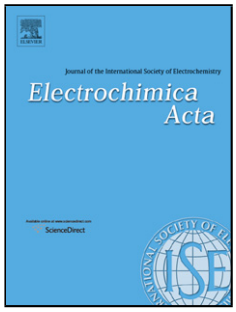

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L-lactate selective impedimetric bienzymatic biosensor based on lactate dehydrogenase and pyruvate oxidase

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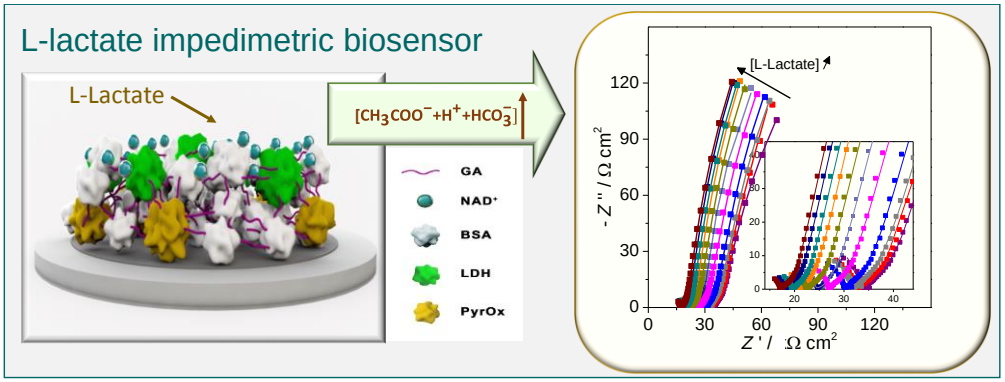
Graphical abstract



HIGHLIGHTS

- Lactate impedimetric biosensor has lactate dehydrogenase/pyruvate oxidase (LDH/PyrOx)
- L-lactate enzymatic oxidation to pyruvate, then to enzymatically generated ions
- Pyruvate oxidation changes impedance characteristics
- Resistance and charge separation at LDH/PyrOx interface for L-Lactate monitoring
- Application to analysis of food samples

Abstract

A new L-lactate selective impedimetric biosensor based on a lactate dehydrogenase (LDH)/pyruvate oxidase (PyrOx) bioselective membrane is reported, fabricated using enzyme drop coating and cross-linking via glutaraldehyde vapours. The sensing strategy is based on the measurement of the change in impedance caused by the generation of charged ions (CH_3COO^-, H^+ and HCO_3^-), from PyrOx catalysed pyruvate oxidation, the pyruvate resulting from L-lactate oxidation catalysed by LDH + nicotinamide adenine dinucleotide (NAD^+). The experimental conditions, pH and buffer concentration, were optimized for best biosensor response, and operational/storage stability and selectivity/specificity were investigated. The limit of detection for L-lactate, was 17 and 20 µM, considering changes in resistance and charge

separation parameters at and close to the LDH-NAD^+/PyrOx interface, respectively. The biosensor was successfully employed for the determination of L-lactate in yogurt samples.

Keywords: impedimetric biosensor; electrochemical impedance spectroscopy; L-lactate; lactate dehydrogenase; pyruvate oxidase.

1. Introduction

Biosensors are nowadays used in many analytical applications, ranging from medical diagnostics, food safety, process control and environmental monitoring, to defense and security [1, 2]. The development of sensitive and selective electrochemical biosensors commonly requires innovative approaches that couple different modification/amplification processes [3–6]. In this work, we report a simple, selective, and sensitive impedimetric biosensor for L-lactate detection. L-lactate is a key metabolite formed from the anaerobic metabolism of glucose in muscles, its production being accompanied by an increase in proton concentration inside the cells; if the rate of lactate production is sufficiently high, the cellular proton-buffering capacity may be exceeded, the cellular pH decreases, and this may result in cell acidosis which disrupts the performance of muscles [7]. In humans, the physiological blood concentration of lactate ranges from 0.6 to 2.0 mM but can rise rapidly up to 20–30 mM during physical activity [8]. Lactate is chiral and has two optical isomers, the L-Lactate being the predominant stereoisomer in mammals, D-lactate representing only 1–5% of the total lactate concentration. The determination of lactate concentration is very important in a variety of fields such as clinical diagnosis [9, 10], sports medicine [11, 12], and food quality analysis, in the monitoring of dairy product fermentation, determination of food freshness/preservation [13, 14].

Various approaches have been used to measure lactate concentration such as high performance liquid chromatography (HPLC) [15], ultraviolet spectrophotometry [16], and refractive index

detection [17]. However, these methods are time-consuming because of the pre-treatment required and the complicated procedure involved. Therefore, the demand for a sensitive, simple, and accurate method has arisen. Among the various analytical methods proposed for lactate determination, electrochemical biosensors offer many advantages such as rapidity, high specificity, low cost and possible miniaturization/integration into circuits to perform automatic sensing [18, 19]. Those developed up until now have been almost entirely amperometric as can be verified in [18, 19] often with the addition of nanomaterials, it being necessary to apply a positive potential to perform the fixed potential amperometry experiments.

