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A New Amperometric Glucose Biosensor with Naphthol Green B as Mediator

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Abstract: Naphthol green B was used, for the first time, as a new mediator in an amperometric glucose biosensor. It is a good mediator, promoting electron transfer from glucose oxidase to graphite electrode. The biosensor shows high sensitivity to glucose at low potential with response time of 30 seconds. The linear range is from 1.5 to 18 μmol/L glucose with detection limit of 0.5 μmol/L glucose.

Keywords: Naphthol green B, mediator, glucose oxidase, biosensor.

Since the work of Clark[1], electrochemical biosensors have attracted a great deal of interest and have become the subject of intense investigations. The electrochemical biosensors perform simple, fast, specific and accurate monitoring with compact and user-friendly instrumentation to transform traditional methods of analysis.

The first successful electrochemical biosensor is the amperometric glucose biosensor using the Clark-type oxygen electrode with direct electron transfer from the glucose oxidase to the surface of the electrode[1]. However, there are some disadvantages in using the oxygen electrode-based glucose biosensor. Electron transfer mediators are involved to circumvent these disadvantages[2]. The mediators include ferrocene and its derivatives[2-4], potassium hexacyanoferrate[5] and tetrathiafulvalene[6]. Water-soluble dyes such as methylene blue, neutral red, toluidine blue and methyl viologen have also been used as mediators in solution because of their high electron transfer efficiency and low cost[7-9].

1-Nitroso-2-naphthol-6-sodium sulphonate ferric salt (naphthol green B) possesses excellent redox characteristics[10]. In this letter, we report the preliminary result that naphthol green B can be used successfully as a mediator for detecting glucose. A possible use of naphthol green B for constructing amperometric biosensors is proposed.

Experimental

Cyclic voltammetry experiments were performed with a three-electrode system using an electrochemical workstation (IM6e, ZAHNER Co., Germany) equipped with THALES

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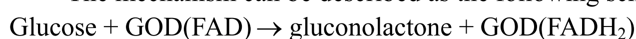
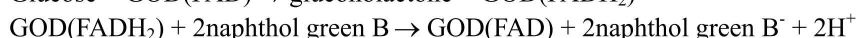
software 6.88. Homemade spectrographic graphite electrodes (6 mm diameter) were used as working electrodes. Saturated calomel electrode (SCE) was used for potential reference. A platinum wire served as the auxiliary electrode. Glucose oxidase (GOD) (EC 1.1.3.4 type X-S, from Aspergillus niger) with an activity of 245,900 U/g and uric acid were supplied by Sigma (Sigma Chem. Co., St. Louis, MO, USA). All solutions were prepared from analytical grade reagents in double distillated and deionized water. Supporting electrolyte was 0.1 mol/L Na_2HPO_4 adjusted to the required pH with $HClO_4$.

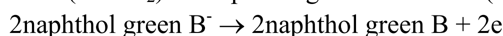
Results and Discussion

Figure 1 shows cyclic voltammograms of the graphite electrode in phosphate buffer (pH 6.0) containing 4 mmol/L naphthol green B and 50 mmol/L glucose without glucose oxidase (a) and with 1.5 mg/mL glucose oxidase (b). Upon addition of glucose oxidase to the solution, a dramatic change in the voltammogram occurs (**Figure 1 b**). The obvious catalytic reaction appeared, accompanied by the increase of oxidation current. The typical catalytic oxidation current shows that the naphthol green B can act as an efficient electron transfer mediator between the $FAD/FADH_2$ centers of glucose oxidase and the graphite electrode surface.

The mechanism can be described as the following scheme:

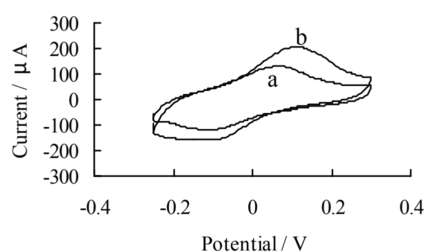

Glucose + GOD(FAD) $\rightarrow$ gluconolactone + GOD($FADH_2$)


GOD($FADH_2$) + 2naphthol green B $\rightarrow$ GOD(FAD) + 2naphthol green B^- + $2H^+$


2naphthol green B^- $\rightarrow$ 2naphthol green B + 2e

Where GOD(FAD) and GOD($FADH_2$) represent the oxidized and reduced form of flavin adenine dinucleotide within GOD.

Figure 1 Cyclic voltammogram of naphthol green B (4 mmol/L) at pH 6.0 and 25°C.

