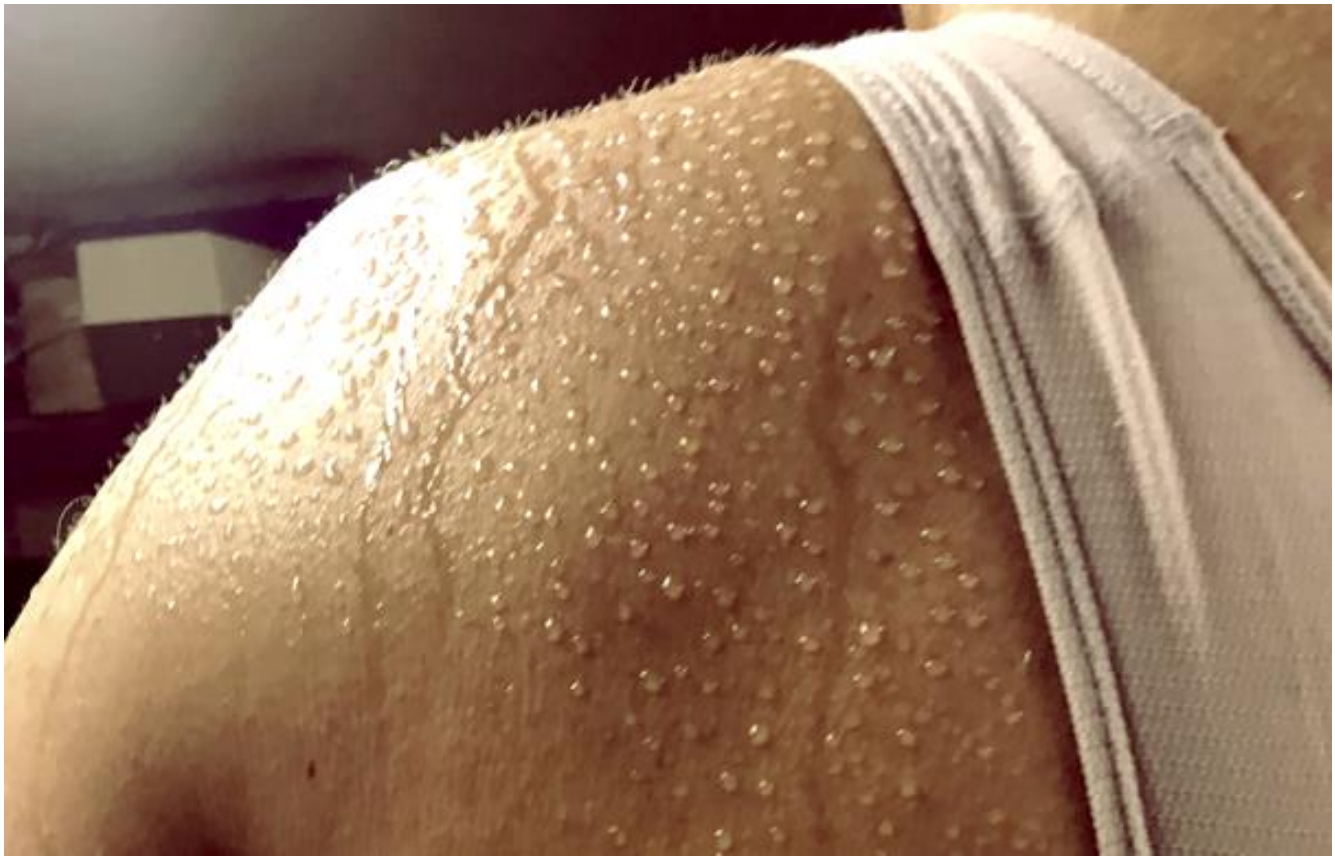
CHALMERS
UNIVERSITY OF TECHNOLOGY



Development of a Non-enzymatic, Non-invasive Sensor for Detection of Lactate in Sweat.

Master's thesis in Biotechnology

ANNIE LJUNGH

Department of Physics
CHALMERS UNIVERSITY OF TECHNOLOGY
Gothenburg, Sweden 2018

Master's thesis 2018

DEVELOPMENT OF A NON-ENZYMATIC, NON-INVASIVE SENSOR FOR DETECTION OF LACTATE IN SWEAT.

ANNIE LJUNGH

Department of Physics
Division of Condensed Matter Physics
CHALMERS UNIVERSITY OF TECHNOLOGY
Gothenburg, Sweden 2018

Development of a Non-enzymatic, Non-invasive Sensor for Detection of Lactate in Sweat.
ANNIE LJUNGH

Supervisor: Gunnar Westman, Department of Chemistry and Chemical Engineering
Examiner: Magnus Karlsteen, Department of Physics

Master's Thesis 2018
Department of Physics
Chalmers University of Technology
SE-412 96 Gothenburg
Telephone + 46 (0)31-772 1000

Cover: Sweet drops assembled on part of the back after training.

Printed by Chalmers Printing Service
Gothenburg, Sweden 2018

Development of a non-enzymatic, non-invasive sensor for detection of lactate in sweat.
ANNIE LJUNGH

Department of Physics
Chalmers University of Technology

Abstract

An athlete's goal is to perform the best way possible and in many cases the level of lactate in blood is an indication of performance possibilities. Blood lactate measurement is conducted to obtain knowledge of the lactate threshold (LT) which is the intensity at which blood lactate starts to increase. Lactate can also be found in sweat and some studies have reported a correlation between lactate in sweat and blood, implying for the possible usage of sweat instead for these types of measurements. Using sweat result in non-invasive measurement, both suitable for the discomfort of the athlete tested and to not affect the training while testing. Most non-invasive sweat sensor are based on degrading of lactate with enzymes and detection with amperometric sensors. For endurance sport, such as marathon's, an enzyme-based sensor can't measure for the total performance time without losing effect.

The aim of the thesis was to produce a sensory device for evaluating the lactate concentration in sweat. Molecular imprinted polymers (MIP) made of overoxidized polypyrrole (oPPy) using ammonium persulfate ($(NH_4)_2S_2O_8$) as an oxidative agent was successfully drop coated onto screen printed carbon electrodes (SPCE) modified with gold nanoparticles (AuNP). By evaluating the peak current observed by cyclic voltammogram between -0.5 V to -1.5 V at a scan rate 0.05 V/s a decreasing peak could be observed at -1 V to -1.5 V with a higher lactate concentration. By data analysis both with modified and unmodified SPCE-AuNP, showed a linear relationship between lactate concentration and peak current. The electrodes were used to evaluate the lactate level in a real sweat sample. The unmodified electrodes overestimated the lactate level with approximately 10 percent while the modified underestimated by approximately 30 percent.

Key words: Lactate, Sweat, Molecular imprinted polymers, Pyrrole, Screen printed carbon electrode.

Acknowledgement

This master thesis project has been conducted at the division of Condensed Matter Physics during the autumn of 2018. The work has been part of Chalmers sport and technology.

First of all, I want to express gratitude towards my supervisor and examiner **Magnus Karlsteen** for helping me start the background to this work in the spring of 2017 and my supervisor **Gunnar Westman** for helping me take the project further. You both have been my guiding stars throughout this work and I'm truly grateful. I want to give a special thanks to **Parveen Deralia** for helping me in the lab and for all the advice.

I want to give a big thank you to all the people at **KMF**, for all the laughs and interesting discussion during lunch. My family and friends, I'm deeply grateful for your support throughout this experience and for allowing me to talk about sweat with you. **Jessica Fredriksson** your input and support has been extremely valuable for me, thank you.

A special thanks for all persons that been helpful during the project.

Jonathan Weidow

Aline Maire Du Poset

Tiina Nypelö

Aktivitus

Annie Ljungh, Gothenburg, December 2018

Content

List of figures

List of tables

Abbreviations

ACS	American Chemical Society
AT	Anaerobic threshold
ATP	Adenosine triphosphate
AuNP	Gold nanoparticles
C.E.	Counter electrode
CV	Cyclic voltammetry
ECP	Electronically conducting polymers
FT-IR	Fourier transform infrared spectroscopy
GXT	Graded exercise test
HI	High intensity
LDH	Lactate dehydrogenase
LER	Lactate excretion rate
LI	Low intensity
LO	Lactate oxidase
LSR	Local sweat rate
LT	Lactate threshold
MIP	Molecular imprinted polymers
MLSS	Maximal lactate steady state
OBLA	Onset of blood lactate accumulation
oPPy	Overoxidized Polypyrrole
PPy	Polypyrrole
R.E.	Reference electrode
SEM	Scanning electron microscopy
SPCE	Screen printed carbon electrodes
SWD	Sweet rate dependent
TBW	Total body weight
W.E.	Working electrode
WBSR	Whole body sweat rate

1. Introduction

During training many people experience ache in their muscles, this is a result of accumulated lactate in the muscles. For the ordinary person the ache is just a negative part of training but for elite athletes it is saying more about how the body is reacting to the training. An elite athlete wants to make sure that the training program is conducted to increase the strength and performance. A commonly used method for evaluating the possible performance as well as a tool for planning practice is the measurement of lactate concentration in blood during increasing intensity(1). This is made by taking multiple blood samples as the intensity of the training is increasing. Something that can be uncomfortable for the athlete and not letting them fully concentrate on the task of the graduating increasing exercise.

