Universitat Autònoma de Barcelona

Doctoral thesis proposal

Development of electrochemical biosensors for the measurement of physiological parameters in sweat for wearable applications

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1. Objectives of the thesis

In recent years, sweat has emerged as an alternative, being an easily accessible, biofluid valid for non-invasive chemo-sensing. Apart from water (99%), it contains electrolytes (sodium, chloride, potassium …), metabolites (glucose, lactate, ethanol…) and small quantities of sugars, proteins, and metal ions [1]. Therefore, sweat presents a wide range of analytes of clinical interest. Compared to other non-invasive biofluids, sweat analysis is the most promising option to monitor the temporal evaluation of parameters of interest for prolonged periods of time. The correlation of sweat parameters with gold-standard blood seems crucial to provide relevant data to clinicians [2].

The advancements in a wide variety of fields, ranging from material science and sensors to the design and fabrication of microsystems and flexible electronics, leaded to the fabrication of biosensors and low-power electronics capable of wireless communication on flexible substrates. This sensor-on-skin approach still has to overcome a great number of limitations inherent to traditional sweat analysis (sample evaporation, appropriate sampling device and no need of trained staff) and has to allow for continuous monitoring of sweat parameters during on-body trials. Nowadays few commercial devices dedicated to sweat sensing use delicate external instruments to measure a particular analyte of interest.

For this reason, in this project, a solution is presented as a further important step towards promoting longer and healthier lives thanks to a non-invasive monitoring biosensor. The developed sensor is intended to be integrated within a monitoring device for a non-invasive, continuous, and remote operation through sweat analysis.

Therefore, this doctoral thesis is framed as a part of a wider multidisciplinary project.
The main goal of this research is the development of a series of technologies and procedures for the design, implementation, and industrialization of electrochemical sensors for their measurement in sweat. The establishment of these technologies and procedures is key to obtain sensors that are able to accurately and selectively measure a number of key analytes in monitoring and treatment protocols.

Once these technologies are developed, they will be used for the development of sensors for specific applications. At this moment have been identified 4 key sensors within the medical industry for development: lactic acid, glucose, sodium and potassium.

Each one of these sensors helps to cover a need within telemedicine protocols in different fields such as cardiology, nephrology, endocrinology, etc. The operation and structure of the sensor must be compatible with the characteristics of a sweat collection fungible and electronics already developed by other members of Onalabs.

The following research plan will be followed to develop the sensor:

- Design and fabrication of the screen-printed electrodes of the biosensor. Fabrication of the functionalization of the working electrode of the sensor with the enzymatic membrane to analyse the specific metabolite.
- Determination of the analyte concentration in order to identify the sensitivity, detection limit and linear range of the sensor.
- Determine the repeatability of the measurements over time and lifetime of the sensor.
- Determine the selectivity of the sensor by testing the response of the sensor in the presence of various interferents present in the sweat.
- Determine the effect of external parameters such as ambient temperature and pH.
- Perform the same electrochemical measurements but integrating the sensor into the microfluidic channel.
- Implementation of the portable sweat sensor and characterization of its response in laboratory model samples.
- Final validation of the device in patients. Possible clinical validation.

2. Methodology

WORKING PLAN	
Non-invasive, highly sensitive wearable platform for analysis of metabolites in sweat will be develop and validated. The wearable device will be designed, developed, tested, and benchmarked against commercial tests, by comparing the specifics metabolites levels measured in blood. **Specific objectives**: 1. Establishment of the guidelines and specification with the view of end-user scenario. 2. Develop and fabricate an electrochemical biosensor. 3. Validation and optimization in artificial sweat. 4. Integrate the sensors in the sweat capture system. 5. Validation: in-vitro and in-vivo laboratory testing.	
Working Packages	**PERIOD**
WP1. Definition of requirements and user specifications	**3 months**
WP1 defines the device specification to meet the user. This task involves understanding all the gaps and issues of the value chain (from the final user to stakeholders' needs) to generate the requirements of all levels of users. This evaluation will be evaluated to implement the full system specifications and drive the appropriate technical development. Based on feed-back from end-users, clinicians Valeria will collect the information and define the specifications for the chemical/physical procedure for sample treatment, data management and validation procedures.	

WP2. Develop and fabricate an electrochemical biosensor	**15 months**
This WP will focus on developing a three-electrode system (working, counter, and reference electrodes). It will be designed and fabricated as part of the skin patch to perform the electrochemical measurements. The appropriate electrodes of the skin patch will be functionalized by an in-house redox mediator formula to facilitate the specific metabolite detection at lower applied potentials.	
WP3. Validation and optimization in artificial sweat.	**6 months**
The electrochemical sensor will be validated in the laboratory using artificial sweat in order to find the parameters that affect its performance. The purpose of using the previous mixture as sample matrix is to mimic sweat properties in a laboratory setting and observe any interferent affecting sensor response.	
WP4. Integrate the sensors in the sweat capture system.	**6 months**
These electrochemical sensors must meet specific design requirements in order to be integrated into the microfluidic architecture of the final device. It will be integrated in a microfluidic architecture with an embedded chrono sampling circuit for the detection of specific metabolite levels in sweat will be developed.	
WP5. *Validation: in-vitro* and *in-vivo* laboratory testing	**3 months**
Each of the modules and the integrated device will be periodically tested and validated both in-vitro (by means of buffer and artificial sweat) and in-vivo (ONALABS volunteers) in the laboratory. During this task, samples with increasing complexity up to on-line monitoring will be evaluated: detection limit, response time, sensitivity >95%, selectivity >90%, positive and negative predictive value.	
WP6. Thesis Writing and Defence (3 months)	

3. Project resources

PhD students will be offered access to the facilities that UAB, or CNM-CSIC where the thesis is carried out (Electrochemistry and Green Chemisry Group, UAB) and "Grup de Transductors Químics (GTQ)", IMB-CNM del CSIC) and will allow to use use specific facilities, resources, machinery or materials, it should be stated here.

Foccusing on the Electrochemistry and Green Chemistry group, different facilities are available in the group to develop Valeria's PhD research project: i) synthetic labs for the preparation of new compounds and materials, including a GC instrument; ii) an electrochemistry lab with potentiostats, electrodes, electrochemical cells and a Faraday cage. In addition, to support the PhD research, several services that the UAB offers (such as Chemical Analysis, Nuclear Magnetic Resonance and Microscopy services) will be used. Finally, due to our collaboration with other institutions (ICN2-BIST, CNM-CSIC, ICMAB-CSIC) and companies (Leitat) in the surrounding area, we can also access their facilities in the UAB Campus and use their resources for the fabrication and analysis of materials (e.g. mechanical testing systems, rheometers, gas sorption analysis).

4. Planning: Tentative work plan or chronogram

WP	Year 1				Year 2				Year 3			
	T1	T2	T3	T4	T1	T2	T3	T4	T1	T2	T3	T4
1	X											
2		X	X	X	X	X						
3							X	X				
4									X	X		
5											X	
6												X

5. Basic references

[1] Xu J, Fang Y, Chen J. Wearable Biosensors for Non-Invasive Sweat Diagnostics. Biosensors (Basel). 2021 Jul 23;11(8):245. doi: 10.3390/bios11080245. PMID: 34436047; PMCID: PMC8391966.

[2] Qiao, Y.; Qiao, L.; Chen, Z.; Liu, B.; Gao, L.; Zhang, L. Wearable Sensor for Continuous Sweat Biomarker Monitoring. Chemosensors 2022, 10, 273.