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Theory of Polymer Entrapped Enzyme Ultramicroelectrodes: Fundamentals

Peter A. Kottke^a, Christine Kranz^b, Yong Koo Kwon^a, Jean-Francois Masson^b, Boris Mizaikoff^b, and Andrei G. Fedorov^{c,*}

^a Georgia Institute of Technology, G. W. Woodruff School of Mechanical Engineering, Atlanta, GA 30332-0405

^b School of Chemistry and Biochemistry, Atlanta, GA 30332-0400

^c G. W. Woodruff School of Mechanical Engineering & Petit Institute for Bioengineering and Bioscience, Atlanta, GA 30332-0405

Abstract

We have developed a theoretical description of the amperometric response of ultramicroelectrode (UME) biosensors formed via enzyme entrapment. Our model allows for multiple enzymes and co-substrates, and results in a closed-form analytical expression for the steady-state current response of the disk ultramicroelectrode. It captures the effects of enzyme-entrapment domain size, species transport properties (which can be different in the polymer and surrounding electrolyte), enzyme kinetics, and axisymmetric diffusion. Assumptions inherent in the derivation are carefully explained, as are the resulting limits on the applicability of the results. The ability to theoretically predict the response of enzyme entrapped UMEs should enable improved design, operation, and data interpretation for this important class of biosensors.

1. Introduction

Ultramicroelectrodes have revolutionized the field of analytical electrochemistry, leading to novel methodologies and making new time and space scales accessible to experimentation [1,2]. For instance, the goal of (electro)chemical imaging with submicron spatial resolution and sub-millisecond temporal resolution has resulted in considerable interest in the use of scanning electrochemical microscopy (SECM), in which UMEs are employed to measure local fluxes of molecules and ions [3–7]. In amperometric operation, the UME, a small electrode (diameter 50 nm–25 μ m), induces an electrochemical reaction, and the resulting Faradaic current is proportional to the net flux of a redox species to the electrode surface [8,9]. An oft-stated advantage of this technology is the ability to predict or interpret the measured electrode current based on a first principles theoretical description of the relevant phenomena [1,9].

For biological applications the versatility of the UME as a probe is dramatically improved when combined with biosensor technology [5]. A biosensor is characterized by a biological recognition element in contact with the transducer surface. Enzymes are frequently chosen as the recognition element due to their remarkable specificity and inherent biocatalytic signal

*Corresponding author: andrei.fedorov@me.gatech.edu.

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amplification [10–12]. Some biologically relevant analytes require a complex biosensing interface, with co-immobilization of multiple enzymes [5,13,14]. Current state-of-the-art immobilization strategies include (i) crosslinking (e.g., attachment of the biological recognition element based on biotin/avidin chemistry [15] or antigen-antibody binding [16]), (ii) covalent attachment to self assembled monolayers [17,18], and (iii) polymer entrapment. Enzyme entrapment in polymer matrices has been demonstrated in conducting polymers [19–21], sol gels [22], and hydrogels [23], including electrodeposited electrophoretic paints (EDPs) [24], which are polymer suspensions deposited via a pH shift induced desolubilization. Electrophoretic paint entrapment exhibits favorable characteristics, including ease of implementation, retention of enzyme activity, and extended sensor stability [13,25]. The model presented in this work was developed to describe the response assuming a probable geometry and likely dominant physics for micro-disk electrodes and polymeric matrices formed via pH shift induced polymer deposition (see section 2.1) [13,24,25].

Theoretical prediction of the amperometric response for miniaturized biosensors utilizing polymer entrapment requires knowledge of the permeable polymer domain shape and transport properties, reaction pathway(s), and enzyme kinetics. Even with known properties and reaction parameters, there is currently no comprehensive theoretical description for predicting the current signal at the microelectrode for a given target molecule concentration. This is because most available models such as those of Savéant and co-authors [14,26–29] and others [30–37] are based on 1-D planar diffusion, usually with the enzyme reaction confined to a monolayer. But 3-D steady-state diffusion dominates in amperometric UME operation [38], and to date analytical models to describe these conditions are limited to extremely large [39] or infinite [40] entrapment domains, which electrodeposition is intended to avoid. Hence, a first principles theoretical model describing the electrodeposited polymer-entrapped enzyme microelectrode experiment is missing, and both interpretation of results and design of the microprobes are at present entirely empirical.

In this contribution an analytical description of entrapped enzyme ultramicroelectrode (UME) steady state response is presented. The formulation is particularly suitable for micro-disk electrodes and polymeric matrices formed via pH shift induced polymer deposition [24]; however, the model may be extended to biosensors based on different enzyme entrapment methods. Experimental validation of the theory and demonstration of its application are provided in a companion paper [41].

