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Application of lactate amperometric sol–gel biosensor to sequential injection determination of L-lactate

Sofia Piedade Gomes [a], Martina Odložilíková [b], M. Gabriela Almeida [c], Alberto N. Araújo [a], Cristina M.C.M. Couto [a,d,*], M. Conceição B.S.M. Montenegro [a]

[a] REQUIMTE, Departamento de Química-Física, Faculdade de Farmácia, Universidade do Porto, Rua Anibal Cunha, 164, 4099-030 Porto, Portugal
[b] ZENTIVA. a. s., U Kabelovny 130, 102 37 Prague, Czech Republic
[c] REQUIMTE, Departamento de Química, Faculdade de Ciências e Tecnologia, Universidade Nova de Lisboa, 2829-516 Monte da Caparica, Portugal
[d] ISCS-Norte-Rua Central da Gandra-1317, GRD Paredes, Portugal

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Abstract

This work describes the construction and evaluation of lactate sol–gel biosensors to accomplish the determination of lactate in pharmaceutical products. Lactate oxidase was incorporated in a porous sol–gel film placed onto a platinum-based electrode. Acid and basic catalysis were assessed. When coupled to a sequential injection system (SIA) the biosensor, based on (3-aminopropyl)trimethoxysilane, 2-(3,4-epoxycyclohexyl)ethyl-trimethoxysilane, deionised water, polyethylene glycol 6000 and acid catalyst, presented a range of linearity of 5×10^{-5} to 5×10^{-3} M. The analytical usefulness of the developed biosensor was evaluated through analysis of commercial pharmaceutical products containing lactate with a sampling rate of 40 samples h^{-1}. The enzyme remained active for at least 30 days, enabling about 700 determinations without sensitivity decrease.
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Keywords: LOx amperometric biosensor; Sol–gel; SIA; Pharmaceutical products

1. Introduction

Measurement of lactate using biosensors is gaining importance for food analysis [1–6] since it is very useful for assessing the freshness and stability of milk, dairy products, fruits, vegetables, sausages and wines. Its clinical determination [1–3,5–8] is helpful in the monitoring of respiratory insufficiency, shocks, heart failure and metabolic disorders. Lactate quantification is also valuable in the quality control of pharmaceuticals containing this compound.

The demand for prompt, reliable and continuous control of chemical species in all analytical fields has evolved the need for small, easy to handle and inexpensive analytical sensors. Therefore, there is currently a large interest in the development of biochemical-specific methods that can be used to monitor the concentration of several metabolites, especially by enzyme immobilization. Distinct strategies have been reported, such as covalent binding, encapsulation within a polymer, physical adsorption, entrapment into semi-permeable membranes, or cross-linking to a suitable supporting matrix [1,9]. Recently, the use of silicate glasses obtained by the sol–gel method for biomolecules immobilization has attracted considerable research. In this context, the immobilization of enzymes at the surface of electrodes with amperometric transduction is highlighted by sol–gel technology [1,2,9–15]. Sol–gel derived biocomposites provide a series of significant advantages relative to the former immobilization schemes, such as low temperature and mild chemical processing conditions yielding rigid and inert materials. The final characteristics of the membranes can

Abbreviations: LOx, lactate oxidase; SIA, sequential injection analysis; 3-APTMS, (3-aminopropyl)trimethoxysilane; EETMS, 2-(3,4-epoxycyclohexyl)ethyl-trimethoxysilane; PEG, polyethylene glycol; HC, holding coil; RV, multiposition valve; P, peristaltic pump; Pt, platinum; K_m, intrinsic Michaelis–Menten constant; K'_m, apparent Michaelis–Menten constant; sat., saturated; R.S.D., relative standard deviation

* Corresponding author. Tel.: +351 22 2087132; fax: +351 22 2004427.
E-mail address: couto_cristina@hotmail.com (C.M.C.M. Couto).

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be tailored using a wide range of starting monomer species thus allowing the entrapment of a large number of different biomolecules. Such materials also tend to preserve enzymatic activity, initially and during long-term storage, and in some cases can lead to an improvement of stability for labile biomolecules [9,10].

