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采用粉末微电极技术改善电流型酶电极的输出性能①

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摘要 由于酶反应的特异性,酶电极在检测生物底物的工作中得到了较广泛的应用. 然而电流型酶电极有以下的缺点,包括响应电流低,活性组份流失造成的电极使用寿命下降以及响应电流的非线性等. 本文根据粉末酶电极模型进行了动力学分析,得出在两种极端情况下粉末酶电极的电流响应动力学公式,以及由响应电流估算表观米氏常数的方法. 采用了两种粉末酶电极(C-PU-GOD 和 C-AQ-DBFc-GOD)对理论分析进行了验证. 实验结果与理论推导较好地吻合,均表明采用粉末微电极技术可显著地提高酶电极的响应电流和扩展酶电极响应的线性范围.

关键词 葡萄糖氧化酶,酶电极,粉末微电极

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Improvement of Output Characteristics of Amperometric Enzyme Electrode by Using the Powder Microelectrode Technique①

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The unique selectivity of the enzyme reactions has made the enzyme electrodes highly prospective for the detection of bioactive molecules. However, the amperometric enzyme electrodes also have their inherent problems, especially the relatively low output current density, the unavoidable gradual deterioration of the enzyme activity, and the non-linearity of the response of the electrode.

We have found that the powder microelectrode technique is a very effective way of improving both the output current density and the linear range of response of the amperometric enzyme electrode. The general aspects of the powder microelectrode technique have been presented in detail in [1].

The performance of the powder enzyme microelectrode can be analyzed with the schematic diagram shown in Fig. 1. On the surface of the current collector (1) is the porous matrix (2) composed of an electron-conducting matrix of particles surface-modified with immobilized enzyme molecules and an intervening liquid matrix of electrolyte containing substrate of enzyme reaction. The enzyme reaction takes place at the interface between the solid and liquid matrices, and the current is collected via the solid matrix and the current collector.

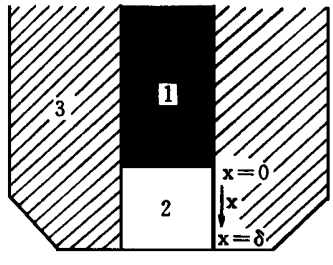

Fig. 1 The powder microelectrode
1. current collector
2. porous matrices
3. glass capillary

Starting from the Michaelis-Menten equation and assuming that a fixed proportion of the reaction product can react at the surface of electrode to create the amperometric signal (I), then the response of the electrode in term of current density can be expressed as:

$$I = I_{max}\frac{c_s^0}{K_m' + c_s^0} \quad (1)$$

in which I_{max} is the limiting current density, c_s^0 is the concentration of substrate at the sur-

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face of the electrode and K_m' is the so-called " apparent Michaelis constant ". Usually K_m' is singificantly smaller than K_m of the same enzyme system in bulk of solution, probably due to the partial deactivation of the immobilized enzyme and the restriction of mass transfer processes within the modifying layer. From Eq. (1) it is clear that the relation between I and c_s^0 can be approximately linear only in concentration range $c_s^0 \leq K_m'$. Therefore, the response of the conventional enzyme electrode can be linear only in low concentration range mainly in the sub-mM region.

Assuming that Eq. (1) is applicable at the solid/liquid interface within the porous layer, then, by combining it with the Fick's second law of steady-state diffusion, we have:

$$nFD_s'\left(\frac{d^2c_s}{dx^2}\right) = SI_{max}\frac{c_s}{K_m' + c_s} \tag{2}$$

in which D_s' is the effective coefficient of diffusion of substrate molecules in the porous matrix, S is the specific surface area of the porous matrix, defined as the total area of solid/liquid interface within unit volume of porous matrix (cm^2/cm^3), c_s is the local concentration of the substrate within porous matrix, and n is the number of electrons involved in the electrochemical oxidation of the reaction product of one substrate molecule. Since $\left(\frac{d^2c}{dx^2}\right) = \frac{1}{2}\frac{d}{dc}\left(\frac{dc}{dx}\right)^2$, Eq. (2) can be transformed into

$$\left(\frac{dc_s}{dx}\right)^2 = \frac{2SI_{max}}{nFD_s'}\int\frac{c_s dc_s}{K_m' + c_s}$$

$$= \frac{2SI_{max}}{nFD_s'}[K_m' + c_s - K_m' \ln(K_m' + c_s)] + constant \tag{3}$$

