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Parameters Regulating Kinetics of Amperometric Biosensor Made of Enzymatic Carbon Paste and Nafion® Gel

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An amperometric glucose sensor (β-D-glucose) using the biocatalytic properties of the enzyme, glucose oxidase (GOD), and the membrane properties of an hydrophobic Nafion® gel is fabricated. The kinetic features of the current produced by the sensor are also investigated.

1. Introduction

Carbon paste electrodes have often been used as mediated amperometric enzyme electrodes.[1] These electrodes may be used without further modification,[2] but they are usually membrane-covered in which case enzyme and redox mediator are trapped at the electrode behind the membrane.[3] Nafion®, a perfluorosulfonic cation-exchange polymer has widely been investigated as a permselective coating for enzyme electrodes.[4, 5] In this paper, we describe a modified electrode consisting of an aqueous carbon paste containing both the enzyme and the mediator, but which is protected by a layer of hydrophobic Nafion® gel. Nafion® gel is a concentrated solution of Nafion® in a fatty hydrophobic

solvent such as trialkylphosphate or a trialkoxyphosphate.[6, 7] In our device, it ensures glucose transport from the external medium to the paste.[8]

2. Fabrication of the Modified Electrode

The biosensor base is a vitreous carbon electrode set in Teflon. It is extended by a Teflon chamber which includes an aqueous carbon paste containing GOD and redox mediator. The entire setup is covered by hydrophobic Nafion® gel which serves as an interface between the external medium and enzymatic chamber (Fig. 1). The enzyme is kept with the mediator in a favorable aqueous environment where it can react with the substrate. Two mediators are used at various concentrations in the enzymatic paste: one is hydrophobic, ferrocene [FeC_5H_{10}], the other hydrophilic, ferrocene methylene trimethyl ammonium chloride [$FeC_5H_9CH_2N(CH_3)_3Cl$]. Similary, two types of gels, using tris(2-ethylhexyl)phosphate (T2EHP [$CH_3(CH_2)_3CH(C_2H_5)CH_2O)_3P(O)$]) or tris(2-butoxyethyl)phosphate (T2BEP [$(CH_3(CH_2)_2OCH_2CH_2O)_3P(O)$]) as solvents, are tested.

3. Results and Discussion

3.1 *Electrochemical response of the electrode*

As the electrode was immersed in a buffer solution, a voltammogram was recorded showing cathodic and anodic waves due to the electrochemical reaction of the mediator (Fig. 2(a)). The redox peaks increase with increasing mediator (FeC_5H_{10} or $FeC_5H_9CH_2N(CH_3)_3Cl$) concentration in the enzymatic paste. When glucose is added to the buffer solution, an increase of the oxidation peak and a disappearance of the reduction peak are observed, indicating a biocatalytic effect induced by GOD (Fig.2(b)).[9] Glucose diffuses through the gel and is oxidized by GOD, then the redox mediator is successively reduced by the enzyme and reoxidized at the electrode.

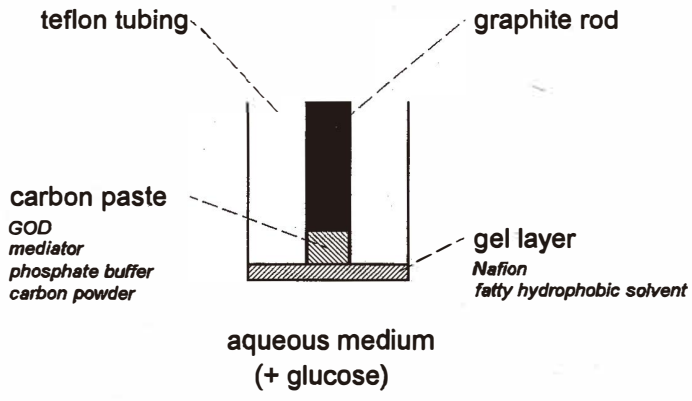


Fig. 1. Electrode scheme.