Lactate oxidase (LOx) from *Pediococcus* sp. was the biological element of the constructed biosensors. LOx is an unstable type of flavin enzyme that acts as catalyst in the oxidation of lactate to pyruvate. Different immobilization techniques, such as through glutaraldehyde coupling onto polyaniline-co-fluoroaniline film [7], Prussian blue co-immobilization in a Nafion layer [3], entrapment in cationic polyelectrolyte complexes [16], physical inclusion in a graphite–Teflon matrix [4], cross-linking with tetrathiafulvalene-tetracyanoquinodimethane charge transfer complex [8] and entrapment in an osmium-based three-dimensional redox hydrogel [17] were previously adopted for the development of LOx based biosensing devices. Despite the great variety of strategies, limited research has been carried out on LOx immobilization in sol–gel matrices [2,14,15].

In this work, the immobilization of LOx in sol–gel films, cast on a platinum support, was carried out. The biosensors characteristics were evaluated concerning catalytic activity, robustness and stability. Their analytical usefulness was also studied by incorporating them into a sequential-injection system enabling the determination of lactate on pharmaceutical products. Resorting to this automatic system, easier samples and reagents solutions manipulation, high sampling rates and cleaner determination procedures were achieved.

2. Materials and methods

2.1. Reagents and solutions

Deionised water (conductivity lower than $0.1\,\mu S\,cm^{-1}$) was used in the preparation of all solutions. Analytical grade chemicals were used without any additional chemical purification. Reagents used in the preparation of sol–gel biosensors were (3-aminopropyl)trimethoxysilane (Fluka), 2-(3,4-epoxycyclohexyl)ethyl-trimethoxy-silane (Fluka), polyethylene glycol 6000 (Fluka) and graphite <20 μm (Sigma).

The buffer solution used in the biosensors evaluation consisted of $H_2PO_4^-/HPO_4^{2-}$ (pH 7.4; 0.1M). This solution was also used as carrier in the developed sequential injection system (SIA).

Lactate oxidase from *Pediococcus* sp. lyophilized was obtained from Sigma.

The lactate standard solutions used in the characterization of the biosensors were prepared by rigorous weighing of the solid DL-lactic acid sodium salt (Fluka) and by subsequent dissolution in the buffer.

The enzyme solution used for biosensors construction was prepared by suitable dissolution of a LOx vial content with potassium phosphate buffer, until the enzymatic activity was 39 units mL^{-1}.

2.2. Apparatus, electrodes and sequential injection system

All electrochemical measurements were carried out using a potentiostat (PGSTAT10 Echochemie/Autolab) controlled by GPES v.4.8 software. For general batch enzymatic studies, a Metrohm 663 VA Stand was used, consisting of a platinum disk (3 mm diameter) with a sol–gel film working electrode, an Ag/AgCl, KCl (sat.) reference electrode and a glassy carbon auxiliary electrode.

For pH measurements, a Metrohm ES20 potentiometer was used with a combined glass electrode of the same brand.

The developed SIA system (Fig. 1) comprised a multiposition 8-port fluid selecting valve (RV) from Valco Instruments (Houston, USA), model Cheminert™ C15-3118E, a 4-channel Gilson Minipuls 3 peristaltic pump (P) (Villiers-le-Bel, France) for the intercalation of sample and buffer solutions and a flow cell (wall-jet 656 VA Metrohm Stand electrochemical detector) with three electrodes—lactate sol–gel sensor (working electrode, WE), Ag/AgCl, KCl saturated (reference electrode, RE) and Au (auxiliary electrode, AE). The SIA manifold involved PTFE connection tubing (0.8 mm i.d.). The holding-coil (HC) between the peristaltic pump and the rotatory valve was 400 cm long and was coiled over a plastic net. A PC using the homemade software developed in Quick Basic 4.5 language controlled the multiposition valve, the timing of the different steps composing the analytical cycle and the movements of the peristaltic pump.

