Kinetic Analysis of an Enzyme-containing Polymer Modified Electrode[*]

ZHU Kan[* *] and WU Hui-huang

(*State Key Laboratory for Physical Chemistry of Solid Surfaces, Department of Chemistry, Xiamen University, Xiamen*, 361005)

A theoretical model for enzyme-entrapped conducting polymer modified electrodes is proposed and appropriate expressions are derived for the steady-state current response of the enzyme electrode. More attention has been paid to the role of conducting polymer in electron transfer and the effect of mass transport. On the basis of kinetic analysis, the performance and optimum design of the second and third generation enzyme electrodes are discussed.

Keywords   Enzyme, Electrode, Polymer, Current response

Introduction

The electrochemical immobilization of enzyme has become a suitable method for the preparation of enzyme electrodes, the immobilization mechanism and the kinetic behavior of the resulted enzyme electrode were reviewed[1—3]. It has been recognized that the electro-deposited polymers such as polypyrrole and polyaniline not only act as the support of immobilized enzyme but also facilitate the electron transfer of the soluble mediator[4] or the direct electron transfer of enzymes with the electrode[5,6]. Furthermore, the method has the advantage of fabricating the so-called reagentless biosensors because of the possibility of entrapping the mediator in the polymer as a dopant[7—9] or by covalent fixation on the monomer[10].

To understand the enzyme reactions within polymer film and to correlate the performance of enzyme electrodes to the conditions of electrochemical immobilization, one way is to model the combined effects of mass transport and bioelectrochemical kinetics and then to compare the theoretical predictions with experimental observations. Thus, Bartlett and Whitaker[11] described a theoretical model for an insulating polypyrrole/glucose oxidase electrode, Marchesiello and Genies[4b] took into account the conductivity of polypyrrole and proposed that the

oxidation of artificial mediator may occur inside the polymeric matrix. In these models, the effect of mass transport in solution was neglected. Recently Gros and Bergel[12] have suggested that the adsorption of glucose oxidase at both the electrode surface and the polypyrrole / solution interface should be considered, aside from the diffusion of substrates in solution involved in the kinetic model. However, the polymer was assumed insulating and their numerical analyses only presented the implicit relations between some of the physical parameters. In addition, a number of work on the kinetic analysis of amperometric enzyme electrodes are instructive to the knowledge of bioelectrocatalytic process in insulating polymers[13—17]. The aim of this paper is to re-examine the kinetic consequences of enzyme processes in conducting polymer films, more attention will be paid to the role of conducting polymers in electron transfer and the effect of the mass transport in solutions. On the basis of kinetic analysis, the performance and design of the second and third generation enzyme electrodes are discussed.

Mathematical Modeling

1   Enzyme Entrapped Conducting Polymer Modified Electrodes with a Soluble Mediator in Solutions

Fig. 1 depicts schematically the processes occurring at an amperometric enzyme electrode, where the enzyme-containing polymer film with a thickness of L is coated on the surface of a metal electrode (at $x = 0$). The steps involved are the following:

(1) the diffusion of substrate(denoted as S) and oxidized mediator (denoted as A) from the bulk of the solution to the polymer/solution interface with the respective diffusion coefficient D_{js}.

Fig. 1   The general kinetic scheme for the enzyme-entrapped conducting polymer modified electrode.

(2) The permeation of S and A into the polymer film and the subsequent diffusion toward the metal surface. The partition equilibriums exist across the polymer/solution interface, with the equilibrium constant $k_j = C_{out} / C_{in}$. C_{out} and C_{in} are concentrations at the solution and the polymer side of the interfaces respectively.

(3) The enzyme reactions take place within the polymer film, $i. e.$, S is transformed into the product (denoted as P) and the enzyme (denoted as E_1) into its reduced state E_2, then E_2 is oxidized into E_1 by mediator A and the latter into its reduced state B:
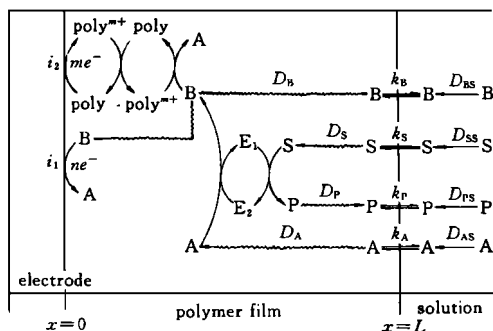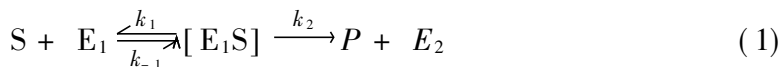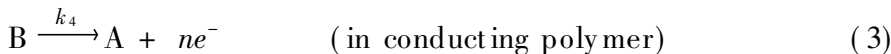
$$S + E_1 \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} [E_1S] \overset{k_2}{\longrightarrow} P + E_2 \tag{1}$$

