Continuous Lactate Sensor

Design Project Report

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Abstract

A lactic acid sensor helps measure fatigue in exercising muscle. By making a sensor that detects lactate in sweat, this can be measured in a non-invasive way. The sensor works by allowing an enzyme-coated working electrode to react with lactic acid in the well. This reaction in turn produces a current that is converted to a voltage with a potentiostat and amplified with a trans-impedence amplifier. This device was tested on solutions of lactate ranging from 25 mMol to 100 mMol, and it outputted voltages from 4.8 mV to 6.59 mV. The amplifier could not produce a high enough output to be read by an Arduino, so lactic acid change over time was not able to be measured.

Keywords: lactate, lactate oxidase, sweat, training, threshold.

Context

Athletes need a way to continuously measure their lactate levels in order to be able to adjust their workouts accordingly. Lactate levels indicate the fatigue within exercising muscle, since lactate is produced when muscles undergo anaerobic respiration. Measuring lactate levels is important for quantitatively determining exercise intensity so that athletes can adjust their exercise program based on their response. These measurements allow them to maximize the efficiency of their training efforts and recovery to prevent overtraining by maximizing training at threshold pace.

Current solutions on the market are primarily blood lactate monitors, in which the user pricks their finger with a strip and inserts it into a device which outputs the molarity of lactate in the blood. This method is invasive and interruptive, requiring a blood sample in the middle of a workout, and it only provides non-continuous data, not showing changes in lactate levels over time. Continuous sweat lactate monitors are in development but not on the market. Our solution, a continuous lactate measurement via sweat, intends to address the invasiveness and non-continuity of blood lactate monitors' data. Using this data, one can adjust their exercise programs based on measured lactate levels.

Description

Our design features a tray for holding the sweat solution and electrodes, a potentiostat for conservation of voltage, and a transimpedance amplifier for converting current to voltage.

We used a multimeter to measure our circuit's output voltage for more precision instead of an Arduino due to the voltage being too small. In the future, we could add another amplifier so that the Arduino would work here.

Module	Feature	Specification
Sweat collection tray	Grooves to fix distances between electrodes	0.17" between counter and working electrodes 0.03" between working and reference electrodes
Sweat collection tray cont.	Container for solution	Holds max of 0.088914 cubic inches of solution
Potentiostat	Conserves voltage	0.6 V
Transimpedance amplifier	Provides amplification	R_f = 1kOhm

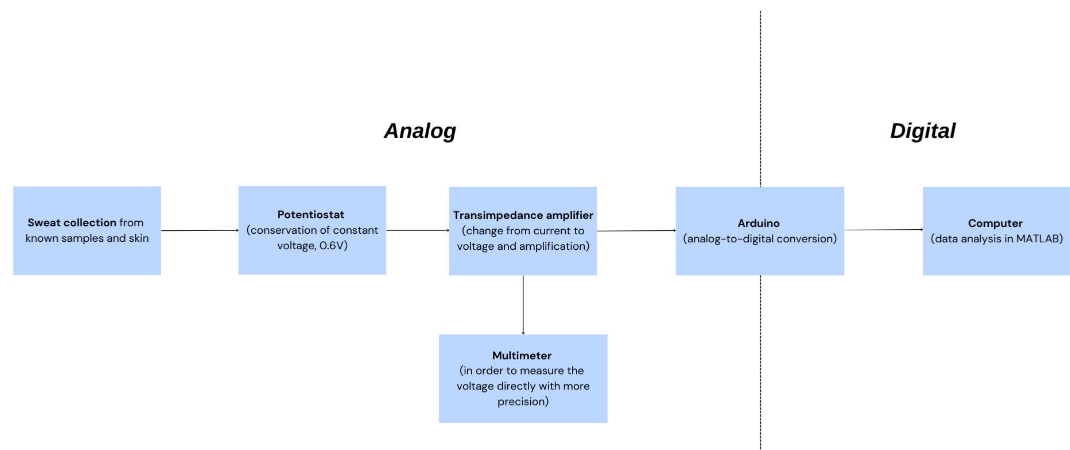


Figure 1. Block diagram

Design Files

Design file name	File type	Comments
Electrode Box ramp.sldprt	CAD model for electrode box	Solidworks
Electrode_Box_ramp.stl	3D printing file for electrode box	
Block_diagram.png	png image to for build instructions	
Circuit_diagram.png	png image to for build instructions	
Breadboard_conexions.png	png image to for build instructions	