Lactate is a product produced from the metabolism within our cells. During training the cells needs to produce more energy in the form of adenosine triphosphate (ATP)(2) to be able to keep performing the training. Because of the increasing demand of energy and the decreasing oxygen level during exercise, the cells have to use different pathways to keep up the energy level. When the energy is produced during anaerobic conditions one of the products is lactate, which starts to accumulate in the blood(3). Through gradually increasing the intensity of the training and measure the concentration of lactate the intensity where lactate starts to build up can be found using multiple different concepts(4). One of these concepts, the lactate threshold (LT) is the intensity of training where lactate starts to accumulate. LT is close to but not the anaerobic threshold (AT) which is the level where the oxygen uptake can account for the energy the cells need(5).

Sweat is a biofluid used for thermoregulating the body and contains small levels of different analyte that can be found in the blood(6-8). Through the common discomfort of blood sampling an increasing trend in production of sweat sensors has started, where analytes usually evaluated from blood is evaluated in sweat. This allowing for a non-invasive measurement. In sweat the lactate level is an indirect indication of increasing physical exertion. The lactate concentration in sweat is both from the blood plasma and from production in the sweat glands(9). Sweat sensors used for lactate measurements have previously used enzymes for detection of the degrading lactate but have shown some problems regarding stability as enzymes is highly affected by pH and temperature(10-12). By using chemical modified electrodes, a non-enzymatic sensor for lactate detection in sweat has been conducted. These sensors show a better storage stability and is not effected in the same extent by environmental changes(13).

The positive aspects of enzymes specificity can be mimicked in molecular imprinted polymers (MIP). MIPs are polymers constructed to have a cavities specific for the analyte of interest and therefore mimic the lock and key mechanisms found in enzymes(11). These can be produced by synthesis either by an oxidative agent or by potential through electropolymerization(14). During the project MIP where evaluated to determine if it could be a suitable solution for a non-enzymatic sweat sensor. This by comparing unmodified electrodes with electrodes modified with MIP. The specificity of the sensor was evaluated as sweat is a complex solution with a great matrix effect.

Therefore, the aim of the project was to produce a sensory device, using a non-enzymatic solution to obtain quantitative measurement of lactate from sweat. The focus was placed on creating the active electrodes responsible for detection of lactate as well the collection of sweat. The project is focused on detection of lactate, this due the influence lactate concentration has on the performers of athletes.

2. Theoretical background

The chapter aims to discuss the different concepts and methods used in this project, describing the use of lactate measurement in exercise and provides information about the bio rich fluid, sweat. Additional information about the concept of sweat based sensors and how these are evaluated is presented. The chapter also describes the basics of how sensors is constructed, tested and modified.

2.1 Lactate

Lactate is the conjugated base of lactic acid, as one carboxy group has been deproteinised(15). In the body, 99% of lactic acid is reduced to lactate and hydrogen ions(16), therefore lactic acid and lactate are often used as synonyms within sport context. The relationship between lactic acid and lactate is displayed in Figure 1. Lactate is produced in muscles during anaerobic conditions from pyruvate(3). The accumulation of lactate in our muscles during training results in ache(3) and is a biomarker of fatigue(17).

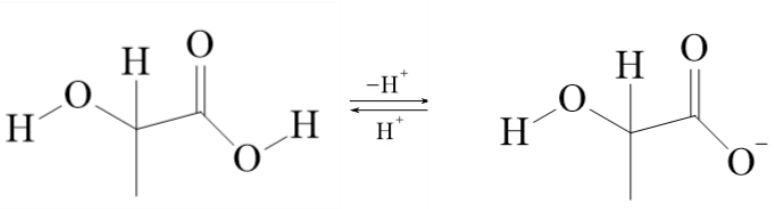


Figure 1, relationship between lactic acid and it's conjugated base lactate.

Pyruvate is produced in glycolysis, an important pathway our cells for creating energy, from glucose. A part of glycolysis is the production of ATP which is used as energy within the cells. Pyruvate is further degraded to acetyl-CoA or lactate in our cells. Acetyl-CoA is used in the Citric Acid Cycle where more energy in form of ATP equivalents is produced. These are then used in the electron transport chain where a proton gradient is made by multiple redox reactions. The proton gradient is used as energy by the membrane enzyme ATP synthase which produces more ATP (3).

Muscle uses energy in form of ATP to produce movement and force, during activity, e.g. exercise, the ATP consumption is increasing. ATP is found in an almost constant level in the body, when it is hydrolysed it also needs to be resynthesized in a similar rate. During exercise the hormones produced, e.g. adrenaline, regulates glycolysis to increase the flux. However, when the oxygen level starts to get low, the pathways which needs oxygen is supressed and pathways that work in anaerobic conditions is used instead. Pyruvate is then converted to lactate by lactate dehydrogenase (LDH) to reoxidized NADH to NAD+(18), a coenzyme used in glycolysis(19). Through reverse glycolysis called gluconeogenesis glucose can be created from pyruvate. Lactate that is created in the muscle cells from glucose is shuttled to the liver via the blood where the LDH converts lactate back into pyruvate, this can be observed in Figure 2. During anaerobic conditions lactate starts to accumulate in the bloodstream (3).

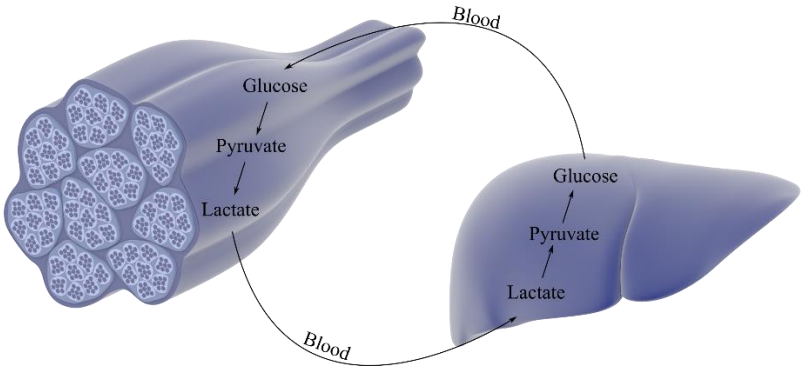


Figure 2, schematic picture of the simplified pathway between the muscles and liver.

2.1.1 Lactate measurement in exercise

Lactate measurement can be used for multiplied purpose in a sport context, to evaluate and predict performance as well as a training tool(1). There are multiple concepts used for measurement of athletes fitness, e.g. aerobic-, anaerobic-, aerobic-anaerobic- and lactate threshold, onset of blood lactate accumulation (OBLA), maximal lactate steady state, individual anaerobic threshold and ventilatory threshold(1, 5). Some of these concepts are defined in Table 1. These different concept are ways to detect the intensity of exercise related to the anaerobic threshold(5).

Table 1, definition of different concepts used for sport analysis.

Concept	**Definition**
Anaerobic threshold (AT)	The oxygen uptake can account for the energy requirement need for the high intensity, i.e. where the blood lactate remains at a steady state (5).
Maximal lactate steady state (MLSS)	Where there is an equilibrium between the rate of transport into the blood and removal of lactate. Not same as AT when there still is an accumulation in the muscles while the blood concentration is constant(5).
Lactate threshold (LT)	The intensity of exercise that result in a considerable increase in blood lactate during an exercise test(5).
Onset of blood lactate accumulation (OBLA)	The intensity which the lactate concentration in blood reached 4 mM. Based on that AT is the same as that concentration (5).

There are multiple ways to evaluate the presented concepts, commonly used is the graded exercise test (GXT), where the intensity of the exercise is gradually increased in a controlled time while vital parameters are measured e.g. lactate concentration in blood and heart rate. The intensity is often referred to as the power (W) of the exercise, e.g. how fast the treadmill is going or the resistance from the cycle (4). The different concepts have both positive and negative aspects. OBLA ignores variability between individuals due to the use of a set concentration(5). The use of this concentration is due to the value reflecting lactate accumulation within the active muscle (1). MLSS, on the other hand, needs to use multiple testing but takes the variability between individuals into account. LT can be defined by multiple principles, as the intensity of training where the resting blood lactate level is increased by 1 mM, when the blood concentration starts to withdraw from the linearity as well as OBLA. These concepts are often close to AT but not equal to(5).

