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Study and modelling of the pH-ElecFET microsensors for the lactate detection

N.Aoun[(a)*], H. Belmabrouk[(a)]

[(a)] Laboratory of Electronics and Microelectronics, University of Monastir, Monastir 5000, Tunisia

**Corresponding author. E-mail : nejibaaoun@yahoo.com*

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Abstract

Lactic acid is one of the most important metabolites in clinical analysis and the food industry. Its detection is an important clinical assay for the diagnosis of numerous human disease conditions. As a result, the analysis of lactic acid and its related lactate ion was widely studied and detection methods based on the lactate oxidase (LOx) enzyme were finally proposed. For its monitoring, the development of smart lactate-biosensors based amperometric and potentiometric ElecFET (Electrochemical field effect transistor) at the microscale is required. Lactate and pyruvate concentration profiles within the sensor were obtained by solving reaction coupled mass conservation equations using a finite element analysis (FEA) software called COMSOL Multiphysics and the currents were calculated from the hydrogen peroxide flux at the electrode surface. In presence of lactate ions, it is responsible for the production of hydrogen peroxide H_2O_2 that is oxidized on the electrochemical microelectrode, leading to the production of proton H^+ and finally for a local pH decrease. The proposed model points out the role of the ElecFET design, i.e. the number of enzymatic units per volume unit n_{enz}, the L-lactate oxidase Michaelis constant K_M and the lactate concentration $[S_1]$.The ElecFET concept is extended to the detection of lactate [1-6 mM] concentration range. The sensitivitie is 13 mV/mM.

Keywords: *lactate-biosensors based ElecFET, solving, current, electrochemical microelectrode, pH.*

1. Introduction

Lactic acid (C3-CH-OH-COOH) is a well-known chemical species involved in many biochemical and biological processes related to life, health and food domains. For food chemistry, it is useful to assess the freshness and stability of milk, milk products, fruit, vegetables and wines.

Lactate detection is through the use of four enzymes: lactate dehydrogenase (LDH), lactate oxidase (LOx), monooxidase lactate (LMO) and cytochrome b2 (Cyt b2). The process in all three cases leads to pyruvate and in the case of LMO conduit acetate. Nevertheless, in all cases, the detection is based on the enzymatic reaction of lactate oxidase [1]. This has been done successfully through the realisation of LOx-based amperometric microsensors [2 3]. The detection principle is based on the use of a metallic work microelectrode on which was immobilized enzyme layer containing lactate oxidase. Based technologies, various metal electrodes were used (platinum [1 4 5 6], graphite [1], carbon [1]) and various enzyme

immobilization materials (hydrogels [1 4], Nafion membrane [1], photosensitive polymers PVA or PVC [1 4]). By analogy with the glucose sensor based on the glucose oxidase enzyme and the monitoring of the hydrogen peroxide H_2O_2 production, amperometric detection principles were studied for the lactate detection. As a result, through the use of different immobilization processes, amperometric biosensors based on a platinum (ultra)microelectrode modified with lactate oxidase were successfully developed, evidencing promising detection properties. This paper presents the development of the ElecFET (Electrochemical field effect transistor) concept for the lactic acid detection in liquid phase (Fig.1). This concept is associated to the combination of amperometry and potentiometry at the microscale. It results from the functional integration of an electrochemical platinum microelectrode around the sensitive gate of a pH-based enzymatic field effect transistor (pH-EnFET) microdevice on a single chip. Lactate is reduced to pyruvate and hydrogen peroxide (H_2O_2) by the enzyme lactate oxidase immmobilisé on the biosensor. The liberated hydrogen peroxide is oxidized at the platinum electrode to a bias 0.7 vs. Ag/AgCl [7], leading to the production of protons H^+ and finally a local increase in pH. These phenomena result in the variation of the threshold voltage of the ElecFET measurable change using a potentiostat and representative of the concentration of lactate ions in the solution. We then focus to study the influences of the main parameters, i.e. (i) number of enzymatic units per volume unit n_{enz}, (ii) L-lactate oxidase Michaelis constant K_M and (iii) lactate concentration [S_1].