The enzymes used in lactate biosensors are lactate oxidase (LOD) or lactate dehydrogenase (LDH). Both LOD and LDH catalyse lactate oxidation to pyruvate (Eqs. 1–2).

$$\text{L-lactate} + O_2 \overset{\text{LOD}}{\Longrightarrow} \text{Pyruvate} + H_2O_2 \quad (1)$$

$$\text{L-lactate} + NAD^+ \overset{\text{LDH}}{\Longleftrightarrow} \text{Pyruvate} + NADH + H^+ \quad (2)$$

$$\text{Pyruvate} + O_2 \overset{\text{PyrOx}}{\Longrightarrow} CH_3COO^- + H^+ + HCO_3^- + H_2O_2 \quad (3)$$

In a LDH-based biosensor, the enzyme catalyses the oxidation of lactate to pyruvate, the presence of the co-factor nicotinamide adenine dinucleotide (NAD^+) being necessary. This latter approach has some advantages, not depending on oxygen and being more selective than LOD for lactate; however, drawbacks include reagent instability [20] and the high reversibility of the reaction between lactate and pyruvic acid in the presence of NADH coenzyme. The use of a second enzyme, such as pyruvate oxidase (PyrOx), has been proposed to solve this issue, by catalysing pyruvate conversion into CH_3COO^-, H^+ and HCO_3^- (Eq. 3). The production of ions in this reaction causes a conductivity increase, which is the working principle of the biosensors developed here.

Different methods to attach enzymes onto the sensing layer have been reported, including adsorption [21], cross-linking [22], covalent attachment [23, 24], conducting polymer

entrapment [25, 26], and confinement in sol-gel matrices [27]. Among these techniques, cross-linking with glutaraldehyde (GA) is one of the simplest methods, which produces biosensors with very good stability and reproducibility [8, 25, 28, 29]. In this work, a novel and original methodology of combining LDH and PyrOx for L-lactate determination is proposed. The biosensor is prepared by drop coating LDH and NAD^+ (external layer) and PyrOx (internal layer) on the surface of screen-printed carbon ink electrodes (SPCE) using GA vapours. Incorporation of NAD^+ in the bioselective layer allows the determination of lactate without adding NAD^+ to the sample for each analysis and shows good sensitivity, stability and reproducibility [30]. The biosensor operation mechanism is based on the generation of charged ions (CH_3COO^-, H^+ and HCO_3^-) in the enzymatic layer, as a result of L-lactate oxidation via LDH/PyrOx catalysed reactions, which consequently produces changes in the impedance of the assembly, and is monitored by electrochemical impedance spectroscopy (EIS). The dependence of the developed biosensor output signals on pH and buffer concentration as well as biosensor operational/storage stability and selectivity/specificity were investigated. The LDH-NAD^+/PyrOx biosensors were used for L-lactate determination in yogurt samples and the results were compared to those from a commercial assay. The developed impedimetric biosensor can significantly simplify and improve the monitoring of L-lactate (LA) in food, in the pharmaceutical industry, and in biotechnology.

2. Experimental

2.1. Reagents and solutions

L-lactate dehydrogenase (LDH, EC 1.1.1.27, 90 U mg^{-1} solid extracted from *E. Coli*), pyruvate oxidase (PyrOx, EC 1.2.3.3, 35 U mg^{-1} lyophilized powder extracted from *Aerococcus* sp.), bovine serum albumin (BSA) (Vfraction), 50% aqueous solution of glutaraldehyde (GA) and L(+)-lactic acid (90%), ethanol, glucose, lactose, fructose, urea and uric acid were from Sigma-

Aldrich Chemie (Germany); glycerol (>99%) was from Acros Organics. Other non-organic compounds were of analytical grade.

Measurements were done in Tris-HCl buffer solution. Stock solutions of L-lactate as well as of interference test substances were prepared in 1 mM Tris-HCl buffer (pH 7.4) and stored at 4 ºC until use. LDH-BSA-NAD^+-glycerol and PyrOx-BSA-glycerol solutions used to modify working electrodes were stored at −20 ºC when not in use.

2.2. Instrumentation and methods

Working electrodes were screen printed carbon ink electrodes (SPCE) (Dropsens, Spain, geometric area 0.126 cm^2), and served as substrates for biosensor construction. The auxiliary electrode was a platinum wire and the reference electrode was Ag/AgCl (3.0 M KCl).

Electrochemical impedance spectroscopy (EIS) experiments were carried out with a potentiostat/galvanostat/ZRA, (Gamry Instruments, Reference 600, USA). An rms perturbation of 10 mV was applied over the frequency range 65 kHz to 0.1 Hz, with 10 frequency values per decade at 0.0 V vs. Ag/AgCl. Fitting of the spectra to equivalent electrical circuits was performed with ZView 2.4 (Scribner, USA) software.

The pH measurements were carried out with a CRISON 2001 micropH-meter (Crison Instruments SA, Barcelona, Spain) at room temperature. All experiments were carried out at room temperature (25±1 ºC).

2.3. Preparation of the biosensors

Before use, the transducers were cleaned with ultrapure water and ethanol. A volume of 2 µL of an enzyme solution of 1% (m/v) PyrOx in 20 mM Tris-HCl buffer pH 7.4 with 5% (m/v) BSA and 10% (m/v) glycerol was deposited on the working electrode, and was allowed to dry for 30 min at room temperature. Then, a second layer was deposited, following the same

protocol, in which the enzyme solution contained 5% m/v LDH + 5.0 mM NAD^+ instead of 1% PyrOx (Fig. 1). The amount of NAD^+ was in large excess in order to avoid any effects of concentration depletion. BSA, a lysine-rich protein with no enzymatic activity, was used as cross-linking co-reagent to help forming LDH and PyrOx bioactive matrices and to protect both enzymes from excessive reaction with GA, which might substantially alter their activity [28]. Glycerol helped achieve homogeneity of the enzyme/BSA solutions and limited enzyme activity loss during storage at −20 ºC. After deposition of the enzymes, the sensors were placed for 30 min in a saturated GA vapour atmosphere, then were dried at room temperature for 1 h to obtain LDH-NAD^+/PyrOx biosensors. The biosensors were kept dry at 4 ºC after preparation, reconditioned in 1 mM Tris-HCl buffer for at least 30 min before the first measurement and afterwards stored in 20 mM Tris-HCl buffer pH 7.4 at 4 ºC.