Firstly, holding coil (HC) and conveyance paths between multiposition valve (RV) and the detection system were filled with the carrier solution by selecting port 8 and positioning the peristaltic pump (P) in the propulsion mode. This configuration was kept until the achievement of a stable current baseline. Sample analyses were performed by aspirating solutions (90 μL for 15 s) from the first port of RV into the HC of the sequential injection manifold. Then the direction of the carrier stream was reversed and a sample zone was propelled towards the detector for 75 s at a $0.5\,mL\,min^{-1}$ flow rate.

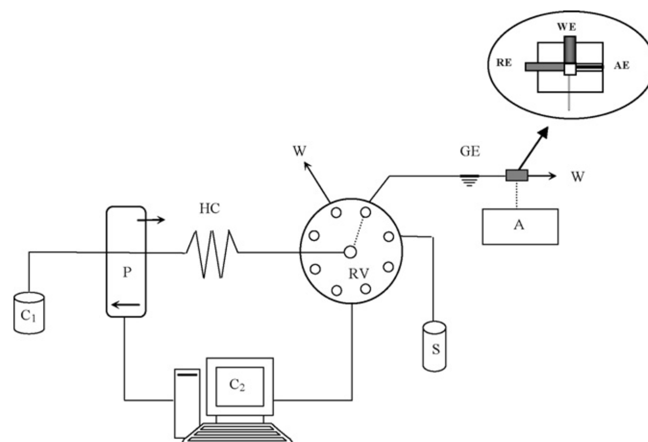

Fig. 1. Scheme of SIA manifold used for the determination of L-lactate. C1, carrier solution (phosphate buffer 0.1 M, pH 7.4); P, peristaltic pump; RV, rotatory valve; HC, holding coil; GE, ground electrode; RE, reference electrode; WE, working electrode; AE, auxiliary electrode; C_2, computer; A, autolab; S, sample; W, waste.

2.3. Sample preparation

Lactacyd® liquido samples were prepared by dilution of 2.500 g up to 100.0 mL with phosphate buffer solution and subsequent mechanical stirring until a homogeneous solution was obtained. After centrifugation, the supernatant was filtered. A volume of 1.00 mL of the filtrate was diluted up to 10.00 mL with the phosphate buffer solution and then introduced into the system. Concerning Calmurid® creme, an aliquot of approximately 500.0 mg of Calmurid® creme was diluted to 100.00 mL with buffer solution. Then the mixture was stirred, centrifuged and then filtered. A volume of 1.00 mL of the filtrate was once more diluted to 10.00 mL with the same buffer solution and introduced into the system. The dilution of each sample was established according to its initial lactate labeled content, in order to obtain a final concentration that fitted in the linear analytical range of the developed biosensors ($\approx 3.00 \times 10^{-4}$ M).

The results were confirmed by using the European Pharmacopoeia method [18]. Lactacyd® liquido and Calmurid® creme samples were prepared firstly by dilution of 1.000 g of Lactacyd® liquido or Calmurid® creme with 10.00 mL of water and 20.00 mL of NaOH 1 M, then the mixture was set aside for 30 min. After this time, the mixture was titrated with HCl 1 M in the presence of 0.5 mL of phenolphthalein R solution until disappearance of the pink colour.

2.4. Membrane preparation and electrode construction

For the biosensor construction an electrode body made from Teflon containing a platinum base with a recessed depth of approximately 1 mm was used. The top of a platinum disk (3 mm diameter) was exposed to the solution.

Before use, the platinum was manually polished with diamond spray Kemet® and polishing paper Kemet® and finally washed with deionised water.

