

Mathematical model of whole cell based bio-chip: An electrochemical biosensor for water toxicity detection

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Abstract

Whole cell biosensors, which have been genetically engineered to respond to environmental stress, trigger a sequence of processes, which leads to generation of electrical current. This work presents a mathematical model describing the kinetic properties of the bacterial enzymatic reactions in response to toxic chemicals, and the resulting electro-active molecule diffusion to the electrode in a miniaturized electrochemical cell. The model characterizes the generated electrical current as a function of bacteria and toxicant concentrations, electrochemical cell dimensions and electrode dimensions. A model was framed for a 100 nL spherical electrochemical cell. Its performance was simulated and compared to experimental data. This simulation results agreed well with the measured data and therefore it should be useful in predicting current variations in similar systems with different geometries, materials and biological components.

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1. Introduction

Bacteria based biosensors systems can be defined as electronic measurement devices that use living cells as sensing elements. They can detect a signal and also record, process and analyze the obtained information. The significance of these ‘whole cell’ biosensors lies in their capability to provide functional physiological information regarding the effect of chemicals on living systems. Conventional sensors, such as molecular, enzymes or DNA sensors, are based on specific interactions and structural recognition within the biological material, thus, they can only identify and quantify anticipated chemicals.

The bacteria based biosensors can be applied in many fields, including health care and medical applications, pharmaceutical screening and environmental monitoring.

Several detection methods, suitable for a specific biological assay, can be employed, including colorimetric, fluorescent, luminescent, and electrochemical detection [1]. In this present study, we utilized the electrochemical detection method, which proved to be simple, sensitive, provides fast response time and can be performed using compact and mobile equipment [2]. The portability of such a system is essential for on site environmental monitoring, and for various medical applications.

In this work, we present a mathematical model of an experimental biosensor system [3]. The experimental system composed of a 100 nL electrochemical cell that was fabricated on a silicon chip containing genetically engineered *E. coli* bacteria in an aqueous solution. These bacteria have been tailored to respond to general chemical stress. The cascade of mechanisms by which *E. coli* bacterial reactions to toxic chemicals are electrochemically converted into electronic signals have been previously reported [4–6] and are illustrated in Fig. 1.

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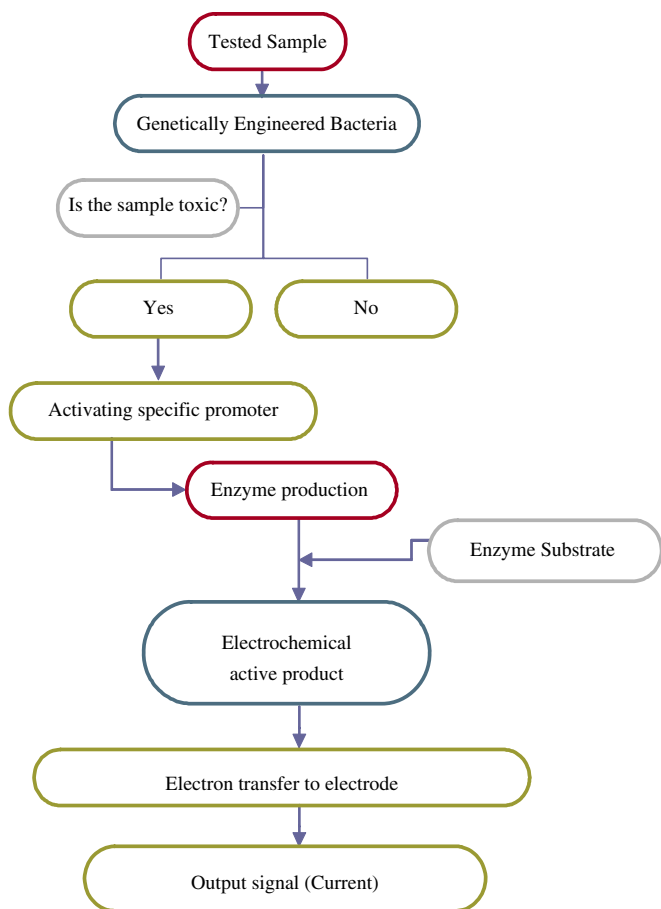


Fig. 1. Flowchart of the bio-electro-chemical reactions: water sample to be tested is introduced to genetically engineered bacteria. The bacteria react to a presence of a toxin by activating specific promoter (regulatory DNA sequence). This promoter induces the production of the reporter enzyme β -galactosidase. The enzyme reacts with the PAPG substrates to produce two different products: *p*-aminophenol (PAP) and β -D-galactopyranoside. The electrochemical active products, the PAP molecules, are oxidized on the working electrode in the electrochemical cell at 220 mV. In this experiment, we used the Amperometric technique.