3. Results and discussion

EIS is a powerful method for analysing the complex electrical properties of a system, and is very sensitive to surface phenomena. Impedance spectra can be analysed with an equivalent electrical circuit to extract the values of the electrical circuit components, which model each interfacial phenomenon. Impedance spectra at LDH-NAD^+/PyrOx/SPCE biosensors were recorded in 1 mM Tris-HCl, pH 7.4 at 0.0 V vs. Ag/AgCl, this potential being chosen from cyclic voltammograms, as that which showed the most significant increase in current upon addition of L-Lactate (data not shown), displayed in the complex plane plots in Fig. 1a together with those at bare SPCE. As seen in Fig 1a, modification of the electrodes with the bioselective layers LDH-NAD^+/PyrOx leads to a significant decrease of the magnitude of the impedance.

The impedance spectra were fitted to the equivalent circuit presented in Fig. 1b. The first element, R_Ω, is the cell resistance, R_{IM} represents the resistance at the interface between the LDH and PyrOx layers and within the PyrOx layer where ions are generated, Z_W is the diffusional

Warburg element representing diffusion of pyruvate from the LDH layer to the PyrOx layer, CPE_{IM} and CPE_{MS} are constant phase elements representing the charge separation at the LDH/PyrOx layer interface and at the LDH membrane/solution interface, respectively. The $CPE = [(Ci\omega)^{\alpha}]^{-1}$, is modelled as a non-ideal capacitor, due to the porosity and non-homogeneity of the layers and interfaces, with $0.5<\alpha<1$. The Warburg element, resulting from the equation $Z_W=R_D\text{cth}[(\tau i\omega)^{\alpha}]\times(\tau i\omega)^{-\alpha}$, where $\alpha<0.5$, is characterized by a diffusional time constant (τ), a diffusional pseudocapacitance (C_D) and a diffusion resistance ($R_D = \tau/C_D$) [31]. The cell resistance value, R_Ω, measured from the impedance spectrum as the intercept with the real part, remains nearly unchanged upon enzymatic modification and substrate addition.

The elements that exhibited a linear dependence with the L-lactate concentration were the high-frequency parameters i.e. the semi-circle diameter, R_{IM}, and CPE_{IM}, which decreased and increased, respectively, due to charged species' generation in the enzymatic reactions at the LDH/PyrOx layer interface. The circuit elements used to fit the lower frequency range of the spectra, Z_w and CPE_{MS}, did not exhibit a linear dependence on L-lactate concentration, as would be expected, and were not used for analytical purposes.

3.1. Optimization of working conditions for L-lactate determination

The pH and Tris-HCl buffer molar concentration are parameters that play an important role both on PyrOx and LDH activities and on the efficiency of their sequential action. Therefore, the influence of these parameters on the biosensor response was studied first. The LDH and PyrOx concentrations were kept constant in the bioselective layer at 5% and 1% (m/v), respectively, which corresponds to an amount of 100 and 20 µg deposited on the electrode substrate, respectively, with theoretical activities of 9 and 0.7 U; the NAD^+ concentration was 5 mM. Biosensor steady-state responses were recorded at pH values ranging from 6.0 to 8.5 for a L-lactate concentration of 0.2 mM. Fig. 2a shows the R_{IM} values plotted vs pH and, as

observed, the optimal pH for the biosensor is close to 8.0. For further work pH 7.4 was chosen, since it is very close to the optimal pH and corresponds to the normal physiological pH value.

A change in concentration of buffer solution automatically results in a change of ionic strength and buffer capacity, which can affect the impedimetric measurements [32]. The concentration of Tris-HCl buffer was varied between 1.0 and 10.0 mM and the change in the relative R_{IM} values is displayed in Fig. 2b. Below 1 mM Tris-HCl concentration the measurements were not sufficiently reproducible, which can be partly attributed to too low a buffer capacity. As seen, the biosensor response decreases significantly as the Tris-HCl buffer solution concentration becomes higher, as would be expected. The electrical changes at the interface due to proton generation in the enzyme reaction are less significant for high concentrations of the buffer solution, due to its increased buffer capacity. Since the highest signals for lactate were obtained for a concentration of 1.0 mM Tris-HCl, this concentration was chosen for further experiments.

3.2. Analytical parameters of lactate biosensors

Impedance spectra recorded in 1.0 mM Tris-HCl, pH 7.4 at SPCE based biosensors are shown as complex plane plots in Fig. 3a, for L-lactate concentrations of 0 up to 400 µM. The values of the equivalent circuit parameters after fitting the impedance spectra to the appropriate equivalent circuits are given in Table 1.

As already mentioned, the circuit elements Z_w and CPE_{MS} did not exhibit a linear dependence on L-lactate concentration. The Z_w values decrease considerably after the first LA injection, from 376 to 20.2 Ω cm^2, having a value of ~55 Ω cm^2 for higher LA concentrations. The diffusional time constants increased from ~1 ms to ~3 ms for LA higher than 100 µM and the α_2 values decreased from 0.45 to ~0.23 for LA concentrations higher than 10 µM, probably due to non-uniformity inside the enzymatic layer in the presence of LA, some of the active sites of the enzyme being occupied by the substrate. CPE_{MS} values increased initially in the presence

of low LA concentrations, from 193 to 232 (100 µM), decreasing afterwards for higher LA concentrations, down to 137 µF cm^{-2} $s^{\alpha-1}$ (350 µM).