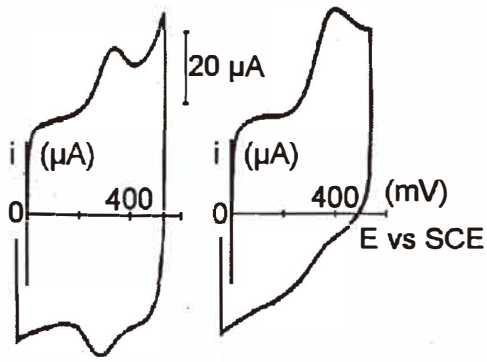


Fig. 2. Cyclic voltammogram of the electrode (v = 20 mV/s). (a) 0.1 mol/L phosphate buffer, (b) same solution as (a) + 10 mmol/L of glucose.
Nafion® gel layer: 6 mg (1/3 Nafion®, 2/3 T2BEP, in weight)
Enzymatic paste: 2 mg (150 mg carbon powder, 0.75 ml 0.1 mol/L phosphate buffer containing 0.036 mmol/L of GOD and 0.02 mol/L of $FcCH_2N(CH_3)_3Cl$)

3.2 *Behavior of the mediator*

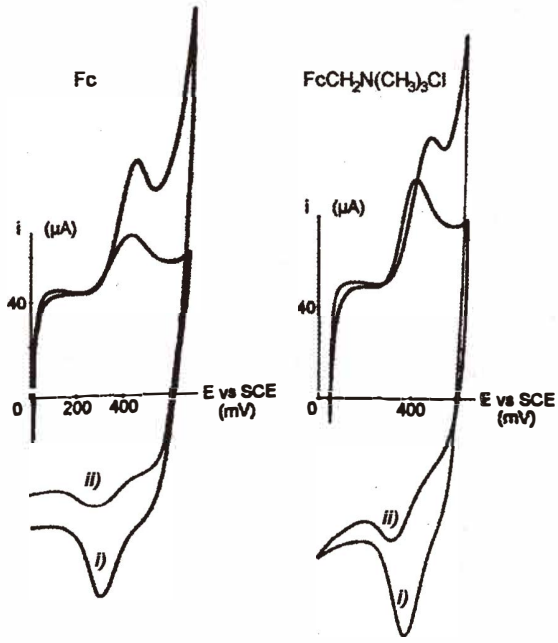
The voltammograms of the ferrocene-mediated electrode indicate a decrease in the anodic and cathodic peaks of this species with time, reflecting a drain of the hydrophobic compound from the aqueous paste to the organic gel (Fig. 3(a)). In the case of the ferrocene methylene trimethyl ammonium chloride-mediated electrode the decrease is less abrupt (Fig. 3(b)), suggesting that the use of a hydrophilic mediator is preferable.

Figure 4 shows the dependence of amperometric steady-state current on mediator concentration in the enzymatic paste. It can be seen that in spite of the drain phenomenon, for a fixed amount of GOD at the electrode, the amperometric response does not increase when the threshold mediator concentration is reached in the enzymatic paste; therefore, it is possible to saturate the enzyme with the electroactive species, so as to optimize the velocity of the enzymatic reaction.

3.3 *Influence of the gel*

T2HEP gels have good mechanical properties (of stiffness and hardness) but induce low amperometric responses, compared to T2BEP gels which are softer. Thus, in subsequent experiments T2BEP gel was used to cover electrodes.

The calibration curves of two electrodes, covered by thin and thick gels, are plotted in Fig. 5. The thin-gel-covered electrode produces a signal of 2.0 μA for 1.5 mmol/L of glucose in the external medium and has a linear range of 10 mmol/L, while the thick-gel-covered one shows a 20 mmol/L linear range and a current of 0.7 μA for 1.5 mmol/L of glucose. Results of a more careful study agree with both curves, as it can be seen in Figs. 6 and 7, where amperometric steady-state current and linear range of the current are plotted



(a) (b)

Fig. 3. Cyclic votammogram of the electrode ($v = 20$ mV/s) in 35 ml of 0.1 mol/L phosphate buffer. (a) mediated by Fc, (b) mediated by $FcCH_2N(CH_3)_3Cl$. i) immediately after fabrication, ii) 4 hours later.
Nafion® gel layer: 6 mg (1 / 3 Nafion®, 2/3 T2BEP, in weight)
Enzymatic paste: 2 mg (150 mg carbon powder, 0.75 ml 0.1 mol/L phosphate buffer containing 0.036 mmol/L of GOD and 0.02 mol / L of mediator)

against the amount of gel covering the enzymatic electrode. The gel has a typical membrane behavior:[10] when the gel thickness increases the amperometric response decreases, but the linear range of this response increases.