2. Principles

The amperometric response of the electrode is related to the net rate of diffusional mass transport of redox species R to the electrode, $J_{E,R}$, by

$$I = \pm nFJ_{E,R} \quad (1)$$

where the determination of the algebraic sign of the current signal is based on the direction of electron transfer, n is the number of moles of electrons transferred per mole of electroactive species oxidized or reduced, and F is the Faraday constant. For unmodified disk UMEs with sufficiently thick insulating sheaths $J_{E,R}$ depends upon the electrode size, (radius a), the diffusivity of the redox species in the electrolyte, D_R , and its bulk concentration, $C_{\infty,R}$. At steady-state, assuming a diffusion-limited electrochemical reaction, the resulting current is theoretically predicted to be $I = \pm 4nFaD_R C_{\infty,R}$ [1].

Amperometric microbiosensor response can also be described by Eq. (1), but determination of the net rate of mass transfer of the electroactive species R to the electrode surface, $J_{E,R}$, is more

complicated (Fig. 1). For a polymer entrapped enzyme UME, the production of R occurs via enzyme-catalyzed reactions confined to a polymer domain adjacent to the electrode surface. The rate of species R production depends upon the bulk concentration of the target analyte, $C_{\infty,A}$, on enzyme reaction kinetics, and on mass transport in two linked domains (polymer and electrolyte solution). Example enzyme catalyzed strategies linking redox species production to analyte species concentrations are depicted in Table I. For three of the example strategies (Uni Uni Reaction, Bi Bi Reaction, and Consecutive Bi Bi Reactions) an increase in $C_{\infty,A}$ results in an increased rate of generation of electroactive species R and consequently an increase in magnitude of current I . Competitive reaction electrodes, such as the last example in Table I, utilize a strategy whereby the electrode current has a nonzero baseline value in the absence of the analyte because the electroactive species, R , is produced by an enzymatic reaction that does not involve the analyte; therefore, the effect of the analyte, A , is to reduce the current, due to participation in a competitive enzyme catalyzed reaction. An advantage of our theoretical description of polymer entrapped enzyme UMEs is its ability to accurately predict the current for electrodes based on many strategies, including all of those listed in Table I.

Our formulation is based on a number of standard assumptions frequently used in modeling of electrochemical processes. These assumptions include Fickian diffusion with uniform transport properties (within a phase) and negligible double layer and migration effects [1]. The electroactive species R has a specified uniform bulk concentration, $C_{\infty,R}$ (which may be zero). Sufficient overpotential is applied on the electrode so that the concentration of the electroactive species, C_R , is brought to zero on that surface. Local equilibrium of species' concentration at the polymer/electrolyte interface is assumed, so that at the polymer/electrolyte interface, the concentration inside the polymer is related to the concentration in the solution by the partitioning coefficient θ_R . For the electroactive species, with diffusivity in the electrolyte D_R , the diffusivity in the polymer is described using a proportionality constant α_R , which is the ratio of the diffusivity in the polymer compared to its diffusivity in the electrolyte. Inside the polymer, volumetric generation of the electroactive species due to the enzyme reaction occurs at a rate given by S_R , which is generally a function of position due to spatial variation in concentrations of the species which participate in the reaction. At steady state the concentration of the electroactive species is therefore described by the coupled reaction-diffusion equations:

$$D_R \nabla^2 C_R = 0 \text{ in the electrolyte} \quad (2)$$

and

$$\alpha_R D_R \nabla^2 C_R + S_R = 0 \text{ in the polymer} \quad (3)$$

The boundary conditions prescribe the bulk concentration

$$C_R = C_{\infty,R} \text{ in the bulk solution} \quad (4)$$

the concentration on the electrode

$$C_R = 0 \text{ on the electrode} \quad (5)$$

and no flux on the insulating surfaces

$$\frac{\partial C_R}{\partial n} = 0 \text{ on insulating surfaces} \quad (6)$$

where $\frac{\partial}{\partial n}$ denotes a derivative in the direction normal to the surface. Boundary conditions also link the two domains. They are the continuity of flux condition

$$\alpha_R \left(\frac{\partial C_R}{\partial n} \right)_{\text{polymer}} = \left(\frac{\partial C_R}{\partial n} \right)_{\text{electrolyte}} \text{ on the polymer/electrolyte interface} \quad (7)$$

and a statement of local thermodynamic equilibrium

$$(C_R)_{\text{polymer}} = \theta_R (C_R)_{\text{electrolyte}} \text{ on the polymer/electrolyte interface} \quad (8)$$

The net flux of the electroactive species to the electrode, $J_{E,R}$, is given by the area integral

$$J_{E,R} = -\alpha_R D_R \int_{\text{electrode}} \frac{\partial C_R}{\partial n} dA \quad (9)$$

Equations (1)–(9) comprise a general model for predicting the current, I , measured at an electrode due to a redox reaction with generation of reactant R limited to a finite domain adjacent to the electrode surface. The current depends on the bulk concentration of redox species R , its transport properties in the polymer and electrolyte, the geometry of the system, and the source term for species R in the polymer, S_R . It is through the source term, S_R , that the analyte species A affects the electrode response. Hence, the prediction of the electrode current, I , as a function of bulk analyte concentration, $C_{\infty,A}$, requires at minimum knowledge of the parameters listed in Table II. Some of the parameters, if not known initially, can be determined via a calibration procedure presented in a companion article [41]. The electrode current prediction also requires evaluation of the source term, S_R , which will depend upon the concentrations of other species. In general, the steady-state concentrations of these species will be governed by diffusion-reaction equations analogous to those for species R . Thus, with the prerequisite parameters in hand, the steady-state current can be obtained by solution of a set of reaction-diffusion equations (inhomogeneous partial differential equations and associated boundary conditions) in linked domains (see Supporting Information).

