescent labels, or gold and latex particles as signal generators and mostly provide yes/no-type answers or semiquantitative results based on visual or colorimetric detection formats.[19]

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10.1021/ac991213d CCC: $19.00
Published on Web 03/23/2000

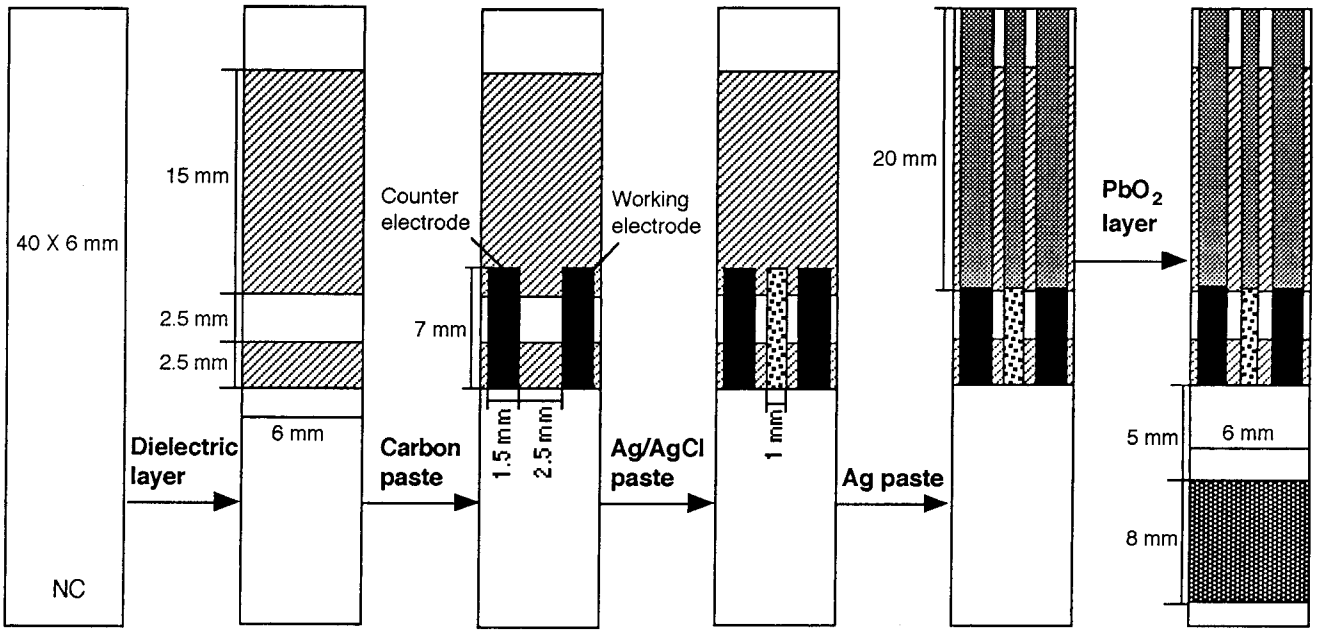

Figure 1. Fabrication of the amperometric nitrocellulose electrode strip having an oxidation layer.

One of our long-term goals has been to devise immunochromatographic strip assay systems based on electrochemical detection. Compared to optical detection systems, electrochemical sensors are very sensitive and usually exhibit a wide linear range.[19] We have investigated the feasibility of combining amperometric thick-film electrode technology with chromatographic strip assay systems. This could be done by connecting or placing a chromatographic NC strip onto screen-printed electrodes typically formed on a nonporous substrate (e.g., PVC,[1,2] polyester,[5,20] and ceramic,[8,21]). However, the most effective transfer of electroactive species generated from the reagent NC strip to the electrode surface may be realized if the electrodes are formed directly on a NC strip (see Figure 1). The lower part of such an amperometric sensor strip then can be used to introduce various zones for sample pretreatment processes.

The aim of this work is to demonstrate the analytical utility of such amperometric sensors screen printed on a porous NC strip. As a model system, a glucose biosensor is constructed by introducing a glucose oxidase (GOx) layer (or band) on a NC-based electrode strip. Glucose biosensors based on amperometric thick-film strip electrodes have been investigated extensively.[8−11] Many of these sensors rely on detection of hydrogen peroxide generated by the GOx-catalyzed reaction.[9] However, direct amperometric detection of hydrogen peroxide requires a relatively high oxidation potential (i.e., >+0.7 V vs Ag/AgCl) and, thus, may suffer severe interference from readily oxidizable species such as ascorbic and uric acids. Several approaches have been reported in order to overcome such problems with interference. These include the use of mediators (e.g., ferrocene and its derivatives,[22] cobalt phthalocyanine-modified electrode[23]) to lower the applied potential and the use of permselective films (e.g., cellulose acetate,[1] Nafion[24]) to discriminate hydrogen peroxide from interfering species.