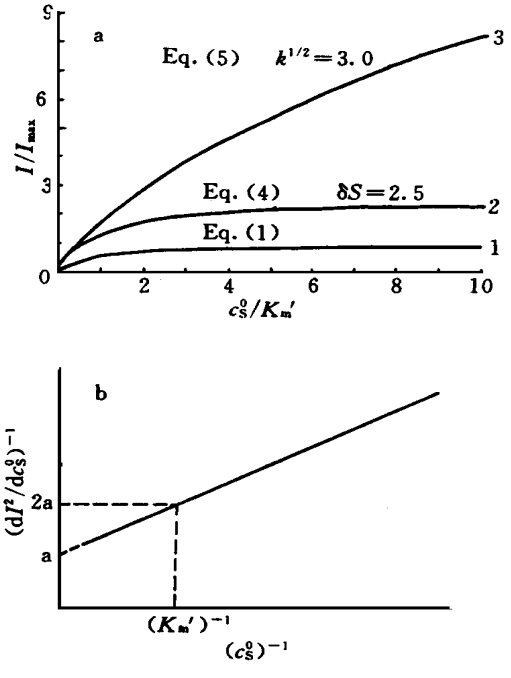

Fig. 2 (a) Theoretical responses of the amperometric enzyme electrodes:
1. Planar enzyme electrode (Eq. (1))
2. Thin porous enzyme electrode (Eq. (4), $S\delta = 2.5$)
3. "Sufficiently thick" porous enzyme electrode (Eq. (5), $k^{1/2} = 3.0$)
(b) The $(dI^2/dc_s^0)^{-1}$ vs $(c_s^0)^{-1}$ plot, and the estimation of K_m'

There are two extreme cases of the concentration polarization of the substrate within the porous matrix. Firstly, for sufficiently thin powder electrode, there is practically no concentration polarization within the powder matrix, so the response of an enzyme powder microelectrode can be directly derived from Eq. (1) as:

$$I = SI_{max}\delta\frac{c_s^0}{K_m' + c_s^0} \tag{4}$$

in which δ is the thickness of the powder layer.

The other extreme case will be realized if the porous layer is sufficiently thick so that the substrate is totally consumed before it can reach the innermost layer of the porous matrix adjacent

to the current collector. Assuming that concentration polarization of the substrate occurs only within the porous matrix and all the generated product of enzymatic reaction within the porous layer can be collected amperometrically (i.e. no loss of signal due to leaching-out of reaction product), the response of the porous enzyme electrode can be expressed as:

$$I = nFD_s\left(\frac{dc_s}{dx}\right)_{x=\delta} = I_{max}k^{1/2}\left(\frac{c_s^0}{K_m'} + \ln\frac{K_m'}{K_m' + c_s^0}\right)^{1/2} \quad (5)$$

in which the dimensionless constant $k = 2nFD_sK_m'S/I_{max}$.

In Fig. 2 the theoretical responses of the planar enzyme electrode (Eq. (1)), the thin porous enzyme electrode (Eq. (4)) and the thick porous enzyme electrode (Eq. (5)) are compared. From Fig. 2 it is clear that, by using the thick porous electrode technique, both the output current density and the range of quasi-linear response can be very effectively improved.

Besides, Eq. (5) can be squared and differentiated with respect to c_s^0 to obtain:

$$\frac{dI^2}{dc_s^0} = \frac{I_{max}^2k}{K_m'} - \frac{I_{max}^2k}{K_m' + c_s^0} \quad \text{or} \quad \left(\frac{dI^2}{dc_s^0}\right)^{-1} = \frac{K_m'^2}{I_{max}^2k}\frac{1}{c_s^0} + \frac{K_m'}{I_{max}^2k} \quad (6)$$

Eq. (6) shows that the $\left(\frac{dI^2}{dc_s^0}\right)^{-1}$ vs $(c_s^0)^{-1}$ plot is a straight line with slope $s = K_m'^2/(I_{max}^2k)$ and intercept $a = K_m'/(I_{max}^2k)$. Since $c_s^0 = K_m'$ when $\left(\frac{dI^2}{dc_s^0}\right)^{-1} = 2a$, so K_m' can be easily estimated from the plot (see Fig. 2(b)).

Eq. (5) and Eq. (6) were tested with two types GOD powder microelectrodes: Type 1, carbon black modified with glucose oxidase (GOD) embedded in polyurethane (PU) (the C-PU-GOD electrode). Type 2, carbon black modified with GOD and dibutylferrocene (DBFc) embedded in Kodak AQ polymer (the C-AQ-DBFc-GOD electrode). The typical responses of the two types of powder enzyme microelectrodes and the planar electrodes modified with analogues procedures are shown in Fig. 3(a) and Fig. 4(a). From these figures it is clear that very significant increase of the output current density and extension of the linear range of response curve can be achieved by using the powder microelectrode

