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# Geometrical effects of nanowire electrodes for amperometric enzyme biosensors

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ABSTRACT

Enzymatic biosensor reactions follow the Michaelis–Menten kinetics, coupled with diffusion. The diffusion reaction processes for amperometric enzyme biosensors have been simulated to explore the geometrical effects of nanowire array electrodes (NWAEs) and nanowire array stack electrodes (NWASEs) from the viewpoint of enhanced mass transport and increased reaction surface area in two limiting cases. For practical analysis considering sensor fabrication, most samples are assumed to have the same unit square (1 cm × 1 cm) footprint. In the reaction-controlled case, the surface area increment improves the sensitivity regardless of electrode geometry. However, in the diffusion-controlled case, well-controlled NWAE or NWASE geometries as well as the increased surface area improve the sensitivity when the peak current at an early stage of the reaction is measured. Peak current engineering by adjusting the geometric parameters of NWAEs and NWASEs will result in a highly sensitive amperometric enzyme biosensor in the diffusion-controlled case. In contrast to previous micro- and nanoelectrode array studies, we investigated NWASEs representing entangled nanowire network electrodes, and report significant improvements in both limiting cases.



## 1. Introduction

Electrochemical biosensors are classified according to their operating principles as potentiometric (including field-effect transistor), amperometric, and impedimetric (or conductometric) [1,2]. Most contain enzymes that supply the electroactive substances used for signal detection [1,2]. Amperometric enzyme biosensors, which are useful for clinical diagnostics and environmental monitoring, are sensitive and suitable for commercial development [1,2]. With regard to the development of amperometric enzyme biosensors, there is a great deal of interest in nanomaterials as assisting agents or high-performance electrodes [3–6]. Nanotechnology is one of the most attractive research topics in science and engineering, including in electrochemical biosensors, due to the fascinating properties of nanomaterials based on their nanoscale dimensions. The nanoscale geometry provides a number of advantages, such as enhanced mass transport toward the nanoelectrodes and large surface-to-volume ratios. Many researchers, including Campton's group in Oxford, have investigated the benefits of micro- and nanoelectrodes and their arrays in voltammetry and chronoamperometry based on numerical simulations and experiments [7–13]. In contrast to previous studies, we focused on enzymatic amperometry with nanowire array electrodes (NWAEs) and nanowire array stack electrodes (NWASEs). Specifically, investigation of NWASEs confirmed the benefits of entangled nanowire network electrodes (three-dimensional nanowire network electrodes). In this study, the geometric effects of nanowire electrodes as amperometric enzyme biosensors were explored by two-dimensional simulation of enzymatic reactions coupled with diffusion. Only diffusion was considered for substrate (analyte) and product transport as this is the primary mode of mass transport in an amperometric enzyme electrode, even in the presence of natural convection and extensive stirring [13,14]. First, we investigated the basis of the sensitivity improvement using entangled nanowire network (nanocomposite) electrodes [15–23]. Although the improvement was suggested to be due mostly to the increased reaction surface area, a systematic investigation of enhanced mass transport and increased surface area was performed. Nanowire arrays and their stacks, representing nanowire network electrodes, were evaluated by comparison with planar electrodes under two limiting conditions of reaction- and diffusion-controlled cases.

## 2. Sensing model and simulation

The glucose sensor with immobilized glucose oxidase (GOx) as an enzyme is assumed for the simulation. GOx monolayer is

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immobilized on the electrode surface with linker layer in between [15]. GOx monolayer which is about 5 nm in diameter is considered as 1 nm-thick layer with a certain concentration of enzyme. The simulation in this work is not particle-based but concentration-based, therefore the layer thickness should be minimized to mimic the monolayer of enzymatic reaction surface.

The glucose–GOx enzymatic reaction is given by the following scheme and the glucose concentration is detected by oxidizing enzymatic product hydrogen peroxide ($H_2O_2$) at the electrode surface. The relatively high potential enough to oxidize $H_2O_2$ is assumed for amperometric detection. The reaction rate is manipulated by changing the rate constants.

$$\text{Glucose} + O_2 \xrightarrow{\text{GOx}} \text{Gluconic acid} + H_2O_2$$

$$H_2O_2 \longrightarrow 2H^+ + O_2 + 2e^-$$

The general model of enzymatic reaction kinetics is given by the following scheme proposed by Michaelis and Menten in 1913. The catalytic reaction is divided into two processes. The first step depicts the reversible formation of the enzyme-substrate complex $E_1$ with no chemical changes, while the second step depicts the irreversible chemical formation of the product $P$ [24,25]. $E$, $S$, $E_1$, and $P$ represent unoccupied enzyme, substrate, occupied enzyme, and product, respectively, or their concentrations. $k_1$, $k_{-1}$, and $k_2$ are the rate constants, which are assumed to be time-independent.

$$E + S \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} E_1 \xrightarrow{k_2} E + P$$

Fig. 1(a) shows the schematic of the electrode structure and the concentrations of substrate and hydrogen peroxide ($H_2O_2$; reaction product) during amperometric measurement. The "product" in this paper is hydrogen peroxide because the $H_2O_2$ detection method was used. The amperometric enzyme electrode system used in this study was composed of three layers: the immobilizer layer (2 nm thick) in contact with the electrode surface with the amperometric boundary condition, in which the product produced in the enzyme layer diffuses toward the electrode; the immobilized enzyme layer (1 nm thick), in which the enzymatic reaction occurs; and the bulk solution layer, which is large enough not to be affected by substrate depletion at the far end, where the substrate diffuses toward the enzyme layer and the product diffuses out to the bulk end.