Build Instructions

First, it was necessary to have a list with all the materials required for the project. The components can be grouped into different categories based on what part of the project they were used in: one silver two platinum wires, lactate oxidase (LOx), sodium lactate, PBS, glutaraldehyde, Clorox bleach and distilled water for the coating and test solutions; PLA for the 3D-printed container; and two TLC2272 op amps, one ARDUINO UNO, one 6.2k and two 1k resistances for the circuit.

The circuit consisted in a potentiostat and a transimpedance amplifier (TIA), which controlled the voltage between the reference (RE) and the working (WE) electrodes, and transformed the current coming out of the WE into voltage, respectively. The counter electrode (CE) should be placed further apart from the other two as seen in Figure X in order to ensure that all the current generated is going through the WE. This electrode must be coated with LOx following the procedure beneath:

1. Obtain 100 Units of 98% Lactate Oxidase and dissolve in 0.5 mL of PBS on Spin Coater to create LOx
2. Mix 63 microliters of 0.5% Glutaraldehyde solution with 125 microliters of LOx
3. Spin the solution on for 5 minutes to make sure it is well- mixed on Spin Coater at the highest speed
4. Let the Platinum electrode sit in the solution for 2.5 hours at room temperature
5. Remove the electrode from the solution and add it to the circuit

Thanks to this coating and the constant voltage maintained (~ 1 V), the hydrogen peroxide produced by the oxidation of lactic acid to pyruvate, reaction catalysed by LOx, will produce two electrons for each molecule of lactate catalysed (see Figure 2), thus having a direct connection between lactate produced and current generated.

$$\text{Sodium-L-Lactate} + O_2 \xrightarrow{\text{LOX}} \text{Pyruvate} + H_2O_2 \rightarrow 2H^+ + O_2 + 2\,e^-$$

Figure 2. Reactions taking place at the WE

In the initial phase of the project it was necessary to check whether the circuit was working. For this the electrical part of the project was divided into three smaller parts for testing efficiency: potentiostat, TIA, and whole circuit without samples. As shown in figures

X, X and X, all the electrical part of the project was working since for the potentiostat the voltage at the CE was matching that of the RE, for the TIA the current entering was the same as the one coming out, and for the whole circuit there was a linear relationship between current and voltage (see figures 3, 4, and 5). It is important to note that for the testing of the whole circuit without samples the circuit was closed using resistances of 1k and of 1Meg between the WE-RE, and RE-CE, respectively.