Various mathematical models have been developed for different electrode geometries and for different biosensors [7–9]. However, a mathematical model describing the diffusion of electroactive molecules in 3D electrochemical cell, resulting from the reaction of recombinant bacteria to toxicant in water has not so far been reported.

In this paper, we modeled a unique structure of whole cell electrochemical biosensor in an effort to establish the fundamental system parameters that will allow designing with different cell structures and electrode geometries.

This model is also essential in the process of understanding and verification of the experimental results. Moreover, the proposed model can be beneficial in planning other bacterial based biosensors for different applications.

1.1. Biochip system

The system that is modeled in this paper includes two units: (1) a disposable silicon chip containing an array of

nano-volume electrochemical cells that house recombinant bacteria, and (2) a reusable unit that includes a multiplexer and a potentiostat connected to a pocket PC for sensing and data analysis. In this paper we model a single 100 nL electrochemical cell that contained many (typically 10^7) microbes. A fixed potential was applied between the embedded working and reference electrodes by the potentiostat, and the output current signal was measured. An aqueous solution containing the bacteria, bacterial nutrients, a substrate that enabled the enzymatic reaction (*p*-aminophenyl- β -D-galactopyranoside (PAPG)) and the toxicant was placed in the electrochemical chamber on the chip. The toxicant interacts with the bacteria, and as a result, the recombinant *E. coli* bacteria produce the enzyme β -galactosidase, which reacts with the substrate *p*-aminophenyl- β -D-galactopyranoside (PAPG). The electrochemically active product of the enzymatic reaction, *p*-aminophenol (PAP) departs from the bacteria and oxidizes on the working electrode. The oxidation at the electrode is translated to a current signal (for more details see Ref. [10]).

1. The starting point ($t = 0$) of the experiment is when the solution was inserted into the chip chambers. The solution was inserted immediately (~ 1 min) after mixing the bacteria, the substrate PAPG and the toxicant.
2. We neglected drift, as the molecules are neutral, and convection since the solution is static and there is no fluid flow during the short measurement time.
3. We will consider all the reactions that occur in the bacterial system using a set of state equations that determine the system response. This approach assumes that the biological material response is determined by the various inputs and the state of the microbes, i.e. we assume a deterministic system. This is an approximation since the microbes themselves can vary between batches. However, we assume that the average response can be treated using a system approach. The system's inputs are the bacterial concentration, substrate (PAPG) concentration and toxicant concentration. The system's outputs are the time dependent PAP concentration and the measured current under fixed potential.

2. Kinetic model of biological reactions

In order to characterize the generated current in the biosensor system, first we express the kinetics in which the PAP molecules are generated. Next, the diffusion model of the PAP molecules towards the working electrode will be developed. The integral of the flux over the electrode area is the total current $I(t)$.

The Michaelis Menten model [11] describes an enzyme–substrate interaction and its kinetic characteristics. Using this model, the enzyme–substrate reaction is described as follows:



where E is the enzyme β -galactosidase, S is the substrate PAPG, ES the enzyme–substrate complex and P is the product PAP + D-galactose. k_1 is the rate constant in which E combines with S to form the ES complex. The ES complex has two possible reaction paths. It can dissociate to E and S, with a rate constant k_2 , or it can proceed to form a product P, with the rate constant k_3 . The rate of the reaction (V) is defined as

$$V = k_3[\text{ES}] \quad (2)$$

The concentration of the uncombined enzyme, $[\text{E}]_{\text{Free}}$, is equal to the total enzyme concentration, $[\text{E}]_{\text{Total}}$, less the concentration of the enzyme–substrate complex $[\text{ES}]$.

