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IMMOBILIZED GLUCOSE OXIDASE CARBON ELECTRODE AS AN AMPEROMETRIC BIOSENSOR

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The electrochemical response of the immobilized glucose oxidase glassy carbon electrode is studied using controlled-potential amperometry. The effect of parameters such as ionic strength and pH on the electrode response is examined.

Key words: Immobilized glucose oxidase, glassy carbon electrode, biosensors

INTRODUCTION

Over the last decade there has been an increasing interest in the development of efficient enzyme electrodes for determination of the concentration of clinically and physiologically relevant substances. Successful methods for such biochemical analysis have been reported by coupling an immobilized enzyme layer with an electrochemical sensor. Among the available immobilization techniques, covalent attachment of an enzyme onto an electrode surface seems satisfying. Glucose oxidase which can be used for a rapid determination of blood glucose levels [1-8] has been frequently used. Previously the covalent attachment of glucose oxidase on glassy carbon electrodes using the carbodiimide activation method has been reported [8]. The same procedure is used in this work. The activity of immobilized glucose oxidase is measured from the catalytic current produced at controlled potential by coupling the following reactions [6,7],

$$\text{glucose} + \underset{(BQ)}{\text{benzoquinone}} \xrightarrow{GOD} \text{gluconic acid} + \underset{(HQ)}{\text{hydroquinone}}$$

$$HQ \longrightarrow BQ + 2H^+ + 2e^-$$

(where glucose oxidase is abbreviated in GOD). Benzoquinone acts as shuttle between the electron-transfer sites of the enzyme and the electrode surface. In this work, the effect of parameters such as ionic strength and pH on the catalytic current is examined.

EXPERIMENTAL

Materials

Glucose oxidase (type VII from *Aspergillus niger*) N-cyclohexyl-N'- (2- morpholinoethyl) carbodiimidemetho-p-toluenesulfonate (CMCT) puriss, and D-glucose were obtained from standard companies. All other chemicals were reagent grade. Stock solutions of glucose were allowed to mutarotate overnight before use. Distilled demineralized water was used. A PAR 175 universal programmer and a PAR 173 potentiostat coupled with a Sefram X-Y recorder were used to obtain controlled-potential amperograms. The working electrode was a glassy carbon (GC; grade GC-A, AIMCOR, Tokyo, Japan) electrode made from a cylinder of 3 mm diameter inserted in a resin casing; (exposed area was 0.071 cm^2). The auxiliary electrode was a platinum wire and the reference electrode was a Metrohm Ag/AgCl (saturated NaCl) electrode.

Procedure

The Gc electrode was polished using diamond paste of 3 and 1 μm size, then 0.5 and 0.05 μm alumina slurry. After each polishing, the GC surface was subjected to ultrasonication in a demineralized water bath for 2 min. An extra ultrasonication in methanol was performed after the 1 μm diamond paste polishing to eliminate oil traces. Following the final ultrasonication, the electrode was transferred to a cell containing 10% HNO_3 + 2.5% $K_2Cr_2O_7$ solution and oxidized at 2.3 V for 20 s; the anodic current density being about 300 $mA.cm^{-2}$. After a careful washing with water, the electrode surface was treated with 0.1M (CMCT) in 20 mM acetate buffer at pH 4.6. Immobilization of the enzyme was then performed with 1 $mg.cm^{-3}$ GOD in 10 mM acetate buffer at pH 5.6 at 277 K for one night.

The presence of GOD covalently immobilized on the electrode surface was demonstrated by measuring the catalytic current Δi as previously described [8]: the modified electrode was placed in 5 cm^3 of 10mM acetate buffer at pH 5.6 containing BQ and the poised at 0.70V. After the background current decayed to a steady-state value, a 200 μl aliquot of 2M glucose solution was added. The fast current increase Δi observed after the glucose addition corresponds to the catalytic current [3,7,8].

When necessary, oxygen was purged from the solutions by bubbling with U-grade nitrogen before the experiments. The experiments were performed at 298K.

RESULTS AND DISCUSSION

Detection of immobilized GOD

To enable electron transfer from redox centers of immobilized glucose oxidase to the glassy carbon electrode, it is necessary to add to the solution an electron-shuttling species. Several electron acceptors employed in amperometric glucose sensors with GOD present in the solution could be used [6]. In this study, the responses obtained when using ferrocene monocarboxylic acid, hexacyanoferrate, hexammineruthenium (III) and benzoquinone as mediators are compared. Benzoquinone gives a fast response although it adsorbs on glassy carbon surface; the other systems investigated here yield only sluggish catalytic currents. Several reasons can be invoked to account for such poor responses as differences in the size, the concentration of mediators and unfavourable electrostatic effects [9]. A typical response of the immobilized GOD electrode in the presence of BQ which has been

used in the course of this work is shown in Fig 1 (curve 1). The corresponding curve obtained with hexacyanoferrate (curve 2) is given for comparison. Further, the response of the immobilized GOD electrode in the presence of BQ is found to be linear with glucose concentration over the range 0-20 mM (0-4g.l^{-1}) which points to the suitability of this immobilized electrode for determination of glucose concentrations.