The concepts should be evaluated based on their context of use(5), the set-up during testing needs to be taken into consideration as there are multiple physical factors that can affect the result(1). Athletes want a high AT as this implies that the body can handle a higher intensity of exercise before the body needs to use anaerobic processes. As mention the use of these thresholds is often for design of training programs. An exercise below the intensity related to the threshold is mild, while one above is high and there in-between moderate(5).

2.2 Sweat

Sweat is used to thermoregulate the body, which is one of the fundamental activities for our bodies (6-8). It mainly consists of water (99%) and low concentrations of analytes found in plasma, where the main metabolites are lactate, different amino acids and lipids(6, 20). Other biomarkers found in both sweat and blood includes Sodium (Na^+), Chloride (Cl^-), Potassium (K^+) and Glucose(9). Sweat is dependent on multiple features, it differs between individuals and body parts(6). The different biomarkers have different relationship between sweat and blood. Due to the transport process the relationship can be passive, active or self-generating(9).

2.2.1 Sweat glands

Sweat is a result of the eccrine sweat glands secretion. The glands are found over large part of the body but with a changing density depending on the body part and individual(8). The sweat glands are located in the skin, see Figure 3A. The gland consists of three parts, the upper coiled duct, which ends at the skin surface and where the sweat is emerged, the dermal duct where some biomarkers is transported into sweat and the secretory coil, the base of the gland and where additional biomarkers is incorporated (9). This is showed in Figure 3B.

Water is introduced to the sweat gland in the secretory coil, as well as Na^+ and Cl^-. Na^+ and Cl^- are later reabsorbed in the dermal duct. These ions have an active transport in the gland and are sweat rate dependent, a concept explained further below. In the secretory coil the transport of metabolic origin is also included into the fluid that is sweat. These molecules with metabolic origin e.g. lactate, is likely both produced in the gland and transported from the plasma(9). The transport of these molecules can be observed in Figure 3B.

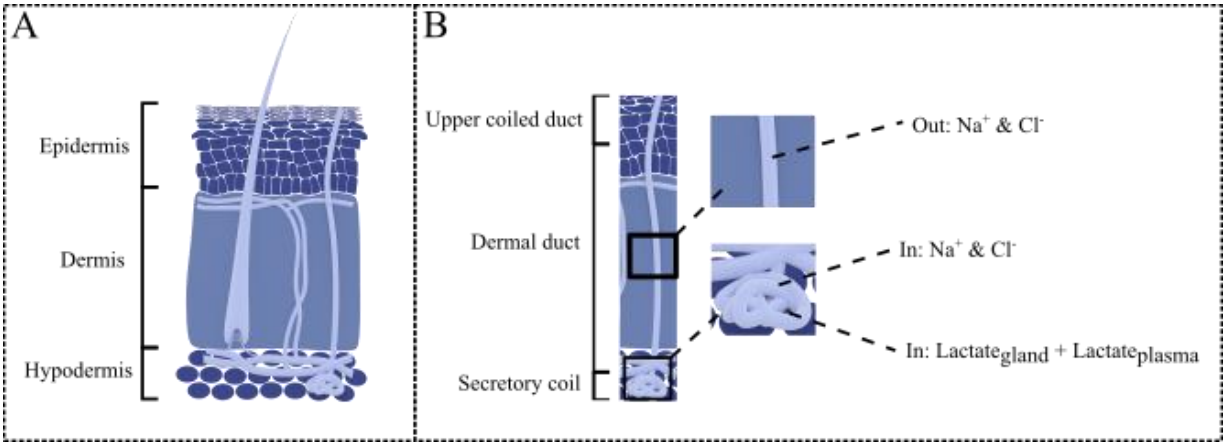


Figure 3, A: illustrative picture of the skin cell with a hair coil, blood vessels and sweat gland. B: the parts of the sweat gland and the transport of lactate, sodium and chloride from the secretory coil and dermal duct (9, 21).

2.2.2 Sweat rate

Sweat rate is the rate in which sweat is secreted. It can be calculated both from local or whole-body perspective, the different calculations have different aspect in regards of sweat sensing(22). It can be found in different units depending on the calculations, when the density of the sweat gland is taken into

consideration, ml/min/m^2 (8) and where it is calculated based on the whole body, l/h (22). Sweat rate is also found to have a relationship with the exercise intensity(9, 23).

Local sweat rate (LSR) can be measured through mass changes in a collection system over a specific body part. For collection system filter paper, patches, pouches, cotton products can be used. To be sure that the sweat collection is representative, testing should be made when sweating reaches a steady-state. For avoiding changes in the sweat rate due to environmental variations, the test should be done during limiting time(22). Studies have shown that there is a high local sweat rate on the head, finger/hand and back(lumbar) of their participants (24).

To calculate the whole body sweat rate (WBSR), the change in body mass during training can be evaluated. This by measuring the nude weight before and after training and correlate by the non-sweat body mass changes during the training e.g. consumption of food, void bladder, water consumption. Using nude body weight minimize the error of sweat trapped in clothes. Additional changes in mass due to metabolic and respiratory mass loss should be corrected to if the test was set in a challenging environment (high intensity or dry environment) or for a long time period (2-3h). The test should be under circumstances where the athlete usually exercise and the calculation can be made according to the Equation 1 (22).

$$WBSR = \frac{m_{\text{before exercise}} - (m_{\text{after exercise}} - m_{\text{fluid intake during exercise}} - m_{\text{urine output during exercise}})}{t_{\text{exercise}}} \quad (22, 25) \qquad \text{Eq. 1}$$

For ions e.g, Na^+ and Cl^-, the sweat rate affects the reabsorption back to the sweat gland. This is for the body to keep its electrolyte levels(26). The concentration of these electrolytes in sweat reflect the water loss in blood during physical exercise. Ring M, et al, showed a significant correlation between Cl^-, concentration and total body weight (TBW) loss during a 2 h interval training, this can be applied to Na^+ as Na^+ and Cl^- is correlated. This sets a base of being able to quantify TBW loss from electrolyte concentrations (26). In further extent these concentrations can be used for calculation of SR as a higher concentration correlates to a higher sweat rate(8, 9, 27). The forearm is a suitable site for these types of measurement as concentration of sodium has been shown to correspond to the concentration in whole body sweat(22).

2.2.3 Lactate in sweat

Lactate can be found in a concentration of 5-60 mM in sweat(9), coming from both the blood plasma but as well from the eccrine gland(9, 28). The transport from plasma is not fully understood but it is thought to relate to the flux from the sweat gland but also passive or active transport from the plasma. Lactate is sweet rate dependent (SWD) and can therefore be found in higher concentration in sweat compared to blood. However the level of lactate is an indirect indicator of physical effort(9). Due to the complexity regarding SWD there are different studies both arguing for and against if lactate in sweat correlate to blood and exercise intensity. Changes in concentration is dependent on the parameters used, testing site and test conditions(7).

Sakharov D. A. et al. found a relationship between lactate in sweat and blood, by evaluating the percentual increment of lactate above the working muscle(28). Buono M. J, et al. suggested an additional way of analysing lactate concentrations in sweat, using lactate excretion rate (LER) (nmol/cm^2/min) instead of concentration of lactate. LER is defined as the sweat lactate concentration and multiplied with the sweat rate at the test site. Taking LSR into account minimizing the error a dilution effect as the intensity of the exercise increases (23).

2.2.4 Sweat collection

Using sweat as a biofluid for sensing allows for the sensor to come close to the newly produced liquid(9). As discussed previously there are multiple ways to collect sweat for measurement of e.g. LSR. In addition, there are sweat collection system using the sweat glands itself as a hydraulic pump. Liu et al,

created a sweat collector by 3D printing a round collector with a curvature of 1 mm sagitta and a hole (d=2mm) touching the skin. The sweat was transported through the hole to a tube where they used the sweat for conductive measurement(29). Sweat collectors can be placed on multiple body parts e.g. forearm(29) and under the chest(26).

2.3 Electrochemistry

Electrochemistry involves the relationship between electrical and chemical reactions. When conducting electrochemical measurement a potentiostats is used to control the electrochemical cells(30).

2.3.1 Cyclic voltammetry

Cyclic voltammetry (CV) is a widely used electrochemical method(30) that can be used to get a fingerprint of chemical reactions conducted at the surface of an electrode. Just with a few calculations a lot of information regarding the measurement can be obtained, e.g. thermodynamic and kinetic parameters(31). The analyte will move to the electrodes surface by convention, migration and diffusion. Through experimental setup the convention and migration movement can be kept to a minimum. The convention movement is the result of e.g. stirring or vibrations, migration is due to electrodes moving due to an electric field(30).