$$E_2 + A \overset{k_3}{\longrightarrow} E_1 + B \tag{2}$$

Of course, Eqs. (1) and (2) should agree with the actual mechanism of enzyme reactions. For instance, in the case of benzoquinone as mediator of glucose oxidase the enzyme reaction has been known to obey the Ping-Pong mechanism[4], Eq. (2) should be rewritten accordingly.

(4) The electrochemical oxidation of B in conducting polymer (rate constant k_4) and on metal surface (rate constant k_5):

$$B \overset{k_4}{\longrightarrow} A + ne^- \qquad \text{(in conducting polymer)} \tag{3}$$

$$B \overset{k_5}{\longrightarrow} A + ne^- \qquad \text{(on electrode surface)} \tag{4}$$

(5) Diffusion of P and B, in film, towards the polymer/solution interface and then into the bulk of the solution. Owing to the parallel reactions (3) and (4), the current response of enzyme electrode, i, consists of the Farady current at metal surface i_1 and at the polymer film i_2, i.e., $i = i_1 + i_2$:

$$i_1 = nFD_B \left(\frac{\partial b}{\partial x} \right)_{x=0} \tag{5}$$

$$i_2 = nF \int_0^L k_4 b dx \tag{6}$$

Obviously the knowledge of the distribution of reacting species in the polymer film is necessary to calculate currents. In principle, the steady-state concentrations of various species in the film can be worked out with the following differential equations:

$$\frac{\partial s}{\partial t} = D_S \frac{\partial^2 s}{\partial x^2} - \frac{k_2 s e_1}{K_M} = 0 \tag{7}$$

$$\frac{\partial a}{\partial t} = D_A \frac{\partial^2 a}{\partial x^2} - k_3 a e_2 + k_4 b = 0 \tag{8}$$

$$\frac{\partial b}{\partial t} = D_B \frac{\partial^2 b}{\partial x^2} + k_3 a e_2 - k_4 b = 0 \tag{9}$$

$$\frac{\partial e_2}{\partial t} = \frac{k_2 s e_1}{K_M} - k_3 e_2 a = 0 \tag{10}$$

where the letters s, a, b and e stand for the concentrations of the species in film denoted by the corresponding capital letters. $K_M = (k_2 + k_{-1})/k_1$ is the Michealis constant for S. From the enzyme balance, i.e., the total concentration of enzyme species $e_\Sigma = e_1 + e_2 + [E_1S]$ (the last term, $[E_1S]$, is the concentration of complex E_1S), Eqs. (8) to (10) will be rewritten as:

$$D_S \frac{d^2 s}{dx^2} - R = 0 \tag{11}$$

$$D_B \frac{d^2 b}{dx^2} - k_4 b + R = 0 \tag{12}$$

$$D_A \frac{d^2a}{dx^2} + k_4b - R = 0 \tag{13}$$

$$R = \frac{k_2 e_\Sigma}{1 + k_2/(k_3a) + K_M/s} \tag{14}$$

It should be noted that the form of R is dependent on the mechanism of enzyme reaction, for the Ping-Pong mechanism the term (k_2/k_3a) in Eq. (14) will be substituted by $(K_{M,A}/a)$, in which $K_{M,A}$ is the Michealis constant for A. To solve the equations, it is essential to establish appropriate boundary conditions. Since the operation potential of the enzyme electrode is usually set at such a value that the oxidation of B is diffusion-limiting and other reactions can not occur, we have

$$b_{x=0} = 0 \tag{15a}$$
$$(ds/dx)_{x=0} = 0 \tag{15b}$$
$$(da/dx)_{x=0} = 0 \tag{15c}$$