The addition of L-lactate leads to a decrease in the high-frequency semi-circle diameter, R_{IM}, and to an increase in the CPE_{IM} value and, since both variations were linearly dependent on the increase in L-lactate concentration, calibration curves for lactate determination were constructed considering both R_{IM} and CPE_{IM}. The biosensors, Fig. 3b, exhibited linear ranges for both R_{IM} and CPE_{IM} between 0.01 and 0.25 mM LA, with the equations R_{IM} (Ω cm^2) = 62.7 – 220.1*[LA] (R^2 = 0.996) and CPE_{IM} (µF cm^{-2} $s^{\alpha-1}$) = 0.2 + 0.98*[LA] (R^2 = 0.995). The detection limits, calculated as three times the signal to noise ratio, were 17 and 20 µM, considering the R_{IM} and CPE_{IM} calibration curves, respectively.

There have been many reports of amperometric biosensors for L-lactate as can be seen from the reviews in [18, 19]. The more sensitive ones have detection limits similar to those obtained here, especially with the incorporation of nanomaterials in the modified electrode architecture, but it is normally necessary to apply a positive potential to maximise the response that can increase the influence of interferents. The impedimetric biosensor developed here operates at 0.0 V, which could be an important advantage.

Useful comparison can be made with non-enzymatic impedimetric sensors for polyols, including lactate, based on boronate-substituted polyaniline films used a sensing strategy based on the conductivity increase during the course of polymer self-doping. In these cases, there is a decrease in the value of the polymer film resistance upon increasing the L-lactate concentration [33, 34]. A conductometric sensor based on flavocytochrome b_2 or L-lactate: cytochrome *c* oxidoreductase immobilized together with $K_3Fe(CN)_6$ in the membrane layer by using GA vapours had a linear range from 0.1 up to 15 mM, with a much higher detection limit of 0.1 mM and comparable sensitivity of ≈ 350 µS cm^{-2} mM^{-1} [35]. In the case of a bi-enzymatic conductometric biosensor, having the enzymes L-lactate oxidase together with horseradish

peroxidase co-immobilized in GA vapours, the linear range was up to 0.2 mM with a detection limit of 0.5 µM and a sensitivity of 770 µS cm^{-2} mM^{-1} [36].

The impedance spectra for L-lactate determinations reported above were recorded over the frequency range 65 kHz down to 0.1 Hz (5 min experiment), but since only the high frequency part of the spectra is needed to complete the high frequency semicircle for the construction of the calibration plots, i.e. from 65 to 0.5 kHz, the measurement time can be reduced to less than 1 min.

3.3. Storage, operational stability and "memory effect"

To investigate the storage stability of the lactate SPCE biosensor, impedance spectra were recorded at the three biosensors every 3-4 days over a one-month period using a 0.2 mM L-lactate solution, see results in Fig. 4a. When not in use, the modified electrodes were stored at 4 ºC in 20 mM Tris-HCl buffer pH 7.4. As observed, there is a loss of 5% of the signal during the first 3 days of storage, followed by a period of 15-20 days of constant signal. After 20 days, the signal begins to decrease linearly having 75% of the initial signal at the end of one month.

In order to assess the operational stability of the LDH-NAD^+/PyrOx/SPCE biosensors, three biosensors were tested 13 times during 48 h, see Fig. 4b. Repetitive measurements (n=14) show a standard deviation of 3.1 %.

To investigate the "memory effect" of the LDH-NAD^+/PyrOx/SPCE biosensor, EIS measurements were done for L-lactate concentrations in the range from 0.1 to 0.9 mM. After each of these, spectra were recorded in blank solutions, after different periods of immersion in buffer solution from 3 min upwards in steps of 3 min. It was found that 15 min immersion time is required to eliminate the "memory effect" of the biosensor at the highest lactate concentration tested of 0.9 mM.

3.4. Selectivity

A number of compounds were tested as potential interferents for L-lactate determination in food products and serum samples: glucose, fructose, lactose, urea, uric acid and ascorbic acid and a mixture of all of them. The fact that the impedance spectra are recorded at 0.0 V vs Ag/AgCl represents an important advantage in minimising interference effects. The study was performed by first recording the spectrum for 0.2 mM L-lactate followed by mixtures of L-lactate with one of the interfering compounds in a concentration ratio of 1:1 L-lactate/interferent. As depicted in Fig. 5, glucose, fructose and lactose did not affect the biosensor signal while urea and uric acid decreased it slightly to 93 and 94% respectively; ascorbic acid had the greatest effect on the biosensor signal, decreasing to 85% in its presence. Finally, a mixture of all interferent compounds was tested and the biosensor response was 78% in the presence of all compounds.

3.5. Determination of L-lactate in yogurt samples

The use of the LDH-NAD^+/PyrOx/SPCE biosensor was further evaluated for the determination of lactate in yogurt samples. Prior dilution of the samples was necessary for the concentration in the sample to fall within the linear range of the biosensor and enable buffering of the sample pH at a suitable value. A mass of 2.25 g of yogurt was diluted in 20 mL of 1 mM Tris-HCl buffer pH 7.4. The samples were than centrifuged for 10 min at 8000 rpm and were ready for use. The standard addition method is useful to analyse complex samples when the matrix effect can be substantial and was applied to the determination of L-lactate concentration in a plain and in a probiotic yogurt. The standard addition procedure consisted in injecting a chosen volume of the prepared food sample followed by four equal injections of standard solution. The determined concentration of L-lactate and the recovery factors of the biosensor are shown in Table 2. It was found that the plain yogurt contained 812±5 mg L-lactate per 100 g of product while the probiotic one had a slightly higher content of L-lactate of 923 ±6 mg per 100 g of

product, in agreement with [37]. The successful determination of L-Lactate in complex matrices with good recovery values, underlines the applicability of the newly developed impedimetric biosensor for food quality analysis or clinical diagnosis.