In general, the solution of this problem can only be accomplished numerically, using, for instance, finite volume, finite element, or finite difference techniques, implemented through either commercially available or user-written computer codes (e.g., see Supporting Information). When using numerical simulation, for any given combination of input parameters and species concentrations, a single electrode current prediction is obtained. The resulting parametric space, even after nondimensionalization, is large, filling it is time consuming and tedious, and extracting useful insight from the results is difficult. To be useful for the broad community of experimentalists, a theoretical tool must be accurate yet simple enough not to require special skills for implementation, and expressed in terms of meaningful and accessible

physical parameters. Analytical solutions are therefore profoundly more useful than numerical simulations, and it is often worth introducing carefully validated simplifications to obtain them.

To this end, our main original result is a closed-form analytical description of the relationship between the steady-state Faradaic current, produced by the oxidation or reduction of the electroactive species on the UME surface, and the bulk concentration of the analyte species, $C_{\infty,A}$. The functional relationship is expressed in terms of an equivalent redox species bulk concentration, K :

$$I = \pm 4nFaD_r K \quad (10)$$

The equivalent redox species bulk concentration, K , which captures effects of geometry and enzyme kinetics, depends upon the bulk concentrations of the analyte species and all co-substrates, and the parameters listed in Table II. The derivation of Eq. (10) is provided as Supporting Information. Much of the present work is devoted to explaining the algorithm(s) by which K can be determined. First we explain the assumptions made in the derivation and the conditions under which they should be reasonable.

2.1. Model Applicability

A subset of conditions exists for which the general model can be solved exactly, and for which numerical simulation is not necessary to predict the electrode current. These conditions are satisfied when the assumptions listed in Table III are appropriate. Some of these assumptions are standard and their limits are well understood. Others require explanation. The first such condition is that the polymer domain shape is accurately approximated as an oblate hemispheroid. The assumption of this oblate hemispheroid geometry is partially justified by consideration of the fact that in electrophoretic paint entrapment the polymer is deposited due to diffusion of protons from the electrode into the solution and isoconcentration surfaces in steady state diffusion from a disk into a semi-infinite medium are oblate hemispheroids [42]. In our analysis, after nondimensionalization of all lengths with the disk electrode radius a , the assumed oblate hemispheroid shape and size are specified by a single dimensionless parameter, Z (see Supporting Information). It is worth noting that with a small Z the entrapment domain is a thin film just covering the electrode.

Another necessary condition for the presented analyses is the requirement of disparate length scales. Specifically, for all species other than the electroactive species R , the ratios of the length scales for changes in their concentration due to enzymatic action in the polymer to the length scale for molecular transport in the polymer must be either much greater than or much less than unity. When the ratio is small, the limiting reactant for the relevant reaction will be entirely consumed close to the polymer/electrolyte interface and an approximation of total consumption of the limiting reactant on the polymer surface is applied. We call this approximation the **heterogeneous reaction approximation**. In contrast, if the length scale ratio is large there will be little variation in concentrations of the non-electroactive species, and therefore little variation in the magnitude of source terms within the polymer. Thus the source terms can be approximated as uniform throughout the polymer domain. We call this approximation the **uniform homogeneous reaction approximation**. An illustration of the effect of these approximations on predicted concentrations is provided in Fig. 2.

3. General Application

Figure 3 provides a guide for application of the polymer entrapped enzyme microbiosensor model. In addition to obtaining all parameters listed in Table II and checking the assumptions of Table III, one of the steps is the determination of the relevant species for the analysis. This

is accomplished by examination of the enzyme reaction mechanism(s), which should reveal those species that affect the reaction rate: they are at least the substrate and co-substrates, and include products if the reaction is reversible. Next, equations for source terms are obtained for steady-state conditions. These source terms can be derived by writing species conservation equations for differential control volumes, or, equivalently, by applying the schematic method of King and Altman [43]. Source terms for some irreversible reactions are included in Table I. Examples with enzyme reaction mechanisms that yield more complicated source terms are included in the companion paper [41]. Once these initial steps are completed, Fig. 3 with the following explanation provides sufficient guidance to predict electrode current. Considerable detail on the derivation of the methodology is available in the Supporting Information.

i. Determination of Approximation Applicability

The source term expressions mentioned above are needed to determine whether the conditions that allow for analytical solution are met. This determination requires calculation of the nondimensional parameters,

$$\Lambda_{ji} \equiv Za \sqrt{|S_{j,s}/(\alpha_i \theta_i D_i C_{\infty,i})|} \quad (11)$$

which are the ratios of the length scale for variation of concentration due to diffusive transport of species i in the polymer, to the length scale due to the variation of concentration of species i as a result of its production or consumption in reaction j , described by the source term S_j . In Eq. (11) Z is a dimensionless parameter specifying the size of the polymer domain, a is the radius of the disk electrode, D_i is the diffusivity of species i in the electrolyte, α_i is the polymer to electrolyte diffusivity ratio for species i , θ_i is the coefficient for equilibrium partitioning of species i at the polymer/electrolyte interface, and $C_{\infty,i}$ is the bulk concentration of species i . Each source term scale, $S_{j,s}$, is a characteristic volumetric rate of production of species by enzyme-catalyzed reaction j , and can be obtained by replacing the concentrations in the source terms using $C_i \sim \theta_i C_{\infty,i}$.

