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Original Paper

The usage of a bismuth film electrode as transducer in glucose biosensing

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Abstract. A second generation glucose biosensor was developed by using neutral red (NR) as a mediator and a bismuth film electrode (BiFE) as a transducer along with immobilized glucose oxidase. The linear range was between 0.2 and 2.5 mM, and a correlation coefficient of 0.999 was obtained with this electrode. The standard deviation (at 1 mM glucose for $n = 4$) and the coefficient of variation were calculated as ± 8.07 μM and 3.4%, respectively. The biosensor was used for the determination of glucose in wine samples.

Keywords: Bismuth film electrode (BiFE); neutral red; glucose oxidase; biosensor

Since its introduction as an alternative electrode material to mercury film electrode (MFE) [1], the bismuth film electrode (BiFE) has been extensively used for various applications including trace metal analysis [1–11] and organic compound detection [12–15].

BiFE can easily be formed by electrodeposition of a proper amount of Bi on suitable substrates such as glassy carbon [1–6], carbon paste [7, 13], pencil-lead [8], copper [16] carbon fiber [1, 17, 18] or graphite epoxy composite electrode (GECE) [19–23] without applying any deaeration. The promising electroanalytical performance of this less toxic electrode material has been proved in many studies; in the field of trace metals and recently organic material analysis as mentioned above. However, in order to extend its scope and explore its power and limitations, the electroanalytical behavior of BiFE in different applications needs to be examined. The application of this electrode as a biosensor transducer is one of these areas.

As it is well known, the success of an electrochemical sensing process relies mainly on the proper choice of the working electrode. Solid electrodes such as gold, platinum and carbon-based electrodes were used as electrode transducers. Apart from these, mercury thin film electrode (MTFE) based biosensor where the laccase enzyme was utilized as biological component was developed by our group [24].

The electrochemical indication of oxidase-catalyzed enzymatic biosensors is possible by measuring either the consumption of oxygen or the oxidation of hydrogen peroxide [25]. In other words, these biosensors (called 'first generation biosensors') are dependent on the concentration of dissolved oxygen in the bulk solution [26]. On the other hand, 'second generation biosensors' involve the usage of artificial electron acceptors, mediators, where the reduction and oxida-

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tion of this material occurs instead of dissolved oxygen [27, 28]. In these systems, all the substances that have a conversion potential lower than the electrode potentials contribute to the overall electrochemical signal. This approach leads to a considerable reduction of electrochemical interferences and the development of mediated biosensors. Thus, mediated biosensors can be constructed with the enzymes that can donate electrons to electrochemically active artificial electron acceptors [29].

The development of second generation biosensors involves a two-step procedure in which the enzyme takes part in the first redox reaction with the substrate and is in turn reoxidized by a mediator. Finally the mediator is oxidized by the electrode as shown below [30].

$$\text{Glucose} + \text{GOx/FAD} \rightarrow \text{Gluconolactone} + \text{GOx/FADH}_2 \quad (1)$$

$$\text{GOx/FADH}_2 + 2\text{M} \rightarrow \text{GOx/FAD} + 2\text{M}^* + 2\text{H}^+ \quad (2)$$

$$2\text{M}^* \rightarrow 2\text{M} + 2\text{e}^- \quad (3)$$

where FAD represents a flavin redox center in GOx. M and M^* are the oxidized and reduced forms of the mediator. Ferrocene, ferricyanide etc., have been the commonly used mediators [31].

Neutral red (NR) is a phenazine dye which had been used as a spectroscopic and voltammetric probe for the detection of DNA in solution [31] as well as biofuel cell applications [32].

The present work examines the performance of BiFE as a biosensor transducer. For this particular application, the electrode was modified with glucose oxidase (GOx) enzyme by means of a gelatin membrane which was then cross-linked by glutaraldehyde, and the obtained system response to glucose was monitored by following the oxidation of NR which was added to the reaction medium as a soluble mediator.