On the other hand, the steady fluxes of various species across the polymer/solution interface $(x = L)$ must be satisfactory to the continuity condition:

$$D_S \left(\frac{ds}{dx} \right)_{x=L} = h_S(s - k_S s_L) \tag{16}$$

$$D_A \left(\frac{da}{dx} \right)_{x=L} = h_A(a - k_A a_L) \tag{17}$$

$$D_B \left(\frac{dB}{dx} \right)_{x=L} = h_B(b - k_B b_L) \tag{18}$$

The mass transport coefficient in the solution h_j is the ratio of respective diffusion coefficient D_j to the thickness of the Nernst diffusion layer δ_j. In the concentration terms the subscripts and L indicate the bulk of the solution and the inner side of the film $(x = L)$, respectively. The equations from (11) to (13) are nonlinear and difficult to obtain analytical solutions. Approximate analytical treatments need to identify suitable limiting cases, and various cases can be assumed on the basis of different kinetic limits, various cases can be assumed. For our purposes, only the following common cases are considered in accordance to the practical measurements:

(1) If reaction (1) is rate-limiting and $s \ll K_M$ so that $(K_M/s) \gg [1 + (k_2/k_3a)]$, then $R = (k_2 e_\Sigma s/K_M)$ and Eqs. (11) and (12) can be reduced to Eqs. (19) and (20):

$$D_S \frac{d^2s}{dx^2} - \frac{k_2 e_\Sigma s}{K_M} = 0 \tag{19}$$

$$D_B \frac{d^2b}{dx^2} - k_4b + \frac{k_2 e_\Sigma s}{K_M} = 0 \tag{20}$$

As seen in appendix, if the following dimensionless parameters are introduced, i.e., $\theta = (k_4 L^2/D_B)^{1/2}$, $\alpha = (k_2 e_\Sigma L^2/k_M D_s)^{1/2}$, and β_i $D_i/h_i k_i L$; then we obtain that when θ α, the total current is

$$i = \frac{nFD_Bb \ \theta\mathrm{ch}\theta}{k_BL(\mathrm{sh}\theta + \theta\beta_B\mathrm{ch}\theta)} +$$
$$\frac{nFD_ss \ \alpha\theta[\alpha - \alpha\mathrm{ch}\theta(\mathrm{ch}\alpha + \alpha\beta_B\mathrm{sh}\alpha) + \theta\mathrm{sh}\alpha(\mathrm{sh}\theta + \theta\beta_B\mathrm{ch}\theta)]}{k_sL(\theta^2 - \alpha^2)(\mathrm{sh}\theta + \theta\beta_B\mathrm{ch}\theta)(\mathrm{ch}\alpha + \alpha\beta_s\mathrm{sh}\alpha)} \tag{21}$$

(2) If the reaction (1) is rate-limiting and $s \gg K_M$ so that $R = k_2e\Sigma$, then we have

$$i = \frac{nFD_Bb \ \theta\mathrm{ch}\theta}{k_BL(\mathrm{sh}\theta + \theta\beta_B\mathrm{ch}\theta)} + \frac{nFD_sK_M\alpha^2}{L}\left[1 - \frac{\mathrm{ch}\theta - 1}{\theta(\mathrm{sh}\theta + \theta\beta_B\mathrm{ch}\theta)}\right] \tag{22}$$

2 Reagentless Enzyme Electrodes

Reagentless enzyme electrodes made of conducting polymers have been reported[6, 18—20]. Because of the absence of natural or artificial mediator in solutions, the direct electron transfer between enzyme and polymer is sometimes assumed although the mechanism of the process is open. In this case the enzyme reaction is still represented by Eq. (1), however, the regeneration of E_2 is formally represented with an electrochemical step taking place inside the polymer:

$$E_2 \xrightarrow{k_5} E_1 + ne^- \tag{23}$$

Therefore the current response is

$$i = nF \int_0^L k_5e_2\mathrm{d}x \tag{24}$$

Analogously one has the differential equation Eq. (25) and the same boundary conditions as Eq. (15b) and Eq. (16):

$$D_s \frac{\mathrm{d}^2 s}{\mathrm{d}x^2} - R(7) = 0 \tag{25}$$

$$R(7) = \frac{k_2e\Sigma}{1 + K_M/s + k_2/k_5} \tag{26}$$

The current expressions in two limiting conditions are obtained:

(1) when $(K_M/s) \gg [1 + (k_2/k_5)]$, then $R(7) = (k_2e\Sigma s/K_M)$,

$$s = \frac{s \ \mathrm{ch}(\alpha x/L)}{k_s(\mathrm{ch}\alpha + \alpha\beta_s\mathrm{sh}\alpha)} \tag{27}$$

$$i = \frac{nFD_ss \ \alpha\mathrm{th}\alpha}{Lk_s(1 + \alpha\beta_s\mathrm{th}\alpha)} \tag{28}$$

(2) when $(K_M/s) \ll [1 + (k_2/k_5)]$, then $R(7) = k_2e\Sigma/[1 + (k_2/k_5)]$

$$i = nF \int_0^L k_5e_2\mathrm{d}x = \frac{nFe\Sigma L}{1/k_5 + 1/k_2} \tag{29}$$

Discussion

1 Role of Polymer Matrix

As shown in the approximate analytical solution for an enzyme entrapped conducting polymer electrode with a soluble mediator in a solution, only when reaction (1) is rate-limiting and $S \ll K_M$, can the current response be obtained to be proportional to the concentration of the substrate. For a given concentration

of S, the current response is strongly dependent on α, β and θ. Among the dimensionless parameters, α^2 is the ratio of the rate of enzyme catalytic reaction to the rate of diffusion in the polymer the film. β^2 is the ratio of the mass-transport coefficient in the polymer film to that in the solution, it reflects the relative importance of these two mass transport steps in the whole kinetic process. θ^2 is the ratio of the reduction/oxidation rate of mediator to its mass transport rate in the film, its value depends on the electroactivity, permeality of the polymer film and the potential controlled on detecting. It is expected that the better electrochemical reactivity of the polymer film will result in a larger rate constant k_4, hence θ. In the extreme condition the polymer film is non-electroactive, then $k_4 = 0$ and $\theta = 0$. By introducing $\theta = 0$ into Eqs. (21) and (22) one has

$$i_1(7) = \frac{nFD_B b}{k_B L (1 + \beta_B)} + \frac{nFD_s s \ (1 - 1/\text{ch}\alpha + \alpha\beta_s\text{th}\alpha)}{k_s L (1 + \beta_B)(1 + \alpha\beta_s\text{th}\alpha)} \tag{21a}$$

$$i(7) = \frac{nFD_B b}{k_B L (1 + \beta_B)} + \frac{nFD_s K_M \alpha^2 (1/2 + \beta_B)}{L (1 + \beta_B)} \tag{22a}$$

When the mass transport coefficients in solutions are infinite (i. e., $\beta = \beta_B = 0$) and when $b = 0$, Eq. (21a) is in agreement with equation (19) given by Bartlett et al. [11a].

To further estimate the dependence of the current response on θ, $b = 0$ is assumed and from Eq. (21) one has the dimensionless current ξ:

$$\xi = i / \left[\frac{nFD_s s}{k_s L} \right]$$

$$= \frac{\alpha^2\theta[1 - \text{ch}\theta(\text{ch}\alpha + \alpha\beta_B\text{sh}\alpha) + \theta\text{sh}\alpha(\text{sh}\theta + \theta\beta_B\text{ch}\theta)/\alpha]}{(\theta^2 - \alpha^2)(\text{sh}\theta + \theta\beta_B\text{ch}\theta)(\text{ch}\alpha + \alpha\beta_s\text{sh}\alpha)} \tag{30}$$

