LOW-COST ENZYME-BASED BIOSENSOR FOR LACTIC ACID AMPEROMETRIC DETECTION

Electrical Modeling and Validation for Clinical and Food Processing Applications

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Keywords: Lactate oxidase, Amperometric detection, Low-cost, Clinical applications, Food processing applications.

Abstract: In this work we present the preliminary resulting measurements of an enzyme-based biosensor for the amperometric detection of lactic acid (LA). The sensor is based on low-cost gold electrodes on polymeric substrate. The redox catalytic enzyme used for analyte amperometric detection is lactate oxidase (LOx) from *Pediococcus* sp. This enzyme has been immobilized over electrodes surfaces by direct adsorption methodologies. Analysis of the enzyme-modified electrodes have been carried out by means of Electrochemical Impedance Spectroscopy (EIS) and with the development of an equivalent electrical model, in order to improve the adsorption process. Biosensors performance have been evaluated with Cyclic Voltammetry (CVM) measurements in different lactic acid solutions with concentrations from 1 μM up to 300 mM. The lactate sensitivity of this disposable biosensor results in about 6.24 μA mM^{-1} cm^{-2}.

1 INTRODUCTION

Lactic acid detection with low-cost devices represents a growing need both in medical and food processing applications, where large-scale screenings are required (Castillo and Gáspár, 2004).

In food processing applications, LA measurements are an easy and effective way to determine food quality because lactate is correlated with degradation processes in milk products (Palmisano, Quinto et al., 2001). Lactic acid is noticeable in biotechnology, health care, and sports medicine applications because its levels are tightly correlated with mortality of patients in shock status or during hypoxia. Normal physiological blood lactate concentrations are below 2 mM, and lactate levels exceeding 7–8 mM are usually associated with multiple organ failure (Sung, Bae et al., 2006).

The most widely used technique for LA detection is a colorimetric and chromatographic analysis, which are expensive and time-consuming due to sample pre-treatment. Conversely, both amperometric (Boujtita and Chapleau, 1996) and potentiometric (Lee, Wu et al., 2008) electrochemical analysis allow high sensitivity and fast response. Two principal electrochemical enzyme-modified sensors refer to L-lactate oxidase (LOx) and L-lactate dehydrogenase. This work investigate LOx-modified gold electrodes for LA detection. The related catalytic reaction pathway is (Gamero and Pariente, 2010):

$$\text{L-lactate} + (\text{LOx})_{OX} \rightarrow \text{pyruvate} + (\text{LOx})_{RED}$$

$$(\text{LOx})_{RED} + (\text{Med})_{OX} \rightarrow (\text{LOx})_{OX} + (\text{Med})_{RED}$$

$$(\text{Med})_{RED} \rightarrow (\text{Med})_{OX} + e^{-}$$

where ferricianyde/ferrocianyde has been used as redox mediator (Med). EIS measurements and an equivalent electrical model have been used to analyze enzyme adsorption on gold surfaces and its role on sensor efficiency.

2 DEVICE AND MEASUREMENTS SET-UP

EIS measurements have been performed with a Solartron SI1260 impedance analyzer in a frequency range between 1 Hz and 1 MHz. An Ag/AgCl in KCl 1 M (CHI111, CH Instruments Inc., US) has

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been used as reference electrode. A custom-built Labview program has been developed to drive the instrumentation and acquire measurements data. CVM measurements have been performed with a CHI 440A potentiostat (CH Instruments Inc., US) with the electrochemical cell composed by the previous Ag/AgCl electrode and a platinum counter electrode (CHI129, CH Instruments Inc., US). The potential range investigate was -0.20 V to +0.70 V for $Fe(CN)_6^{4-/3-}$ with a scan rate of 50 mV/s. All data have been analyzed with MATLAB 7.9, while fitting results have been obtained with ZVIEW 2.80 (complex fitting, data-modulus weighting) software. LOx, PBS (Phosphate Buffer Saline), redox mediators and other chemicals have all been provided by Sigma Aldrich. LOx (50 units/mg) has been obtained as lyophilized powder. All solution has been prepared with Millipore water.

The biosensor consists of circular 50 nm thick gold electrodes with 1 mm diameter and of a gold contact pad both on polymeric substrate. Electrodes have been contacted through micro-positioned probes (Wentworth Laboratories).

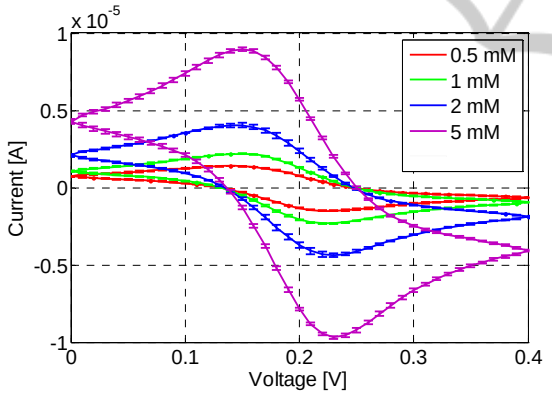

Figure 1: CVM measurements on 1 mm diameter electrodes for different redox mediator concentrations with scan rate of 50 mV/s.