4. Conclusions

New impedimetric LDH-NAD^+/PyrOx biosensors have been developed on screen printed carbon ink electrode substrates. Electrochemical impedance spectroscopy enabled the evaluation of analytical parameters of the developed biosensors, under the optimized experimental conditions of 1 mM Tris-HCl buffer pH 7.4. The values of resistance at the interface between the two enzyme layers, R_{IM}, and the corresponding capacitance, represented as CPE_{IM}, enabled the construction of linear calibration plots for LDH-NAD^+/PyrOx/SPCE biosensors, with a close to 1.0 correlation coefficient, the detection limits being 17 and 20 µM, for R_{IM} and CPE_{IM}, respectively. The biosensors exhibited a very high operational and storage stability, and the high selectivity towards interferent compounds makes them suitable for the determination of L-lactate in food samples, demonstrated by its successful determination using the standard addition method in yogurt samples. The excellent recovery factors demonstrate the applicability of the device for the detection of L-lactate in complex samples.

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FIGURE CAPTIONS

Fig. 1. a) Complex plane impedance spectra recorded in 0.1 mM Tris-HCl, pH 7.4, at 0.0 V vs Ag/AgCl at SPCE before and after LDH-NAD^+/PyrOx enzyme layer deposition; the lines represent equivalent circuit fitting; b) equivalent circuit used to fit impedance spectra.

Fig. 2. Influence of Tris-HCl a) pH and b) concentration on LDH-NAD^+/PyrOx/SPCE biosensor response to 0.2 mM LA; measurements were performed in a) 1 mM Tris-HCl and b) pH 7.4 Tris-HCl buffer, at 0.0 V vs Ag/AgCl.

Fig. 3. a) Complex plane impedance spectra recorded in 1 mM Tris-HCl, pH 7.4, at 0.0 V vs Ag/AgCl at LDH-NAD^+/PyrOx/SPCE for concentrations of L-lactate of 0, 10, 25, 50, 100, 150, 200, 250, 300, 350 and 400 µM; the lines represent equivalent circuit fitting; b) Calibration curves for LDH-NAD^+/PyrOx/SPCE.

Fig. 4 a) Storage stability and b) operational stability of LDH-NAD^+/PyrOx/SPCE biosensors; measurements were performed using a 0.2 mM solution of LA in Tris-HCl buffer pH 7.4, at 0.0 V vs. Ag/AgCl.

Fig. 5. Interferent effect on LDH-NAD^+/PyrOx/SPCE biosensor recorded in 1mM Tris-HCl buffer solution, pH 7.4, applied potential 0.0 V in the presence of interfering compounds; [LA] = 0.1 mM in a 1:1 ratio of interferent:LA.

Scheme 1. Schematic representation of LDH-NAD^+/PyrOx biosensor

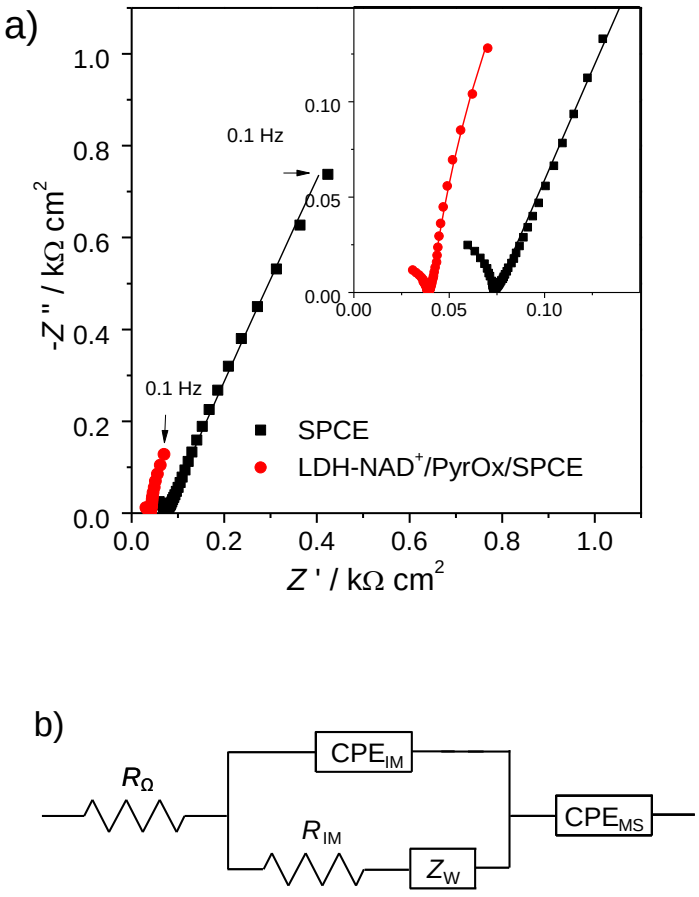


Fig. 1. a) Complex plane impedance spectra recorded in 0.1 mM Tris-HCl, pH 7.4, at 0.0 V vs Ag/AgCl at SPCE before and after LDH-NAD$^+$/PyrOx enzyme layer deposition; the lines represent equivalent circuit fitting; b) equivalent circuit used to fit impedance spectra.

