UNIVERSIDAD DE BURGOS
ESCUELA DE DOCTORADO

***"PLAN DE INVESTIGACIÓN"*[1]**

Programa de Doctorado: Electroquímica, ciencia y tecnología

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Plan de investigación (Título): Screen printing of electrochemical biosensors on flexible substrates. Development of electronic prototype devices.

Fecha de presentación:

Justificación del tema objeto de estudio:

Diagnosis of various diseases and monitoring analytes are of extreme importance, once some of that analytes are known to be specific for a given disease and hence can be helpful to monitor its progress, or even as indicators of quality of products from food and pharmaceutical industries [1]. Several molecules have been recognized as key analytes, among which L-lactic (LA) is of our interest. Determination of LA plays an important role in areas such food industry, clinical diagnosis or sports medicine [2]. In food industry the lactate level is an indicator of fermentative process and is related to stability, storage quality and freshness in products such as wine, tomato sauces, milk products, fruits and juices, where can be a marker for fermentation process by lactic acid bacteria [4,11]. In health, its determination is helpful for monitoring respiratory insufficiency, shocks, heart

[1] La Escuela de Doctorado de la Universidad de Burgos articulará los protocolos pertinentes para la tramitación y evaluación de este documento por vía telemática, garantizando la validez de su presentación, confidencialidad y efectos jurídicos de su contenido.

failure and metabolic disorders [3]. Any change or increase of blood lactate is an indication of survival capability. In sports medicine, LA displays a role in training status and fitness [4]. The production of such metabolite from the anaerobic metabolism of glucose in muscles, is accompanied of a pH decrease, which exceeds the buffering capacity of the body, affecting the muscles performance by cell acidosis. This process is known as "lactate acidosis" and is closely related with the physical condition of the individuals. Thus, is required LA monitoring for purpose of training and evaluation of the performance endurance [5].

The presence of LA in sweat is a function of eccrine gland energy metabolism, an increase in the exercise intensity leads to increased production of sweat lactate. Perspiration is a suitable biological sample for the monitoring of such metabolite, in a noninvasive way [6].

Among other methods, LA can be assayed using high performance liquid chromatography [7], fluorometry [8], chemiluminescence [9] and magnetic resonance spectroscopy [10], or even by enzymatic test kits. Those methods are time-consuming, require the sample pre-treatment, are expensive and require educated personnel [6, 11]. Also, those methods add the disadvantage of the need of sample collection, being not able to measure the analytes directly *in situ*. By far, for reasons of simplicity, integration, and portability, electrochemical lactate biosensors have gained the most significant developments.

The determination of that analyte using enzymatic biosensors has been reported by several authors for many applications [12-16], contrasting the disadvantages of conventional methods. The developed biosensors are based on electrochemical transduction, that have been greatly used due to the recognized robustness, simplicity, economic fabrication, broad range of linearity, ideal response time [17] and the possibility to combine these advantages with the inherent selectivity of biological recognition processes [18]. Among those biosensors, the ones that use the screen-printing (SPE) technology provides additional advantages such as small size, geometric uniformity, small volume sample required and an automated and easy fabrication.

Briefly, the fabrication of SPE is based on the deposition of different inks/pastes layers that allow the printing onto a substrate of a desired pattern for each one of the functional parts of the sensor. The inks/pastes are divided into conductive and insulating. The conductive inks are made of a resin and organic solvent mixture, on which the conductive material is dispersed. The Ag/AgCl ink is employed in the pseudo-reference electrode

printing, while the working and auxiliary electrodes are composed by graphite, gold, platinum, etc., where the incorporation of nano and biomaterials for the electrochemical reaction is performed. The insulating inks are made of polymeric or ceramic materials that allow the correct electric insulation of the conductive constituents of the electrodes. On the other hand, the materials commonly used as substrate for printing are, the PVC, nitrocellulose, polycarbonates and ceramics [19].

Considering the potential of the SPE technology, its manufacture has been transferred to the development of flexible electrochemical sensors, by the use of non-traditional substrates [20]. The most widely employed materials as flexible substrats are the Kapton (polyimide), the polyethylene naphthalate (PEN), polyethylene terephthalate (PET), and Teflon (polytetrafluoroethylene) [30], which offer characteristics like plasticity, hydrophobicity, suitable dielectric and insulating properties, thermal stability and low thermal expansion coefficient.

The adaptation of the SPE technology for flexible substrates, increases the applicability for the sensors on different fields. The use of flexible biosensors are described in the literature for the determination of different biomarkers and sugars in samples such as tears [22, 23] and also on the determination of chemical species in skin, by using the flexible sensor as temporary tattoo [16, 24].

Another potential field for application of flexible sensors is the intelligent packaging, which aims to monitor conditions of the packaged product providing information about the quality during transportation and storage. These smart labels are also suitable to acquire information about physiological, chemical and microbiological aspects of the products [25]. In this case the packaging gives us information about its quality and the events that have marked its processing, acting as an indicator for possible degradation, as well as maintenance, transportation or improper distribution.