3 REDOX MEDIATOR CONCENTRATION

During gold electrodes surfaces preliminary electrical characterization, EIS and CVM measurements have been performed in "fresh" condition, i.e. gold electrodes without any biological coverage. The aim of these measurements was to determine the best trade-off between SNR (Signal-to-Noise Ratio) of interfacial charge transfer and redox mediator concentration (see Figure 1). From these tests the measurement solution has been chosen as ferri/ferrocyanide 1 mM in PBS 100 mM.

4 COVERAGE STUDY AND FITTING TECHNIQUE

An equivalent electrical model has been developed to assess electrodes enzymatic coverage (see Figure 2). The main feature of this model is the *a*-weighted contribution of two different electrical impedances (Huang, Nguyen et al., 2004).

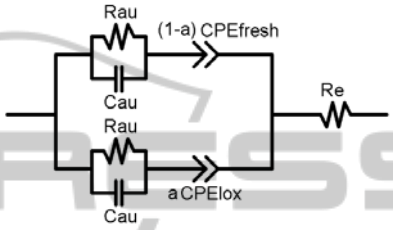

Figure 2: Equivalent electrical model for electrode coverage studies. Parameter *a* is the numerical impedance multiplier. Free electrode interface equivalent element CPE_{fresh} is multiplied for (1-*a*), while full enzyme-covered electrode impedance for *a*.

Enzyme-free electrodes have been measured with EIS in PBS and results have been fitted with an equivalent electrical model composed of a series between a Constant Phase Element CPE (Onaral, Sun et al., 1984), CPE_{fresh}, and a parallel between R_{Au} and C_{Au}: the former is related to electrochemical interfacial processes between electrodes and electrolyte, while the other takes into account the electrodes material. Impedance frequency dependence is modeled with $CPE_{fresh} = 1/C_{fresh}(j2\pi f)^{nfresh}$, where f is the frequency and n_{fresh} is a number between 0 and 1. Values for R_{Au} and C_{Au} (see Table I) have been obtained using COMSOL Multiphysics 3.4 (AC/DC In-plane electric currents module) to simulate the sensor electrical impedance (2 cm length, 50 nm thick) with 1 mm diameter active circular area and 100 μm probe contact area. Gold electrical parameters have been set to conductivity $\sigma = 4.5\ 10^7$ S/m and relative dielectric permittivity $\varepsilon_r = 6.9$. Electrolyte spreading resistance R_e value has been obtained by fitting EIS measurements of PBS. To obtain second branch model parameters values, electrodes surfaces have been full covered with 25 mg/mL LOx in PBS 100 mM solution. The immobilization protocol consisted in the deposition of 1 μL of enzyme solution directly onto the electrodes for 1 hour and then rinsed with

de-ionized water (Parra and Casero, 2006). Functionalization data have been fitted with similar equivalent circuit with CPE_{LOx}. In the complete electrical model the weighting parameter *a* is the only fitting variable. The behavior of *a* as a function of immobilized LOx concentration C_{LOx} has been obtained from EIS measurements: each curve depicted in Figure 3 has been fitted using the coverage model, obtaining *a* values described in Figure 4.

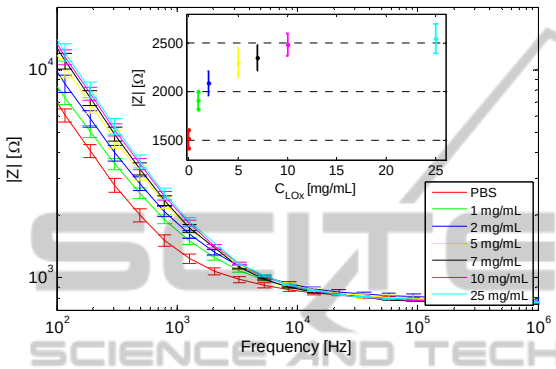

Figure 3: EIS measurements on round gold electrodes (1 mm diameter) with different concentrations of immobilized LOx. Box: EIS response |Z| at 1 kHz.

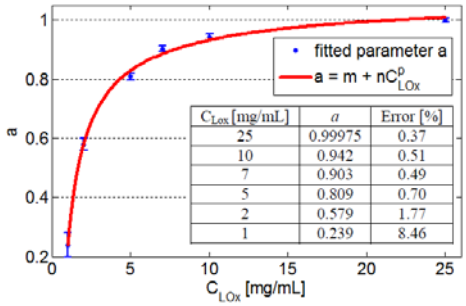

Figure 4: Variation of coverage parameter *a* as a function of enzyme concentration C_{LOx}. Fitted parameters are: m = 1.086, n = -0.7414, p = -0.8473. With the described power function the goodness of fit is $R^2 = 0.9979$.

As can be seen from Figure 5, parameter *a* trend reaches a saturation level for LOx concentrations above about 10 mg/mL, which corresponds to *a* = 0.94, therefore in these conditions an adequate electrode coverage degree is obtained. Moreover, sensors electrical response as a function of C_{LOx} has been evaluated with CVM measurements in order to assess the electric transduction efficiency. Figure 5 shows CVM reduction currents peaks with 1 mM and 100 mM LA in measurement solution: the trade-off between SNR and electrodes coverage can be obtained for C_{LOx} = 10 mg/mL.