The sol–gel membrane was prepared using an optimum concentration of (3-aminopropyl)trimethoxysilane (3-APTMS) (70 μL), 2-(3,4-epoxycyclohexyl)ethyl-trimethoxysilane (EETMS) (20 μL), deionised water (700 μL), polyethylene glycol 6000 (PEG 6000) (700 μL) and a catalyst. As acid catalyst, an HCl 0.1 M (7 μL) solution was used, according to previous work [12]. Basic catalysis was also studied resorting to a tetramethylammonium hydroxide ∼25% (v/v) in water solution (7 μL), to enable the basic environment. Three different formulations were tested, two of them using the acid catalyst (types A and C) and one using the basic one (type B). Graphite powder (2 mg mL^{-1}, <20 μm) was incorporated in type C formulation.

The components of the sol–gel membrane were mixed thoroughly by vortexing for 3 min and ultrasonicating for 10 min. Then, 15 μL of the homogenized solution was placed on the platinum base of the electrode body. Sol aging took place at room temperature for approximately 60 min. Before gelation was complete, 14 μL of enzyme solution (39 units of enzyme per millilitre) were added to the layer of the aged sol and left for a 24 h aging period at room temperature.

The electrodes were washed with phosphate buffer (pH 7.4; 0.1 M) and stored in the same buffer at 4 °C, when not in use.

3. Results and discussion

3.1. Biosensor characterization

The sol–gel process involves the development of an inorganic matrix through the formation of a colloidal suspension (sol) and gelation of the sol to form a wet gel, which upon drying forms a xerogel [10,19]. The properties of the porous sol–gel matrix formed, namely the substrate and oxygen diffusion within the matrix and its porous structure, are affected by variables, such as pH, Si:H_2O ratio and enzyme concentration. The main precursors selected for gelation were 3-APTMS and EETMS, according to results previously described in the literature [11,12], since the obtained gel was found to be suitable for enzyme entrapment and strongly attached to Pt surfaces. PEG 6000 was included in the formulations since it affects the sol–gel membrane porosity and resistance to cracking, increases the wetted area and also reduces the interaction between enzyme and surface silanol groups of the forming sol–gel membrane [11–13]. In order to optimize the LOx sol–gel immobilization procedure different formulations were prepared using the same starting monomers, PEG 6000 and Si:H_2O ratio, but varying the catalyst type (acid/basic catalyst) and adding graphite powder as matrix modifier.

The electrochemical activity of the constructed LOx biosensors was verified by cyclic voltammetry and amperometry in batch conditions with 15.0 mL phosphate buffer (pH 7.4; 0.1 M) in absence and presence of different lactate concentrations. By cyclic voltammetry the potential was scanned from 0 to +1 V versus Ag/AgCl, KCl (sat.) with scan rates between 0.050 and 0.500 V s^{-1}. These studies were compared with a control electrode prepared in the same way but without the enzyme addition. There was an increase in the anodic current (Fig. 2) corresponding to the electrochemical oxidation of hydrogen peroxide produced in lactate catalytic oxidation. Performing the cyclic voltammetry at different scan rates (from 0.050 to 0.500 V s^{-1}), a linear correlation between the scan rate and peak current intensity (I_p) was observed ($R^2 = 0.991$), suggesting a typical adsorption-controlled electrochemical process [20,21].

The process of immobilizing an enzyme usually disturbs the microenvironment of its active site as revealed by the modification of the enzyme kinetic characteristics. Therefore, it is common to observe a shift from the intrinsic Michaelis–Menten constant of a soluble enzyme (K_m) to an apparent Michaelis–Menten constant (K'_m). This difference might be the result of either microenvironment charge–charge interactions between substrate and enzyme or environmental (electrostatic or hydrophobic) constraints imposed by the supporting polymer on the enzyme structure [22]. Catalytic activity might also be biased by limitations of mass transport, which happen when substrate or product transport rate superimpose the catalytic reaction rate. After setting the working potential of the biosensor versus Ag/AgCl, KCl (sat.) reference electrode to 0.700 V, K'_m values and biosensor lifetimes (corre-