The diffusion of substrate and product is described by Fick's second law through all layers. The enzymatic reaction terms based on the Michaelis–Menten kinetics are included in the enzyme layer without any steady-state assumption, as follows [26].

$$\begin{aligned} \nabla \cdot (D_S \nabla S) + \{-k_1 E_T S + (k_{-1} + k_1 S) E_1\} &= \frac{dS}{dt} \\ k_1 E_T S - (k_1 S + k_{-1} + k_2) E_1 &= \frac{dE_1}{dt} \\ \nabla \cdot (D_P \nabla P) + k_2 E_1 &= \frac{dP}{dt} \end{aligned} \quad (1)$$

$E_T$ is the total immobilized enzyme concentration assumed to be a constant that is equal to $E + E_1$. $D_S$ and $D_P$ are the diffusion coefficients of the substrate and product, respectively, which are assumed to be identical throughout all layers. Only the amperometric boundary condition ($P = 0$) at the electrode surface is set for simulations. For the bulk solution layer, no boundary condition is needed because of its infinite thickness. The current is calculated at the electrode surface via Eq. (2) [26,27]:

$$I(t) = nAD_P F \nabla P(t)_{\text{at electrode surface}} \quad (2)$$

where $n$ is the stoichiometric number of electrons involved in an electrode reaction, $A$ is surface area, and $F$ is the Faraday constant.

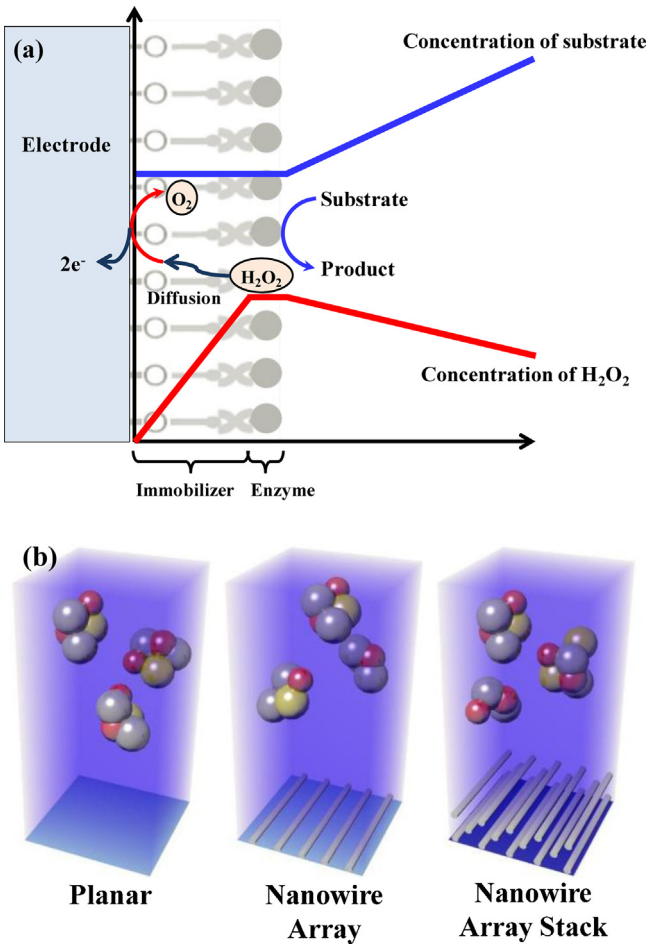


**Fig. 1.** (a) Schematic of an amperometric enzyme biosensor electrode and (b) the three types of electrode assessed in this study. The substrate (analyte) coming from the bulk solution layer reacts and produces hydrogen peroxides in the enzyme layer. Substrate depletion by the reaction induces a continuous supply of substrates by diffusion from the bulk solution layer. The hydrogen peroxides produced by the reaction can diffuse in either direction (toward the electrode or the bulk solution), but most move to the electrode surface and participate in the amperometric reaction, which generates the electric current. Quantitatively, more than 99.9% (numerical simulation result) of hydrogen peroxide can be converted into the electric current in this sensor system due to the extremely steep hydrogen peroxide concentration gradient toward the electrode surface. All types of electrode have the same unit square (1 cm × 1 cm) footprint for pragmatic evaluation considering sensor fabrication.

Enzymatic reactions are categorized into two limiting cases; *i.e.*, reaction-controlled (fast diffusion) and diffusion-controlled (fast reaction) cases. The dimensionless parameter $V$ (Eq. (3)) suggested by Mell and Maloy in 1975 [14] compares the maximum rate of the enzymatic reaction ($k_2 E_T$) with diffusion through a thin layer of membrane ($D_S K_m / d^2$), where $d$ is the thickness of the enzyme layer and $K_m$ is the Michaelis constant defined by $(k_{-1} + k_2)/k_1$ [24]. In Table 1, the case R with small $V$ and the case D with large $V$ represent a reaction-controlled case and a diffusion-controlled case, respectively. All electrode geometries in this work were simulated and explained separately for both limiting cases with $V$ in Eq. (3).

$$V = \frac{k_2 E_T d^2}{D_S K_m} \quad (3)$$

The typical reaction parameter values [24,28] and the diffusion coefficient of glucose in water were used for the reaction-controlled case (case R). Some parameters were modified for the highly diffusion-controlled case (case D). The reaction and diffusion parameter values employed in this study are summarized in Table 1.