The method consists of a triangular change in potential(32), from the initial potential a linear increase of potential is made until reaching the switching potential, where the potential is switched and decreased to the starting value(31). The rate of the potential changes from the initial to the final potential is called the sweep rate(31) or scan rate(30), this variable controls the time for the experiment(31, 32). This result in a potential profile as seen in Figure 4. The molecules charge changes depending on the potential. The diffusion layer is depending on the number of molecules with a changed charge that can be found at the electrodes surface. If the potential is scanned negatively positively charged ions will be reduced to its neutral form. The more molecules that have been reduced create a larger diffusion layer and make it harder for the non-reduced molecules to reach the electrode surface(30). In addition, the diffusion layer depends on the scan rate, a high rate results in higher currents as an result of a smaller diffusion layer(30).

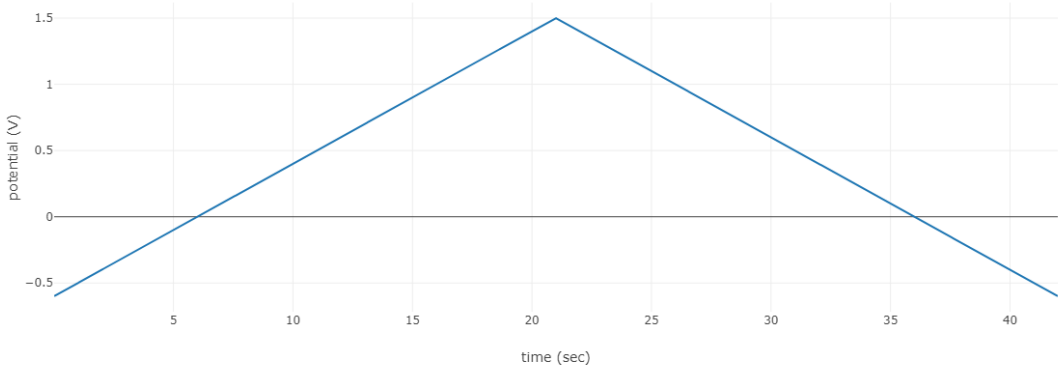


Figure 4, CV made on dummy cell between -0.6 V and 1.5 V with the scan rate of 0.1 V/s. Graph show the change in potential over time.

From a CV scan the relationship between the potential (E) and the resulting current (i) (30, 32) is of interest. During scanning when the potential is reduced it is called negative or cathodic direction, the peak current in this area is referred to as $i_{p,c}$. On the contrary when the potential is increased it is called positive or anodic direction, where the peak current is $i_{p,a}$ (30). These peaks together with the equivalent

peak potential is important parameters obtain from the test(32). The peak current can be used to find a relationship with concentration(33).

The experimental parameters for CV measurement include the scan rate, but also information about the solvents used as well as the electrodes. The analyte needs to come in contact with the surface of the electrode and this is made by the supporting electrolyte. A supporting electrolyte reduces the solutions resistances which helps the movement of the electrodes. The supporting electrolyte is a salt dissolved in a solvent suitable for the experiment conditions. A high level of electrolyte maximizes the movement of analyte through migration to the electrodes(30).

2.3.2 Electrochemical cells

There are multiple electrode setups, often used is a three-electrode setup, containing of a working electrode (W.E.), counter electrode (C.E.) and reference electrode (R.E.). The working electrode is where the main electrochemical reaction happens, the surface should be well defined as the measurements depends on the surface interaction with the molecules. The reference electrode is used as a reference point during electrochemical measurement, therefore, the applied potential is often referred versus the reference used. The counter electrode is used to complete the electrical circuit. Therefore the current can be measured between the W.E. and C.E. (30).

Screen printed electrodes

The use of screen printed electrodes allows for a minimization of the setup for electrochemical measurements, require less maintenance and no need for large sample volumes(33, 34). DropSens provides screen printed carbon electrodes (SPCE) made with a three-electrode configuration. The different electrodes are printed onto a ceramic substrate, with a W.E. and C.E. of carbon, pseudo R.E. and electric contacts of silver. Over these an insulated layer is printed leaving only a volume of 50 µL uncovered, called the working area (33). A SPCE can be seen in Figure 5.

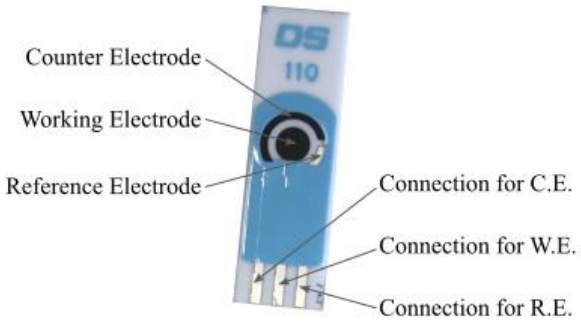


Figure 5, picture of a screen-printed carbon electrode with gold nanoparticles from DropSens with the different electrodes and connections are pointed out.

2.4 Selective sensor

To minimize the interference from the complex solutions, sensors can be made to be selective for the analyte of interest. There are both enzymatic and non-enzymatic selective sensors and they have different advantages depending on the area of use.

2.4.1 Enzyme based sensor

Most non-invasive sweat sensors are based on degrading of lactate with enzyme (35-41) and detection often by amperometric sensors. The amperometric sensor measure the change in hydrogen ions and the signal can be converted to a lactate concentration. Two types of enzymes can be used for these types of

measurement, lactate oxidase (LO) and LDH(42). The mechanisms of the breakdown can be shown in Figure 6.

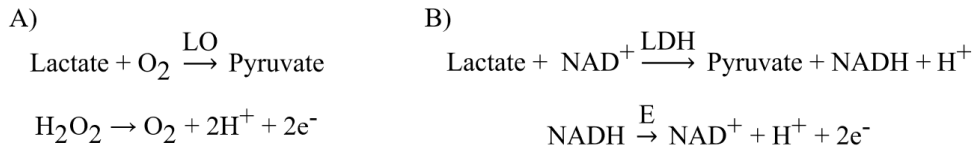


Figure 6, showing reaction for the enzyme A: LO and B: LDH in contact with lactate, (42).

The usage of enzymes provides a specific and sensitive method, however there are some negative aspects. Enzymes are very sensitive to different conditions as heat and pH(10-12), the change of conditions can result in inactivation of the enzyme, and therefore a non-functional sensor. During training, the temperature increases which impact the characteristics of sweat, meaning usage of the sensor can affect the enzyme negatively. Enzyme based sensors have a restricted effective time, meaning that the usage of these will be limited to two hours (38) and that is not enough time for a long-distance sport. In addition, the sensors need to be stored in the cold to not loose sensitivity during long storage(38).

2.4.2 Non-enzyme based sensor

A non-enzymatic sensor needs to be able to have similar specificity as the enzymatic sensor while providing better storage and stability over time.

Molecular imprinted polymers

A possible alternative to enzymes is molecularly imprinted polymers (MIP) that mimic the lock and key mechanism of enzymes. MIPs mimic enzymes active site by being synthesis to form cavities matching the analyte of interest, therefore making them specific(11). The target can bound back to the MIPs and result in a change that can be picked up depending on sensor. In comparison with enzymes MIPs are more stable for extreme pH, temperature and pressure(43).

The target, often referred to as template in the synthesis process, should not be affected by the polymerization and temperature changes. The template binds to functional monomers to create the complex which later create the cavities (44). These functional monomers is to enhance the selectivity of the target(12). For use in electrochemical measurement, it is suitable to use monomers that can form non-conducting films or conducting films(14). Cross-linkers is then used to stabilize the polymer matrix created around the template and functional polymers. (44). The function of MIPs can be observed in Figure 7. When modifying an electrode with MIPs the thickness of the layer is important, a too thick layer can negatively affect the sensing capability while a too thin layer does not provide the desired specificity and stability(12).

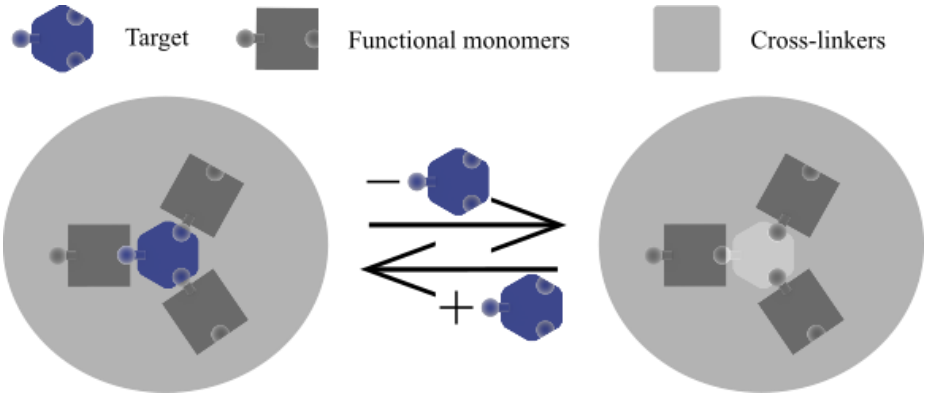


Figure 7, illustrative picture of MIPs where a cavity is filled or unfilled depending on if the target molecule is present.