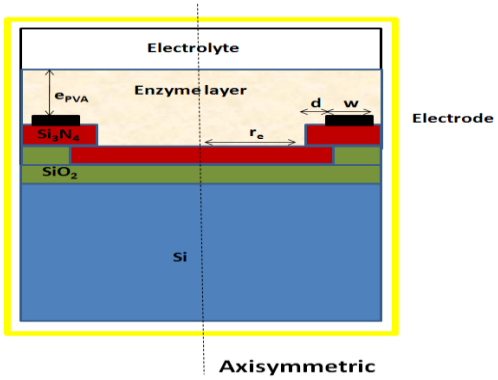

Figure 1. Schematic of the simulation geometry for a structure lactate ElecFET in axial coordinate

Order to do so, detection strategies have relied on the lactate oxidase (LOx) enzyme whose enzymatic reaction can be resumed as following (Fig.1):

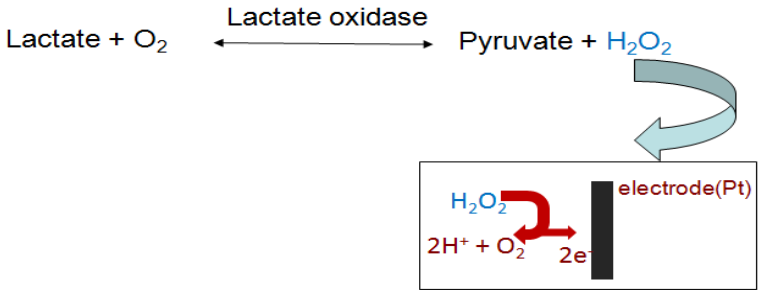

Figure 2. Lactic acid-catalyzed reaction and the lactate oxidase dissociation reaction of hydrogen peroxide on the platinum microelectrode

2. Presentation of the simulation model

The modelling approach used takes into account the different chemical, electrochemical and physical phenomena occurring in the Lactate-ElecFET detection principles:

- Oxido-reduction on the integrated microelectrode

- Diffusion phenomena of S_1 (substrate1=lactate), S_2 (substrate2=O_2), P_1((product1=pyruvate), and P_2 (product =H_2O_2)
- Constant diffusivities for lactate, O_2, pyruvate and H_2O_2;

The Lactate-ElecFET detection properties were thus modeled by studying the diffusion phenomena of the main chemical species in the enzymatic reaction, and finally by analyzing the pH detection properties of the silicon nitride Si_3N_4 ChemFET gate.

2.1. Modelling of the electrochemical reactions (H_2O_2)

The Lactate-ElecFET concept was used of the hydrogen peroxide (H_2O_2) oxidation in water based solutions:

H_2O_2 oxidation ($V_P > E_1^+$): $H_2O_2 \text{----->} 2H^+ + 2e^- + O_2$ (1)

where V_p is the polarization voltage on the microelectrode and E_1^+ is equilibrium potentials of the O_2/H_2O_2 redox couple respectively ($E_1^+ \approx 0.7$ V [7]).

2.2. Modelling of the mass transport phenomena

The modelling is focused on the mass transport phenomena into the channel of the main interfering chemical species, i.e. lactate $C_3H_5O_3^-$, pyruvate $C_3H_3O_3^-$, O_2 , H_2O_2 , H^+ and OH^-:

$$\frac{\partial C_i}{\partial t} = D_i\left(\frac{\partial^2 C_i}{\partial r^2} + \frac{1}{r}\frac{\partial C_i}{\partial r} + \frac{\partial^2 C_i}{\partial z^2}\right) + \varepsilon g_i \qquad (2)$$

where t denotes the time, C_i is the concentration of the studied chemical species, D_i is its diffusion coefficient and εg_i term represents the enzymatic consumption/production phenomena per time unit, the integer parameter ε being representative of the number of molecules consumed ($\varepsilon < 0$) or produced ($\varepsilon > 0$) according to the enzymatic reaction studied. In the case of lactate oxidase enzyme (equation 1) and according to the Michaelis-Menten equation, g_i is given by:

$$g(r,z,t) = a_M n_{enz} \frac{[S](r,z,t)}{[S](r,z,t) + K_M} \qquad (0 \le z \le z_{PVA}) \qquad (3)$$

where a_M is the maximal activity ($a_M = 16.67\times10^{-9}$ mol/s for one enzymatic unit), n_{enz} is the enzymatic units number per PVA volume unit, [S](r,z,t) is the substrate concentration distribution in solution and K_M is the enzyme Michaelis constant.

In order to solve the mass transport equation using finite element model, the following initial and boundaries conditions have been chosen:

Mass transport of i species across the electrode surface is given by:

$$-D_i \frac{\partial C_i}{\partial z} = \frac{\nu_{i,j}}{nF} i_j \quad (z = z_e \text{ , } r = d) \qquad (4)$$

where n is the number of electron, the stoichiometric coefficient and F is Faraday's constant.

i_j denotes the current density given from the kinetic equations for the electrochemical reactions at the electrode surface based on the Butler–Volmer expression [9]:

$$i_j = i_{0\,j;ref}\left\{\prod_i\left(\frac{c_{i,0}}{c_{i,ref}}\right)^{pi,j} \exp\left(\frac{\alpha_{a,j}F}{RT}\eta_j\right) - \prod_i\left(\frac{c_{i,0}}{c_{i,ref}}\right)^{qi,j} \exp\left(-\frac{\alpha_{c,j}F}{RT}\eta_j\right)\right\} \qquad (5)$$

Where $i_{0j,ref}$ is the exchange current density due to reaction "j" at the reference concentrations in A/cm^2, $c_{i,0}$ is the concentration of species "i" adjacent to the surface of electrode in mol/cm^3, $c_{i,ref}$ is the reference concentration of species "i" in mol/cm^3, α_{aj} and α_{cj} are respectively the anodic transfer coefficient for reaction "j" and the cathodic transfer coefficient for reaction "j", p_{ij} and q_{ij} represent respectively the anodic

reaction order of species "i" in reaction "j" and the cathodic reaction order of "i" species in reaction "j", R is the gas constant, T is the temperature and η_j is the overpotential of reaction "j" in V (volt), and it is measured with respect to a reference electrode of a given kind in a solution at the reference concentrations.

The overpotential for electrochemical reaction "j", (η) in Eq. 5 is given by

$$\eta_j = V_p - E_0 \quad (6)$$

where V_p is the polarization voltage on the microelectrode, E_0 is the equilibrium potential for the H_2O_2 oxidation.

The total current at the electrode is obtained by:

$$I(t) = \int_A i_j \, dA \quad (7)$$

where A is the surface area of the electrode per electrode volume

The concentration gradients are equal to zero on the Si_3N_4 insulative surface:

$$-D_i(n\nabla C_i) = 0 \quad (8)$$

The concentrations are constant at the top boundary surface:

$$C_i = C_{0,i} \quad (9)$$

Initially, the concentrations of each species are as follows (pH = 7):

$$\begin{cases} C_{S_1}(t,r,z) = Cte \\ C_{S_2}(t,r,z) = Cte \\ C_{P_1}(t,r,z) = 0 \\ C_{P_2}(t,r,z) = 0 \end{cases} \quad ;0 < z, 0 \le r, 0 < t \quad [H^+]=10^{-7}M, [OH^-] =10^{-7}M \quad (10)$$

Finally, by solving the mass transport equations (Eq. (2)) system related to each interfering chemical species, their different concentration distribution in solution C(r,z,t) have been defined. In the case of the lactate-ElecFET based on the L-lactate oxidase enzymatic reaction (Eq. (1)), this modelling has been applied thoroughly for lactate (ε =−1), H_2O_2 (ε = +1) and pyruvate (ε = +1) and O_2 (ε = -1) in order to determine the following concentration distributions into the channel: $[S_1](r,z,t)$, $[S_2](r,z,t)$, $[P_1](r,z,t)$, and $[P_2](r,z,t)$.