If $\beta_i = 0$, then Eq. (30) can be reduced to form equivalent to $I_T = I_P + I_s$ in the literature[4b]. The relations of ξ to θ and α in Eq. (30) are shown in Fig. 2, indicating that ξ increases with θ and α. It is noticeable that the significant effect of θ on ξ manifests itself only when α has larger values. Fig. 3 is the plot of ξ against θ at various values of α, it can be seen that when α is small (for example, $\alpha < 0.5$), ξ will be almost independent of θ. If ξ_{act} is the dimensionless current of an electroactive polymer, ξ_{non} is the dimensionless current of a non-electroactive polymer and θ_0 is defined as the value of θ when $(\xi_{act} - \xi_{non})/\xi_{non} = 10\%$, then the relation of θ_0 to α can be represented in Fig. 4. Apparently, in the case
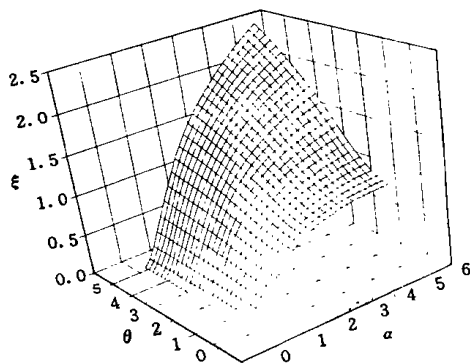

Fig. 2　Plot of the dimensionless current ξ against α and θ, according to Eq. (30) when the mass transport in solution is neglected($\beta_i = 0$).
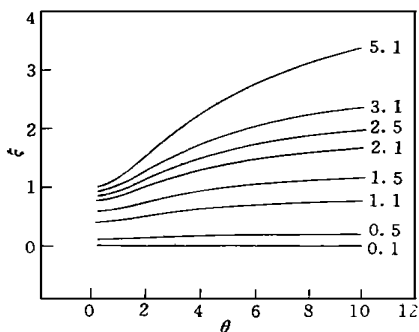
Fig. 3 Effects of θ on current responses of the enzyme-entrapped conducting polymer modified electrode.
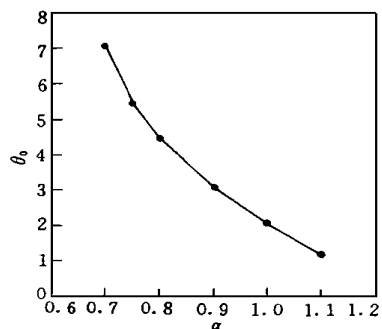The figures at the curves are the values of α.

Fig. 4 Relation of θ_0 (the critical value of θ) to α.

of $\alpha < 1$ the polymer does not exhibit the ability of facilitating electron transfer unless $\theta > 2$. Marchesiello et al.[4b] concluded that for their glucose oxidase/ polypyrrole electrode with quinone as the mediator the oxidation of reduced mediator hydroquinone occurs inside the conducting polymer, in fact, in their work $\alpha = 1$—4 and $\theta > 10$ could be found. On the other hand, Bartlett et al.[11b] suggested that the oxidation of reduced mediator H_2O_2 on poly-N-methylpyrrole was inefficient, where α was only about 0.1.

2 Effect of Mass Transport in Solutions

As seen in Eqs. (21) and (28), the current responses of the enzyme electrode are strongly dependent on the dimensionless mass-transport coefficient β. Very small β implies the negligible effect of mass transport in solutions, on the contrary, very large β indicates the controlling step associated with the mass-transport in solutions. According to Eq. (21) and assumed $\beta_s = \beta_B = \beta$, the relationship between the dimensionless current ξ and β as well as α is shown in Fig. 5 for a set values of θ. It is clear that the effect of mass transport is particularly significant for the polymer films with larger θ. In general, at given θ and α, ξ increases as β decreases and reaches its limit value when β approaches zero. The ξ − α curves at β change dramatically with the increase of θ, but the curves at β 10 for different θ are almost identical [see Fig. 5(A) (B) and (C)]. Therefore, the intensive mass transport in solutions is necessary to obtain a higher response current and to examine the role of polymer in electron transfer, unless the mass transport in the polymer film is slow (i.e., in the case of lower D_s and larger L). For this purpose, the RDE technique is usually applied. Fig. 6 shows the relationship of dimensionless current of a reagentless enzyme electrode, for the comparison, the analogous plot is given in Fig. 7 for the insulating polymer electrode with a soluble mediator. The effect of mass transport can be analyzed in the
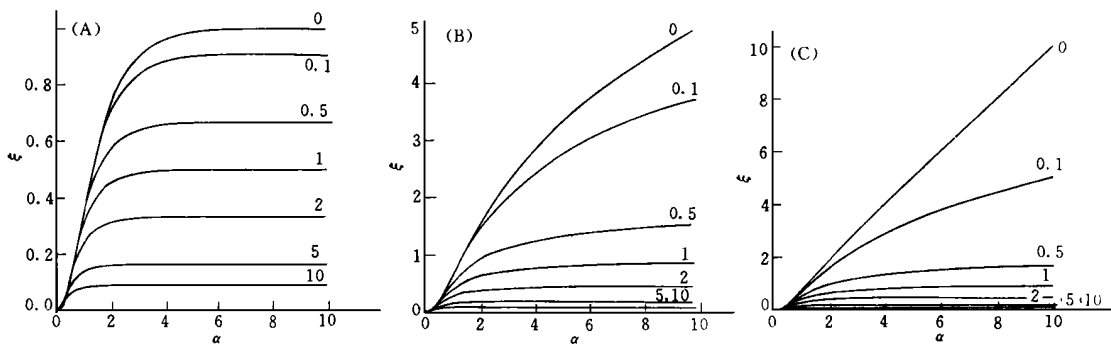
same way as the mentioned above.