In this report, a new method of minimizing such an interference problem is devised for a glucose biosensor formed on a NC electrode strip. A layer of strong oxidizing reagent (i.e., PbO_2) is introduced at the sample loading area below the GOx reaction band of the NC strip sensor; i.e., a PbO_2 layer, a GOx layer, and screen-printed electrodes are placed serially on the NC strip. Samples applied are carried chromatographically via the PbO_2 band to the GOx layer, where glucose is catalyzed to liberate hydrogen peroxide that is detected at the electrode surface. However, interfering species are destroyed (i.e., oxidized) during their diffusion through the PbO_2 band before they reach the electrodes (see Figure 2). In this contribution, the analytical performance of this NC strip-based glucose sensor is discussed.

EXPERIMENTAL SECTION

Materials and Apparatus. The sources of reagents and materials used were as follows: glucose oxidase (type VII, from *Aspergillus niger*, 151 000 units/g of solid), β-D(+)-glucose, ascorbic acid, uric acid, acetaminophen, and Triton X-100 from Sigma (St. Louis, MO); lead dioxide, disodium hydrogenphosphate, and sodium dihydrogenphosphate from Kanto (Tokyo, Japan); poly(vinyl alcohol) (PVA; MW 22 000) from Fluka (Buchs, Switzerland); carbon paste (TU-15ST, lower resistance type), silver paste (LS-506J), and insulator paste (CR-10) from Asahi Chemical Research Laboratory (Tokyo, Japan); silver/silver chloride paste from Gwent Electronic Materials (Pontypool, U.K.); and nitrocellulose (8 μm, membrane supported with polyester) from Whatman (Rotenburg, Germany). The PbO_2 paste was prepared by mixing 40 g of PbO_2 powder with 2.5 g of PVA in 7.5 mL of water, using a homogenizer (Daisan Science, Seoul, Korea). A custom manual screen printer was used for screen printing all pastes. All chronoamperometric measurements were carried out with an EG&G PAR model 273A potentiostat/galvanostat.

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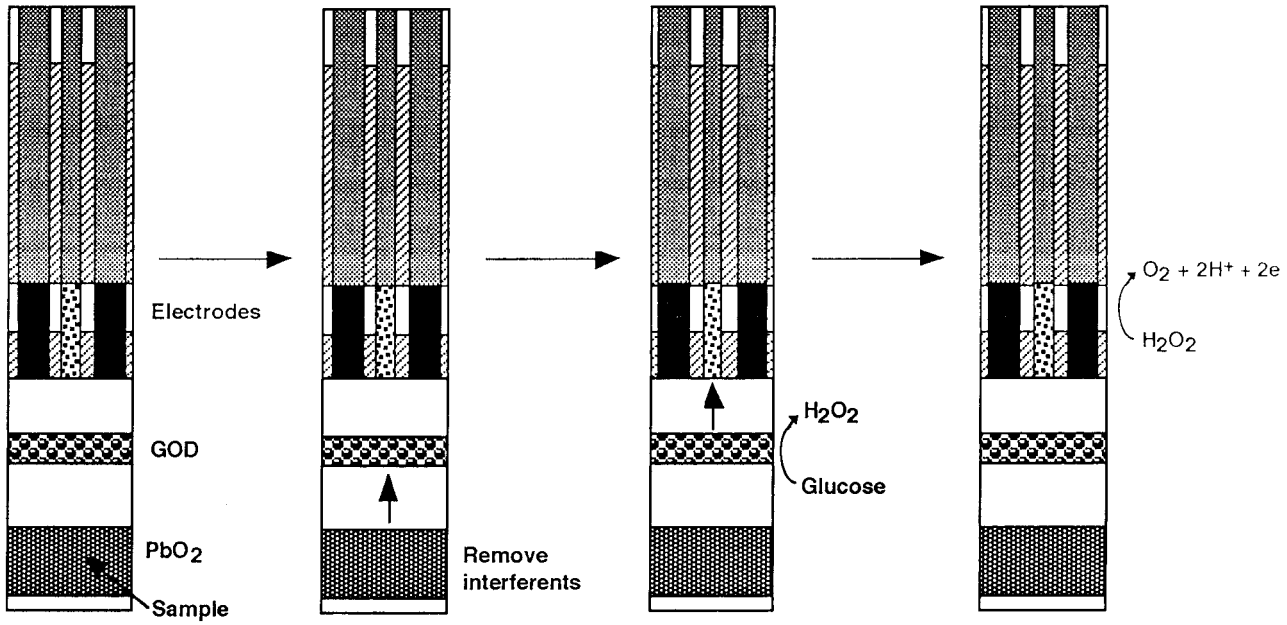