By using MIPs for the sensor similar features as with enzymes can be achieved. A molecular imprinted sensor have good limit of detection and because the sensory system can be small it is suitable for smaller sensory as handhold devices(11). Previously studies have used this technique to detect lactate in sweat (13).

2.4.3 Modification of screen printed carbon electrodes

SPCE can be modified both with biological samples e.g. strings of nucleic acids and proteins as well as non-biological samples. The use of these modified electrodes is wide, electrodes have been specified to e.g. ethanol(33) glucose(12) and lactate(13). In addition, gold nanoparticles (AuNP) are often electrodeposited on SPCE to increase the stability and sensitivity. This due to gold's electrocatalytic activities and compatibility with multiple biomolecules (45).

The MIPs can be integrated to the electrode by different methods, e.g. drop-coating and electropolymerization (14). Drop coating is made by dispersion of a solution with the chosen molecules on the working electrode and then left to dry(33). Electropolymerization uses the potential from the electrode to build the polymer structure onto the electrode(46). This speeds up the time of an otherwise long process(14). For verifying that there is polymers left after cleaning on the modified electrode, a CV measurement is often made. This to see how a modification change the cyclic voltammogram e.g. characterization of the sensor probe(12) as well as determination of the new surface(33).

Often used for sensors is electronically conducting polymers (ECP) due to their electronic properties. An example of ECP is polypyrrole (PPy) synthesised by oxidation through an oxidative agent. During synthesis of MIPs a problem is often related to the removal of template before usage. When synthesising MIPs using ECP overoxidation of the polymer structure cope with this problem. Overoxidized Polypyrrole (oPPy) is an ECP used in multiple MIPs for chemical based sensors. Pyrrole is a common used functional monomer during productions of MIPs(47). PPy has been used previously for production of MIP due to its stability both over time and during different pH. As the oxidation of PPy to oPPy is irreversible and related to the high stability(48). Using a one-step preparation technique MIP imprinted with lactic acid of oPPy has been synthesis for used for detection of amino acids. By different oxidative agent as well as additional polyvinylpyrrolidone (PVP) to they were able to produced MIP of oPPy colloids(49).

2.5 Characterisation methods

For characterisation multiple methods can be used. To evaluate the change of surface behaviour for sweat collector contact angle measurement can be used. Chemical characterisation of the modified material can be observed by analysing the surface as well as the chemical bonds, which can be made

using contact angle measurement, SEM and FT-IR. For a complex matrix, standard addition is a method to evaluate the concentration of an analyte taking the matrix into consideration.

2.5.1 Contact angle measurement

To investigate the character of a surface, surface contact angle can be measured. This by measuring the angle between the liquid and solid when a droplet of liquid is placed onto the surface. A lower contact angle is corresponding to a more hydrophilic surface(50).

2.5.2 Fourier transform infrared spectroscopy

Fourier transform infrared spectroscopy (FT-IR) gives insight in the functional groups of the molecules, as it detects the properties of the bonds within the molecules. The infrared energy able to pass through the sample is plotted against the wavelength (often showed in wavenumber(cm^{-1}), the number of wavelengths in one centimetre). The peaks observed in the spectrum correlate to a bond within the molecule(51).

2.5.3 Scanning electron microscopy

Scanning electron microscopy (SEM) is a method for detection the topology of a surface. This by scanning every position of a x-y grid made over the sample. Each placement of the grid is hit by the electron beam and analysis of the scattering from the point is evaluated. Depending on the relative strength of the measured signal the part of the grid is varying from black to white(52).

2.5.4 Standard addition

When the matrix effects of a sample are specific for the sample standard addition is a method where the matrix effect is taken into account. In standard addition the concentration of an analyte (X) is evaluated dependent on a known added standard (S). A known volume (V_0) of the unknown sample is diluted in increasing volume (V_S) of a known standard solution to a set volume (V). The different dilution is then evaluated, and the response is the total response from both the unknown and known sample. The relationship between the concentration and response (I) from the initial (i) compared to the finale (f) are equal(53) as shown in Equation 2.

$$\frac{[X]_i}{[S]_f+[X]_f} = \frac{I_X}{I_{S+X}} \qquad \text{Eq. 2}$$

$$[X]_f = [X]_i\left(\frac{V_0}{V}\right),\ [S]_f = [S]_i\left(\frac{V_s}{V}\right) \qquad \text{Eq. 3-4}$$

Equation 2-4 can be further elaborated to Equation 5, from this the concentration of the sample ($[X]_i$) in the solution can be calculated as the x-intercept, i.e. when the y-axis is equal to zero(53).

$$I_{S+X}\left(\frac{V_0}{V}\right) = I_X + \frac{I_X}{[X]_i}[S]_i\left(\frac{V_s}{V}\right) \qquad \begin{cases} x-axis = [S]_i\left(\frac{V_s}{V}\right) \\ y-axis = I_{S+X}\left(\frac{V_0}{V}\right) \end{cases} \qquad \text{Eq. 5}$$

3. Method

Multiple aspects of a sweat sensor have been evaluated. Firstly, sweat collection system, how to take the newly created liquid to the sensor. The modification of screen printed carbon electrodes and how they affected the analysis of lactate in sweat. Multiple concentration curves were conducted with increasing matrix complexity, to be able to create a reference from the observed peak current during cyclic voltammogram. Finally, using the obtained modification and concentration curve a pure sweat sample was evaluated to observed which method came closest to the true value found in the sweat sample. Before testing with a person all participants had to sign a consent form and health survey found in Appendix A – Contract with participants.

3.1 Sweat collector

A sweat collector was 3D-printed in PLA (Polylactic acid) by knowledgeable personal according to the design shown in Figure 8. The collector was printed in two different sizes, on smaller (2.9x0.4x2 cm) and one larger (5.5x0.4x4.2 cm) with an O-ring. The design of the larger collector can be seen in Appendix B – Design of sweat collector.

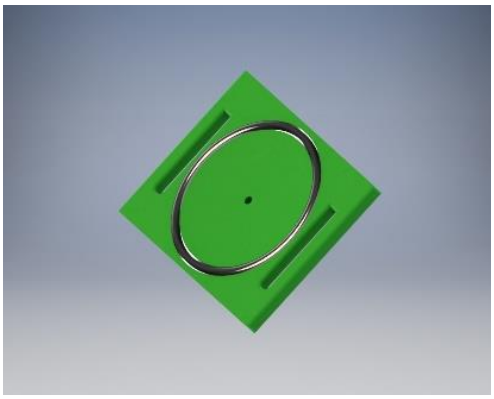


Figure 8, model of sweat collector with O-ring.

3.1.1 Test of sweat collector

Test of the sweat collector's function was made by a participant cycling with the goal to effectively start sweating. The sweat collector was secured with an elastic band around the upper arm of the participant. The heart rate and cadence were measured using Garmin 735 XT with effect pedals and heart rate monitoring belt under the chest. During the exercise the change in physical appearance (e.g. sweating) and if the participant started to feel fatigue was observed. The sweat collector was observed if sweat was participated through the column. After the test the sweat collector was removed and observed underneath.

3.1.2 Modification of sweat collector

To further develop the sweat collector, a modification of the surface was made through coating. A small volume of newly synthesised hemicellulose was dissolved in water during stirring and heating overnight. The solution was then distributed over a flat PLA surface and left to dry for 3 days. Contact angle measurement was conducted by analysing the angle between a water droplet and the uncoated/coated PLA surface using an Attension Theta optical tensiometer by KSV instrument. The mean contact angle between the uncoated or coated PLA and water was compared.

3.2 Sweat collection

Sweat was collected using two different methods, the first using absorptive pads and the second using pipettes. The collected sweat was from a male 50-55 years old with good physic.

3.2.1 Sweat collection with pads

Sweat was collected simultaneously as a lactate threshold measurement where the subject conducted a GXT on bicycle. Sterile pads were attached to the participants leg, neck and forearm with medical tape. Before application of the pads the body part was cleaned with alcogel. The pads on the leg was attached before the test and left during the entire testing period. Pads on the neck and forearm was changed approximately every three minutes to correspond to the blood samples taken for lactate threshold measuring. The pads were removed and stored in plastic containers sealed with parafilm in approximately 4º C until extraction. In total 13 samples were collected, one from the leg and six samples each from the neck and forearm. The pads were centrifuged 3000 rpm for a total of 30 min before additional centrifuge in 10 min at 3500 rpm in tubes with filter to extract the liquid from the pad.