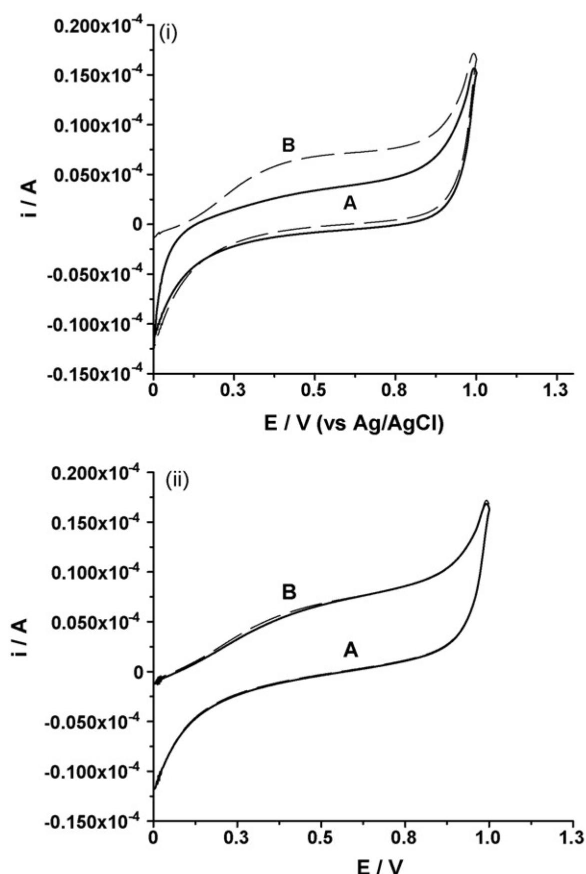

Fig. 2. Cyclic voltammograms of type A biosensor and the same type of biosensor without enzyme solution. (i) Cyclic voltammograms of type A biosensor with a scan rate of 0.050 V s^{-1}. (A) In absence of lactate solution; (B) 1000 μL of lactate solution 1×10^{-2} M. (ii) Cyclic voltammograms of the biosensor without enzyme solution with a scan rate of 0.050 V s^{-1}. (A) In absence of lactate solution; (B) 1000 μL of lactate solution 1×10^{-2} M.

sponding to irreproducibility, reduction or absence of catalytic activity) were evaluated (Table 1). Lineweaver–Burk plots of $1/i_{ss} = (K'_m/i_{max})(1/C) + 1/i_{max}$, where i_{ss} is the current measured for enzymatic product detection under steady state, i_{max} the current measured for enzymatic product detection under substrate saturation conditions and C is the lactate concentration, revealed straight lines for lactate concentrations above 1.0×10^{-3} M. For lower concentrations, deviations from linearity were obtained for all the developed biosensors (Fig. 3), even under strong stirring conditions. This effect became less important after soaking the biosensors in buffer solution for 11 days (Fig. 3b), instead of 5 days (Fig. 3a), suggesting that due to the hydrophobic character of the starting monomers, especially the EETMS, membrane hydration is a slow process. Thus, after suitable bioelectrode

Table 1
Kinetic characteristics of sol–gel based lactate sensors

	14 μL LOx solution	Type A	Type B	Type C
Life time	Hours	30 days	7 days	14 days
K'_m (mM)	1.13	1.26	8.21	6.22

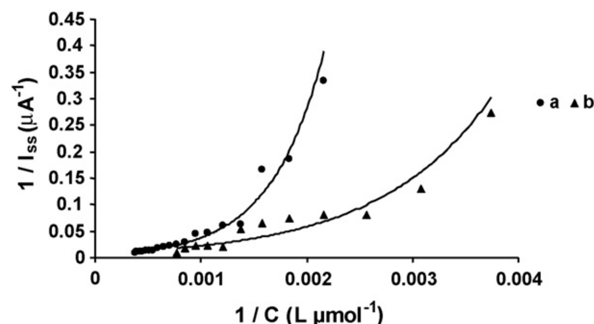

Fig. 3. Lineweaver–Burk plots obtained for type A biosensor. (a) After a 5 days soaking period in the buffer solution; (b) after an 11 days soaking period in the same solution.

soaking, linearity above 3.4×10^{-4} M ($1/C \leq 0.0027$ L μmol^{-1}) of lactate could be obtained.