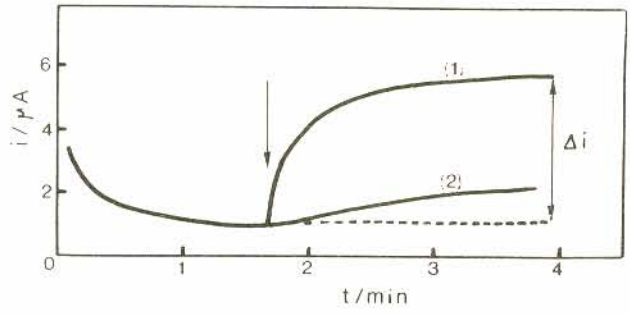

Fig. 1: Current-time curve at a controlled-potential value of E = 0.70 V obtained at the immobilized GOD GC electrode in the presence of (1) 5 mM benzoquinone and (2) 5 mM hexacyanoferrate, in 10 mM acetate buffer at pH 5.6. (The arrow indicates the addition of glucose.)

Effect of the ionic strength

Since the glucose electrode can be utilized in various analytical conditions, it is important to know whether the ionic strength can have an effect on the electrode response. The catalytic current has been detected by varying the nature and the concentration of the supporting electrolyte, as shown in Fig. 2. Δi remains constant when acetate concentration is increased from 10 mM to 1 M, but decreases when adding increasing sodium chloride concentrations. This effect could be specific of the nature of the anion (acetate or chloride).

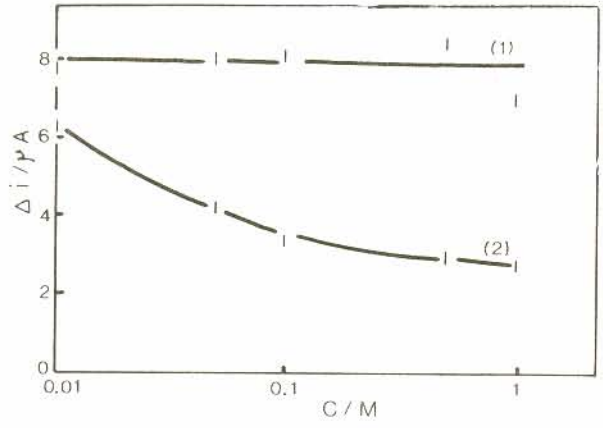

Fig. 2: Dependence of the catalytic current Δi on the concentration of the supporting electrolyte at pH 5.6: (1) sodium acetate (2) sodium chloride (in 10 mM sodium acetate)

Effect of pH

The effect of pH on the catalytic current was studied within the pH range 2.8-10.0 using a mixed buffer composed of 10 mM sodium acetate + 10 mM potassium phosphate + 10 mM sodium borate. Owing to the instability of benzoquinone solutions at alkaline pH, solid BQ was added into the cell just before detection. The pH-activity profile is shown in Fig. 3. The immobilized GOD electrode exhibits an optimum response in the pH range 4.5-8.5. The curve for the immobilized enzyme (curve 1 in Fig. 3) has a rather flat shape compared to that obtained when the enzyme is present in the solution (curve 2) [2,4], with a shift of the optimum pH of approx. 1.5 unit toward the alkaline side. Such an alkaline shift can be attributed to the influence of negative charges on the carbon surface and simultaneously to a loss of positive charge on GOD resulting from the immobilization. It is interesting to note that the optimum-pH range is more extended than that obtained in preceding papers [4,5] when using other supports for immobilization. The immobilized GOD electrode is not damaged after working at extreme acid (3.0) and alkaline (10.0) pH, since the catalytic response is restored after coming back to optimum-pH values.

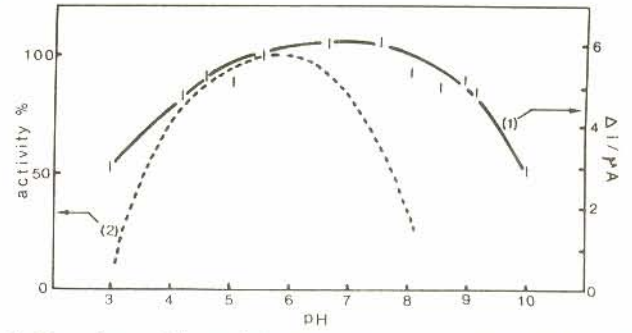

Fig. 3: Dependence of the catalytic current Δi on pH (curve 1) in the mixed (10 mM sodium acetate + 10 mM potassium phosphate + 10 mM sodium borate) buffer. The data obtained for glucose oxidase activity in solution by Cho and Bailey [2] are given for comparison (curve 2).

CONCLUSION

The covalent immobilization of GOD on glassy carbon through carbodiimide activation permits the attachment of stable enzyme layer to the electrode surface. This immobilized GOD electrode is a convenient tool for measuring glucose activity. Such an electrode has a good specificity since it is verified that no response is obtained in the presence of other substrates such as sucrose, D-fructose and D-galactose. It is stable with time. Its response is virtually constant on a relatively large pH range and is slightly affected when modifying the ionic strength.

The results presented in this paper confirm the benefit of using a glassy carbon surface as electrochemical detector and give additional data on the performances of this immobilized GOD electrode.

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