A single species may be produced or consumed by more than one reaction, and each reaction will involve more than one species. Our analytical method requires using Eq. (11) to determine for each reaction j which, if either, of two approximations can be applied. For a given reaction j described by source term S_j , the maximum and minimum Λ_{ji} are found by considering all species i with nonzero bulk concentrations that participate in the reaction. The **uniform homogeneous reaction approximation** should be used if

$$\max(\Lambda_{ji}) \leq 0.6 \quad i:S_j=S_j(C_i) \quad (12)$$

and the **heterogeneous reaction approximation** is valid in the case of

$$\min(\Lambda_{ji}) \geq 3 \quad i:S_j=S_j(C_i) \quad (13)$$

ii. Calculating the equivalent bulk redox species concentration, K

If the source term describing the production of the redox species, i.e. S_R , satisfies Eq. (12) then the uniform homogeneous reaction approximation is applied to the production of species R , and

$$K = T_c \left(C_{\infty,R} + T_v \frac{a^2 S_R}{3D_R} \right) \quad (14)$$

with the evaluation of S_R described in iii (below). If instead S_R satisfies Eq. (13) then the heterogeneous reaction approximation is applied, and

$$K = T_c \left(C_{\infty,R} + \omega_{AR} \frac{D_A}{D_R} C_{\infty,A} \right) \quad (15)$$

where ω_{AR} is a stoichiometric coefficient specifying the number of moles of species R produced per mole of the analyte A consumed in the enzyme catalyzed reaction. In Eqs. (14) and (15), two parameters that capture the polymer shape and transport property effects are used:

$$T_c = \frac{\alpha_R \theta_R}{\frac{2}{\pi} \tan^{-1}(Z)(1 - \alpha_R \theta_R) + \alpha_R \theta_R} \quad (16)$$

and

$$T_v = \left\{ \frac{Z}{2\theta_R \alpha_R} + \left[\frac{\pi}{2} - \tan^{-1}(Z) \right] (1 + Z^2) \right\} Z \quad (17)$$

In the case that the heterogeneous reaction approximation applies to the redox species producing reaction, then Eqs. (15) and (10) are sufficient to predict the electrode current. If, however, the homogeneous reaction approximation is used to describe the generation of the redox species, then Eqs. (14) and (10) cannot be used without first evaluating the uniform source term S_R .

iii. Determining concentrations for evaluation of S_R (uniform homogeneous reaction approximation)

The source term used in Eq. (14) requires an assumption of uniform concentration in the polymer for one or more species. The approximation of the uniform concentrations is obtained by considering a non-uniform concentration in the solution so as to satisfy global mass conservation for species i . Global mass conservation is satisfied by balancing the net rate of transport of species i from the polymer with the net rate of generation of species i in the polymer. The result is a system of algebraic equations for approximate uniform concentrations in the polymer, $C_{p,i}$, that must be solved simultaneously. To obtain this system of equations, first, for all relevant species:

- a. if species i is produced or consumed by the electrode reaction, then we define v_{iR} as the stoichiometric coefficient giving the number of moles of species i produced per mole of redox species R consumed on the electrode;
- b. if species i is produced or consumed in a reaction which is treated under the heterogeneous reaction approximation, then we define ω_{ki} as a stoichiometric coefficient specifying the number of moles of species i produced per mole of limiting reactant k consumed in the reaction;

- c. if species i is produced or consumed in any reaction(s) treated under the uniform homogeneous reaction approximation, then we define S_i as the steady state source term for production of species i by those reactions, and all concentrations used in the expression for S_i are approximated as uniform within the polymer. The determination of these uniform species concentrations in the polymer, $C_{p,i}$, is discussed below. First it is necessary to write an equation that is derived from a statement of species conservation in the polymer for species i :

$$C_{p,i} = \theta_i \left[C_{\infty,i} + \omega_{ji} \frac{D_j}{D_i} C_{\infty,j} + \frac{a^2 S_i}{3D_i} G + \nu_{iR} \left(1 - \frac{2}{\pi} \tan^{-1} Z \right) \frac{D_R}{D_i} T_c \left(C_{\infty,R} + T_v \frac{a^2 S_R}{3D_R} \right) \right] \quad (18)$$

where

$$G \equiv Z \left(1 + Z^2 \right) \left(\frac{\pi}{2} - \tan^{-1} Z \right) \quad (19)$$

Once steps (a)–(c) have been performed for all relevant species, there will be a system of equations of the type given by Eq. (18), written for all species that participate in reactions treated under the uniform homogeneous reaction approximation. The system is generally non-linear, and must be solved numerically, for example, using the method of successive approximations with $C_{p,i} = \theta_i C_{\infty,i}$ as initial guesses.

iv. Electrode Current Calculation

With Eq. (10), the steady-state electrode current is calculated using the equivalent bulk concentration (K) of redox active species R , obtained from either Eq. (14) (uniform homogeneous reaction approximation for S_R) or from Eq. (15) (heterogeneous reaction approximation for S_R).