Fig. 5　The dimensionless current ξ as a function of the dimensionless parameters α and β.

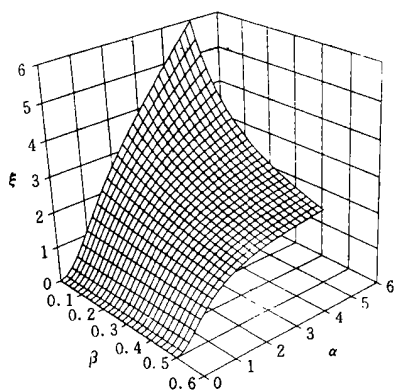The figures at the curves indicating the value of β, (A) $\theta=0$; (B) $\theta=10$; (C) $\theta=\infty$.

Fig. 6　Effects of α and β on the current re-
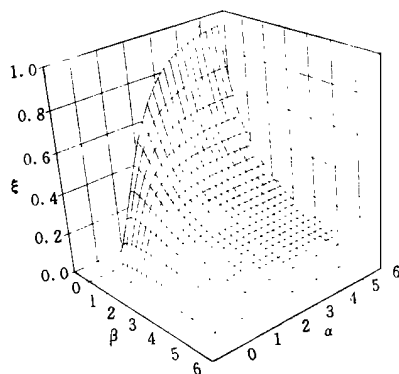sponses of reagentless conducting
polymer electrode.

Fig. 7　Effects of α and β on current re-
sponses of insulating polymer en-
zyme electrode.

3　Optimum of Parameters α and L

The enzyme membrane is the key component of the enzyme electrode. For its optimal design the effect of the parameter α on the properties of the enzyme electrode must be further considered. The dimensionless number α is related not only to the enzyme concentration $e\Sigma$ and the membrane thickness L but also to the kinetic parameters k_2, k_M and D_s, which are determined by the nature of en-zyme and the conditions of immobilization. Since α^2 reflects the ratio of the en-zyme reaction rate to the diffusion rate of the substrate in the membrane, the large α suggests that the mass transport in the membrane is rate-controlling. At the same time, the term $(D_s K_M / k_2 e\Sigma)^{1/2}$ has the dimension of length and can be defined as the effective thickness of a reaction layer, x_R, hence $\alpha = L / x_R$. Under the given condition of enzyme immobilization, $(D_s K_M / k_2 e\Sigma)^{1/2}$ is expected to be constant, and the polymer membrane thickness L will be an independent variable determining α. From Fig. 2 and Fig. 5 it can be found that for the second genera-

tion enzyme electrode with infinite mass transport in solutions, the larger α generally leads to the higher current. If the polymer is non-electroactive, ξ almost remains unchanged when α 5.