Figure 2. Amperometric measurement employing glucose sensor fabricated on a nitrocellulose strip with the PbO_2 layer.

Screen-Printed NC Electrode Strips. NC membranes were immersed in 10% methanol for 5 min, washed with deionized water, and air-dried. Thick-film amperometric electrodes were then fabricated in a group of 20 (with a 2-mm gap between each) by screen printing appropriate pastes onto a NC membrane (100 × 90 mm). As illustrated in Figure 1, the following layers were formed sequentially on each sensor strip: (1) dielectric insulating layers, (2) carbon working and counter electrodes, (3) Ag/AgCl reference electrode, (4) Ag conducting layers, and (5) PbO_2 oxidation layer. The curing temperatures/times were as follows: (1) 130 °C/10 min for dielectric, (2) 140 °C/10 min for carbon paste, (3) 140 °C/5 min for Ag and Ag/AgCl, and (4) room temperature/2 h for PbO_2.

Immobilization of Enzyme on NC Electrode Strips. An enzyme casting solution was prepared by dissolving 25 mg of GOx in 1 mL of 0.1 M phosphate buffer, pH 7.0, containing 0.2% Triton X-100 and 0.1 M sodium chloride. A small volume (typically 3 μL) of this GOx mixture was dispensed onto a NC strip at a gap between the electrodes and the PbO_2 layer. These sensors were kept in a container maintaining a humidity condition of 50−60% for 1 h.

Evaluation of Screen-Printed Sensors. Amperometric measurements with NC strip glucose biosensors are illustrated in Figure 2. Two drops (10 μL each) of sample solution were sequentially applied directly onto the surface of the PbO_2 layer with a 1-min interval. A single drop of 20 μL results in overflow because of a limited diffusion rate of the sample through the PbO_2 layer, while a drop of 10 μL is not sufficient for the sample to reach across the strip to the electrodes. The amperometric reading was initiated at the time the applied sample reached the electrodes chromatically, typically 2.5 min after applying the second drop of the sample. The anodic current data were collected for 15 s at a constant potential of +0.7 V applied between the working and reference electrodes to detect hydrogen peroxide generated from the GOx-catalyzed reaction. The steady-state current values were used to plot calibration curves. The background electrolyte was 0.1 M sodium phosphate, pH 7.0, and all standard and sample solutions were prepared with this buffer solution.

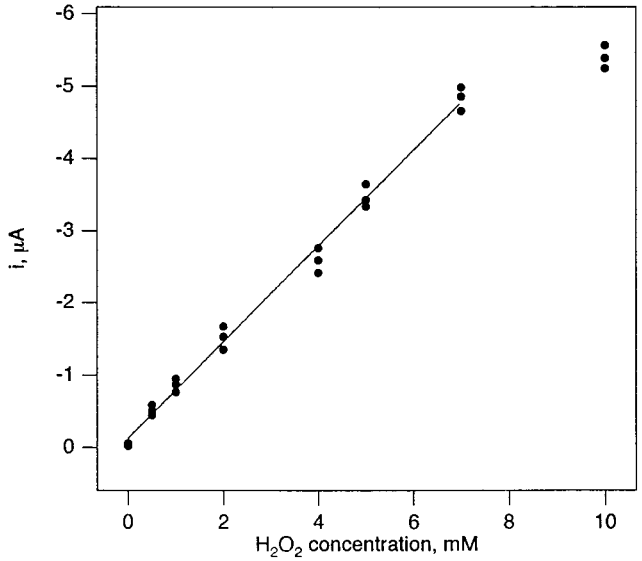

Figure 3. Amperometric response of electrodes screen printed on nitrocellulose strip toward hydrogen peroxide. Three strips were used for the measurement of each concentration.