2.3. Modelling of the acid-base chemical reactions in water

We are interested here primarily to the volume of reactions, namely:

$$H_2O <----> H^+ + OH^- \quad (11)$$

Thus R_i is given by the following expressions:

$$R_i = -k_f([H^+][OH^-]) + k_b[H_2O]) \quad (12)$$

with $i = H^+$ and OH^-

The forward rate constant k_f of the water protolysis reaction (11) is estimated by values taken from literature [13] while the backward rate constants k_b is obtained from where k_e denotes the water dissociation constant. As a result, equation (12) becomes:

$$R_i = k_f(k_e - [H^+][OH^-]) \quad (13)$$

It should be assumed that the buffer properties of the solution in our model are negligible versus to the water protolysis. Such assumption is required to understand ElecFET detection/transduction principles.

2.4. Modelling of the pH-ChemFET response

At last, the pH-ElecFET threshold voltage variation is related to the pH at the silicon nitride Si_3N_4 surface according to the following equation [10,11]:

$$V_T(t) = V_{T0} + s_0.(pH(0,t) - pH_{pzc}) \quad (14)$$

where s_0 is the pH-ChemFET sensitivity ($s_0 \approx 59.2$ mV/pH), pH(0,t) is the pH at the Si_3N_4 sensitive surface when the diffusion phenomena "steady state" is reached and V_{T0} is a constant parameter depending on the SiO_2/Si_3N_4 pH-ChemFET technology [12] (pH_{pzc} was estimated around 4 for Si_3N_4 [9,13]).

In the following, since the V_{T0} value is only related to the pH-ChemFET technological fabrication, it is of no influence concerning the ElecFET detection properties and it will not be taken into account, i.e. it will be chosen equal to zero [14,15].

The sensor sensitivity s_0 is finally given by the Nernst law: $s_0 = \frac{\ln(10)kT}{q} = 59.2mV/pH$

3. Results and Discussions

To optimize the geometric design of the lactate ElecFET microdevices, two-dimensional axisymmetric simulations are implemented (Figure 1). The different geometrical dimensions were therefore defined accordingly: r_e is the gate sensitive radius, d denotes the distance between the gate radius and the electrode, w is the microelectrode ring width, and e_{PVA} is the thickness of enzyme layer. The model was simulated with Femlab/Comsol Multiphysics package 4.3b. The numerical method is based on finite-element method.

The governing equations (Eq. 2, 3, 13 and 14) subject to the given boundary conditions (Eq. 4 and 8-10) are solved using COMSOL Multiphysics. The kinetic parameters and the constants used in this simulation are listed in Table 1. According to theoretical equations, the most influential parameters are the number of enzymatic units per volume unit n_{enz}, the L-lactate oxidase Michaelis constant K_M and the lactate concentration $[S_1]$. The majority of simulations have been devoted to modeling the sensor response, i.e. variation of the threshold voltage. The curves of this threshold voltage, measured in an aqueous solution at pH 7 are shown in Figures 5–7.

Table 1. Physico-chemical input values used in the simulation model.

Parameter	Value	unit
F	96.500	C/mol
D_{H3O+}	9.3×10^{-9}	m^2/s
D_{OH-}	5.3×10^{-9}	m^2/s
D_{P2}	3.1×10^{-10}	m^2/s
D_{S2}	2×10^{-9}	m^2/s
D_{S1}	4×10^{-10}	m^2/s
D_{P1}	1×10^{-9}	m^2/s
e_{PVA}	10	µm
T	300	K
pH_0	7	
z_e	0.5	µm
d	50	µm
r_e	10	µm
w	100	µm
k_f	1.5×10^{11}	L/mol/s
k_e	1×10^{-14}	mol^2/L^2
α	0.5	

3.1. Study of the main chemical species concentrations

Their influences on the lactate-ElecFET sensor detection properties have been studied on ranges appropriate with lactate detection:

- $[S_1]$ (mmol/L): (10)
- $[S_2]$ (mmol/L): (10)
- K_M (mol/L): (10^{-4})
- n_{enz} (unit/cm^3): (10^3)

Figures 3 and 4 represent typical variations of the lactate and pyruvate concentrations $[S_1](r,z,t)$ and $[P_1](r,z,t)$ near the sensor surface. They clearly show the lactate consumption as well as the pyruvate production. However, the main influence of the enzymatic reaction is given by the pH variations pH(r,z,t) in proximity to the pH-ISFET sensitive surface (Figure 4).

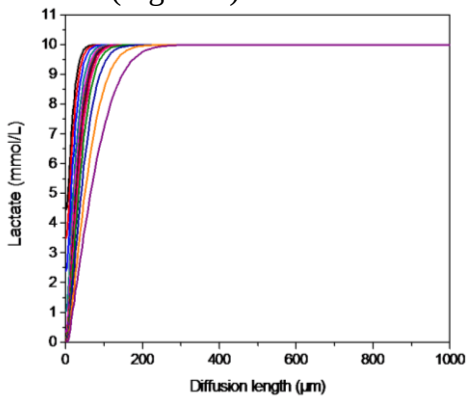

Figure 3. Lactate concentration $[S_1](r,z,t)$

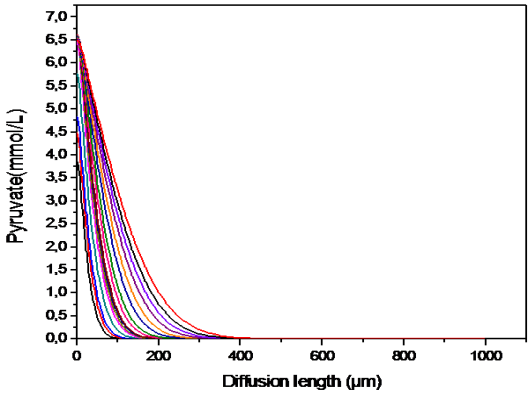

Figure 4. Pyruvate concentration $[P_2](r,z,t)$

3.2. Study of the most influential parameters

Their influences on the lactate-ElecFET sensor detection properties have been studied on ranges appropriate with lactate detection (main values are given in parentheses):

- $[S_1]$ (mmol/L): [1 to 6]
- $[S_2]$ (mmol/L): (10)
- K_M (mol/L): [10^{-4} to 10^2] (10^3)
- n_{enz} (unit/cm^3): [10^4 to 10^7] (10^3)
- $-V_p$: 0.7 V
- $-t_p$: 5 s

Figure 5a shows the threshold voltage variations V_T versus time for different lactate concentrations (V_p=0.7 V and t_p=5 s).When the lactate concentration increases, the V_T value also increases. In fact, the lactate

reaction in the presence of LOx produced hydrogen peroxide H_2O_2. For a potential V_p=0.7 V, the electrochemical molecule was oxidized on the platinum microelectrode, leading to proton H^+ release and therefore to a local pH change measured by the pH-ChemFET. When the polarization is interrupted, the lactate-ElecFET microsensor response follows a return to equilibrium of the system because of the diffusion laws. Plotting the maximal V_T value versus lactate concentrations, typical detection curves were obtained (Figure 5b). However, results enable to determine detection sensitivities about 13 mV/mM for the lactate detection in concentration range between [1–6mM]. Such result is worse than that obtained for conventional amperometry technique [16]. Nevertheless, the ElecFET detection properties can be improved by optimizing the enzymatic layer properties, the microdevice geometry and integration level, as well as the polarization conditions.