4. Examples

We demonstrate aspects of our method using two of the example strategies depicted in Table I: a Uni Uni reaction (one reactant, one product) with A as reactant and R as product; and two Bi Bi reactions competing for a common (shared) co-substrate, with A as one of the reactants for one reaction and R as one of the products of the other reaction. In both cases, we assume that the all necessary parameters are known (Table II) and that the conditions listed in Table III are met. We limit consideration to zero bulk concentration of the redox active species, $C_{\infty,R} = 0$, and irreversible enzyme catalyzed reactions. We do not consider the complication of participation in the electrode reaction by species that also participate in the enzyme catalyzed reaction (other than species R). Note that the methodology can be applied without the latter restrictions as demonstrated in the companion paper [41].

4.1. Uni Uni Reaction

First an irreversible Uni Uni enzyme reaction is considered: $A \xrightarrow{\text{enzyme}} R$. In accordance with Fig. 3, we begin by identifying the relevant species (A and R) and writing expressions for the source terms. The steady state source terms are given in terms of the kinetic constants $V_{\max}$, which is the maximum reaction velocity, and K_A , which is the Michaelis constant:

$$S_R = -S_A = \frac{V_{\max} C_A}{K_A + C_A} \quad (20)$$

Figure 3 indicates that the next step is evaluation of the length scale Λ_A . Substituting Eq. (20) into Eq. (11) and using $C_A \sim \theta_A C_{\infty,A}$ yields

$$\Lambda_A = Za \sqrt{\frac{V_{\max}}{\alpha_A D_A (K_A + \theta_A C_{\infty,A})}} \quad (21)$$

With Eq. (21) one can determine whether the uniform homogeneous approximation applies ($\Lambda_A \leq 0.6$) or the heterogeneous reaction approximation applies ($\Lambda_A \geq 3$).

4.1.1 Uniform Homogeneous Reaction Approximation—The uniform source terms are obtained by replacing C_A in Eq. (20) with $C_{p,A}$. The stoichiometric coefficients ν_{AR} and ω_A are zero, so that Eq. (18), written for species A , is $C_{p,A} = \theta_A (C_{\infty,A} + Ga^2 S_A / (3D_A))$. Combined with the expression for the source term, i.e., Eq. (20) with $C_A = C_{p,A}$, this allows one to solve for the uniform concentration of analyte in the polymer:

$$\frac{C_{p,A}}{\theta_A C_{\infty,A}} = \frac{1}{2\Pi_A} \left[\sqrt{(1 + \Psi_A G - \Pi_A)^2 + 4\Pi_A} - (1 + \Psi_A G - \Pi_A) \right] \quad (22)$$

where $\Pi_A = \theta_A C_{\infty,A} / K_A$ and $\Psi_A = \theta_A V_{\max} a^2 / (3K_A D_A)$. Equation (22) can be used to evaluate S_R , which is then substituted into an expression for electrode current obtained by combining Eqs. (10) and (14)

$$I = \pm 4nFD_R a \left(\frac{a^2}{3D_R} T_c T_v S_R \right) \quad (23)$$

The results can also be described in terms of a measure of enzyme electrode effectiveness, $K' \equiv I / (4nFD_A a C_{\infty,A})$:

$$K' = \frac{\Psi_A T_c T_v}{1 + \Pi_A \left(\frac{C_{p,A}}{\theta_A C_{\infty,A}} \right)} \left(\frac{C_{p,A}}{\theta_A C_{\infty,A}} \right) \quad (24)$$

4.1.2. Heterogeneous Reaction Approximation—In the opposite limit of consumption of the analyte by the redox-species-producing reaction very close to the polymer surface, Eq. (15) is used. For a Uni Uni reaction the stoichiometric coefficient is unity, i.e. $\omega_{AR} = 1$, so that from Eq. (15) one obtains $K = T_c D_A C_{\infty,A} / D_R$, which, with Eq. (10), gives $I = \pm 4nFa T_c D_A C_{\infty,A}$. Rewriting this expression in terms of the previously defined measure of enzyme electrode effectiveness, K' , yields

$$K' = T_c \quad (25)$$

4.1.3. Transition between Regimes—The motivation behind consideration of the uniform homogeneous reaction approximation was the recognition that as entrapped enzyme electrodes become smaller, the ratio of the length scale for concentration variation as a result