$$[\text{E}]_{\text{Total}} = [\text{E}]_{\text{Free}} + [\text{ES}] \quad (3)$$

In the present model we have an excess amount of molecular substrate (PAPG), thus, most of the time the enzyme binding receptors are saturated with substrate. Therefore, the enzymes appear as enzyme–substrate complex $[\text{ES}]$, and the amount of the free enzyme $[\text{E}]_{\text{Free}}$ is negligible. Therefore

$$[\text{E}]_{\text{Total}} = [\text{ES}] \quad (4)$$

Combining Eqs. (2) and (4) yields the velocity of the enzymatic reaction

$$V_{\text{max}} = K_3[\text{E}]_{\text{Total}} \quad (5)$$

The same equation is obtained according to the Michaelis Menten model, in the extreme case when the enzyme sites are saturated with substrate, which means that $[\text{S}]$ is much greater than Michaelis constant (K_m), which is defined as $K_m = (k_3 + k_2)/k_1$. In that case $[\text{S}]/([\text{S}] + K_m)$ approaches 1. Thus, $V_{\text{max}} = k_3[\text{E}]_{\text{Total}}$.

Note that the Michaelis Menten model assumes steady-state. In steady state, the rate of formation and breakdown of the ES complex are equal and not time dependent. In our model, the time constant of the enzymatic reaction (between the enzyme and the substrate) is much shorter than the time constant of the enzymes production rate.

Consequently, the velocity of the enzymatic reaction is linearly dependent on the enzyme concentration. The rate of the enzyme β -galactosidase $[\text{E}]$ production is unknown. A simple model would assume that the enzyme production rate is constant and proportional to the toxicant concentration, in some concentration range. A further logical assumption is that the enzyme production rate reaches its maximal level at maximum toxicant concentration. Above this critical toxicant concentration, cell function deteriorates until they die. The following equations resulting from combining this assumption with the previous kinetics equations:

$$\frac{d[\text{E}]}{dt} = \alpha \cdot (\text{cells}) \cdot [\text{toxicant}] \quad (6)$$

where α is the rate constant with 1/s units. The enzyme production rate is a function of the toxicant concentration, and the bacteria concentration.

The product concentration depends on the velocity of the enzymatic reaction:

$$\frac{d[\text{P}]}{dt} = V \quad (7)$$

where $[\text{P}]$ is the product concentration $[\text{PAP}]$ and V is the velocity.

Combining Eqs. (2), (4) and (5)

$$\frac{d[\text{P}]}{dt} = K_3[\text{E}_{\text{T}}] \quad (8)$$

Combining Eqs. (6) and (8)

$$\frac{d[\text{P}]}{dt} \approx K_3 \cdot \alpha \cdot [\text{cells}] \cdot [\text{toxicant}] \cdot t \quad (9)$$

The product concentration is time dependent and proportional to the maximum rate of the reaction (V_{max}), bacterial concentration and toxicant concentration.

The optimal activity of the bacteria is under their “log phase” conditions [12]; in that case, the signal is proportional to the bacterial concentration. In addition, we assume that the bacterial concentration remains constant during the experiment (up to 10 min).

3. Kinetic model of the electrochemical reactions

3.1. Electrolyte diffusion simulation

In order to calculate the current flow as function of time, we simulated the diffusion of the PAP molecules $[\text{P}]$, with concentration $C_0(r, z, t)$, in a chamber where a disk electrode lays at the origin. Those molecules are oxidized on the working electrode surface, and consequently, generate a current signal. Each PAP molecule discharges two electrons. The concentration of the electro-active species is assumed nearly zero at the electrode surface. We also assume that the current is totally controlled by mass transfer and by the kinetics of the reactions in the solution away from the electrode.

The electrochemical cell has a cylindrical symmetry; therefore, we can reduce the system from 3D to 2D by writing the diffusion equation in cylindrical coordinates.