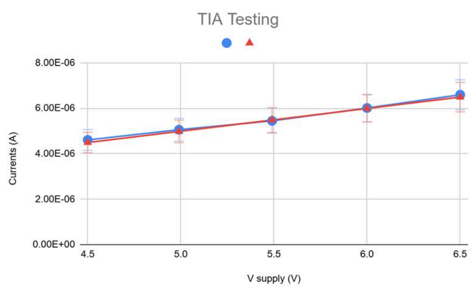


Figure 3. Potentiostat testing graph

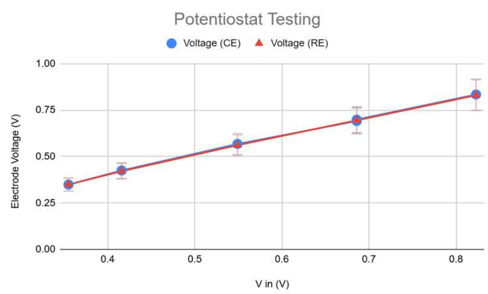


Figure 4. TIA testing graph

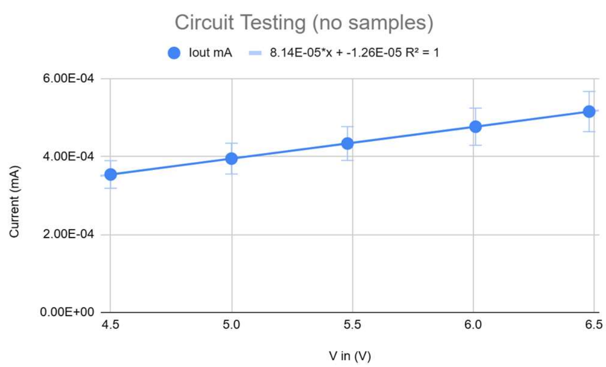
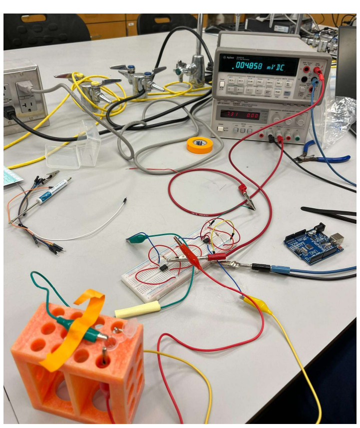


Figure 5. Circuit testing graph

Once all sections were checked, the circuit was integrated with the electrodes (see Figure 7), being first tested with steel electrodes before using the platinum and silver ones. The goal of this experiment was to check that compounds used will not interfere with the reaction by placing several solutions and recording twice the voltage out of the circuit.



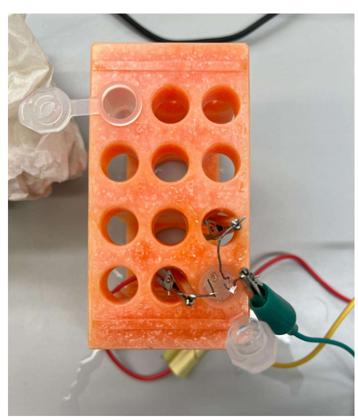


Figure 6. Overview of the prototype *Figure 7. Electrodes placements (**CE**, **WE** and **RE**)*

Operation Instructions

In order to use this device it is necessary to make the connections shown in Figure 8. It is key to fix the multimeter probes to point A and ground using alligator clips, otherwise we would not be able to get a precise reading for the voltage (with this circuit design, adding an additional amplification stage would have solved this issue and ARDUINO UNO would have been able to read the values).

Furthermore, it is important to emphasize that there are no safety concerns as the voltage applied is very small and one of the main goals of the device was to make it non-invasive.