3.2.2 Sweat collection with pipettes

During a training session sweat was collected from the back by pipettes. The participated cycled for 1.5 h in a low intensity pace before doing additional cycling for 1 h in a high intensity pace. The pace was set according to participant's anaerobic threshold. After the first part of the session approximately 0.5 ml of sweat was collected by a pipette and transferred to an Eppendorf tube. Approximately 10 minutes before the participant stop the session the existing sweat on the back was dried of with a sterile pad, after the session was done approximately 0.5 ml of sweat was again collected into a new Eppendorf tube. The Eppendorf tubes were closed and covered in parafilm and instantly chilled. The samples were kept below 0º C until testing.

3.2.3 Analyse of lactate concentration in sweat.

Using the standard addition method, the collected sweat was analysed to find the concentration of lactate in the sweat. For samples collected during low intensity exercise the sample was diluted according to Table 2, while sample collected during high intensity was diluted according to Table 3. The standard solution was 1 M of lactic acid solution created by diluting American Chemical Society (ACS) graded Lactic acid (85%) solution with distilled water.

Table 2, preparation of the solutions for standard addition testing at sweat collected at low intensity. Water is referring to distilled water.

	V_0 (µl)	V (µl)	$[S]_I$ (M)	V_s (µl)	V_{water} (µl)
Blank	0	500	1	0	500
1	50	500	1	0	450
2	50	500	1	25	425
3	50	500	1	50	400
4	50	500	1	100	350
5	50	500	1	150	300
6	50	500	1	300	150

Table 3, preparation of the solutions for standard addition testing at sweat collected at high intensity. Water is referring to distilled water.

	V_0 (µl)	V (µl)	$[S]_I$ (M)	V_s (µl)	V_{water} (µl)
Blank	0	500	1	0	500
1	50	500	1	0	450
2	50	500	1	50	400
3	50	500	1	150	300
4	50	500	1	300	150
5	50	500	1	350	100
6	50	500	1	450	0

The samples were further diluted by 10 (50 µl of solution in 450 µl of distilled water) to obtain a response within the limits of the system. This was included in the calculations of the true value of lactate within the sample. The samples were tested as described in 3.5.1 Concentration curve and analysed according to Appendix D.2 Data analysis of CV and D.4 Data analysis for standard addition.

3.3 Modification of SPCE

Based on previously reported one step preparation of MIP of oPPy (49), four different synthesis were made, exact volumes can be observed in Table 4. All containing pyrrole and ACS graded lactic acid (85%), two with poly(vinyl alcohol) (PVA) and with either potassium ferrocyanide ($K_4Fe(CN)_6 \cdot 3H_2O$) or ammonium persulfate ($(NH_4)_2S_2O_8$) as an oxidative agent. 0.0086 g of PVA (99%) was dissolved in distilled water during heating (60º C) and occasional stirring. Additional distilled water was added during the heating due to water losses. After 2 h most of the PVA had dissolved but some small undissolved parts could still be found in the solution.

Table 4, the different chemicals used for the four different synthesis of MIP of oPPy.

	A	B	C	D
Py	0.108 g	0.107 g	0.103 g	0.1 g
LA 85%	0.2 ml	0.2 ml	0.2 ml	0.2 ml
PVA solution	-	1.5 ml	-	1.5 ml
Distilled H_2O	10 ml	8.5 ml	10 ml	8.5 ml
$K_4Fe(CN)_6 \cdot 3H_2O$	0.4226 g	0.4226 g	-	-
$(NH_4)_2S_2O_8$	-	-	0.2284 g	0.2283 g

Pyrrole and lactic acid were first dissolved in room temperature distilled water creating a clear solution. The solution was left for 30 min before adding the PVA solution. After 2 hours the solution had turned lightly pink. The solutions were placed on a magnetic stirrer before the oxidative agent was added. In the solutions containing ammonium persulfate there was an instant precipitate resulting in that the solution becoming black whereas there was no noticeable product in the solutions containing potassium ferrocyanide. The pH was modified to pH 4 with 0.1M NaOH(aq). The synthesis was then carried out in room temperature for 16.5 hours. After synthesis all solutions had a precipitate and had turned dark green.

After synthesis the solution was centrifuged twice for 15 min and 4300 rpm. The solutions were decanted before 30 ml of distilled water was added and additional centrifuge for 15 min and 4300 rpm was conducted. The solutions were decanted again before addition of 5ml of distilled water, final centrifuge of 15 min and 4300 rpm (twice for the solution containing ammonium persulfate due to the precipitate still dissolved in the supernatant). The solutions were decanted carefully using a pipette and the pellets was left to dry overnight in room temperature. The black powder was then additional dried in 40º C for 1.5 h. 0.006 g of powder was then diluted in 2 ml of distilled water to create a polymer solution. The SPCE-AuNP was then drop-coated with the polymer solution and dried in room temperature for 3 days.

The powder was analysed by FT-IR using a Spectrum One FT-IR Spectrometer (Perkin Elmer instruments). 600 mg of dried potassium bromide (KBr) was grinded together with three equal parts of the sample powder (approximately 6 mg in total). The powder was then divided into three and pressed into pellets used for analyses. The analyse was conducted with 16 scans with a 4 cm^{-1} resolution from 4000-400 cm^{-1}, and background scan together with scan of pure KBr pellet as reference. Both unmodified SPCE-AuNP and the modified SPCE-AuNP were imaging using SEM on a LEO Ultra 55 (Gemini).

3.4 Calibration with dummy cell

For the electrochemical measurement an open source potentiostats(54), Rodeostat (IO Rodeo), was used together with screen printed carbon electrodes with gold nanoparticles (DRP-110GNP, DropSens). These were connected with an adapter (B, IO Rodeo) and the potentiostat was connected to a computer where the provided Rodeostat software app was used. The Rodeostat was set up according to protocol that can be found in Appendix C.1 Set-up of Rodeostat before testing.

Calibration of the Rodeostat was made following the protocol that can be found in Appendix C.2 Calibration of Rodeostat, based on the method suggested in a google document(55) from IO Rodeo. The potentiostat was connected to the computer and a 50 kΩ resistor. A cyclic voltammogram was taken between -0.6 V and 1.5 V with the scan rate of 0.1 V/s, for each of the available current range. The data was processed according to the code specified in Appendix D.1 Data processing of calibration with dummy, the applied potential was converted to current using ohm's law as the resistant of the dummy cell was known. The converted current was plotted against the obtain current from the test and a fitted line was obtained.

3.5 Screen printed carbon electrode testing

In total four different types of solutions were used for measurement with increasing complexity, 0.1 M potassium chloride (KCl) in distilled water or tap water, only tap water or artificial sweat. Tap water was used without any further modification. A simple artificial sweat was made containing Na^+, Cl^- and K^+ and glucose according to previously artificial sweat formulation (56). In an Erlenmeyer flask containing 50 ml of heated water (approximately 37º C) 0.0031 g of Glucose, 0.0456 g of KCL and 0.0099 g of Sodium chloride (NaCl) were added and stirred during heating. The pH was checked to be in-between 5-6 before filling the flask to 100 ml. This resulted in a concentration of 6.1 mM K^+, 1.7 mM Na^+, 7.8 mM Cl^- and 0.17 mM Glucose, which is in the range of concentration found in sweat. The solution was then left to cool done to room temperature before used for lactic acid stock solutions.

From a stock solution of 1 M lactic acid a 0.1 M solution was made, this was further dilute according to Table 5 to create multiple samples of known lactic acid concentrations.

Table 5, dilution table for creating different concentration of lactic acid solution.

Finale lactic acid concentration [mM]	Diluted from what solution [mM]	Volume of lactic acid solution[µl]	Volume of solvent [µl]	Total volume [µl]
2.5	25	100	900	1000
5	25	200	800	1000
10	100	100	900	1000
15	100	150	850	1000
20	100	200	800	1000
25	100	250	750	1000
30	100	300	700	1000
60	100	600	400	1000

3.5.1 Concentration curve

To create a concentration curve the electrode was inserted into the adapter and connected to the Rodeostat. 50 µl of the known lactate acid concentration was dispersed over the working area, making sure to cover the R.E. Cyclic voltammogram was taken between -0.5 V to -1.5 V with the scan rate of 0.05 V/s for each of the concentration and background scan. Between each measurement the electrode was rinsed with distilled water and dried carefully with a paper towel. Measurement was done in triplets by using three different electrodes. The current obtained from the measurements was corrected according to the calibration curve, the current was then background subtracted and filtered before analysed for local minimum and maximum. The mean of the peak currents was then used for linear regression and creation of a concentration curve. One-sided ANOVA testing was made to observe if there were any large differences between the electrodes. This was processed according to code that can be found in appendix D.2 Data analysis of CV and D.3 Data analysis for creating concentration curves. For the unmodified electrodes all different solutions were used and for the modified electrodes only artificial sweat was used for scanning concentration curve. Both electrodes where used for real sweat samples. For the modified electrodes only one scan was made per concentration and all scans was conducted on different electrodes.

4. Results and discussion

The results obtained by testing of the sweat collector, sweat collection, modification of electrodes and the finale sweat sensor will be presented and evaluated.