The type of catalyst affects the enzyme immobilization. In acid catalysis conditions the sol–gel process leads to randomly branched and linear-like polymers with small pores while in basic catalysis sol–gel results in highly branched clusters, with larger pores that facilitate the substrate entry [19]. When comparing the different constructed biosensors (Table 1), one may conclude that the basic catalysis rendered the biosensors membranes with lower affinity for lactate (higher K'_m), shorter lifetime and that gave irreproducible results (not shown). A possible explanation is that the enzyme addition to the basic aged sol might have caused some LOx denaturation. Despite this, a higher sensitivity was observed due to the increased pore size obtained with this catalysis. Membrane A, obtained by acidic synthesis of the sol–gel membrane, only led to a slightly increased K'_m value when compared to values obtained for the enzyme assays in solution. This membrane also showed a longer operational time. Although some data in the literature suggested that the presence of graphite powder facilitates the electron transfer within the sol–gel matrix and increases the electrochemical active area and capacitive current [11], the K'_m value obtained by type C biosensors was higher than the solution K'_m. The apparent lower LOx affinity for the substrate might be explained by the presence of an interfering substance (graphite) or by diffusion problems. If graphite powder acted as a diffusion barrier for O_2, lactate or H_2O_2, the intensity of the oxidation current would be lower and thus, a higher K'_m value would be expected.

Due to its lower K'_m value and longer lifetime, type A biosensor was chosen for analytical application (Fig. 4). A mean period of 50 s was observed between each lactate addition and the achievement of the corresponding steady state signal.

3.2. SIA system optimization

For analytical assays the developed biosensor was coupled to a conventional SIA system (Fig. 1) using phosphate buffer, pH 7.4, as carrier solution. Some flow parameters were optimized before lactate analytical determinations, namely the influence of the hydrodynamic parameters on the peak height, reproducibility and accuracy of the results.

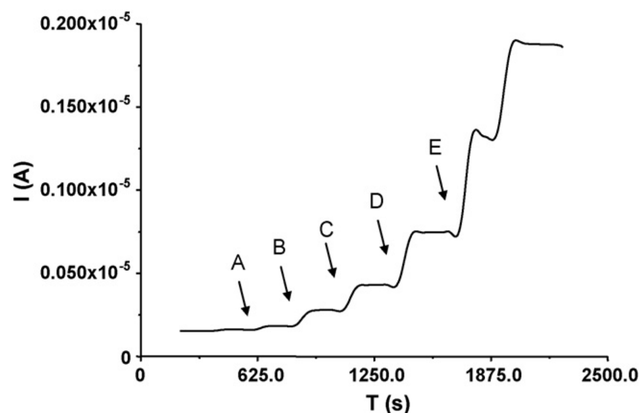

Fig. 4. Current–time recording obtained at type A biosensor after the addition of successive aliquots (A, 100 μL; B, 100 μL; C, 200 μL; D, 400 μL; E, 800 μL) of a 0.011 M lactate solution.

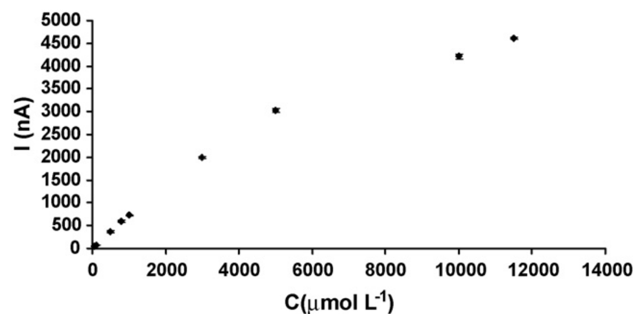

Fig. 5. Calibration curve of type A lactate biosensor.