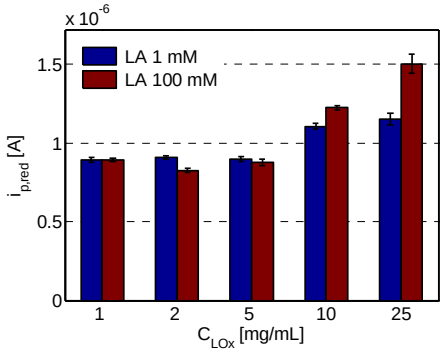

Figure 5: CVM peak currents with different concentrations of immobilized LOx and 1 mM (blue) and 100 mM (red) LA. For C_{LOx} below 10 mg/mL the solutions cannot be properly detected.

Table 1: Electrical parameters for Figure 2 model.

Parameter		Value	Error [%]
R_{Au}		0.6078 Ω	-
C_{Au}		$2.62\ 10^{-18}$ F	-
CPE_{fresh}	C_{fresh}	$4.4\ 10^{-7}$ F	1.82
	n_{fresh}	0.89784	0.35
R_e		821 Ω	0.94
CPE_{LOx}	C_{LOx}	$2.3\ 10^{-7}$ F	1.71
	n_{LOx}	0.88817	0.31

5 LACTIC ACID DETECTION

The enzymatic biosensor has been tested with LA racemic mixture at different concentrations. First 2 mm diameter gold electrodes have been used to detect LA concentration range between 70 µM up to 1.2 M in order to verify transduction saturation levels. Results of CVM measurements are depicted in Figure 6. In order to study sensor lower detection limits and sensitivity, CVM measurements have been carried out in a lower LA concentration range, i.e. down to 1 µM, with 1 mm diameter electrodes. Figure 7 depicts reduction peaks currents as a function of LA concentration and sensor linear response range comparable with other works in literature (Gamero and Pariente, 2010). The corresponding LA sensitivity is about 6.24 µA mM^{-1} cm^{-2} (Jena and Raj, 2006).

6 CONCLUSIONS

Low-cost gold electrodes-based sensors have been functionalized with immobilized lactate oxidase enzymes using direct adsorption protocols. Electrodes surface coverage has been investigated

with EIS measurements in concentration range between 1 mg/mL up to 25 mg/mL. An equivalent electrical model based on a weighted contribution approach has been developed in order to obtain a numeric parameter that is related to the electrode coverage. Both this methodology and CVM measurements have identified in 10 mg/mL the enzyme concentration that leads to adequate electrodes coverage. The sensors lactic acid detection performance have been evaluated using CVM measurements: the upper saturation levels is reached at about 300 mM, and the sensitivity is about 6.24 µA mM^{-1} cm^{-2}.

This work is part of a University of Padova research project for the development of an innovative cell-based Lab-On-Chip for clinical and food-processing massive screening applications.

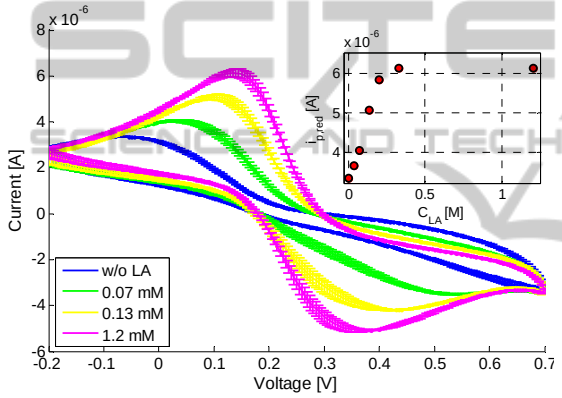

Figure 6: CVM response of 2 mm diameter gold sensors with different LA concentration in measurement solution. Box: CVM reduction peaks currents as a function of LA concentrations. The response saturates for LA concentrations above 300 mM.

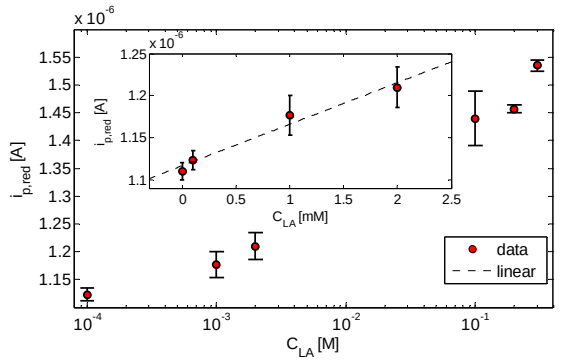

Figure 7: CVM reduction peaks currents as a function of LA concentrations for round gold electrodes (1 mm diameter). The tested LA concentrations vary from 1 µm up to 300 mM. Box: magnification in milli-molar range. Regression line: $i_{p,red} = 4.9\ 10^{-2}\ C_{LA} + 1.1\ 10^{-6}$.

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