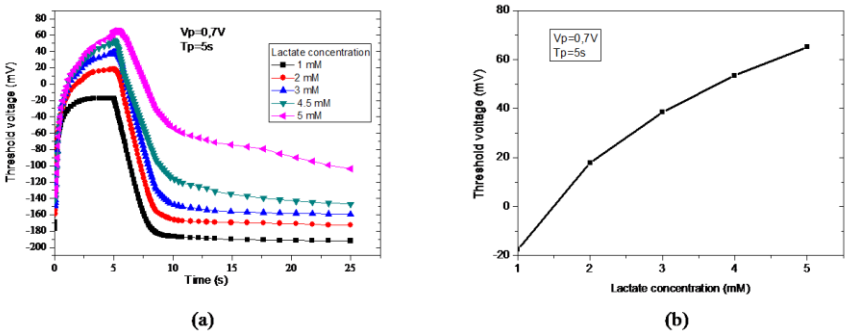

Figure 5. (a) ElecFET microsensor response versus time (V_p=0.7 V) for lactate detection, (b) ElecFET lactate detection

Figure 6 represents typical lactate-ElecFET responses with L-lactate oxidase Michaelis constant K_M. For the lowest values, saturation of the lactate detection properties is evidenced. These saturation phenomena should be related with the lactate diffusion from the electrolyte towards the PVA enzymatic layer. They also allow to define the lactate-ElecFET detection limit around 1 mmol/L. Finally, Figure 6 shows that the K_M increase is responsible for the shift of the lactate-ElecFET detection range towards the higher lactate concentration.

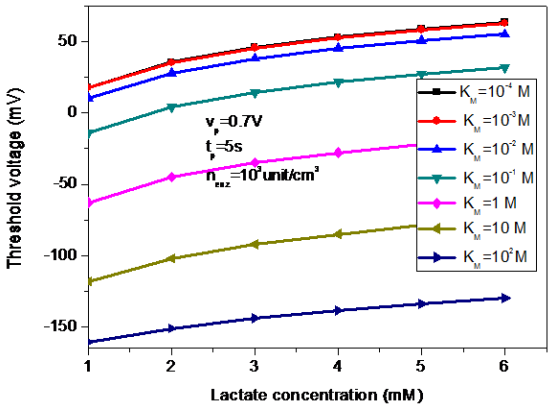

Figure 6. Lactate-ElecFET response with lactate oxidase Michaelis constant K_M.

In the same way, Figure 7 represents typical lactate-ElecFET responses with L-lactate oxidase enzymatic units per volume unit in the PVA layer n_{enz}. As n_{enz} is decreased towards the lower extreme, lactate detection properties are lost. Conversely, as n_{enz} is increased towards the highest extreme, the saturation phenomena of lactate detection are highlighted. These responses were predicted by Eq. 3.

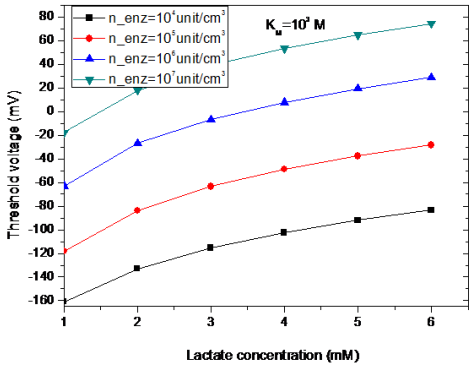

Figure 7. Lactate-ElecFET responses with number of enzymatic units per volume unit n_{enz}.

4. Conclusion

In this study, we have investigated the modeling of the ElecFET microdevice , taking into account enzymatic reactions, chemical and electrochemical. It allows a combination of the pH-ChemFET-metry technique and redox phenomena. The pH-ElecFET techniques were studied for the lactate detection in solution. The second one, related to the enzymatic production of hydrogen peroxide H_2O_2 in solution whose detection principles are based on the H_2O_2 electrochemical oxidation using an integrated platinum microelectrode in order to obtain local pH variations. The influence of the ElecFET design, i.e. the number of enzymatic units per volume unit n_{enz}, the L-lactate oxidase Michaelis constant K_M and the lactate concentration $[S_1]$. So, the obtaining of pH impulsional variations in microvolumes was clarified and the potentiometric for the lactate detection was evidenced in the [1–6mM] concentration range.

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