3.4 *Kinetic features of the electrocatalytic current*

The enzymatic reaction in the enzyme layer and the electrode reaction may be written as

$$GOD_{ox} + \text{glucose} \underset{k_{-1}}{\overset{k_1}{\rightleftarrows}} \langle GOD - \text{glucose} \rangle \xrightarrow{K} GOD_{red} + \text{gluconic acid} \qquad (1)$$

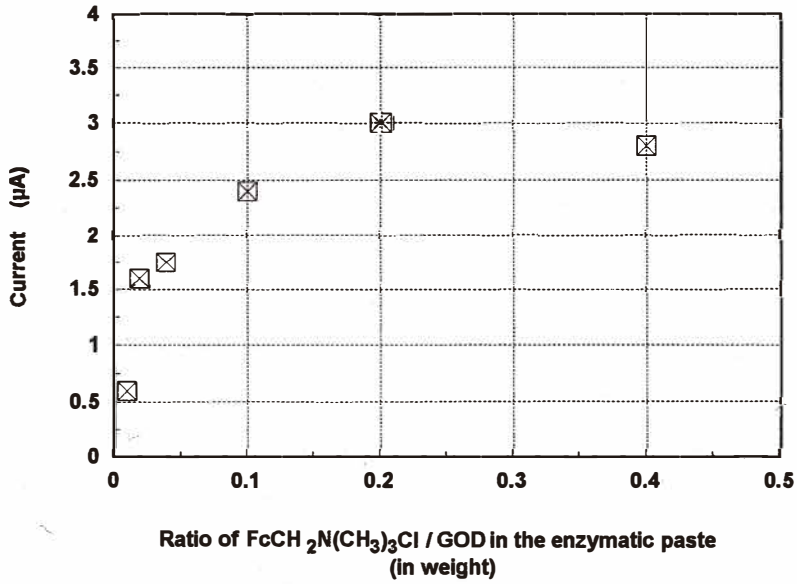


Fig. 4. Amperometric response of the electrode (E = 700 mV vs. S. C. E., ω = 26 rad/s) in 0.1 mol/L phosphate buffer containing 1.4 mmol/L of glucose.
Nafion® gel layer: 6 mg (1 / 3 Nafion®, 2/3 T2BEP, in weight)
Enzymatic paste: 2 mg (150 mg carbon powder, 0.75 ml 0.1 mol/L phosphate buffer containing 0.036 mmol/L of GOD and various amounts of $FcCH_2N(CH_3)_3Cl$)

$$GOD_{red} + 2/n\, M_{ox} \xrightarrow{k} GOD_{ox} + 2/n\, M_{red} \tag{2}$$

$$\text{at the electrode: } 2/n\, M_{red} \longrightarrow 2/n\, M_{ox} + 2e^-, \tag{3}$$

where GOD_{red} and GOD_{ox} are the reduced and oxidized forms of the enzyme, respectively, M_{red} and M_{ox} are the reduced and oxidized forms of the mediator, respectively, (M exchanges 1 e^-, n = 1), ⟨GOD-glucose⟩ is a transitory complex consisting of the enzyme and its substrate, and K is a velocity constant called "turn-over" of the enzyme.[11]

According to Michaelis-Menten enzymatic kinetic theory, if (GOD) is the total concentration of the enzyme [(GOD) = (GOD_{red}) + (GOD_{ox}) + (⟨GOD-glucose⟩)], the velocity of such an enzymatic reaction can be expressed as

$$v = \frac{K(\mathrm{GOD})}{1 + \dfrac{K_M}{(M_{ox})^{2/n}} + \dfrac{K_{glu}}{(\mathrm{glucose})}}, \tag{4}$$

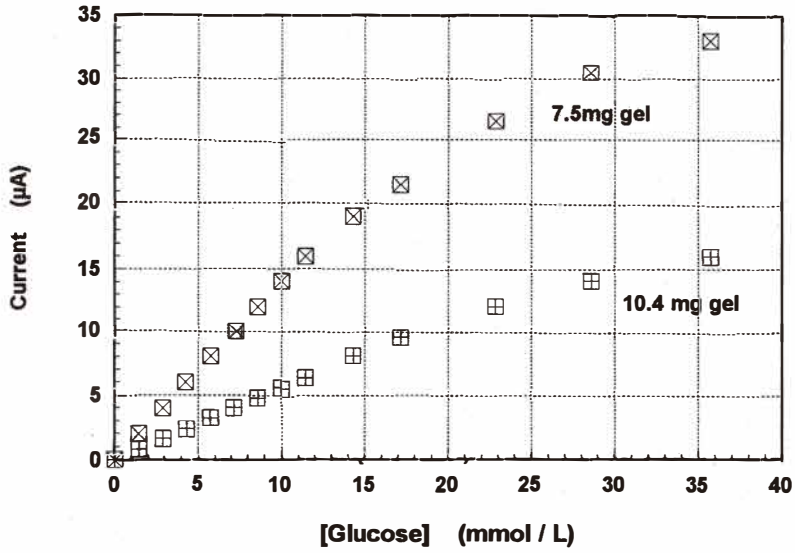


Fig. 5. Calibration curves of two electrodes.
Nafion® gel layer: 7.5 and 10.4 g (1/3Nafion®, 2/3 T2BEP, in weight)
Enzymatic paste: 2 mg (150 mg carbon powder, 0.75 ml 0.1 mol/L phosphate buffer containing 0.036 mmol/L of GOD and 0.04 mol/L of $FcCH_2N(CH_3)_3Cl$)
Initial solution: 0.1 mol/L phosphate buffer

where $K_M = K / k$ is the Michaelis constant for the mediator and $K_{glu} = (k_{-1} + K) / k_1$ is the Michaelis constant for the glucose.