Experimental section

Apparatus

Chronoamperometric and cyclic voltammetric experiments were carried out with an Autolab PGSTAT 12 potentiostat/galvanostat (Eco Chemie B.V, The Netherlands, www.ecochemie.nl). A glassy-carbon (GC) disk (Metrohm, Herisau Switzerland, www.metrohm.com) served as the supporting electrode for the biosensor transducer, with the Ag/AgCl (3.0 M NaCl) and platinum electrode (Metrohm, Herisau, Switzerland) acting as the reference and counter-electrodes, respectively.

Reagents

Glucose was purchased from Merck AG (Darmstadt, Germany) while glucose oxidase (GOx, β-D-Glucose:oxygen 1-oxidoreductase, E.C. 1.1.3.4, Type II-S, 15,000–50,000 units/g solid), Neutral Red, calf skin gelatin and glutaraldehyde were obtained from Sigma Chem. Co. (St. Louis, MO, USA, http://www.sigmaaldrich.com). All other chemicals were of analytical grade. Standard stock solution of bismuth ($1000\,\text{mg}\cdot\text{L}^{-1}$, atomic absorption standard solution) was obtained from Aldrich and diluted as required. An 0.1 M sodium acetate buffer solution (pH 4.5) served as supporting electrolyte.

Sodium acetate buffer solution (100 mM, pH 4.5) was utilized as the supporting electrolyte during bismuth film formation while phosphate buffer (50 mM, pH 7.0) solution was used as supporting electrolyte during the enzymatic reactions.

Electrode preparation

The glassy carbon disc electrode (GCE) was polished by hand using alumina slurry on a felt pad prior to Bi film formation. BiFE was prepared *ex-situ* by following the usual procedure [1–23], which includes introducing Bi solution ($500\,\mu\text{g}\cdot\text{L}^{-1}$) into a working cell containing 10 mL acetate buffer (100 mM, pH; 4.5) with three electrodes. For this process, a deposition potential of -1.1 V was applied for 5 min without applying any deaeration.

GOx (1 mg) and gelatin (10 mg) were mixed at 38 °C in potassium phosphate buffer (pH 7.0, 250 µL). 50 µL of mixed solution was spread over the BiFE surface and allowed to dry at 4 °C for 1 h. Finally, it was immersed in 2.5% glutaraldehyde in phosphate buffer (50 mM, pH 7.0) for 5 min for cross-linkage.

Measurements

All chronoamperometric measurements were carried out in ambient conditions under continuous and constant stirring. After each run, the electrode was washed with distilled water and kept in working buffer solution for 5 min. The measurement was based on the oxidation of neutral red reduced due to the enzymatic reaction on the electrode surface. The current changes that resulted from substrate (glucose) addition into the medium were recorded with a potentiostat at -500 mV for 300 sec. Moreover, the relative (%) biosensor response was estimated by assuming a current value of 100% under the optimal working conditions.

Cyclic voltammetric measurements were conducted in the potential range of -0.200 mV and -0.900 mV, at $100\,\text{mV}\cdot\text{sec}^{-1}$s with a frequency of 50 Hz and an amplitude of 20 mV.

Results and discussion

When electron mediators are involved in biosensor structures, reagentless second-generation amperometric biosensors are obtained. Redox mediators have a special function in biosensors. Their role is to replace the electron transfer performed by oxygen in order to avoid the problems that arise from low oxygen concentrations in some cases [33]. As it is well known, in this system, instead of molecular oxygen, the media-

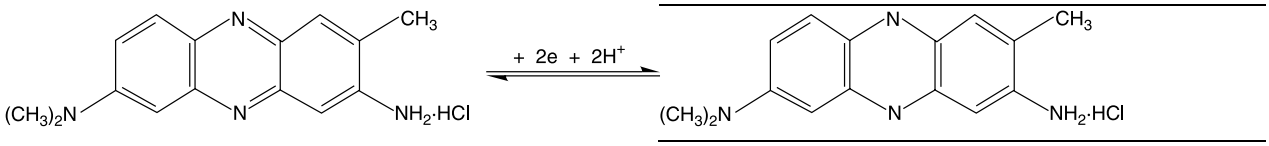
tors accept electrons from the enzyme and become reduced and then oxidized at the electrode surface.