Eq. (10) presents the electrolyte diffusion in a cylindrical coordinates system according to Fick's second law, where D_0 is the electrolyte diffusion coefficient and $f(t)$ is the electrolyte production rate.

$$\frac{\partial C_0(r, z, t)}{\partial t} = D_0 \left(\frac{\partial^2 C_0(r, z, t)}{\partial r^2} + \frac{1}{r} \frac{\partial C_0(r, z, t)}{\partial r} + \frac{\partial^2 C_0(r, z, t)}{\partial z^2} \right) + f(t) \quad (10)$$

To solve Eq. (10) numerically we supply the following initial and boundary conditions;

$$C_0(r, z, 0) = 0 \quad (11)$$

Eq. (11) indicates that at $t = 0$, there were no PAP molecules in the reaction chamber. Assuming full oxidation of the electrolyte at the electrode, we demand Eq. (12), where r_0 is the electrode radius.

$$C_0(r, 0, t) = 0 \quad \text{for } (r \leq r_0) \quad (12)$$

There is no internal or external flux of electrolyte through the chamber edges and no oxidation of the electrolyte outside of the electrode

$$\left. \frac{\partial C_0(r, z, t)}{\partial z} \right|_{z=0} = 0 \quad \text{where } (r > r_0) \quad (13)$$

$$\left. \frac{\partial C_0(r, z, t)}{\partial z} \right|_{z=z_{\max}} = 0 \quad (14)$$

$$\left. \frac{\partial C_0(r, z, t)}{\partial r} \right|_{r=r_{\max}} = 0 \quad (15)$$

The axial symmetry imposes zero radial flux at the z axis

$$\left. \frac{\partial C_0(r, z, t)}{\partial r} \right|_{r=0} = 0 \quad (16)$$

Eq. 10 was solved numerically on a grid of 20×130 cells, with a time step of 0.001 s. Second derivatives were implemented using the four nearest neighbors.

3.2. Electrolyte flux and current calculation

Once the electrolyte concentration is known, we may proceed to calculate the electrolyte flux at the electrode, and the current that is produced when the electrolyte is oxidized at the working electrode.

The electrolyte molecule flux at the electrode $J_0(r, z, t)$ $\left[\frac{\text{molecules}}{\text{cm}^2 \text{ s}}\right]$, driven by the gradient in the electrolyte concentration near the electrode

$$\vec{J}_0(r, z, t) \left[\frac{\text{molecules}}{\text{cm}^2 \text{ s}}\right] = -D_0 \nabla C_0(r, z, t) \quad (17)$$

We can neglect the radial flux component since the electrode height is very small compared to other cell dimensions. Thus, we left just with the z component of the gradient in Eq. (17). This leads to the following derived equation for the flux at the electrode:

$$\vec{J}_0(r, z, t) = -D_0 \frac{\partial C_0(r, z, t)}{\partial z} \cdot \hat{z} \quad (18)$$

We may now translate the electro-reactive molecules flux into an electron electric-flux $J(r, t)$ $\left[\frac{\text{Amp}}{\text{cm}^2}\right]$

$$\vec{J} \left[\frac{\text{Amp}}{\text{cm}^2}\right] = q \cdot n \cdot \vec{J}_0 \quad (19)$$

$$J(r, t) = n \cdot F \cdot J_0(r, t) \quad (20)$$

where q is the electron charge, F is Faraday constant and n is the number of electrons that are exchanged at the electrode per molecule of the electro-reactive by-product (PAP) during the oxidation process. Finally, we integrate the flux over the electrode area to get the total current $I(t)$.

$$I(t) = 2\pi \int_0^{r_0} r \cdot \vec{J}(r, z, t) \cdot \hat{n} \cdot dr \quad (21)$$

where \hat{n} is a unit vector normal to the electrode. This vector is vertical on the top side of the electrode.

4. Results and discussion

4.1. Experimental procedure

Genetically engineered bacteria were used as whole cell sensors to detect acute toxicity in water. We used recombinant *E. coli* bacteria bearing plasmid with one of the following promoters: *Dnak*, *grpE* or *fabA*. These promoters were fused to the reporter enzyme β -galactosidase [3,5,13]. Bacteria cultures of 3×10^7 cells/ml were used for all experiments. Different concentrations of ethanol (0.5–2%) or phenol (1.6–16 ppm) were introduced to the bacterial suspensions in the presence of the substrate PAPG. The substrate, PAPG, was added to a final concentration of 0.8 mg/ml (100 nL total volume). Immediately thereafter (~ 1 s), the suspensions were placed in the electrochemical cells. The response of the bacteria to the toxic chemicals was measured on-line by applying a potential of 220 mV. The product of the enzymatic reaction (PAP) was monitored by its oxidation current. Additional measurements in the absence of the bacteria were performed to exclude the possibility of electroactive species in the nutrients medium, in the substrate, or in the substrate and the nutrients medium mixture, which can contribute to the current response. The substrate was present in an excess amount. Thus, any change in the amount of product formed over a specified period of time was dependent only upon the level of enzyme presented. The amount of enzyme present in the reaction is measured by the activity it catalyzes. The relationship between activity and concentration is affected by many factors such as temperature, pH, etc. In this model, based on experimental results, these factors are optimal and constant. For more experimental details and results see [3]. Fig. 2 shows the chamber dimensions.