RESULTS AND DISCUSSION

Initially, we investigated several different approaches of combining a screen-printed electrode strip (i.e., on a nonporous substrate) with a NC-based reagent strip to devise a disposable chromatographic strip system with the capability of electrochemical detection. However, all of the procedures examined were rather complicated and did not yield sensors with acceptable reproducibility. Efficient attachment of the reagent strip to the electrode strip is best achieved by forming the electrodes directly on a porous reagent strip. Figure 3 demonstrates the feasibility of fabricating such sensors, i.e., screen printing amperometric electrodes on a porous NC strip (see Figure 1 and the Experi-

mental Section for the detailed fabrication process). In this experiment, sensors were prepared without the PbO_2 layer, and one sensor strip was used for a single measurement of hydrogen peroxide by employing the same protocol used for the sensors with the PbO_2 layer. As can be seen from Figure 3, amperometric electrodes formed even on a porous NC strip functioned surprisingly well and showed satisfactory current signals in a wide dynamic range (a slope of $-0.664\ \mu A/mM$ in the range of 0−7 mM hydrogen peroxide). More importantly, sensor-to-sensor reproducibility, which is critical in the fabrication of disposable-type sensors, was greatly enhanced with the proposed approach (standard deviation, $\pm 0.018-\pm 0.16\ \mu A$), particularly compared to those of sensors employing two separate substrates (e.g., a porous reagent strip connected to a nonporous electrode strip).

The proposed NC strip sensor system has the capability of introducing various sample pretreatment layers to the chromatographic NC strip and thus is potentially useful for devising immunoassay-type sensor strips that require antibody/antigen reaction zones. As a preliminary application, however, the sensor strip was utilized to construct an amperometric glucose biosensor by formulating two sample pretreatment zones on the chromatographic NC strip: (1) a catalytic GOx layer that converts glucose to electroactive hydrogen peroxide and (2) a PbO_2 layer that removes interferences (see Figures 1 and 2 and the Experimental Section for details). The most serious problem associated with amperometric glucose sensor devices with an operating potential of +0.7 V vs Ag/AgCl for hydrogen peroxide detection is known to be the interference from readily oxidizable species (e.g., ascorbic acid) present in physiological samples. While various methods have been reported in order to overcome such an interference problem,[1,2,8,22−24] this is the first attempt to investigate the use of PbO_2 for such purpose.

In the present sensor design, the PbO_2 paste screen printed on the NC electrode strip serves as a sample pretreatment layer that removes interference by its strong oxidizing ability: i.e., interferences in samples are oxidized prior to contacting the electrodes, and thus, do not interfere with electrode measurements (see Figure 2). However, hydrogen peroxide generated from the GOx/glucose reaction is not affected by PbO_2 since the GOx layer is formulated between the PbO_2 layer and the electrodes. Having two separate layers of enzyme and PbO_2, rather than depositing PbO_2 directly on the enzyme layer, may be advantageous in that the enzyme activity is not affected by PbO_2. In this sensor format, however, glucose in samples diffuses through the PbO_2 paste and, therefore, should not be oxidized by PbO_2. This is demonstrated in Figure 4 showing amperometric responses of NC strip glucose sensors prepared with (type A) and without the PbO_2 layer (type B) toward glucose (5.5 mM) and/or a mixture of interference species (0.5 mM ascorbic acid/0.5 mM acetaminophen/0.5 mM uric acid): (a) type A/background, (b) type A/glucose, (c) type B/glucose, (d) type A/interference, (e) type B/interference, (f) type A/glucose/interference, and (g) type B/glucose/interference. As can be seen, the amperometric signals of both sensors with (curve b) and without PbO_2 (curve c) were not very different toward glucose, showing that the PbO_2 layer does not seriously affect the concentration of glucose added. As expected, the sensor fabricated without PbO_2 exhibited a huge response (curve e) toward a mixture of oxidizable species (acetaminophen, ascorbic and uric acids). However, such an interfering response signal disappeared (curve d) when the PbO_2-based sensor was used, indicating that the interfering species are oxidized during their diffusion through the PbO_2 layer. This can be further demonstrated by comparing responses of the PbO_2 type toward a sample containing only glucose (curve b) and a sample containing both glucose and interferences (curve f): the steady-state current signals were virtually the same for both samples. It can be seen, however, the signal of the sensor prepared without the PbO_2 layer is additive toward glucose and added interferences (curve g).