of the enzyme reaction to the length scale for molecular transport in the polymer becomes larger. Hence, there is less variation of concentrations within the polymer domain, and therefore less variation of the rates of homogeneous reactions. Conversely, if reaction rates are very high then concentration variation due to reaction can occur over very short length scales, and appreciable reaction rates become confined to a region close to the polymer/electrolyte interface. The consequences of these conclusions are illustrated in Fig. 4. In Fig. 4 predictions of enzyme electrode effectiveness are plotted against polymer domain size, given by the dimensionless parameter Z , for constant $\alpha_R \theta_R$, Ψ_A , and Π_A , using the two approximations given by Eqs. (24) and (25). Also plotted are results of numerical simulations generated using a commercial finite volume method solver [44] (see Supporting Information). The electrode effectiveness predicted from numerical simulation shows a smooth transition with increasing Z from values correctly predicted using the uniform homogeneous reaction approximation, Eq. (24), to values correctly predicted using the heterogeneous reaction approximation, Eq. (25). In Fig. 4, plots of analyte centerline concentration for large and small polymer deposits are presented and clearly illustrate the qualitative difference in concentration variation in the polymer for electrodes well approximated by the different formulations.

The plots in Fig. 4 also confirm experimental observations that an optimal size of the entrapment domain exists [24,45,46]. The existence of a Z corresponding to a maximum electrode effectiveness K' is evident in Fig. 4, and the occurrence of the optimum can be explained in terms of the two approximate models. The uniform homogeneous reaction approximation model exhibits a monotonic increase with Z of predicted electrode effectiveness, while the heterogeneous reaction approximation model displays the opposite trend. The maximum K' occurs at a polymer domain size, with corresponding Z , within the transition regime between the two limiting behaviors. Note that other factors could be more important than maximum effectiveness for actual sensor design. For instance, a smaller Z would provide better temporal resolution. Finally, the plots in Fig. 4 illustrate another application of the results of our analysis to experimental design. For a given sensor, the heterogeneous reaction approximation predicts the maximum possible electrode effectiveness. Thus, Fig. 4 provides an indication of the improvement in effectiveness that is possible by increasing, for instance, enzyme loading while decreasing deposit size. By quantifying the potential for improvement, the results allow an experimentalist to make an informed decision when contemplating attempts to increase sensitivity.

4.2. Competitive Reaction Electrode

We next model sensors with two enzymes co-immobilized to catalyze the following reactions:

The analyte A does not participate in the redox species (R) producing reaction, Reaction 1. Instead, A reduces the production of the electroactive species R due to involvement in a competitive reaction, Reaction 2, which also consumes shared co-substrate, B . Therefore, application of the heterogeneous reaction approximation to the redox species producing reaction, i.e., use of Eq. (15), is not appropriate for competitive reaction electrodes.

Because in this example the reactions are irreversible and none of the products, with the exception of R , participate in the electrode reaction, the relevant species, in addition to R , are

the analyte A , F , and the shared reactant, B . Equations for the source terms for irreversible Bi Bi reactions are derived assuming steady-state conditions. In general, for irreversible Bi Bi reactions, three mechanisms are possible: random; compulsory ordered; and double displacement (ping pong) [47,48]. Regardless of the actual mechanism, the source terms due to Reaction 1 are

$$S_F = S_{B1} = -S_R = -V_1 \frac{C_F C_B}{K_{iF} K_{B1} + K_F C_B + K_{B1} C_F + C_F C_B} \quad (28)$$

where the kinetic constants V_1 , K_{iF} , K_F , and K_{B1} depend on the rate coefficients and the concentration of total active enzyme sites (for a ping-pong mechanism $K_{iF} = 0$). The source terms due to Reaction 2 are

$$S_A = S_{B2} = -V_2 \frac{C_A C_B}{K_{iA} K_{B2} + K_A C_B + K_{B2} C_A + C_A C_B} \quad (29)$$

The source term for the shared reactant, B , has components from both reactions:

$$S_B = S_{B1} + S_{B2}.$$

The values of four length scale ratios must be calculated to determine which, if any, approximation applies to each reaction: Λ_{1B} ; Λ_{1F} ; Λ_{2B} ; and Λ_{2A} . These parameters are calculated by replacing the corresponding concentrations in the source term, i.e., Eq. (28) or (29), with $\theta_i C_{\infty,i}$, leading to Eq. (11), e.g.,

$$\Lambda_{1F} = Za \sqrt{\frac{V_1}{\alpha_F D_F} \frac{\theta_B C_{\infty,B}}{K_{iF} K_{B1} + K_F \theta_B C_{\infty,B} + K_{B1} \theta_F C_{\infty,F} + \theta_F C_{\infty,F} \theta_B C_{\infty,B}}} \quad (30)$$

If the maximum length scale ratio for the reaction that produces the redox active species, Reaction 1, satisfies the criteria for application of the uniform homogeneous reaction approximation, i.e., $\max(\Lambda_{1B}, \Lambda_{1F}) \leq 0.6$, then the presented methodology can be applied. If this criterion is not met, however, including, as noted previously, the possibility that the heterogeneous reaction approximation may apply, then the analytical solution results cannot be used.