For a lactate concentration of 5×10^{-3} M, the temperature of the solutions was varied between 20 and 42 °C. No significant variations were observed in the analytical signal in the range from 25 to 42 °C. As such, it was decided to work at room temperature (25 °C). The pH effect was studied between 6.2 and 7.4, using phosphate buffer as carrier solution. Once again no significant differences in the response of the biosensor were observed. Therefore, a pH of 7.4 was chosen for subsequent work. Ionic strength was evaluated in terms of the carrier solution concentration (potassium phosphate buffer). An optimum response was obtained for a 0.1 M concentration.

The flow rate influence was evaluated by measuring the height and area of the peaks obtained between 0.075 and 0.93 mL min^{-1}. It was observed that the amperometric response decreased with the increase of the flow rate. This behaviour is a consequence of the contact time of the sample zone with the surface of the biosensor. Slower flow rates allow a longer interaction between the substrate and the enzyme. Consequently, a more intense amperometric response might be obtained. The selection of the optimum flow rate value was a compromise between sensitivity, reproducibility and buffer consumption. Therefore, the flow rate of 0.45 mL min^{-1} was selected for the measurement step.

After flow rate optimization, injection volume was determined. The effect of the injected sample volume was investigated by setting different sampling times using a previously defined aspirating rate (0.36 mL min^{-1}) and directing it towards the detector. Increasing the injection volume up to 89.9 μL (sampling time of 15 s), the analytical signal increased. Larger volumes (longer times) negligibly influenced the peaks height but increased the peaks width. Thus, sampling time was set to 15 s as a compromise between reproducibility and sample consumption. The biosensor response showed a typical Michaelis–Menten behaviour (Fig. 5). The response increased up to the concentration of 1×10^{-2} M, beyond which it remained practically constant. The linear response obtained ranged from 5×10^{-5} to 5×10^{-3} M, with a detection limit of 1×10^{-5} M. Successive calibrations were performed obtaining the following equation signal I (nA) = 38(±16) + 177(±0.03)C (μmol L^{-1}).

Experiments were conducted to evaluate the biosensor stability over time. The variation in analytical signal obtained from the injection of a 5×10^{-3} M lactate standard solution was verified during a period greater than 30 days corresponding to approximately 800 injections. The amperometric signal was stable for a period of about 30 days (2.18×10^{-6} to 2.51×10^{-6} A), decreasing afterwards. Thus, the biosensors constructed permitted more than 700 substrate determinations to be carried out being easily recycled at the end of their useful life.

3.3. Interference studies

Substances tested as potential interferents for lactate in pharmaceuticals were: glucose, fructose, acetic acid, uric acid, ascorbic acid, citric acid, galactose and urea. The effect of the presence of these substances on lactate steady-state amperometric response was checked out for four different concentration levels of the potential interferent compound between 1×10^{-4} and 5×10^{-2} M. None of them, except ascorbic acid and uric acid, affected the biosensor response. Since these substances are not present in the studied formulations, they cannot be considered interferents.

3.4. Analytical applications

In order to evaluate the analytical usefulness of the sequential injection system with amperometric detection, analysis of lactate in some pharmaceutical products were carried out. Each sample (Calmurid® creme and Lactacyd® liquido) was mea-

Table 2
Determination of lactate in commercial products using the proposed SIA amperometric method

Product	L-Lactate concentration (%)[a]	L-Lactate concentration (%) reference method[a]	Recovery test (%)
Calmurid creme	5.66 ± 1.20	5.79 ± 1.22	98.9 ± 0.44
Lactacyd liquido	1.54 ± 0.44	1.86 ± 0.17	99.0 ± 0.48

[a] Average (%) ± standard deviation (n = 4).

sured in quadruplicate (Table 2). The lactate concentration was calculated by linear interpolation. Lactate label concentration values on Calmurid® creme (5%) and Lactacyd® liquido (1%) were confirmed by the obtained results and also by using the European Pharmacopoeia method [18]. The high relative standard deviation values obtained with both the proposed procedure and reference method were due to the high viscosity of the matrices of both samples, making it difficult to reproducibly extract L-lactate.