The NC/PbO_2-based disposable glucose sensor system was further characterized by plotting a calibration curve as shown in

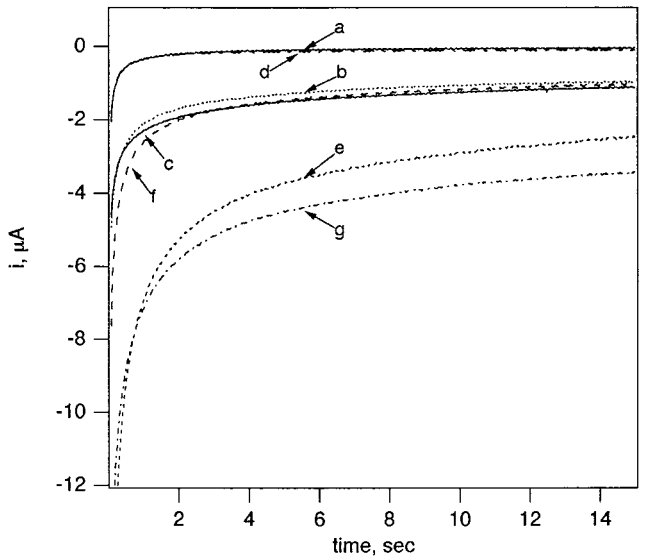

Figure 4. Amperometric response profiles of the nitrocellulose strip glucose sensors prepared with (type A) and without the PbO_2 layer (type B) toward glucose (5.5 mM) and/or a mixture of interference species (0.5 mM ascorbic acid/0.5 mM acetaminophen/0.5 mM uric acid): (a) type A/background, (b) type A/glucose, (c) type B/glucose, (d) type A/interference, (e) type B/interference, (f) type A/glucose/interference, and (g) type B/glucose/interference.

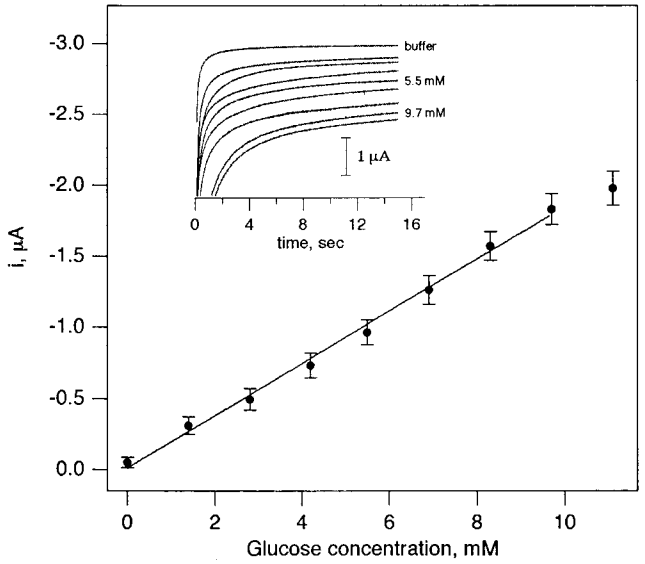

Figure 5. Amperometric dynamic response and a calibration plot obtained with the nitrocellulose/PbO_2-based disposable glucose sensor system. Five sensor strips were used for each glucose concentration.

Figure 5. In this experiment, five sensor strips were used for each glucose concentration, and representative dynamic responses of the sensors toward each concentration are shown also in Figure 5. Sensor-to-sensor reproducibility (standard deviation, ±0.026−±0.086 μA), sensitivity (slope, −0.183 μA/mM), and linearity (correlation coefficient, 0.994 in the range of 0−10 mM) obtained with the proposed sensor system appear to be adequate for glucose measurements in physiological samples.

In this report, we have described two new methods that may prove useful in the fabrication of disposable amperometric sensor strips: screen printing thick-film electrodes on a chromatographic NC strip and the use of PbO_2 as an interference-removing agent. The glucose sensor system constructed by combining the two methods is shown to be insusceptible to interference from oxidizable species. The use of PbO_2 is a generic approach to eliminating interference for amperometric sensors (particularly those based on hydrogen peroxide detection) and could find applications in fabricating other biosensors. Given the promising results, it is likely that the amperometric sensors screen printed on a chromatographic strip will offer a relatively simple method to mass fabricate amperometric immunoassay strip sensors.

ACKNOWLEDGMENT

This study was supported by a grant (HMP-97-E-1-0004) of the Good Health R&D Project, Ministry of Health & Welfare, R.O.K.

Received for review October 25, 1999. Accepted February 3, 2000.

AC991213D