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Amperometric Enzyme Sensor for Glucose Based on Graphite Paste-Modified Electrodes

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ABSTRACT

Amperometric enzyme electrode for glucose is described based on the incorporation of glucose oxidase (GOD) into graphite paste modified with tetracyanoquinodimethane (TCNQ). The incorporated enzyme exhibits high activity and long-term stability over the earlier TCNQ-based glucose sensor (*1*). The sensor provides a linear response to glucose over a wide concentration range. The response time of the sensor is 15–50 sec, and the detection limit is 0.5 m*M*. Stable response to the substrate was obtained during a period of 35 d. Application of the sensor in the plasma analysis is reported.

Index Entries: Enzyme electrode; hydrodynamic amperometry; plasma analysis.

INTRODUCTION

The electrochemical biosensors for glucose measurements have been studied extensively and reviewed during recent years (*2–6*). The important parameters that govern the reliability of the probes in "real sample" analysis are selectivity, wide linearity, high stability, and short response time. Amperometric response of the sensor is based on probing of the enzymic reaction either by oxidation of hydrogen peroxide or by the electron exchange from the active center of the redox enzyme. Amperometric

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signal obtained by the electron exchange from the active center of enzyme and electrode surface considerably increases the selectivity and sensitivity of analysis since the selective inherent properties of the enzyme are monitored directly. The electron exchange from the active center of enzyme like glucose oxidase to the electrode surface is facilitated by incorporating electron transfer relays between the active center of enzyme and electrode surface (*7*). There are a number of reports on the use of organic metal (salts of TCNQ) as an efficient electrocatalyst (*8–10*) for the direct electron exchange from the active center of enzymes. However, Cenas and Kulys (*11*) report that the oxidation of redox enzyme at the surface of organic metal proceeds through mediatory way, and they express the current of enzyme electrode in terms of mediator concentration produced during the slight dissolution of the organic metal salts on the surface of the electrode. Further, Cenas and Kulys (*12*) demonstrate that TCNQ can promote electron transfer between GOD and electrode. Considering these observations, Hendry and Turner (*1*) report glucose sensor utilising TCNQ as a mediator. However, the sensor suffers from the following drawbacks: Short half-life of the electrode (i.e., 1–1.5 h), which may be attributed to either loss of enzyme activity, loss of enzyme, or leaching of TCNQ from the electrode and Limited linear range of the calibration curve (i.e., anodic current is linear up to 20 m*M* glucose and nonlinear up to 75 m*M*).

Recent reports (*5,13,14*) show that GOD alone, GOD-linked mediator, or GOD and mediator can be incorporated into the graphite/carbon paste electrode (graphite particles suspended in Nujol oil) (CPE), which results in an enzyme electrode with enhanced mechanical and electrochemical stability. The extended linearity of the sensor is attributed to diffusion-limited conditions through and within the oily electrode interface (*5*), however, the electrode stability is limited (8 d) related to progressive leaching out of the mediator from the electrode. We considered, therefore, to develop a glucose sensor incorporating TCNQ and GOD into the graphite paste with wide linearity and high stability, since TCNQ is least soluble in aqueous solution. This short paper describes the performance of glucose sensor in plasma analysis based on the incorporation of GOD and TCNQ into the graphite paste.

EXPERIMENTAL

Materials

GOD type X-S, activity 150,000 U/g and dialysis tubing 250-9U were from Sigma (USA). TCNQ and graphite fine powder (Merck grade, < 50 μm)were obtained from Aldrich (West Germany). Nujol oil (spectroscopic grade, 0.85 g/mL) was obtained from E Merck (UK). All other reagents employed were of analytical grade.