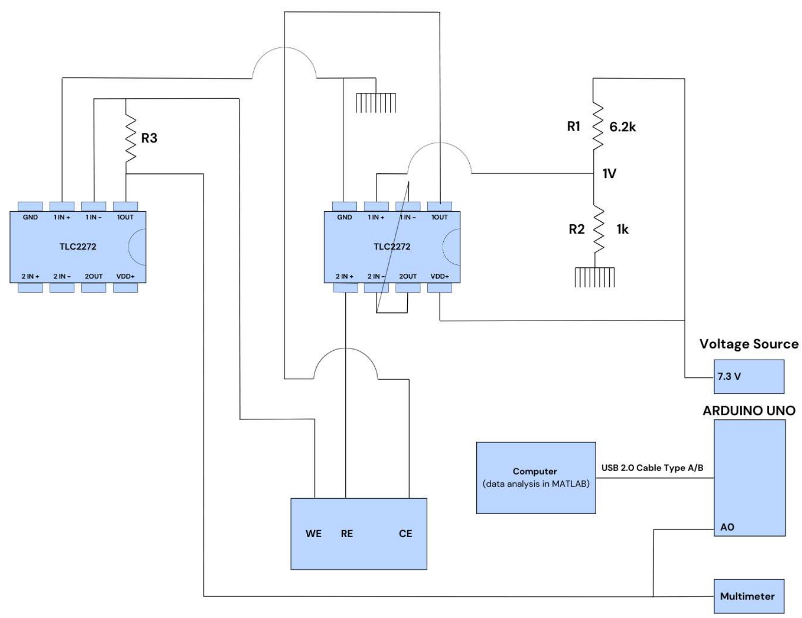


Figure 8. Circuit on breadboard

Testing, Validation and Characterization

To test our device we first coated the electrodes and then placed them at a fixed distance apart to keep the testing between concentrations consistent. At first when the electrode hit the solution it would jump to a high voltage value. This first contact was the reaction first taking place and the outer layers of lactate oxidase on the electrodes reacting. From there the voltage slowly decreased until it reached a stable value for which it would hold consistently. Due to the nature of how this was occurring the group decided to add more lactate oxidase onto the electrode in the unsubmerged part and let it slowly seep into the lactate solution. We thought this "reverse test" would be a good way to simulate sweat going into the system and slowly gaining lactate concentration as it builds up around the electrode. We had to do it this way as the lactate oxidase was our limiting reactant and the jump for it going fully submerged was difficult to measure. What ended up resulting was pretty interesting, at first the voltage would hit a small spike with the initial addition, then it would slowly go down as before, but right when it reached the stable value, the voltage would then start to slowly go up. We are assuming this is due to the slow flow of the oxidase coming down the electrode. This was exciting to see as it mimicked a slow upward change in voltage that we were hoping to get initially. It would continue to go up until it got very close but not quite to the peak value of the initial testing and then go down again to the stable value once the reactions subdued. In the future, a test where a controlled amount of lactate could be slowly added at specific volumes where it would not over saturate the lactate oxidase on the electrode would be the optimal way to test this device.

For one final experiment we started with a very small volume of 20 mmol concentration then stepped our way up through each concentration until we got to 100 mmol to see if this would give us the slow rise, but unfortunately it was difficult to make it consistent so whenever we would step up concentrations, the voltages would also step up and it would not be a slow continuous rise like we hoped. In the end our tests proved that the reaction could be measured, just by finding the best way to do this and model real life situations proved to be difficult.

Below are results we got for stable and peak values. At the end the stable values are used for the calibration curve as these were the repeatable results. These would also most likely be closest to actual sweat situations as the sweat will come in at a controlled manner and not get dumped in like the peak values demonstrate. So unknown concentrations of lactate could be determined from their voltage using this curve when they reach their stable value. We tested this by making 200 mMol lactate and acting as if we did not know and the projected slope of the stable value was 8.6 mV and our measured value was around 8.5 mV so this validated our curve. The peak results are important as they show the range at which these concentrations can output, but they are difficult to repeat and not as precise.

Lactate Concentration	25mMol	50mMol	80mMol	100mMol
Stable Value	4.801mV	5.410mV	5.985mV	6.342mV
Peak Value	7.0mV	7.5mV	8.1mV	8.5mV

