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Design and optimization of a lactate amperometric biosensor based on lactate oxidase cross-linked with polymeric matrixes

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Abstract

The design and characterization of a lactate biosensor is described. The biosensor is developed through the immobilization of lactate oxidase (LOD) in an albumin and mucin composed hydrogel. The enzyme is then cross-linked with glutaraldehyde to the polymeric matrix and entrapped between two polycarbonate membranes. The hydrogen peroxide produced by the reaction of lactate and LOD is detected on a Pt electrode operated at 0.65 V versus Ag|AgCl. The performance of the biosensor was evaluated in matrixes with different amounts of albumin, mucin and glutaraldehyde. The response time of the sensor to 10 μM lactate required 90 s to give a 100% steady-state response of 0.079 μA. Linear behavior was obtained for 0.7 μM < c_{Lac} < 1.5 mM. The detection limit calculated from the signal to noise ratio was 0.7 μM. Only 0.1 U of enzyme was necessary to get a biosensor with a relatively high current flow and an excellent stability over a storage period of 30 days. High reproducibility in the response was obtained when several biosensors were prepared with the same composition.

Keywords: Biosensor; Electrochemical sensor; Enzyme electrode; L-Lactate; Lactate oxidase; Mucin; Albumin

1. Introduction

During the past four decades there has been increasing research on the development of lactate biosensors mainly because of the association of lactate with several severe clinical conditions. Elevated blood lactate concentration can predict multiple organ failure and death of patient with septic shock. Lactic acidosis is known to accompany decreased tissue oxygenation, left ventricular failure, and drug toxicity [1]. Analysis of lactate in saliva can be used as a preliminary diagnostic for cystic fibrosis. Gauging blood lactate is also relevant for the results of exercise and athletic performance.

In addition to the relevance in clinical diagnosis, the determination of lactate is very important in other areas such as fermentation and food analysis [2].

Most lactate amperometric biosensors reported in literature are based on immobilized lactate dehydrogenase (LDH) or lactate oxidase (LOD) [3–10]. In the latter configuration, LOD catalyzes the conversion of lactate to pyruvate and hydrogen peroxide, which can be oxidized at the electrode surface, according to the following reactions:

$$\text{l-lactate} + \text{LOD}_{ox} \rightarrow \text{pyruvate} + \text{LOD}_{red} \quad (1)$$

$$\text{LOD}_{red} + \text{O}_2 \rightarrow \text{LOD}_{ox} + \text{H}_2\text{O}_2 \quad (2)$$

$$\text{H}_2\text{O}_2 \rightarrow \text{O}_2 + 2\text{e}^- \quad (3)$$

Two of the major problems that concern to most biosensors, not only those related to lactate, are operational and storage stability as well as sensor-to-sensor reproducibility. The stability is of great importance for the success of these devices as analytical instruments, and is mainly dependent on the lifetime, or the rate of denaturation or inactivation, of the enzyme employed [2,10,11]. The use of novel immobilization methods [8,9,12–16] or media [17–21] is usually employed in order to enhance the enzyme stability by optimizing the surrounding microenvironment. The sensor-to-sensor reproducibility is also a major problem concerning the reliability of these devices. This issue is rarely addressed [22,23], and when relevant data are presented the numbers are rather discouraging [10,24–26].

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Therefore, immobilization of enzyme on electrode surface is a key step for the construction and performance of biosensors. It worth to mention that one of the best analyte detection limit was achieved for a sol–gel enzyme electrodes that immobilizes the enzyme into an albumin matrix [27,28]. Albumin is well characterized globular protein has several amino groups that could be linked to other species by using bi-functional molecules such as glutaraldehyde [29,30].

Recently, biomaterials such as mucin and chitosan have attracted increasing attention for immobilizing enzymes through the formation of polyelectrolyte complexes between the enzymes and polysaccharide chains [29–32]. These polymers form hydrogels that stabilize the three-dimensional structure of the enzyme by allowing for a sufficient amount of water to be present and by mimicking cytoplasm or cytosol properties [27].

With regards to mucin, it corresponds in fact to a group of proteins that are major components of the mucous that coats the surface of cells lining the respiratory and digestive tracts [33]. They have polypeptide chains with domains rich in threonine and/or serine whose hydroxyl groups are in *O*-glycosidic linkage with oligosaccharides [33]. These extracellular glycoproteins consist in a proteic core surrounded by diverse polysaccharide groups which molecular weight ranges from 0.5 to 20 MDa. Irrespectively of their size, approximately the 80% of the molecular mass corresponds to oligosaccharide chains, providing suitable environment to stabilize the three-dimensional structure of enzymes [34,35].