Recovery studies were also performed by adding 1 mL of a 1.00×10^{-3} M lactate solution to 1 mL of each sample. Mean recoveries obtained are also presented in Table 2 and emphasize the good accuracy of the results provided by the proposed procedure.

4. Conclusions

The undertaken study shows that in comparison with the basic one, acid catalysis enables the development of LOx biosensors with enhanced characteristics. This improvement is associated with an increased lifetime (more than 700 measurements per month) and stability.

The developed biosensor, with the best characteristics, exhibited an increased linear response range when compared with other sol–gel biosensors previously described in literature. The possibility of its incorporation in a sequential-injection system allowed high sampling rates with low reagent consumption and good precision. For all these reasons, the proposed bioanalytical system provides the basis for the construction of a simple, low cost and stable lactate biosensor.

Acknowledgements

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References

[1] B.D. Malhotra, A. Chaubey, Sens. Actuators B 91 (2003) 117–127.
[2] B. Lillis, C. Grogan, H. Berney, W.A. Lane, Sens. Actuators B 68 (2000) 109–114.
[3] R. Garjonyte, Y. Yigzaw, R. Meskys, A. Malinauskas, L. Gorton, Sens. Actuators B 79 (2001) 33–38.
[4] B. Serra, A.J. Reviejo, C. Parrado, J.M. Pingarrón, Biosens. Bioelectron. 14 (1999) 505–513.
[5] M.M.F. Choi, Food Chem. 92 (2005) 575–581.
[6] N.G. Patel, A. Erlenkotter, K. Cammann, G.C. Chemnitius, Sens. Actuators B 67 (2000) 134–141.
[7] S. Suman, R. Singhal, A.L. Sharma, B.D. Malthotra, C.S. Pundir, Sens. Actuators B 107 (2005) 768–772.
[8] S.A.M. Marzouk, V.V. Cosofret, R.P. Buck, H. Yang, W.E. Cascio, S.S.M. Hassan, Anal. Chem. 69 (1997) 2646–2652.
[9] W. Jin, J.D. Brennan, Anal. Chim. Acta 461 (2002) 1–36.
[10] O. Lev, M. Tsionsky, L. Rabinovich, V. Glezer, S. Sampath, I. Pankratov, J. Gun, Anal. Chem. 67 (1995) 22A–30A.
[11] P.C. Pandey, S. Upadhyay, H.C. Pathak, I. Tiwari, V.S. Tripathi, Electroanalysis 11 (1999) 1251–1258.
[12] C.M.C.M. Couto, A.N. Araújo, M.C.B.S.M. Montenegro, J. Rohwedder, I. Raimundo, C. Pasquini, Talanta 56 (2002) 997–1003.
[13] P.C.A. Jerónimo, A.N. Araújo, M.C.B.S.M. Montenegro, D. Satinsky, P. Solich, The Analyst 130 (2005) 1190–1197.
[14] T. Park, E.I. Iwuoha, M.R. Smyth, R. Freaney, A.J. McShane, Talanta 44 (1997) 973–978.
[15] J.A. Cox, P.M. Hensley, C.L. Loch, Microchim. Acta 142 (2003) 1–5.
[16] V.G. Gavalas, N.A. Chaniotakis, Mikrochim. Acta 136 (2001) 211–215.
[17] T.J. Ohara, R. Rajagopalan, A. Heller, Anal. Chem. 66 (1994) 2451–2457.
[18] European Pharmacopoeia 5.0, European Directorate for the Quality of Medicines, Council of Europe, Strasbourg, France, 15 June 2004.
[19] C.J. Brinker, G.W. Scherer, Sol–Gel Science: The Physics and Chemistry of Sol–Gel Processing, Academic Press USA, New York, USA, 1990.
[20] A.M.O. Brett, C.M.A. Brett, Electrochemistry: Principles Methods and Applications, Oxford University Press, New York, USA, 1993.
[21] J. Zen, T. Yu, Y. Shih, Talanta 50 (1999) 635–640.
[22] R.A. Kamin, G.S. Wilson, Anal. Chem. 52 (1980) 1198–1205.