Preparation of the Enzyme Electrode

The enzyme electrode was made by pressing the active paste into the well of a specially designed electrode body made from Teflon™. The paste surface was manually smoothed on clean paper. The paste was prepared by careful mixing of graphite fine powder and TCNQ in a mortar followed by the addition of GOD and Nujol oil. Paste had the composition of graphite powder = 100 mg, TCNQ = 100 mg, GOD = 20 mg, and Nujol oil = 62 mg, which was filled into the well (diameter = 3mm, depth = 2mm) of the electrode body followed by mounting the electrode with a dialysis tubing pretreated according to the manufacturer's instruction and held in place by silicone rubber O-ring.

Electrochemical Measurements

Electrochemical measurements were performed with a Vibrant Electrochemical system (model VSM/MPC/30) connected to a personal computer. EPSON LX-800 was used to print the data. A two compartment cell with a graphite paste working electrode, a saturated calomel reference electrode, and a platinum foil auxiliary electrode was used for the measurements with a working vol of 5 mL. The electrode responses were determined by hydrodynamic amperometry at the constant potential of 220 mV vs SCE. The enzyme electrode was placed in stirred phosphate buffer and the appropriate potential was applied. The background current was allowed to decay to a steady state, varying concentration of glucose was added, and the new steady-state current was recorded. All the measurements were made at $20 \pm 0.2°C$ under nitrogen atmosphere.

Analysis of Plasma Glucose

The plasma samples were analyzed with the enzyme electrode covered with a dialysis tubing. A constant potential 220 mV vs SCE was applied across the enzyme electrode and at the steady-state background current; plasma samples (1 mL) were injected into the reaction cell, and the new steady-state current was recorded. The measurements were made with 5mL, 0.1*M* phosphate buffer pH 7 at 20°C.

RESULTS AND DISCUSSION

Incorporation of enzymes or mediators (*13–18*) into carbon paste electrode results enhanced mechanical and electrochemical stability with low background current (*5*). Since TCNQ acts as an efficient mediator and the redox couple $TCNQ^{\circ}/TCNQ^{-}$ is least soluble in aqueous solution, it is of interest to incorporate TCNQ and GOD into the graphite paste. Figure 1 shows a typical calibration graph of steady-state anodic current vs glucose

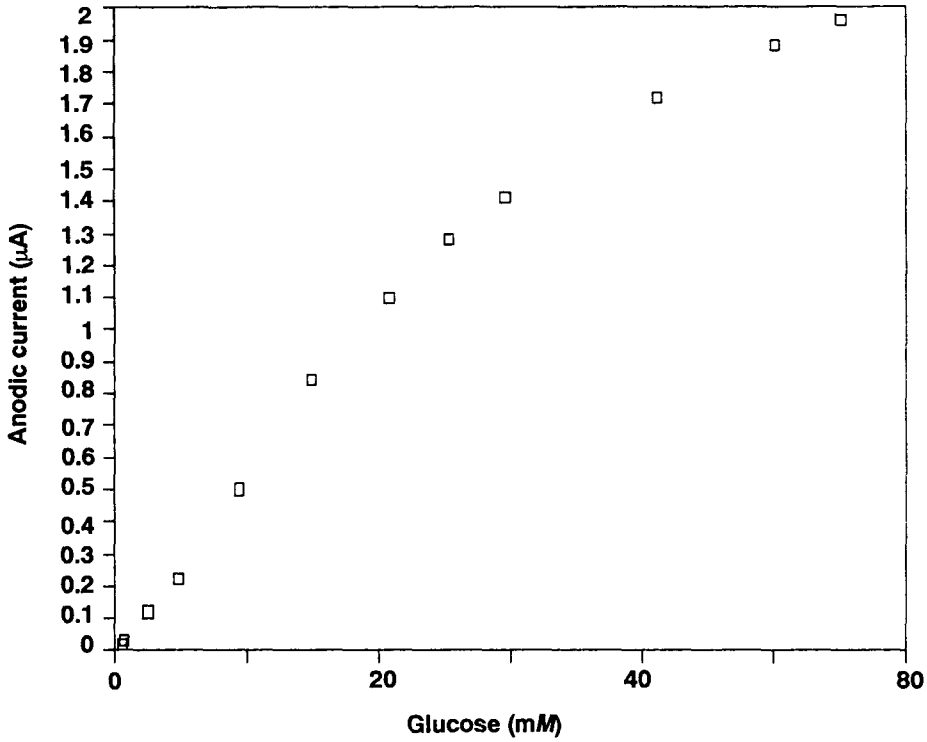

Fig. 1. Calibration curve for glucose analysis. Steady-state current was measured at 220 mV vs SCE in 0.1*M* phosphate buffer pH 7.0 at 20°C.