4.1 Sweat collector

Two types of sweat collectors were 3D printed, one smaller and one bigger version. They were tested so observe is sweat could be collected through the column during exercise.

4.1.1 Sweat collector version 1

The first version of the sweat collector with the size of 2.9x0.4x2 cm can be observed Figure 9.

Figure 9, first version of the sweat collector.

During testing of the sweat collector no sweat was observed to pass through the column. After the exercise when the collector was removed the elastic band used to keep the collector in place was wet and on the inside of the collector sweat drops could be observed. Due to the accumulation of sweat overall on the body, this could be the effect of the collector not sitting tight enough for the sweat gland to fully work as pumps.

4.1.2 Sweat collector version 2

The second version of the sweat collector was made in larger dimensions (5.5x0.4x4.2 cm) and with packing in the form of an O-ring to provide a seal between the skin and the sweat collector. The second version of the sweat collector can be observed in Figure 10.

Figure 10, the second version of the sweat collector with O-ring.

The second version of the sweat collector showed more promising results. After testing a clear sweat accumulation could be seen under the collector as seen in Figure 11, but no sweat was transported

through the entire collector. There was a clear mark on the participants arm of the O-ring suggesting that the collector was sitting tight and allowing the sweat to only transport within the collector. The increased size of the collector could be the result of this as it may take a longer period of time to fill the collector, so the glands can work as pumps.

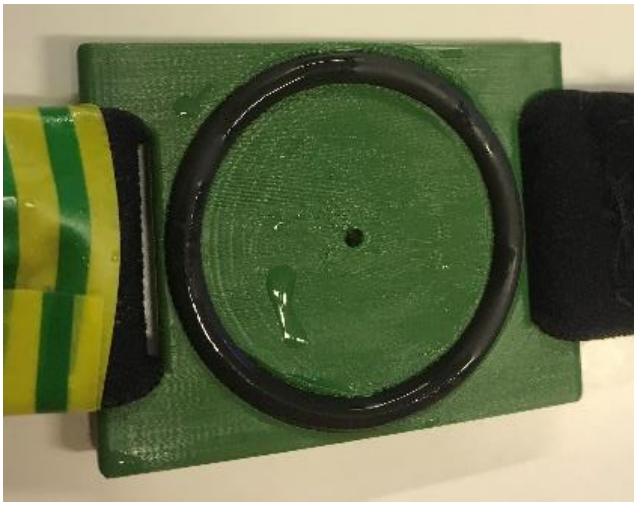


Figure 11, sweat collector ver.2 after test. A small sweat accumulation can be seen on the collector.

4.1.3 Test of modified sweat collector

By coating the material, the contact angle decreases as can be observed in Table 6, this indicates that the surface became more hydrophilic. As a more hydrophilic surface would imply that the surface would be better at driving the sweat forward using capillary force.

Table 6, the results from the contact angle tests. The contact angle decreased with a coating of hemicellulose.

PLA	
62.26 ± 5.15 (5)	mean degree ± SD (n)
PLA with hemicellulose	
44.35 ± 14.08 (6)	mean degree ± SD (n)

4.2 Sweat collection

Sweat was collected using two different collection methods for the aim of using it for lactate determination using the standard addition method.

4.2.1 Sweat collection with pads

During testing the participant showed a large sweat accumulation and that resulted in that the tape release from the skin in the neck. After centrifuging no accumulation of liquid could be observed in the tubes. There are multiple probable causes of this, the adsorption power of the pads was too good or that not enough sweat was collected during the short intervals. This can be the indication why the pads did not become saturated during testing and no fog was observed in the tubes. Due to the failed extraction no results could be obtained from this collection. Further test could be done on the headspace of the pads via HPLC to evaluate the concentrations.

4.2.2 Lactate value in sweat samples

Sweat collection using the second method resulted in approximately 0.5 ml of sweat that could be used for further testing. Using the accumulated sweat through this method the value of lactate within the sweat sample could be evaluated using standard addition. The standard addition curves can be seen in Figure 12 and through calculation the level of lactate was 14.32 and 7.81 mM for low- and high intensity, respectively. The decrease of concentration between low and high intensity may be a result of the dilution effect as sweat rate is affected by the intensity. Due to that SR was not determined at the time of testing the LER could not be calculated. For further sweat testing, SR should be a collected.

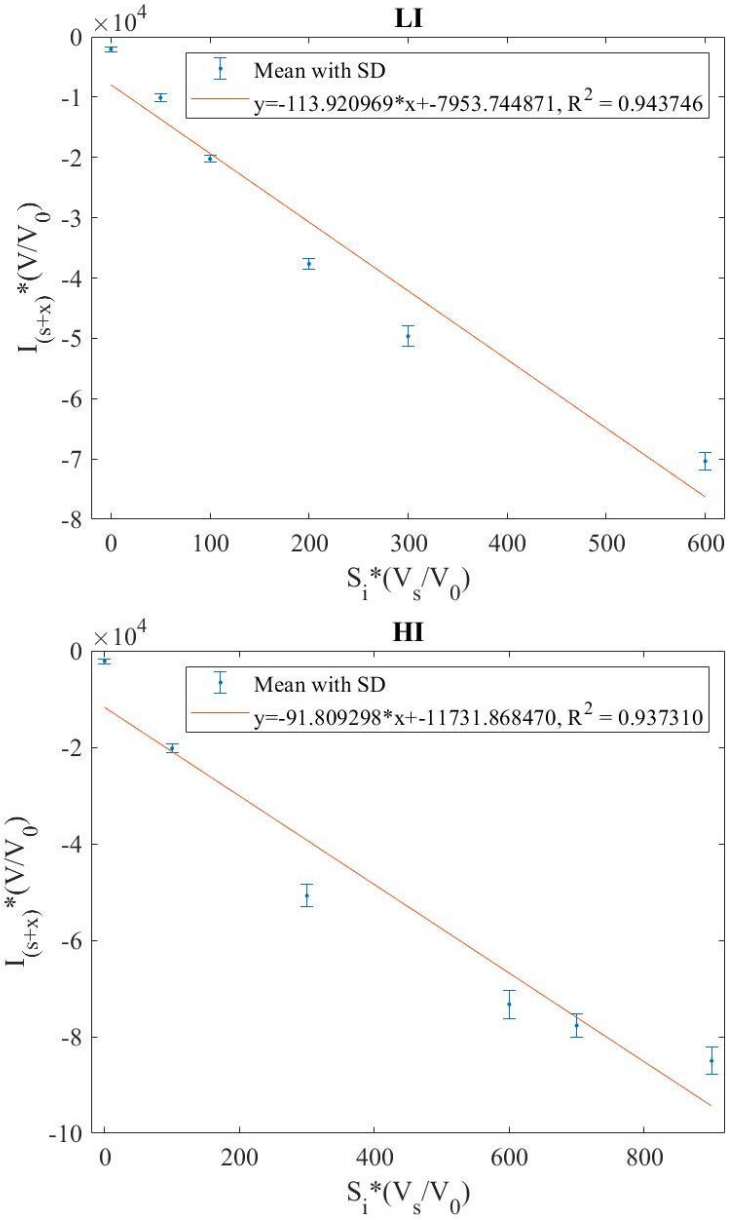


Figure 12, graphs from standard addition for sweat collected at low (LI) and high (HI) intensity.

4.3 Modification of electrodes

During the synthesis of oPPy the solution underwent multiple colour changes from a clear solution to a black powder as observed in Figure 13. Through the separation process not enough powder was formed from the solution using potassium ferrocyanide as oxidative agent. This indicated that there was a too low concentration of oxidative agents in these solutions. Further testing with different concentration of oxidative agent can be done to evaluate the concentration that results in a higher yield.