Fig. 8 shows the plots of (ξ/α) to α, in which a maximum exists on the curves near α 1.5 $(i.e., L$ 1.5$x_R)$ for the second generation enzyme electrodes. The physical meaning of (ξ/α) is the dimensionless current when $L = x_R$, therefore it excludes the thickness of the enzyme membrane from the dimensionless term. Fig. 8 also gives the plot of (ξ/α) to α for the reagentless enzyme electrode, the value of (ξ/α) reaches its limit when $\alpha > 3$ $(i.e., L > 3x_R)$. It follows that the optimum membrane thickness should be different for two kinds of enzyme electrodes.
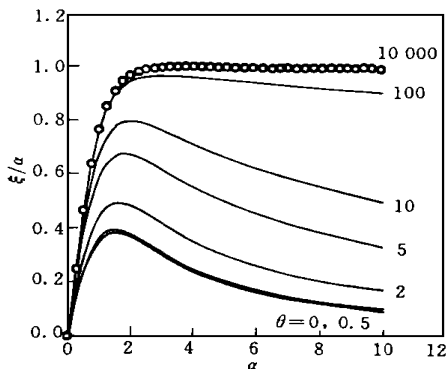

Fig. 8 Plots of (ξ/α) as function of α for an enzyme electrode with a soluble mediator in solution(———) and reagentless enzyme electrode( ). The figures at the curves indicating the value of θ.

Conclusion

The current responses strongly rely on the kinetics at the enzyme electrode, including the rates of the enzymatic reactions and of the electrochemical steps, and the mass transport in the immobilized enzyme membrane and in the solution.

A thorough theoretical treatment is needed to be done, however, the results described above give an insight into the role of conducting polymers in bioelectrocatalytic processes. It is believed that the apparent kinetic parameters and the factors influencing the performances of enzyme electrodes such as sensitivity and the linear concentration range for detection can be predicted from the equations in this work. For demonstration, we will present experimental observations of some typical systems in the further work.

Appendix

In this appendix the expressions are derived for the concentrations of S and B in the film and the current responses of the enzyme electrode.

1) If reaction (1) is rate-limiting and $s \ll K_M$ so that $(K_M/s) \gg [1+ (k_2/k_3a)]$, then $R= (k_2e\Sigma s/ K_M)$ and Eqs. (11) to (13) are simplified as:

$$D_S \frac{d^2s}{dx^2} - \frac{k_2e\Sigma s}{K_M} = 0 \tag{A 1}$$

$$D_B \frac{d^2b}{dx^2} - k_4b + \frac{k_2e\Sigma s}{K_M} = 0 \tag{A 2}$$

The general solution of Eq. (A1) is $s = c_1^s e^{\alpha x/L} + c_2^s e^{-\alpha x/L}$ where dimensionless parameter $\alpha = (k_2 e \Sigma L^2/k_M D_s)^{1/2}$, with boundary conditions (15b) and (16) then

$$s = \frac{s \, \mathrm{ch}(\alpha x/L)}{k_s(\mathrm{ch}\alpha + \alpha\beta_s\mathrm{sh}\alpha)} \tag{A3}$$

with (A1) and (A3) and boundary condition (15a) and (18), when $\alpha \neq \theta$

$$b = c_1^b e^{\theta x/L} + c_2^b e^{-\theta x/L} + \frac{D_s s \, \alpha^2 \mathrm{ch}(\alpha x/L)}{D_B k_s(\theta^2 - \alpha^2)(\mathrm{ch}\alpha + \alpha\beta_s\mathrm{sh}\alpha)} \tag{A4}$$

$$c_1^b = \frac{b}{2k_B(\mathrm{sh}\theta + \theta\beta_B\mathrm{ch}\theta)} - \frac{D_s s \, \alpha^2[(\mathrm{ch}\alpha + \alpha\beta_s\mathrm{sh}\alpha) - (1 - \theta\beta_B)e^{-\theta}]}{D_B k_s(\theta^2 - \alpha^2)(\mathrm{sh}\theta + \theta\beta_B\mathrm{ch}\theta)(\mathrm{ch}\alpha + \alpha\beta_s\mathrm{ch}\alpha)}$$

$$c_2^b = \frac{b}{2k_B(\mathrm{sh}\theta + \theta\beta_B\mathrm{ch}\theta)} + \frac{D_s s \, \alpha^2[(\mathrm{ch}\alpha + \alpha\beta_s\mathrm{sh}\alpha) - (1 + \theta\beta_B)e^{\theta}]}{D_B k_s(\theta^2 - \alpha^2)(\mathrm{sh}\theta + \theta\beta_B\mathrm{ch}\theta)(\mathrm{ch}\alpha + \alpha\beta_s\mathrm{ch}\alpha)}$$