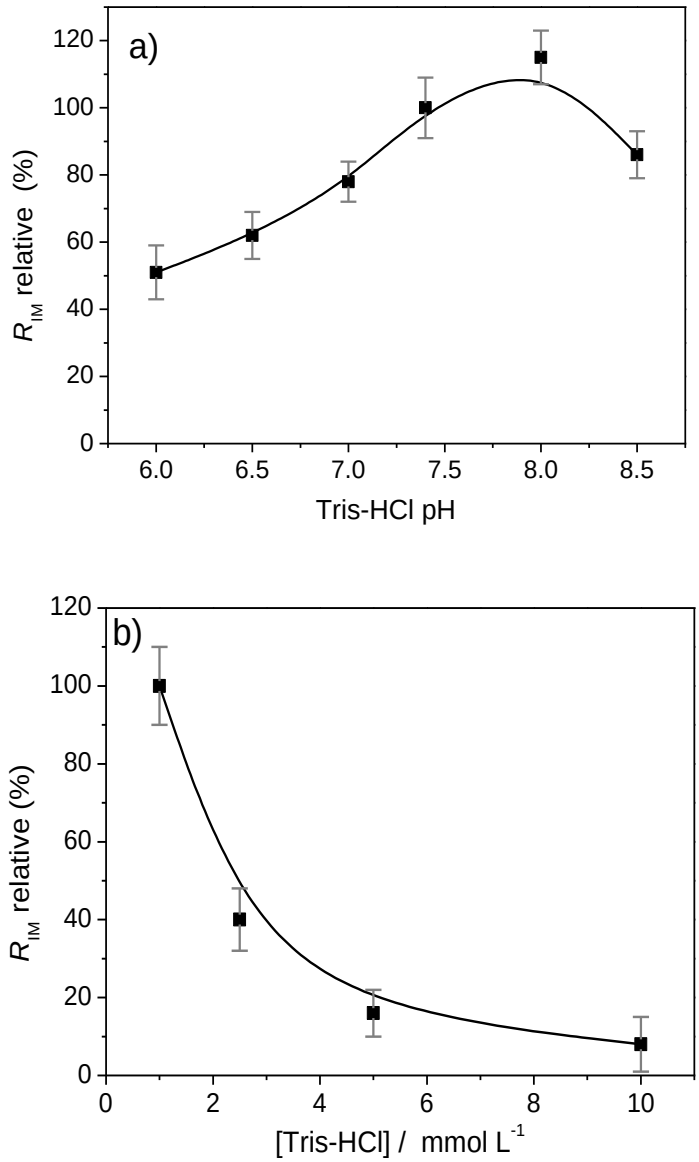


Fig. 2. Influence of Tris-HCl a) pH and b) concentration on LDH-NAD^+/PyrOx/SPCE biosensor response to 0.2 mM LA; measurements were performed in a) 1 mM Tris-HCl and b) pH 7.4 Tris-HCl buffer, at 0.0 V vs Ag/AgCl.