Within the flexible sensors are also included those whose substrate is a textile material. The sensing fabric leads to intelligent textiles that are used in manufacturing of smart clothes. Many smart fabrics are already used in advanced types of clothing, mainly for protective and safety clothing, while at the same time successfully cover fashion concepts, comfort and innovation. The textile materials used are diversified and have been developed to allow the implementation of electronic microcircuits, offering various types of functions. However, the integration of circuits in clothing is more often an adhesion of those circuits rather than a true integration into the fabric structure, representing a problem on mass production and commercialization. The key requirement

that allows the integration of sensors for the improvement of interactive textiles is the development of conductive textiles. So far, there have been some researches developing conductive textiles, however, these studies have only resulted in laboratory demonstrations for early stages of research. Those conductive textiles can be fabricated using the printing technique, which is already applied in the textile industry with a purely ornamental purpose. For this reason, the potential adaptability of printed circuits on fabrics, opens significant expectations in this field.

The use of textiles sensors brings opportunities and advantages that can be exploited for practical applications such as sensor use on body, where the durability integrity to the deformations and weight are basic requirements. These sensors have been developed especially in the last decade and must fulfil certain characteristics such as the small size, simplicity of operation and rapid response. The flexibility provided, is an improvement that allows the monitoring of real-time information, especially when applied in clothes for patients, resulting in smart textiles, whit great importance in biomedical applications by measuring different parameters, diagnosing and identifying health threats.

Several references can be found for distinct applications of textile sensors like the detection of explosives using Gore-TEX [26], or in detection of water contaminants using neoprene [27, 28]. J. Wang proposed the determination of significant biological parameters in underwear using carbon electrodes [29] and also the determination of lactic acid in dermis using a temporary tattoo [16]. The same author has extensively reviewed the screen-printing of sensors onto different textiles substrates [30]. Different textiles used for these sensors may be natural, such as wool and cotton; or synthetic, such as polyester, nylon and other polymers, and must provide inert properties, stable operation for long periods of time, be water-resistant to liquid phase measurements [30], hydrophobic and compatible with the required printing.

In light of the above, one of the focus of this project is the application of the LA biosensors in flexible textile supports, in order to develop noninvasive biosensors for point-of-care monitoring of species in human samples. Another proposed improvement, will be carried out by the research group "Diseño Electrónica Aplicada" of the Technological Institute of Castilla y León (ITCL), whose efforts will be focused in converting the developed textile biosensors into autonomous prototypes based on amperometric biosensors, without the need of a conventional connected potentiostat.

Objetivos y Metodología :

The main objective of this work, is improve knowledge in development of screen-printed systems, using flexible substrates for printing, modified with enzymes and/or with nanomaterials, to be used as an autonomous biosensor without the need of a conventional connected potentiostat.

The work aims to develop flexible textile enzymatic SPE devices in order to determine LA, in body fluids (sweat, blood and urine). The research activity is focused on the construction of miniaturized sensors, using the SPE microfabrication technology applied to textiles substrates, allowing geometric uniformity in a serial massive production. Those devices offer simple, highly sensitive, fast response, robust, miniaturized and low cost biosensors that can be integrated to establish an autonomous measurement device for very small volume samples.

LA will be detected through the enzymatic reaction of lactate oxidase (LOx). The immobilization of the enzyme provides the electrochemical signal to be measured, offering the selectivity for the interest analytes. The electrochemical detection strategy is based on amperometric response of each system, after the evaluation of the ideal potential in presence of the respective enzyme substrates. The measurement of the electric current, which is associated with the electrons involved in the redox process, will be carried out by varying the concentration of the analytes. That variation will affect the electric current onto the working electrode, where the enzyme is immobilized. In that way, is possible to quantify the species by the alteration of the current measured, due to the electrons variation by the resulting redox reaction [31]. The amperometric behavior and the modifications will be implemented and evaluated in both, conventional and textile substrates for the screen-printing technique. The device will be first tested with synthetic samples with a known concentration of the analyte and the biosensor validation will be carried out by using conventional methods for the analytes determination.

On the other hand, is also suggested the development of a prototype, by adapting the produced textiles biosensors into autonomous measurement device without the need of a conventional connected potentiostat. The achievement of this goal of a great importance, once it will allow the integration of this device into clothes and monitoring the analytes directly on the body.

Four specific goals (G) are intended to be reached during the project:

G1 - Development and study of conductivity characteristics of different composites based on nanofibers and commercial graphene, in order to achieve a hybrid material with high conductivity for application as printing material.

G2 - Implementation of a sensorial device on traditional substrates using nanomaterials and/or biomolecules to determine LA. It is intended the identification of optimal experimental conditions for the determination of LA, using a conventional potentiostat connected to the SPE.