4.2. Simulation results

The simulation was run with simulation step of 0.1 ms for 600 s. Comparing the concentration profiles at different

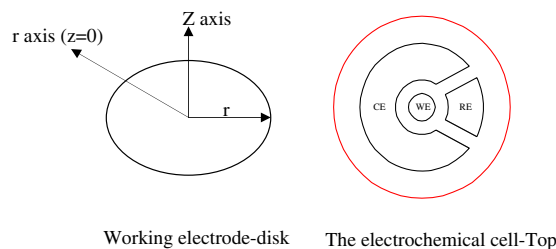


Fig. 2. Cell dimensions: $r_0 = 150 \mu\text{m}$, $r_{\text{chamber}} = 560 \mu\text{m}$, $h_{\text{chamber}} = 100 \mu\text{m}$, working electrode height = 200 nm.

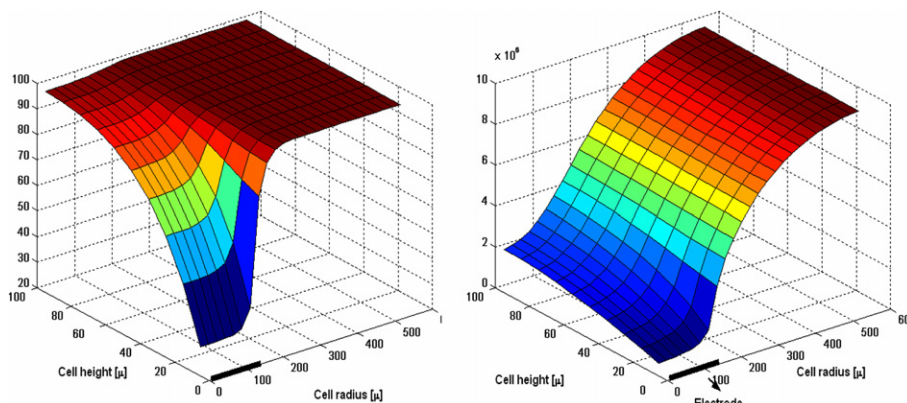


Fig. 3. Concentration profile of the PAP molecules after 1 s (left) and after 600 s (right). Since α and V_{\max} of Eq. (9) are unknown – the concentration units are arbitrary. Chamber dimensions: radius 560 μm ; working electrode radius 150 μm ; chamber height 100 μm ; diffusion coefficient $2 \times 10^{-5} \text{ cm}^2/\text{s}$.

times (Fig. 3) shows that the shape of the profile evolves rapidly – after 1 s, the shape of the concentration profile of the PAP molecules is similar to the shape that stabilizes after 600 s. This indicates that the enzyme production function changes slowly compared to the kinetics of the diffusion, which means that the limiting factor in the reaction is the enzyme production rate.

The model and the experimental results are compared in Fig. 4, which shows the fitting to experiments in which three different concentrations of phenol (1.6 ppm, 8.3 ppm and 16 ppm) were introduced to the recombinant *E. coli* bacteria [3]. We assumed a linear production function. Since the multiplicative factor $\alpha \cdot V_{\max}$ in Eq. (9) is unknown, we fit the data so that the value of the simulation current and the experiment current would be the same after 400 s. This is a fit of a single multiplicative parameter. The diffusion