Provided the uniform homogeneous reaction approximation applies to Reaction 1, then we first note that $v_{iR} = 0; i=B, F, A$. If $\max(\Lambda_{2B}, \Lambda_{2A}) \leq 0.6$ then the uniform homogeneous reaction approximation is applied to both reactions. The system of equations for the uniform concentrations in the polymer is obtained by writing Eq. (18) for A , F , and B :

$$C_{p,F} = \theta_F \left(C_{\infty,F} + \frac{a^2 S_F}{3D_F} G \right) \quad (31)$$

$$C_{p,A} = \theta_A \left(C_{\infty,A} + \frac{a^2 S_A}{3D_A} G \right) \quad (32)$$

and

$$C_{p,B} = \theta_B \left(C_{\infty,B} + \frac{a^2 (S_A + S_F)}{3D_B} G \right) \quad (33)$$

where we have used $S_B = S_A + S_F$. In the expressions for the source terms, the local concentrations are all replaced with uniform concentrations, e.g., $C_B = C_{p,B}$. The system of equations, Eq. (31)–(33) is solved for $C_{p,F}$, $C_{p,A}$, and $C_{p,B}$, which then allows the uniform source term for the redox species, S_R , to be evaluated using Eq. (28). Finally, S_R is substituted into Eq. (14) to obtain the equivalent bulk redox species concentration, K , which is used to obtain the electrode current prediction from Eq. (10).

Alternatively, if $\min(\Lambda_{2B}, \Lambda_{2A}) \geq 3$ then the heterogeneous reaction approximation is applied to Reaction 2. First we must verify that the analyte, A , is the limiting reactant for Reaction 2, a requirement that is met if $D_A C_{\infty,A} < D_B C_{\infty,B}$. The stoichiometric coefficients $\omega_{2B} = 1$, and the mass conservation equation, Eq. (18), is then written for species F and B ,

$$C_{p,F} = \theta_F \left(C_{\infty,F} + \frac{a^2 S_F}{3D_F} G \right) \quad (34)$$

and

$$C_{p,B} = \theta_B \left(C_{\infty,B} + \frac{a^2 S_F}{3D_B} G + \frac{D_A}{D_B} C_{\infty,A} \right) \quad (35)$$

The resulting system of equations is solved, yielding the uniform concentrations of species B and F in the polymer, which allows evaluation of S_R and therefore, evaluation of the electrode current.

In the companion paper [41] we present application of our theory to a real competitive reaction electrode, similar in principle to that explained above, though including some additional complications.

5. Conclusion

We have developed a theoretical model of the steady state amperometric response of polymer entrapped enzyme ultramicroelectrodes. The model is analytical, although not explicit except in the simplest of cases. For realistic situations, solution of a small system of nonlinear algebraic equations is required. Two limiting cases are considered, based on the assumption that (i) homogenous reaction rates can be treated as spatially uniform, or (ii) reactions are confined to a region very close to the entrapment domain boundary

A notable advantage of the presented theory is that UME calibration becomes a measurement of transport properties and enzyme kinetics parameters. Because the results of calibration are quantities with specific meanings in a physics-based electrode model, the resulting description of electrode response can be used to predict the changes in electrode behavior as experimental conditions or electrode parameters are altered, leading to targeted optimization efforts and improved data interpretation.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Fig. 1.

Sensor Schematics. Simplified schematic representation comparing amperometric UME operation (a) to entrapped enzyme amperometric microbiosensor operation (b and c). a) Redox active species R diffuses to the electrode where it loses or gains n electrons, producing a current, I . b) Analyte species A diffuses to the polymer, where an immobilized enzyme catalyzes its conversion to redox active species R . Some of species R diffuses to the electrode where it loses or gains n electrons, producing a current, I , while some of it diffuses back into the electrolyte. c) Analyte species A and shared reactant B diffuse to the polymer, where co-immobilized enzymes catalyzes (1) conversion of B to redox active species R , and (2) co-conversion of A and B to inactive species P . Some of species R diffuses to the electrode where it loses or gains n electrons, producing a current, I , while some of it diffuses back into the electrolyte. The effect of the analyte A is to *reduce* the production of R and hence *lower* the magnitude of I . The predicted current response of the microbiosensors is expressed analogously to that of the disk UME, using an equivalent bulk redox species concentration, K . The presented solution method accounts for effects of geometry, diffusion, transport properties, and enzyme kinetics.

Fig. 2.

Concentration Profiles – actual vs. approximate. Comparison of expected concentration profiles along the symmetry axis for approximations used to develop an analytical solution (dots) to concentration profiles that would be obtained without use of the approximations (solid lines). The top shows analyte species concentrations, and the bottom shows corresponding redox species concentrations. The concentration profiles could be for Uni Uni, Bi Bi, or Consecutive Bi Bi strategies (see Table I).

Fig. 3. Guide to the application of the presented methodology for prediction of electrode current.

Fig. 4.