In this study, albumin–mucin hydrogel is employed as LOD host matrix. The enzyme is cross-linked to the matrix with glutaraldehyde and entrapped between two polycarbonate membranes. The effect of different parameters related to the composition of the proposed biosensor are presented and discussed. Finally, characteristics related to the storage stability and the sensor-to-sensor reproducibility are analyzed for the resulting lactate biosensor prototype.

2. Experimental

2.1. Reagents

All solutions were prepared with ultra pure water (18 MΩ cm^{-1}) from a Millipore Milli-Q system. The base electrolyte solution (0.11 M) consisted in 0.01 M HK_2PO_4/H_2KPO_4 and 0.1 M KNO_3 (all from Merck). This solution was renewed weekly and small amounts of H_2SO_4 (Baker) or KOH (Merck) were used to fix it at pH 7.0. A stock solution of 0.01 M lactate (Sigma) was prepared in the base electrolyte. Different mother solutions of glutaraldehyde (Sigma) were also made in the base electrolyte. 50 U LOD from Pediococus species (Sigma) were diluted in 1000 μL of the base electrolyte. After dissolution, aliquots of 20 μL of enzyme were separated into 50 eppendorf and saved at −20 °C. Thus, every aliquot bears 1 U of LOD. Mucin (Sigma) was powdered and saved as a dry powder at 4 °C. Bovine serum albumin (Sigma) was used as received. All solutions were stored at 4 °C. All chemical reagents were of analytical grade and used as received.

2.2. Apparatus

All electrochemical experiments were performed with an Autolab PGSTAT30 Electrochemical Analyzer (Eco Chemie, Netherlands). The measurements were carried out using a conventional three-electrode system with a Pt wire as the counter electrode, an Ag|AgCl|KCl (3 M) as the reference electrode, and a homemade 3 mm diameter Pt disc as the working electrode. The working electrode was made from a Pt rod fixed into a 10 mm diameter Teflon cylinder. Amperometric detection was obtained under batch conditions with stirring of 120 rpm at a desired working potential after a preconditioning time of 2000 s.

2.3. Preparation of the enzymatic electrode

A total mass of 6.0 mg composed by different amounts of mucin and albumin was dissolved in 40 μL of base electrolyte. Proteins were mixed during 3 min until a colloidal suspension was obtained, and then transferred into an eppendorf containing 1 U of LOD. The 60 μL of the LOD-matrix system were then mixed during 3 min and saved at 4 °C.

To prepare the enzymatic electrode, aliquots of 6 μL of the LOD-matrix system and 3 μL of glutaraldehyde were entrapped between two membranes of polycarbonate that were previously wet with buffer solution pH 7. After this, the enzymatic matrix was placed on the working electrode surface and fixed to it with a suitable cap. After waiting 5 min, buffer solution was added to stop the cross-linkage of the system.

3. Results and discussion

3.1. pH-effect

Fig. 1 shows normalized enzyme electrode responses in 1.0 mM lactate solutions. The LOD-matrix system was made with a hydrogel composed by 100% of mucin cross-linked

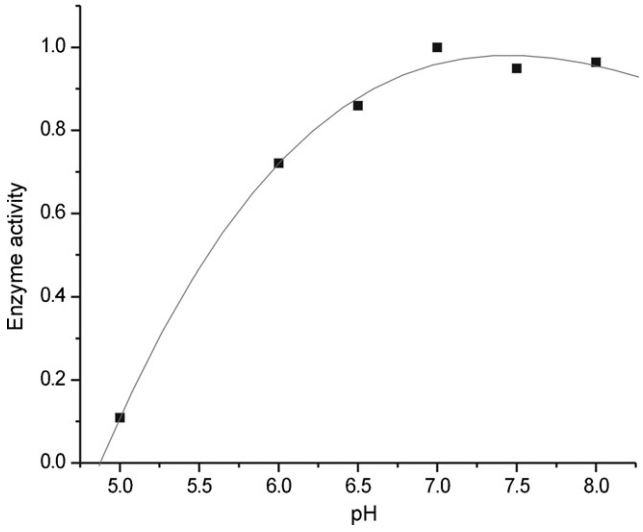

Fig. 1. Normalized electrochemical response of the enzymatic electrode to 1.0 mM lactate solutions at different pH values. LOD-matrix system: mucin and LOD cross-linked with glutaraldehyde (1%, v/v).