G3 - Screen-printing of the conductor composites in flexible substrates to evaluate their use in development of textiles with sensorial ability. It is intended to identify a textile substrate suitable for printing the selected composites and verify if the developed flexible SPE electrodes are able to be used as biosensors with the conditions found as ideals in G2.

G4 – Conversion of developed flexible biosensor to work as autonomous device.

Below is described the methodology and tasks (T) to accomplish the previously defined goals (G1, G2, G3 and G4).

-**G1:**

T1 - Identification and characterization of nanostructured composites. The proprieties of a hybrid material with high conductivity will be study when added in different commercial conductive inks.

T2 - The selected composites will be printed on commercial polyester supports.

-G2:

T3 – Modification with nanomaterials and/or biomaterials and validation of the selected screen-printed electrodes. Evaluation of SPE amperometric response for LA, based on enzymatic reaction of LOx. The electrodes will be connected to a potentiostat system (PalmSens) and the analyte will be evaluated by applying the potential required in each case. In order to achieve better electrochemical response, for each biosensor will be held an characterization based on:

a) Optimization of all parameters related to the amperometric electrochemical response, such as the applied potential required; and also the ones related to the enzymatic system such as, immobilization methods (covalent linkage, physical adsorption, cross-linking, encapsulation and entrapment), pH, enzyme units and the amount of others components used for the immobilization process.

b) Determination of the reproducibility, repeatability, biosensor lifetime, calibration range and determination of the detection capabilities.

c) Interference analysis, with special attention in ascorbic acid since is recognized as major interfering specie in biological fluids.

d) Biosensor application on synthetic samples to determine the analytes.

e) After the achievement of the previously proposed, biosensors validation will be held by using conventional methods.

-G3:

T5 - Screen-printing of the selected composites in different textiles substrates, such as Gore-Tex and neoprene, study of their mechanical properties, adhesion, conductivity, deformation and stability over time.

T6 - Evaluation of the flexible electrodes, which will be evaluated using the ferrocyanide/ferricyanide couple, a reference in redox behavior. The system recognized with the better properties will be then converted into a flexible biosensor to determine LA using the conditions found in G2.

- G4: An electronic system able to measure directly the amperometric response will be adapted to detect LA. The device will be constructed with the products found as ideals in the G1 and G3 taking into account the required potential for each amperometric response, calibration ranges and the detection capabilities from the G2.

T6 - This autonomous measurement textile device will be study and modified to allow determination of the LA. The required potentials and the optimal conditions defined in the G2 will be used. Once the device is prepared, the measurement will be conducted considering the sensitivity and reliability of response.

T7 – Application of the developed prototypes, in clothes to monitor levels of LA in sweat produced during exercise by sportsmen.

For G2 and G4, the first stage of measurements will be made with synthetic sample and after in real human fluids (sweat) to validate the biosensor.

Medios y recursos materiales disponibles:

The research group, where the project will be developed has a DEK 248 since several years, which is the equipment needed to produce the screen-printed electrodes in flexible or solid substrates. This research group also has an industrial microscope ECLIPSE LV100 PLAN BD and various potentiostats and cells:

•Potenciostat Methrom (Herisau, Switzerland) 746 VA Trace Analyzer coupled to a 747

VA Stand with a Metrohm Multimode electrode.

•Potenciostat µAutolab (Echochemie, BV, Utrech, Netherlands) connected to a µAutolab IME 663 unit, coupled to 663 VA Stand with a Metrohm Multimode Electrode.

•Potenciostates µAutolab (Ecochemie) conected to µAutolab IME Type II y Type III units.

•Multipotenciostat model 1030 CH-Instruments (Texas, USA)

•4 Potenciostates Palm Sens

•Flow cell/ (Drop Sens) for measurements on line.

Furthermore, this instrumentation is in our research laboratory but in the university exists a supportive group for investigation that can provide us techniques like Mass-ICP, chromatographic techniques, scanning electron microscopy, confocal microscopy among others, to be used as auxiliary and reference techniques to corroborate the results.

Additionally, we also have available resources and facilities from companies that collaborate in the project. The "Grupo Antolin" has laboratories that are at the forefront of technological offer, which will be used in tasks that involved GA:

• Laboratory of acoustic and vibrations, laboratory of organic and inorganic materials: allow the implementation of new materials, decreasing the needed to be integrated in materials and the laboratory of electronic.

Planificación temporal ajustada a tres años:

Año/Trimestre	**1º**	**2º**	**3º**
1º	Literature Review Characterization of materials.	Electrochemical study/characterization and optimization of LA biosensors.	Study of electrode modification with nanomaterials and/or mediators.
2º	Calibration and validation of the methodology for LA determination.	Interferences study. Application to synthetic samples.	Adaptation to Screen-printed textile substrates
3º	Conversion into autonomous measuring biosensor.	Application of the developed biosensor in human sweat.	Writing of the thesis.

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Lugar y fecha de entrega:

En Burgos, a 15, de Junio de 2015

Firmas:

Doctorando **Director** **Coordinador**