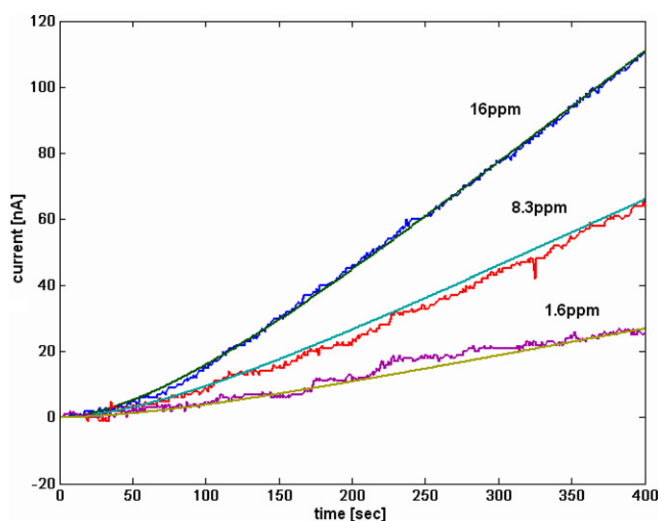


Fig. 4. Comparison between model simulation and experimental results. The experiments were performed using recombinant *E. coli* containing a promoterless *lacZ* gene fused to promoter *grpE* exposed to increasing concentrations of phenol. The bacteria cultures with the substrate PAPG and the phenol were placed into the 100 nL volume electrochemical cells on the chip immediately after the phenol addition.

coefficient $D_0 = 2 \times 10^{-5} \text{ s/cm}^2$ was found to achieve best fit between experiment and simulation; this value agrees reasonably well with possible diffusion coefficient values for bio-chemical solutions such as in our experimental system. This is the only fitting that was done. The fitting results are shown in Fig. 4, and indicates that both the simulation and the experimental results exhibit close to linear behavior after 70–80 s, and both exhibit some bending at the beginning of current production. This bending represents mostly the time it takes for the diffusion processes to follow the rate at which the molecules are produced in the cell. Another possible cause for deviation from linearity at the beginning is the possibility of some variance in the time at which different cells have started their enzyme production, but given the excellent fit of the model to experiment – there is no need to assume such variance in enzyme induction time.

To gain more insight from the model, we investigate the current response to variation of the working electrode radius, chamber height and chamber radius; in each case, we change one parameter while keeping all the others at their original values. We introduce a step function in the electrolyte production rate, which means that for $t < 100 \text{ s}$ there was no enzyme in the chamber and for $t > 100 \text{ s}$ there was a constant level of enzyme in the chamber. In Fig. 5 we show the current response to a step function for different choices of electrode radius. This figure indicates that the larger the radius of the electrode, the response time is faster. It should be noted that the round bending in Fig. 5 and the deviation from linearity in Fig. 4 are both result of the speed of diffusion process. The difference between the figures is a result of supplying different source functions, in Fig. 4 this is a linear function that simulates the kinetics of the system, while in Fig. 5 it is a step function that makes it easy to note the response curve of the system. It is evident that the maximal current is the same for all electrode choices – this makes sense because in the steady state the amount of molecules that are removed at the electrode should be equal to the amount of molecules that are produced in the cell. A meaningful parameter that can be measured is the time it takes to reach

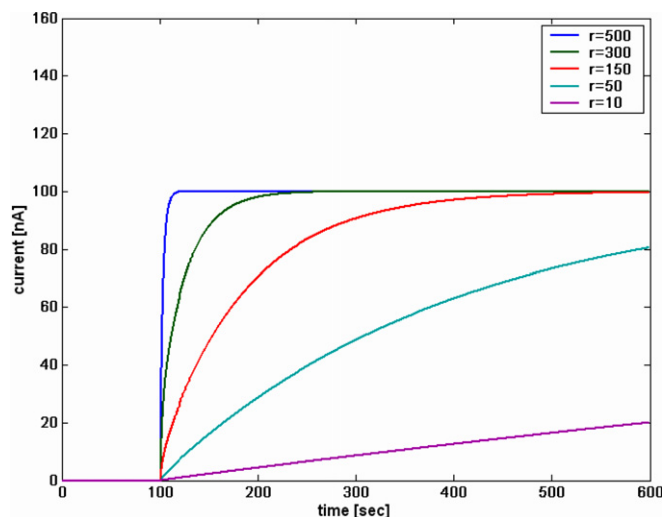


Fig. 5. Current response to a step function for different electrode radius; electrode radius units are in μm .

half the steady state height. It may be seen in Fig. 5 that the bigger the electrode radius – the shorter the response time. The response time of electrode radii of 300, 150 (the actual experimental system) and $50\ \mu\text{m}$ reached half of the height after 14, 55, and 200 s, respectively. Those results are summarized in Fig. 6a.