According to this theory and if we assume there is no concentration polarization of glucose inside the paste, the flux of glucose consumed at the electrode can be expressed as

$$J = \frac{K(\mathrm{GOD})L}{1 + \dfrac{K_M}{(M_{ox})^{2/n}} + \dfrac{K_{glu}}{(\mathrm{glucose})}}, \tag{5}$$

where L is the thickness of the carbon paste cylinder, and (GOD) and (glucose) the glucose and enzyme concentrations, respectively, in the paste (Fig. 8).

As we already have shown, in our device, the enzyme can be saturated with mediator and because the gel acts as a membrane, glucose concentration in the paste is small. That is, $M_{ox}^{2/n} >> K_M$ and $[K_{glu}/(\mathrm{glucose})] >> 1$; thus the above expression (5) can be simplified to

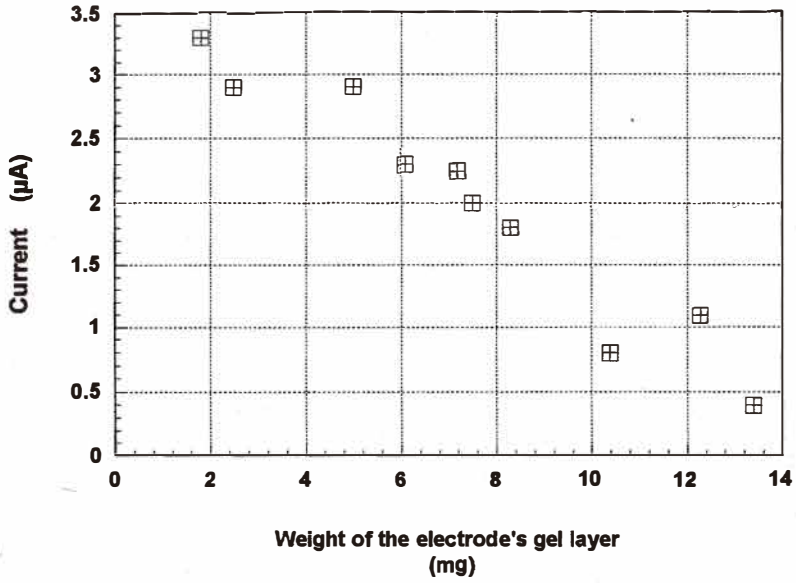


Fig. 6. Dependence of the steady-state limiting current on the thickness of the gel: amperometric answer of the electrode (E = 700 mV vs. S. C. E., ω= 26 rad/s) in 0.1 mol/L phosphate buffer containing 1.4 mmol/L of glucose.
Nafion® gel layer: various amounts (1 / 3 Nafion®, 2 / 3 T2BEP, in weight)
Enzymatic paste: 2 mg (150 mg carbon powder, 0.75 ml 0.1 mol/L phosphate buffer containing 0.036 mmol/L of GOD and 0.04 mol/L of $FcCH_2N(CH_3)_3Cl$)

$$J = \frac{K}{K_{glu}}(\mathrm{GOD})(\mathrm{glucose})L. \tag{6}$$

On the other hand, if we postulate that no kinetic barrier exists for substrate transfer from one phase to another, and that the glucose transport in aqueous phases (aqueous phases being paste and external medium, organic phase being the gel) is faster than the glucose diffusion inside the gel (Fig. 8), the glucose flux penetrating the paste is

$$J = D_{glu}\,\kappa\frac{(\mathrm{glucose}) - (\mathrm{glucose})_\infty}{(L_0 - L)}, \tag{7}$$

where D_{glu} is the glucose diffusion coefficient inside the gel, κ the partition coefficient for glucose between gel and aqueous phases, $(\mathrm{glucose})_\infty$ the glucose concentration in the external medium and $(L_0 - L)$ the gel thickness.