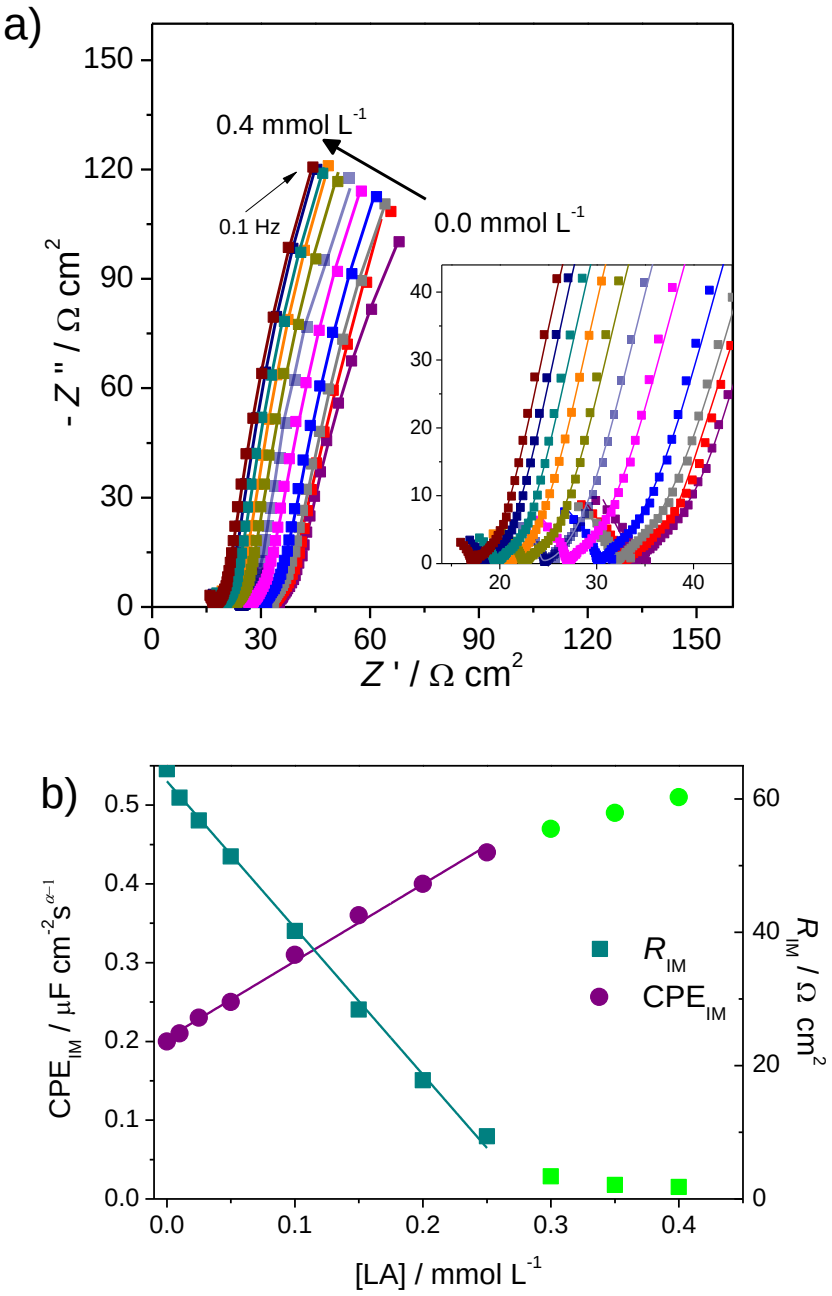


Fig. 3. a) Complex plane impedance spectra recorded in 1 mM Tris-HCl, pH 7.4, at 0.0 V vs Ag/AgCl at LDH-NAD$^+$/PyrOx/SPCE for concentrations of L-lactate of 0, 10, 25, 50, 100, 150, 200, 250, 300, 350 and 400 μM; the lines represent equivalent circuit fitting; b) Calibration curves for LDH-NAD$^+$/PyrOx/SPCE.

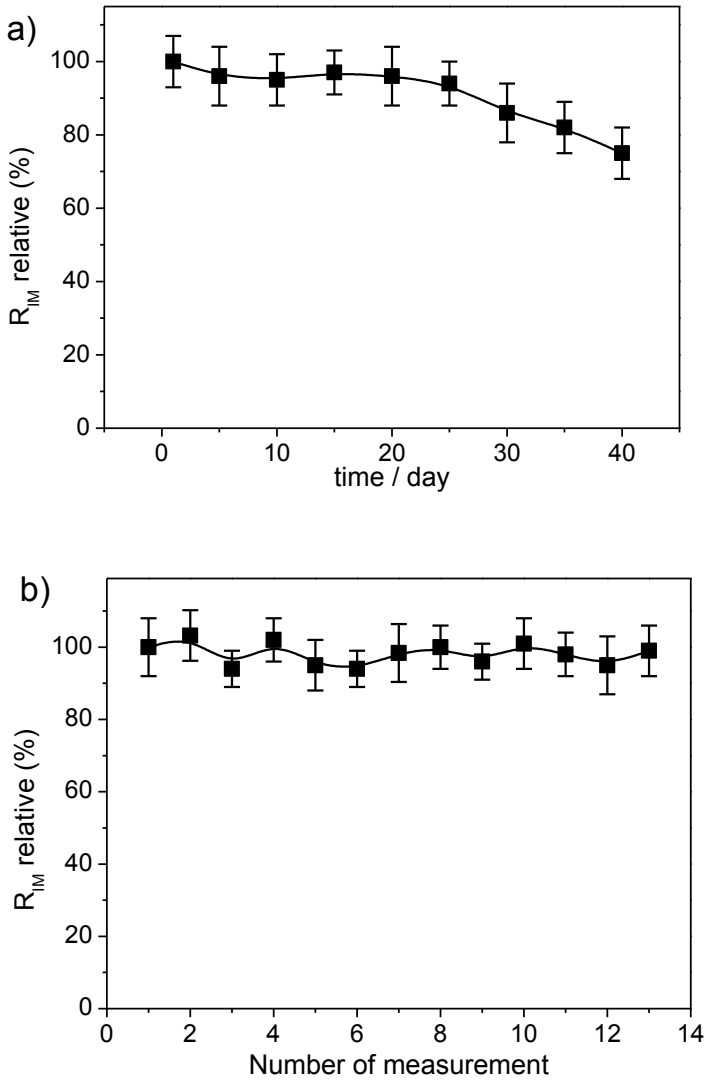


Fig. 4 a) Storage stability and b) operational stability of LDH-NAD^+/PyrOx/SPCE biosensors; measurements were performed using a 0.2 mM solution of LA in 1 mM Tris-HCl buffer pH 7.4, at 0.0 V vs, Ag/AgCl.