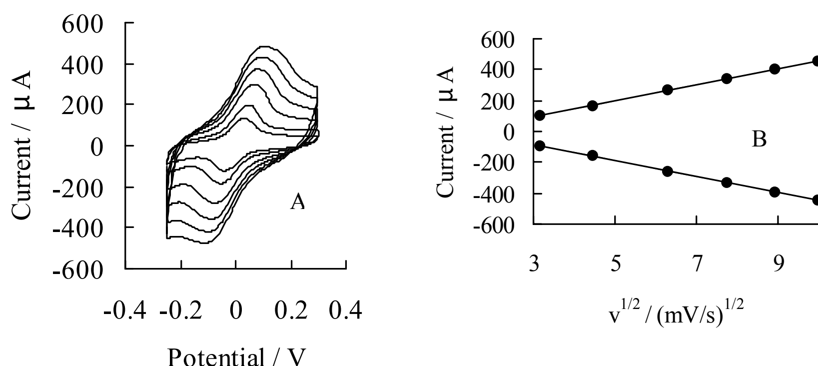


a. In the presence of D-glucose (50 mmol/L) at a scan rate of 25 mV/s.
b. The conditions were as for (a), but with the addition of glucose oxidase (1.5 mg/mL).

Cyclic voltammetry shows quasireversible behavior of naphthol green B. Typical cyclic voltammograms of 4 mmol/L naphthol green B in the phosphate buffer (pH 6.0) at graphite electrode at different scan rates are shown in **Figure 2 A**. Separation of the reduction and the oxidation peaks (ΔE_p) is 60 mV at a scan rate of 10 mV/s and increases with increasing scan rate. The linear relationship of peak current to the square root of

scan rate indicates that the reduction and oxidation of the naphthol green B / naphthol green B^- couple is diffusion-controlled at the graphite electrode (**Figure 2 B**).

Figure 2 (A) Cyclic voltammograms of the graphite electrode at different scan rates (from inner curve to outer curve): 10, 20, 40, 60, 80, 100 mV/s in the phosphate buffer (pH 6.0) containing 4 mmol/L naphthol green B. (B) Plot of the peak current *vs*. the square root of scan rate.



The effect of oxygen on the response signal of the glucose biosensor was investigated by comparing measurement in nitrogen-saturated buffer with that in air-saturated buffer and no detectable changes were noted. It indicated that oxygen dissolved in the solution had no influence on the determination of current response. Therefore, all subsequent experiments were performed in air-saturated buffer.

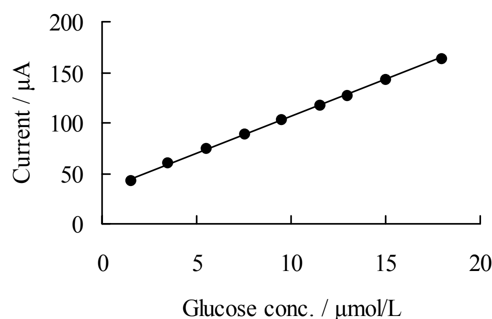
The optimum operating conditions were chosen according to our experiment results and all further experiments were carried out in phosphate buffer (pH 6.0) containing 4 mmol/L naphthol green B and 3 mg/mL glucose oxidase at 25°C.

Calibration data were obtained under the optimum experimental conditions mentioned above. **Figure 3** illustrates calibration curve for the amperometric glucose biosensor. The response time is within 30 s. The calibration curve exhibits a very linear behavior with a correlation coefficient of 0.9997 (n = 9). The sensor gives a linear current response in the range 1.5 ~ 18 μmol/L glucose. The lowest detection limit was found to be 0.5 μmol/L based on a signal-to-noise ratio of 3.

To investigate the reproducibility of the current response of the biosensor, repetitive measurements were carried out in 5 μmol/L glucose, 4 mmol/L naphthol green B and 3 mg/mL glucose oxidase solution. The results of 9 successive measurements show relative standard deviation of 0.42%. The operational stability of the enzyme sensor was measured by monitoring the response for 2 h period of continuous use and almost no decrease in sensitivity was observed. Thus the biosensor exhibits good, reproducible and stable characteristics and can be used for repetitive measurements.

Some common interferences such as ascorbic acid and uric acid at concentrations in the respective physiological concentration ranges did not cause any observable interference to the electrode response to glucose.

Figure 3 Calibration curve for the sensor with 4 mmol/L naphthol green B and 3 mg/mL glucose oxidase in the phosphate buffer (pH 6.0) at 25°C.



Acknowledgments

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