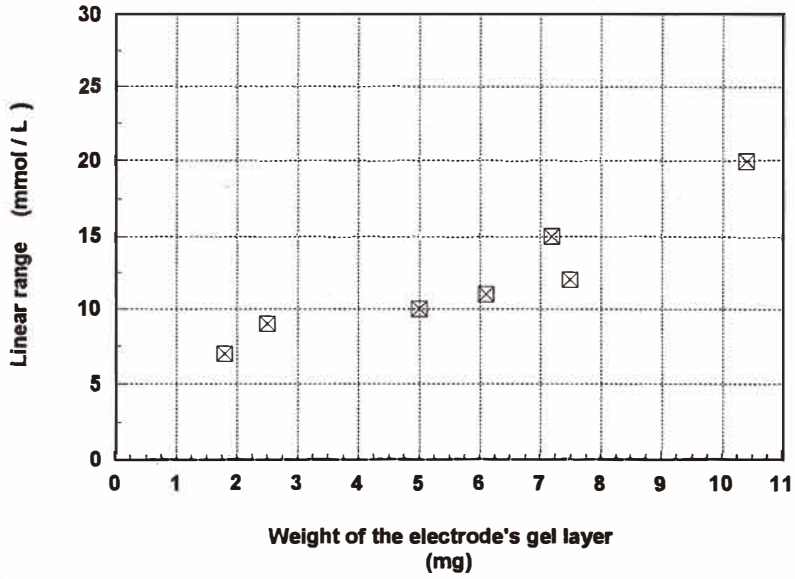


Fig. 7. Dependence of the linear range on the thickness of the gel: amperometric response of the electrode (E = 700 mV vs. S. C. E., ω = 26 rad / s) in 0.1 mol/L phosphate buffer containing 1.4 mmol/L of glucose.
Nafion® gel layer: various amounts (1 / 3 Nafion®, 2 / 3 T2BEP, in weight)
Enzymatic paste: 2 mg (150 mg carbon powder, 0.75 ml 0.1 mol / L phosphate buffer containing 0.036 mmol / L of GOD and 0.04 mol / L of $FcCH_2N(CH_3)_3Cl$)

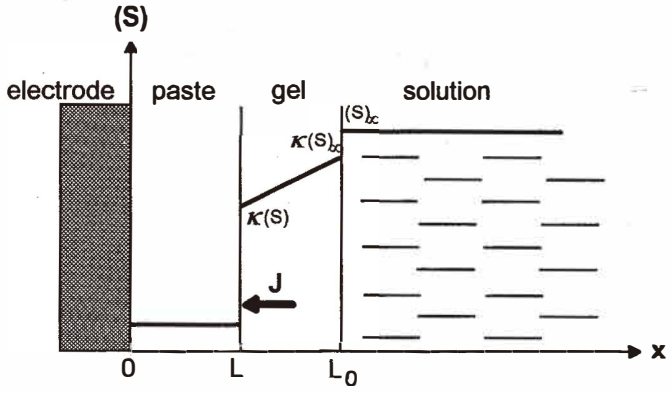


Fig. 8. Concentration profile of glucose. (S is for glucose)

Then eqs. (6) and (7) can be solved to give

$$J = \frac{\frac{K}{K_{glu}}(\mathrm{GOD})(\mathrm{glucose})_{\infty} L}{1 + \frac{K}{K_{glu}} \frac{(\mathrm{GOD})(L - L_0)L}{\kappa \; D_{glu}}}. \tag{8}$$

If the enzymatic reaction rate is fast, i. e. (glucose) << (glucose)$_{\infty}$, the 1 can be neglected in the above expression to obtain

$$J = D_{glu} \frac{\kappa(\mathrm{glucose})_{\infty}}{(L - L_0)} \tag{9}$$

The electrochemical current is

$$i = -2F \; SJ = 2F \; SD_{glu} \frac{\kappa(\mathrm{glucose})_{\infty}}{(L_0 - L)}, \tag{10}$$

where S is the surface of the base of the enzymatic paste cylinder and F = 96500 C the Faraday constant.

Actually, the lack of the glucose diffusion coefficient in the gel and of the partition coefficient for glucose between the gel and the aqueous phases prevents the verification of whether the kinetic features obtained by eq. (10) are in good agreement with the experimental results. Nevertheless, the equation predicts, as shown above, that the current response is mainly controlled by the diffusion rate of glucose in the Nafion® gel layer.

4. Conclusions

From our initial experiments, we have shown that our system has the advantage of high concentrations of GOD and redox mediator at the electrode. Thus, it can deal with a flux of glucose which is of great importance. By acting as a membrane, the gel limits glucose flux and allows the sensor to be more independent from the kinetics of the enzymatic reaction. Further studies are in progress to determine the glucose partition coefficient between water and gel and glucose diffusion coefficient inside the gel.

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