Transition Between Approximation Regimes. (Top) Predictions of enzyme effectiveness, K' , are plotted against polymer domain size, Z , for constant $\alpha_R \theta_R = 0.1$, $\Psi_A = 1$, and $\Pi_A = 1$, using the two approximations for an irreversible Uni Uni reaction, Eqs. (24) and (25), i.e., the uniform homogeneous reaction approximation (thin line) and heterogeneous reaction approximation (thick line), respectively. Also plotted are results of numerical simulations (dots) generated using a finite volume method solver (see Supporting Information for details). The electrode effectiveness predicted from numerical simulation shows a smooth transition with increasing Z from values correctly predicted using the uniform homogeneous reaction approximation to values correctly predicted using the heterogeneous reaction approximation. (Bottom) Plots of

analyte concentration along the system axis of symmetry for large and small polymer deposits clearly illustrate the qualitative difference in concentration variation for electrodes well approximated by the different formulations. Curves correspond to $Z=2$ (thick line), $Z=0.75$ (dotted line), and $Z=0.15$ (thin line).

Table I

Examples of Enzyme Catalyzed Reactions Used to Link Analyte “A” Species Concentration to Redox “R” Species Concentration for Electrochemical Detection

	Schematic Representation	Source Terms
<u>Uni Uni</u>		$S_R = -S_A = \frac{V_{\max} C_A}{K_A + C_A}$
<u>Bi Bi</u>		$S_R = -S_A = -S_B = V_{\max} \frac{C_A C_B}{K_A C_B + K_B C_A + C_A C_B}$
<u>Consecutive Bi Bi</u>		$S_A = S_B = -V_1 \frac{C_A C_B}{K_A C_B + K_B C_A + C_A C_B}$ $S_R = -S_H = V_2 \frac{C_F C_H}{K_F C_H + K_H C_F + C_F C_H}$ $S_F = -S_A - S_R$
<u>Competitive Bi Bi</u>		$S_A = -V_1 \frac{C_A C_B}{K_A C_B + K_{B1} C_A + C_A C_B}$ $S_R = V_2 \frac{C_B C_H}{K_{B2} C_H + K_H C_B + C_B C_H}$ $S_B = S_A - S_R$

Table II

Parameters for Current Prediction

Symbol	Name and Description	Comments
a	Disk electrode radius	Typically obtained through amperometric electrode characterization[49].
Z	Polymer domain size	Dimensionless. Assumes an oblate hemispheroid (see Supporting Information).
D_i	Diffusivity of species i in the electrolyte	
θ_i	Partitioning coefficient for species i	Determination via chronoamperometry is described in the companion paper[41].
α_i	Diffusivity ratio of species i	Ratio of diffusivity in the polymer to diffusivity in the electrolyte. Determination via chronoamperometry is described in the companion paper[41].
$V_{\max}$, K_E , etc.,	Enzyme kinetics constants such as maximum velocity and Michaelis-Menton coefficients.	The enzyme activity in the polymer is determined as a result of the calibration procedure detailed in the companion paper[41].

Table III

Assumptions

The model is applicable to steady-state, amperometric, entrapped enzyme UMEs provided the following assumptions are applicable:

- 1 Electrode Geometry: Disk UME embedded in a thick insulating sheath
 - 2 Polymer Geometry: Polymer domain shape is closely approximated by an oblate hemispheroid.
 - 3 Enzyme Kinetics Description: The reaction mechanism and the kinetic constants are known so that expressions for steady-state source terms can be developed. Enzyme activity in the polymer domain cannot be known *a priori* and must be obtained during calibration [41].
 - 4 Enzyme Kinetics in Polymer: Mechanism and rate coefficients are not altered by entrapment. Enzyme activity is uniform in the polymer. No aging effects are considered.
 - 5 Electrode Kinetics: Sufficient overpotential is applied to the electrode so that the concentration of the electroactive species, R , is brought to zero on the electrode surface.
 - 6 Mass Transport: Steady state diffusion: migration, advection, and transient effects are negligible.[38] Diffusivity for each species is uniform in the electrolyte (D_i) and in the polymer ($=\alpha_i D_i$). Species transport across the polymer/electrolyte interface is rapid, so that local equilibrium prevails and at the interface a partitioning coefficient, θ_i , relates the concentration inside the polymer to that in the solution.
 - 7 Disparate Length Scales in Polymer: The effect of each reaction on the analyte and co-substrates can be described mathematically as a source term that is either uniform throughout the polymer domain, i.e., Eq. (12) is satisfied (**uniform homogeneous reaction approximation**), or is confined to the polymer/electrolyte interface, i.e. Eq. (13) is satisfied (**heterogeneous reaction approximation**).
 - 8 Enzyme Kinetics Approximation: Reactions described using the heterogeneous reaction approximation can be treated as irreversible.
 - 9 Reaction Approximation/Analyte Concentration: If the analyte participates in a reaction described with the heterogeneous reaction approximation, then it is the *limiting reactant* for the reaction, i.e., $D_A C_{\infty,A} < D_i C_{\infty,i}$ for all other reactants i .
-