concentration. The electrode takes less than 15 s to attain 95% steady value on the addition of glucose at low concentration, however, the response time increases as the substrate concentration is increased. The response time is also slightly increased after several operations of the electrode. The response of the enzyme electrode is linear over the range 5–50 m*M* and nonlinear up to 250 m*M*. The response of the electrode reported here is obtained on the second day of preparation and is stable for 1 wk. The electrode is stable for a period of 1 mo with a loss of 25% activity from the second day of preparation.

Assay of Blood Samples

Glucose in plasma samples has been analyzed with enzyme electrode (A) and with a routine spectrophotometric method (B) (*19*). The results obtained from these two methods follow the relation:

$$A = 1.101\,B - 0.195$$

The data are recorded in Fig. 2. The correlation is better over the earlier graphite paste based glucose sensor (*5*).

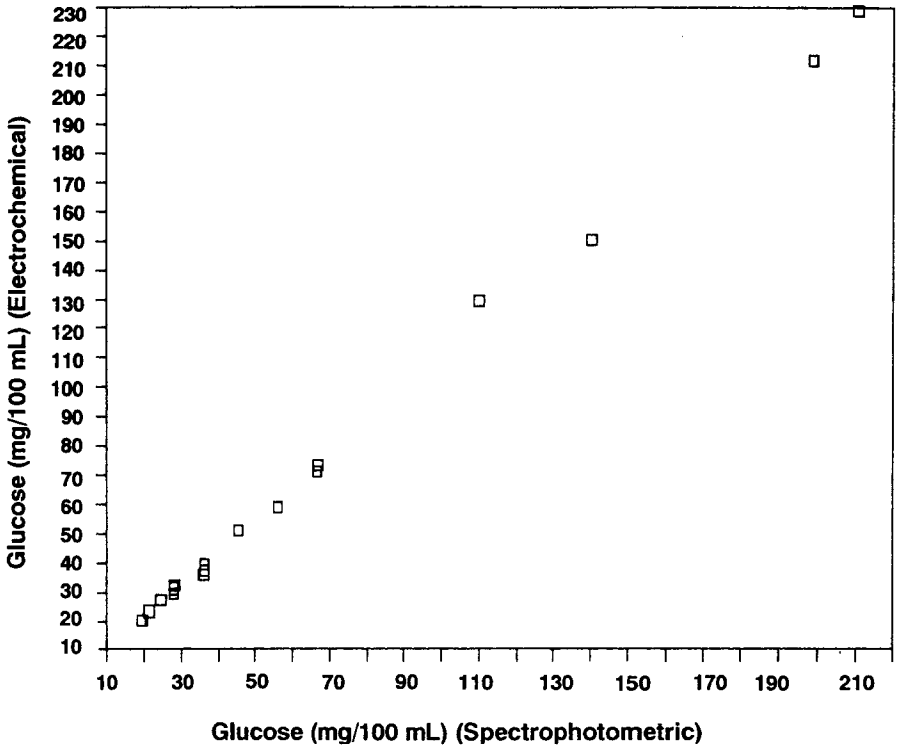

Fig. 2. Results of plasma analysis. The data were obtained with the enzyme electrode and a routine spectrophotometric method (*19*).

CONCLUSION

The graphite paste enzyme electrode modified with TCNQ has high storage and operational stability as compared to earlier carbon paste glucose electrodes (*5*) and offers significant advantages in handling along with possible miniaturization of the probes.

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