One of the advantages of BiFE is that it tolerates the presence of dissolved oxygen. BiFE is not affected with the presence of oxygen in the measuring solution. This is why this electrode material can not be used as a transducer for oxido-reductase enzyme based amperometric biosensors. By using a proper mediator, a 'second-generation biosensor' can be obtained that may pave the way for the utilization of BiFE as biosensor transducer.

For this purpose neutral red, a monomeric soluble mediator, was used as ''electron shuttle'' between the enzyme and electrode. Since oxygen could not be monitored on BiFE, the redox behavior of neutral red was monitored instead of the oxygen consumption for deriving information about the substrate [31].

Electrochemical reactivity

The C=N group in the NR structure is responsible for the electrochemical redox process of the NR mediator. The redox process of this mediator can be expressed using the following equation [34]:



The promising electroanalytical properties of the Bi electrode have been reported for different applications [1–24]. In this paper the cyclic voltammograms of NR (1 mM) obtained with BiFE (a) and a conventional GCE (b) were compared and are given in Fig. 1. For BiFE, the oxidation peak appeared at −0.481 V with a 26.6 μA current value and a reduction peak at −0.659 V with a 58.7 μA current value; while these values were −0.517 V, 15.72 μA and −0.566 V, and 39.20 μA for GCE, respectively. Obviously, BiFE yields better current values (almost 50% higher) which can be expected to have profound effect, such as better sensitivity, on the developed biosensor.

On the other hand, it is known that the oxidation reaction of the mediator rather than the reduction occurs at the second-generation biosensors, since the mediator takes electrons from the enzyme active center and loses them on the electrode surface. For this reason and with the help of the cyclic voltammograms above, −500 mV was chosen as optimum working potential and used for further experiments.

Effect of pH

The effect of pH on the electrode response was investigated at various pH values (6.0, 6.5, 7.0, and 7.5) by using phosphate buffer systems (50 mM) for 1.0 mM glucose (Fig. 2). As can be seen from the figure, the response current of the electrode to glucose increases significantly from pH 6.0 to 7.0, and then decreases sharply at pH values higher than 7.0. As a result, pH

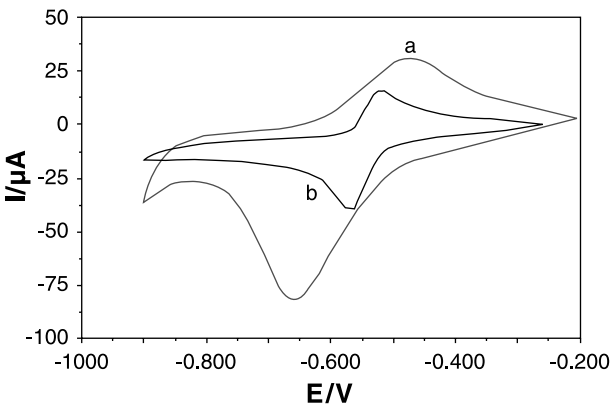


Fig. 1. The cyclic voltammograms of 1 mM NR (*a*) at BiFE, (*b*) at GCE [pH 7.0, 50 mM phosphate buffer system as supporting electrolyte, scan rate 100 mV · sec^{-1}]

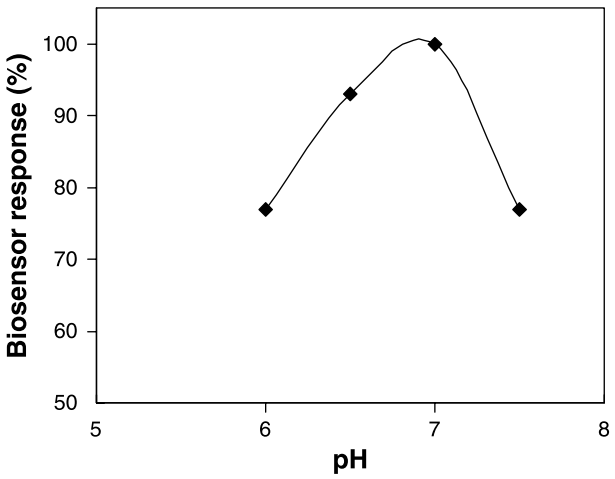


Fig. 2. Effect of pH on the biosensor response with phosphate buffer systems (50 mM) for 1.0 mM glucose in the presence of 1 mM NR

7.0 was chosen as optimum pH and used for further studies.