Figure 9. Lactate Concentration Testing Results

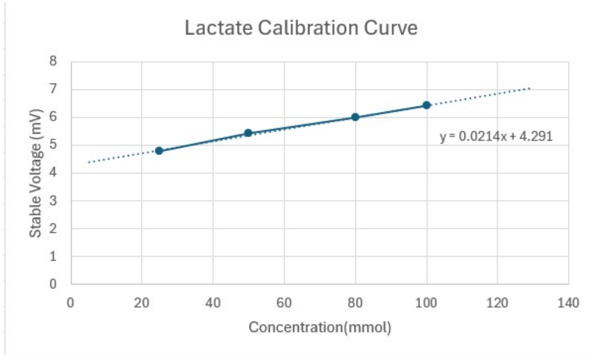


Figure 10. Lactate Concentration Calibration Curve

Future Work

With additional time, we could add an additional amplifier or increase the output of the TIA so that an Arduino can read the sensor's voltage output. This will allow us to measure the lactic acid change over time. Additionally, optimizing the electrode coating so that the reaction between lactic acid and lactate oxidase is longer and therefore easier to measure. Further down the line, the sensor could be made wearable by making it wireless, making the sensor into a patch, and integrating it with an app so users can access this data like with other performance metrics for health and cardio like heart rate. Of course, this will be improved and studied with user-testing on non-athletes and athletes of varying levels.

Author’s Credit

Mary- Circuit testing, electrode coating, chemical solutions, final testing, contacted Georgia Tech researchers and professors, researched enzyme coating, co-wrote the report and presentation

Guillermo - Circuit design, development, building and testing, final testing, co-wrote the report and the presentation, research of enzyme functionality and current generation, contacted research paper authors and GeorgiaTech professors.

Colten- Circuit building and testing, gathered necessary chemicals and supplies, Gave everyone access to Lab, Contacted helpful Tech Professors, Edited and printed container 2, helped with coating/testing and other necessary presentations/reports

Grace- Circuit building and testing, 3D printing design and printing, helped with coating/testing, co-wrote the report and presentation.

References

Caldaroni, M. (n.d.). COSMED - Lactate Pro 2: portable, easy to use and fast lactate monitor. COSMED. https://www.cosmed.com/en/products/cardio-pulmonary-exercise-test/lactate-monitor

Pine Research Instrumentation, Inc. (2021, May 18). What is a potentiostat and how does it work? [Video]. YouTube. https://www.youtube.com/watch?v=pzB122dTij8

Vossen, L. (2024b, February 27). Continuous Lactate Monitors for athletes – explained. Molab. https://molab.me/continuous-lactate-monitors-for-athletes/

Bonaventura, J. M., Sharpe, K., Knight, E., Fuller, K. L., Tanner, R. K., & Gore, C. J. (2015a, January 27). Reliability and accuracy of six Hand-Held Blood Lactate Analysers. https://pmc.ncbi.nlm.nih.gov/articles/PMC4306774/

Lactate testing for athletes: the ultimate resource. (2024b, February 4). Upside Strength. https://upsidestrength.com/lactate-testing-for-athletes/

García-Guzmán, J. J., Sierra-Padilla, A., Palacios-Santander, J. M., Fernández-Alba, J. J., Macías, C. G., & Cubillana-Aguilera, L. (2022). What is left for Real-Life lactate monitoring? Current advances in electrochemical lactate (Bio)Sensors for agrifood and biomedical applications. Biosensors, 12(11), 919. https://doi.org/10.3390/bios12110919

Xuan, X., Pérez-Ràfols, C., Chen, C., Cuartero, M., & Crespo, G. A. (2021b). Lactate biosensing for reliable On-Body sweat analysis. ACS Sensors, 6(7), 2763–2771. https://doi.org/10.1021/acssensors.1c01009

Tigris. (2024b, February 5). Detection of Hydrogen Peroxide 4/5 – with Prussian Blue. PalmSens. https://www.palmsens.com/knowledgebase-article/detection-of-hydrogen-peroxide-with-prussian-blue/

ZimmerPeacock. (2020, August 18). introduction and review on screen printed electrodes (SPE) and their application to biosensors [Video]. YouTube. https://www.youtube.com/watch?v=Sm6sS-yuQgo

ZimmerPeacock. (2020b, September 20). Introduction to electrochemical biosensors [Video]. YouTube. https://www.youtube.com/watch?v=gXw7armpsEw

PKVitality. (2022, September 8). K'Watch Athlete - PKVitality
https://www.pkvitality.com/ktrack-athlete/