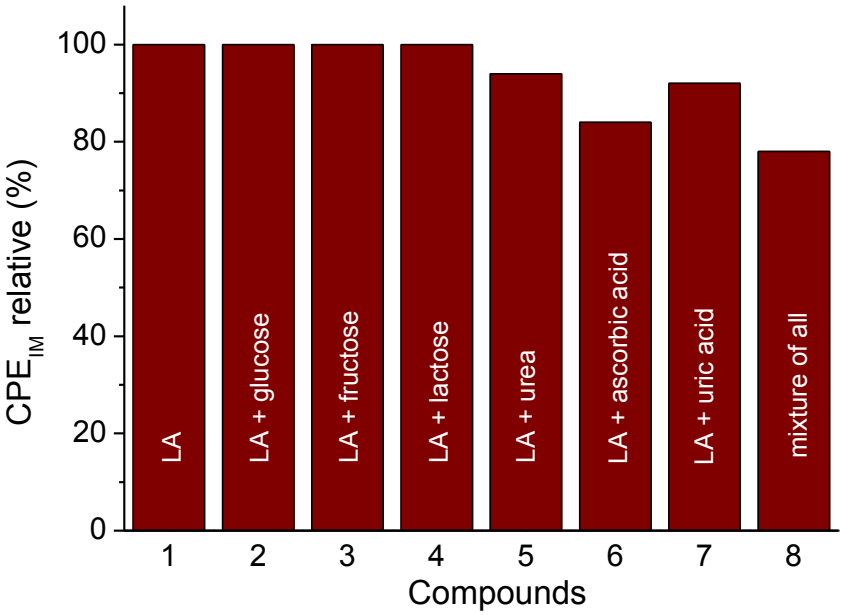
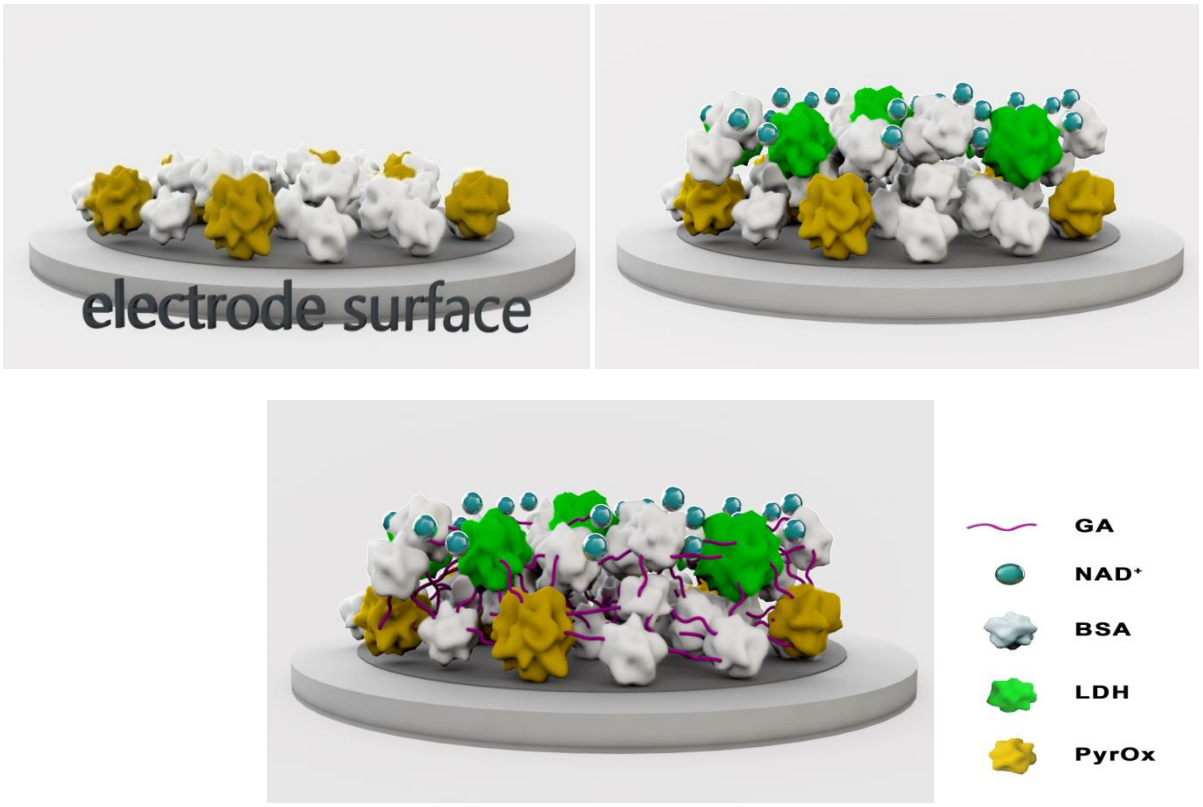


Fig. 5. Interferent effect on LDH-NAD$^+$/PyrOx/SPCE biosensor recorded in 1 mM Tris-HCl buffer solution, pH 7.4, applied potential 0.0 V in the presence of interfering compounds; [LA] = 0.1 mM in a 1:1 ratio of interferent:LA.



Scheme 1. Schematic representation of LDH-NAD^+/PyrOx biosensor

TABLES

Table 1. Equivalent circuit component values for SPCE lactate biosensors extracted by fitting the spectra in Fig. 3 with the equivalent circuit in Fig. 1b.

[LA] / μM	CPE_{IM} / μF cm^{-2} $s^{\alpha-1}$	α_1	R_{IM} / Ω cm^2	Z_W / Ω cm^2	τ / ms	α_2	CPE_{IM} / μF cm^{-2} $s^{\alpha-1}$	α_3
0	0.20	0.80	64.5	376	1.0	0.45	193	0.94
10	0.21	0.81	60.2	20.2	0.8	0.47	196	0.80
25	0.23	0.82	56.8	61.1	1.1	0.26	199	1.0
50	0.25	0.96	51.4	61.3	0.9	0.25	213	1.0
100	0.31	0.98	40.2	51.2	2.6	0.24	232	1.0
150	0.36	0.99	28.4	55.3	2.8	0.23	149	1.0
200	0.40	0.97	17.8	45.9	3.0	0.22	144	1.0
250	0.44	1.00	9.4	53.9	2.8	0.23	143	1.0
300	0.47	0.97	3.4	50.4	3.3	0.22	139	1.0
350	0.49	1.00	2.1	55.3	2.6	0.24	137	1.0
400	0.51	1.00	1.8	56.8	3.3	0.22	319	1.0

Table 2. Determination of L-lactate in yogurt food samples

Sample	Added / μM	Found / μM	Recovery (%)
Plain yogurt	0	100.0 ±2.3	--
	50	146.1±8.7	97.4
	100	198.0±11.0	99.0
	150	250.2±14.0	100.1
	200	302.2±16.0	107.3
Probiotic fruit yogurt	0	117.5±3.3	--
	50	176.3±9.0	105.6
	100	215.1±10.2	99.1
	150	274.8±15.3	102.9
	200	313.6±18.4	98.9