We further investigated additional factors that may influence the response of the system. Fig. 6b shows the dependence of the response time on the chamber height. We note that the bigger the cell height the longer the

response time. An interesting finding is that the log–log graph of the response time deviates from linearity at a height of $150\ \mu\text{m}$ (our default size for the electrode radius), the same phenomenon happens with the electrode radius of $100\ \mu\text{m}$ (our default value for chamber height). We explain both phenomena by the fact that above those values the relevant parameter becomes the dominant factor (either chamber height or electrode radius) and then there is a simple dependence of the response time that is linear in the log–log graph. Below those values the response is dominated by both parameters and hence we do not see a simple relation. Furthermore, it is important to note that the electrode radius affects the response more than the chamber height this can be seen by the values of their respective slopes (-2.45 and 1.16).

Another parameter that we checked is the chamber radius. Here we note a simple linear relationship in the log–log graph (Fig. 6c).

5. Conclusions

The present work describes a numerical model, which developed to describe the kinetic processes in a miniaturized electrochemical biosensor system resulting from specific enzymatic reaction and diffusion field. This model shows excellent agreement with the measured data and therefore, it should be useful to predict further current variations in similar systems with different geometries, materials and biological components. The model

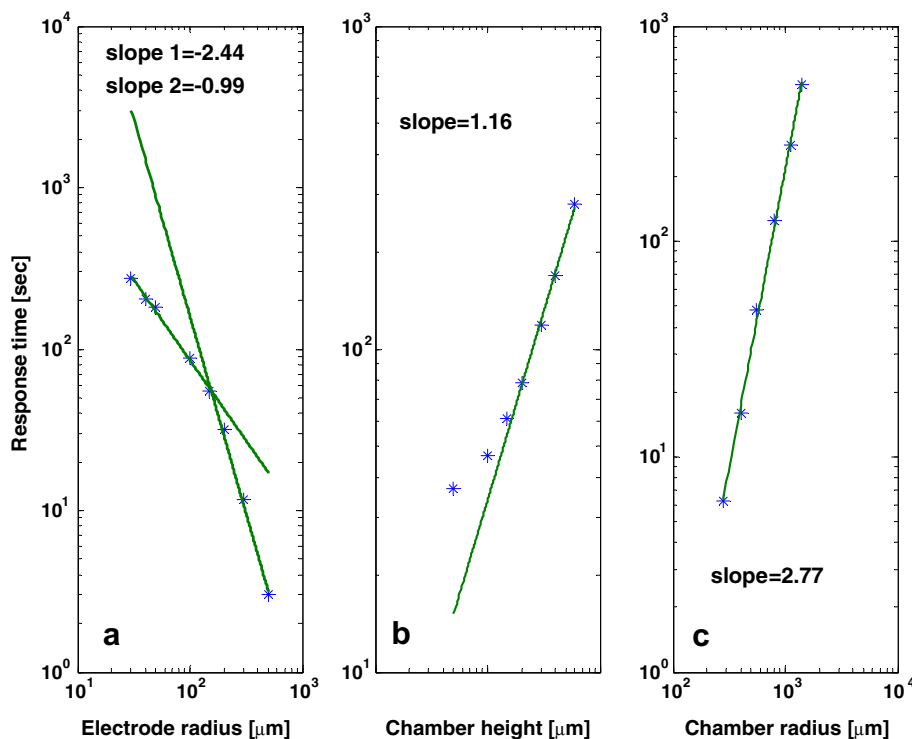


Fig. 6. (a) Variation of response time vs. working electrode radius, (b) variation of response time vs. chamber height, (c) variation of response time vs. chamber radius. Original chamber parameters: working electrode radius $150\ \mu\text{m}$, chamber height $100\ \mu\text{m}$, chamber radius $560\ \mu\text{m}$.

demonstrates the benefit of utilizing a miniature electrochemical cells. The relationship between different chamber parameters (such as cell radius and height and electrode radius) were investigated.

Furthermore, the agreement between simulation and experiment validate of both the model for the kinetics of the PAP production described by Eq. (9) and the model for the diffusion of the PAP molecules.

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