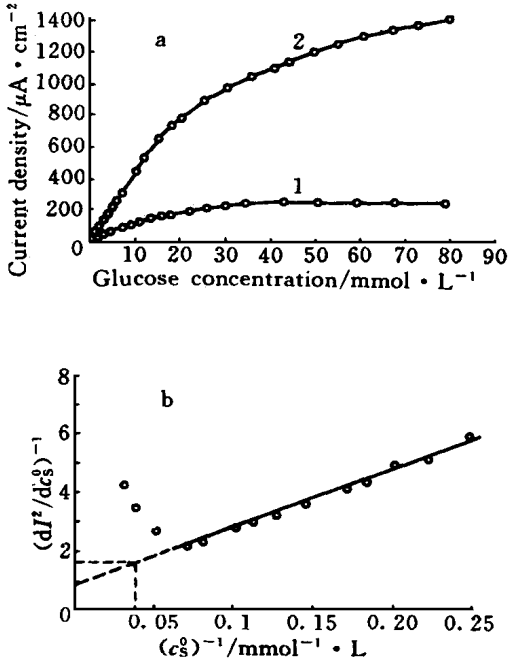

Fig. 3 (a) Responses of the PU-GOD modified enzyme electrodes:
1. Planar Pt microdisk electrode (dia. 0.5 mm) modified with PU and GOD, thickness of modifying layer 2 μm
2. C-PU-GOD powder microelectrode (dia. 0.5 mm, δ = 0.5 mm)
(b) The $(dI^2/dc_s^0)^{-1}$ vs $(c_s^0)^{-1}$ plot, and the estimation of K_m'

technique. The maximal obtainable current density for both types of modified powder microelectrodes can be well above 1 mA/cm^2, significantly higher than the output of glucose sensors employing similar modifying layers [2-6]. The linear range of the response of both types of electrodes is about 0～15 mM, and the current response does not reach its maximum value at glucose concentration of ca. 60～70 mM. Fig. 3(b) and Fig. 4(b) show that the $\left(\frac{dI^2}{dc_s^0}\right)^{-1}$ vs $(c_s^0)^{-1}$ plots are linear in the lower concentration range, as predicted in Eq. (6). The apparent values of Michealis constant K_m' estimated from the Fig. 3(b) and Fig. 4(b) by method shown in Fig. 2(b) are approximately 26 mM for the C-PU-GOD electrode and about 14 mM for the C-AQ-DBFc-GOD electrode. These estimated K_m' values are somewhat higher (especially in the case of C-AQ-DBFc-GOD electrode) than K_m' values obtained from the response curves of the planar electrodes modified with similar procedures, and they are more close to results measured in solution, (for example, K_m' = 33 mM in air-saturated solution[7]).

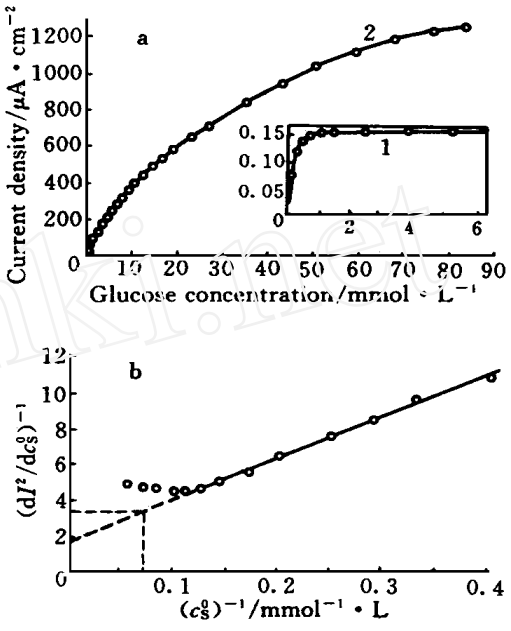

Fig. 4 (a) Responses of the AQ-DBFc-GOD modified enzyme electrodes:
1. GC planar disk electrode (dia. 4 mm) modified with AQ-DBFc-GOD, thickness of modifying layer 2 μm
2. C-AQ-DBFc-GOD powder microelectrode (dia. 0.5 mm, δ = 0.7 mm)
(b) The $(dI^2/dc_s^0)^{-1}$ vs $(c_s^0)^{-1}$ plot, and the estimation of K_m'

The powder GOD electrodes respond rapidly to the change of glucose concentration in solution. Steady-state current can usually be reached in less than 20 s. The long-term stability of the porous GOD electrodes is also fairly good. The interferencing effects of electroactive species such as ascorbic acid, uric acid, L-cystein and acetaminophen on the output of the powder enzyme electrodes were also found to be less than in the case of planar enzyme electrodes.

Key words　Glucose oxidase, Enzyme electrode, Powder microelectrode

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