Analytical characteristics

A linear relationship between the sensor response and the glucose concentration was obtained in a concentration range of 0.2–2.5 mM with the equation $y = 0.3207x + 0.0074$. The correlation coefficient, R^2 was 0.999. For comparison, the same measuring procedure was repeated using non-modified GCE instead of Bi-film electrode (using the same GCE). As a result, linearity was observed in the range of 0.5–1.2 mM with the equation of $y = 1 \times 10^{-4}x + 0.209$, the correlation coefficient, R^2 being 0.983. At higher concentrations, both standard curves deviated from linearity.

Reproducibility of the enzymatic biosensor was tested for glucose (1 mM, $n = 4$). The standard deviation (S.D), variation coefficient (cv) and limit of detection (LOD) values were calculated as ± 8.07 μM, 3.40% and 40.56 μM ($S/N = 3$), respectively.

These values are comparable to the values that were obtained with other NR including glucose biosensors where Polymer Neutral Red (PNR) was deposited on carbon-film and modified with GOx to obtain the biosensor [35]. GOx was immobilized by cross-linking with glutaraldehyde or by means of an oxysilane sol–gel network. In that paper it was mentioned that the usage of flow cells provided better results with a detection limit of 36.0 ± 3.2 μM ($n = 3$) for glutaraldehyde and 62.0 μM for sol–gel, respectively. The linear range was up to 0.9 mM for glutaraldehyde and 0.75 mM for sol–gel.

It is known that the semiconducting films that are prepared from mediators (like PNR) by means of electropolymerization provide high catalytic currents in the electroenzymatic reactions [36]. On the other hand, the combination of BiFE with monomer NR yields a better linear range with comparable detection limit values. From this point, it can be claimed that with BiFE it is possible to obtain more economical and practical second-generation glucose biosensors.

Concerning operational stability, no activity loss was obtained at the biosensor response during 7 h for approximately 20 measurements. To avoid mediator deposition inside the biofilm, which may occur after long time usage of this system, daily prepared enzyme electrodes were used to get more reproducible results.

Sample application

Sirince Asmalibag labeled wine samples from Artemis Sirince Sarapcilik were analyzed using the standard addition method. A known amount of glucose (0.5 mM) was added to the reaction cell from the stock solution that was prepared by diluting a wine sample with 50 mM phosphate buffer, pH 7.0. The recovery of the analytical signal for each of the diluted samples was calculated as 97 and 104%, respectively.

From the recovery values, it can be concluded that the developed biosensor can be utilized for glucose detection even in complicated matrix systems like wine. Also, using this biosensor renders the laborious sample treatment step that is commonly applied before the measuring step in alternative methods (such as spectroscopy and chromatography).

Conclusion

The possibility to work with BiFE in the presence of dissolved oxygen restricts its use as an oxidoreductase biosensor transducer since the measuring process of these biosensors relies on monitoring the oxygen amount in the medium. In this paper, a second-generation glucose biosensor was developed using NR as mediator, and the performance of BiFE as biosensor transducer was examined. The performance of BiFE was compared with that of plain GCE, and as a result more sensitive current values with a wider linear range and better correlation coefficient values were obtained with the film electrode. So it can be concluded that BiFE is a better transducer than conventional GCE for this application. The analytical characteristics of the developed monomer (NR) biosensor are found to be comparable to values of electropolymerized (PNR) glucose biosensors.

The developed biosensor was also applied to wine samples, and good recoveries were obtained. According to our findings, it can be concluded that our system is not affected by the sample matrix. Instead of laborious and time-consuming sample preparations, it is possible to obtain promising results with the developed system simply by diluting the sample solution with working buffer. The preliminary results showed the possibility of future applications in other samples.

Ongoing efforts are being made in our group to extend the use of BiFE in biosensing systems and integrate the mediator inside the sensing layer.

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