$$i_1 = nFD_B\left(\frac{\partial b}{\partial x}\right)_{x=0}$$

$$= \frac{nF\theta}{L(\mathrm{sh}\theta + \theta\beta_B\mathrm{ch}\theta)}\left[\frac{D_B b}{k_B} + \frac{D_s s \, \alpha^2}{k_s(\theta^2 - \alpha^2)} \times \frac{(\mathrm{ch}\theta + \theta\beta_B\mathrm{sh}\theta) - (\mathrm{ch}\alpha + \alpha\beta_B\mathrm{sh}\alpha)}{(\mathrm{ch}\alpha + \alpha\beta_s\mathrm{sh}\alpha)}\right] \tag{A5}$$

$$i_2 = nF\int_0^L k_4 b \, dx = \frac{nFD_B b \, \theta(\mathrm{ch}\theta - 1)}{k_B L(\mathrm{sh}\theta + \theta\beta_B\mathrm{ch}\theta)} + \frac{nFD_s s \, \alpha^2\theta(M_0 + M_1 + M_2)}{k_s L(\theta^2 - \alpha^2)} \tag{A6}$$

$$M_0 = -\frac{(\mathrm{ch}\theta - 1)(\mathrm{ch}\alpha + \alpha\beta_B\mathrm{sh}\alpha)}{(\mathrm{sh}\theta + \theta\beta_B\mathrm{ch}\theta)(\mathrm{ch}\alpha + \alpha\beta_s\mathrm{sh}\alpha)}$$

$$M_1 = \frac{(1 - \mathrm{ch}\theta - \theta\beta_{BS}\mathrm{sh}\theta)}{(\mathrm{sh}\theta + \theta\beta_B\mathrm{ch}\theta)(\mathrm{ch}\alpha + \alpha\beta_s\mathrm{sh}\alpha)}$$

$$M_2 = \frac{\theta\mathrm{sh}\alpha}{\alpha(\mathrm{ch}\alpha + \alpha\beta_s\mathrm{sh}\alpha)}$$

Total current $i = i_1 + i_2$

$$i = \frac{nFD_B b \, \theta\mathrm{ch}\theta}{k_B L(\mathrm{sh}\theta + \theta\beta_B\mathrm{ch}\theta)}$$
$$+ \frac{nFD_s s \, \alpha\theta[\alpha - \alpha\mathrm{ch}\theta(\mathrm{ch}\alpha + \alpha\beta_B\mathrm{sh}\alpha) + \theta\mathrm{sh}\alpha(\mathrm{sh}\theta + \theta\beta_B\mathrm{ch}\theta)]}{k_s L(\theta^2 - \alpha^2)(\mathrm{sh}\theta + \theta\beta_B\mathrm{ch}\theta)(\mathrm{ch}\alpha + \alpha\beta_s\mathrm{sh}\alpha)}$$

(2) If the reaction Eq. (1) is rate-limiting and $s \gg K_M$ so that $R = k_2 e \Sigma$, then we have

$$i_1 = \frac{nF\theta}{L(\mathrm{sh}\theta + \theta\beta_B\mathrm{ch}\theta)}\left[\frac{D_B b}{k_B} + D_s K_M \alpha^2\left(\frac{\mathrm{ch}\theta - 1}{\theta^2} + \frac{\beta_B\mathrm{sh}\theta}{\theta}\right)\right] \tag{A8}$$

$$i_2 = nF\int_0^L k_4 b \, dx = \frac{nF\theta}{L(\mathrm{sh}\theta + \theta\beta_B\mathrm{ch}\theta)}$$
$$\left[\frac{D_B b}{k_B}(\mathrm{ch}\theta - 1) - D_s K_M \alpha^2\left(2\frac{\mathrm{ch}\theta - 1}{\theta^2} + \frac{\beta_{BS}\mathrm{sh}\theta}{\theta}\right)\right] + \frac{nFD_s K_M \alpha^2}{L} \tag{A9}$$

$$i = \frac{nFD_B b \, \theta\mathrm{ch}\theta}{k_B L(\mathrm{sh}\theta + \theta\beta_B\mathrm{ch}\theta)} + \frac{nFD_s K_M \alpha^2}{L}\left[1 - \frac{\mathrm{ch}\theta - 1}{\theta(\mathrm{sh}\theta + \theta\beta_B\mathrm{ch}\theta)}\right] \tag{A10}$$

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