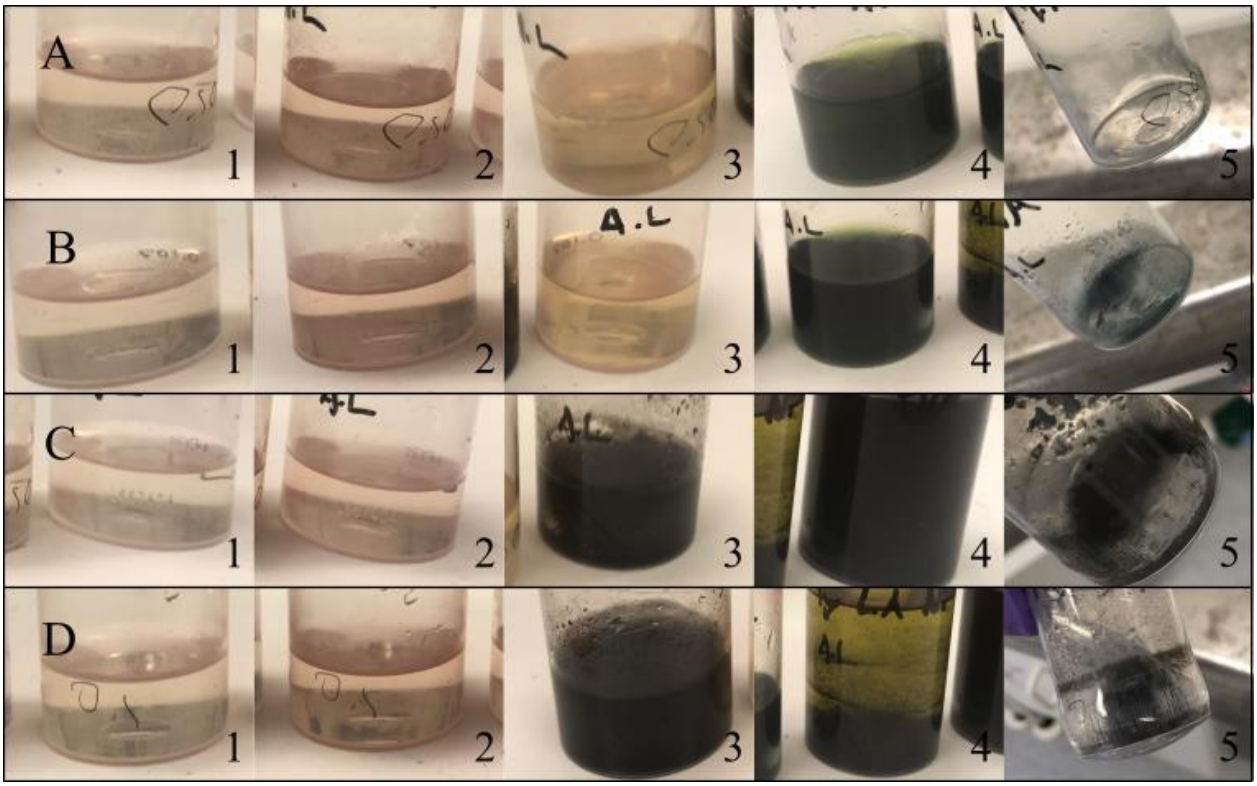


Figure 13, the synthesis process for creation of MIPs of oPPy. The different synthesis A-B had potassium ferrocyanide ($K_4Fe(CN)_6{\cdot}3H_2O$) while C-D had ammonium persulfate ($(NH_4)_2S_2O_8$) as an oxidative agent. B and D had PVA in the solution. Pictures at 1: is after addition of Py and LA solution, the solution is clear, 2: after 2 h, the solution has turned light pink, 3: after addition of oxidative agent, A and B have a yellow colour while C and D has a black colour, 4: after the synthesis was completed, all solutions is dark green, and 5: after decantation of supernatant, a black powder is left.

The synthesis using ammonium persulfate as oxidative agent resulted in a higher volume of powder, which was analysed using FT-IR to evaluate the level of lactic acid left within the polymer network. A lower level of lactic acid results in more empty cavities. The spectra can be observed in Figure 14. For PPy the peak at 1558 cm^{-1} is correlated to the C=C bond(49). The C=O and C-O bond in lactic acid is observed at 1651 and 1292 cm^{-1} respectively(49). There is a negative trend at approximately 1651 cm^{-1} and a peak can be observed at 1294 cm^{-1} correlating to lactic acid. The peak at 1294cm^{-1} is larger for the oPPy produced with PVA, this may be a result of the C-O bond in the PVA molecule. Overall there is no large difference between the polymers, but further testing was conducted using the PVA free oPPy as it may be the one with most cavities.

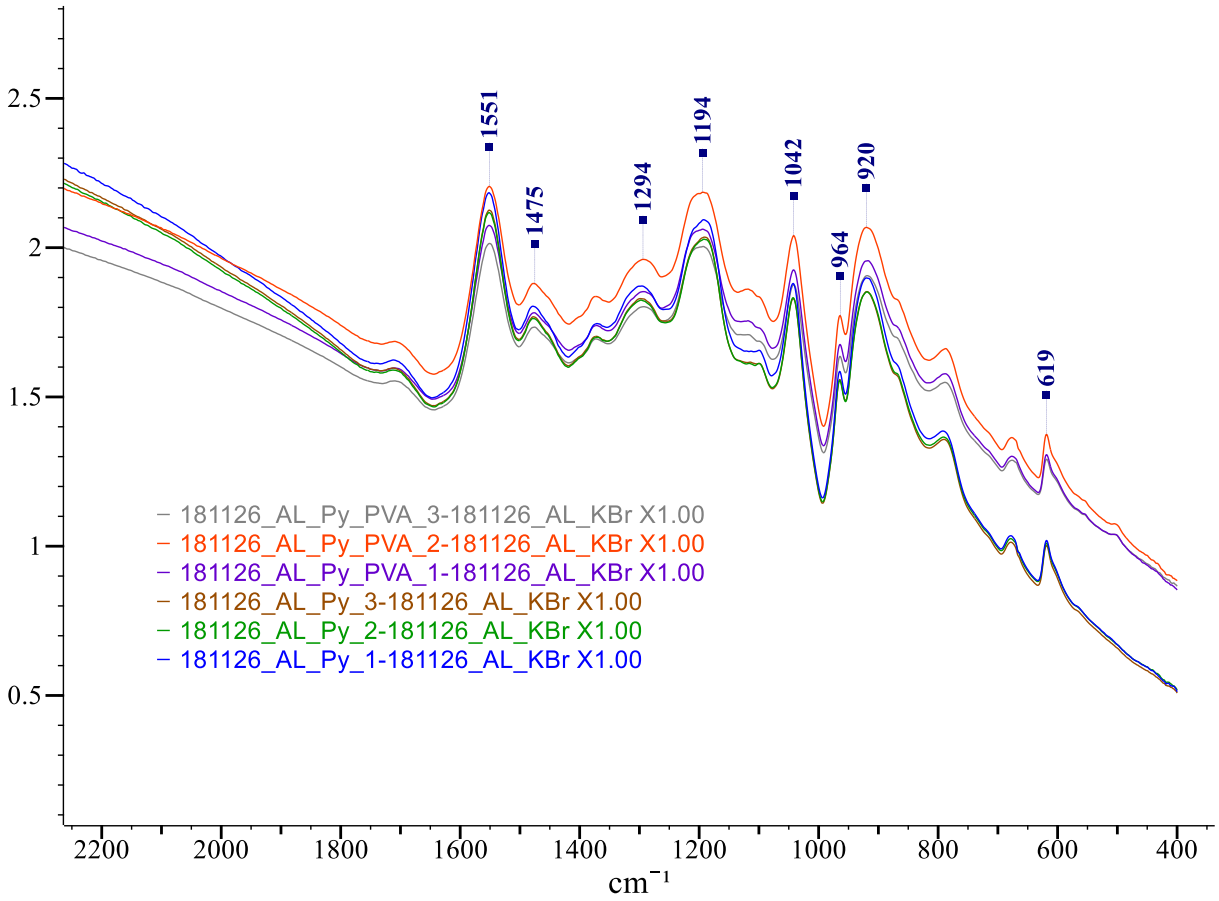


Figure 14, FT-IR spectra of oPPy produced with and without PVA, of the wavenumber (cm^{-1}) versus the absorbance. The background of the KBr has been subtracted.

4.3.1 Drop coating of electrodes

After drying a black layer could be observed on the modified SPCE-AuNP as shown in Figure 15, as further shown in Figure 16 the polymer network can be shown compared with the unmodified electrode. This indicated that there is a layer of MIPs on the surface of the electrode.

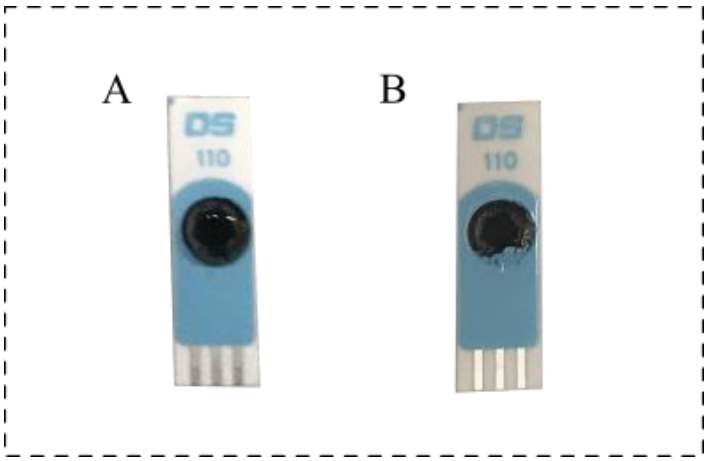


Figure 15, A: picture of SPCE-AuNP after addition of MIP solution, B: picture of the modified electrode after drying.

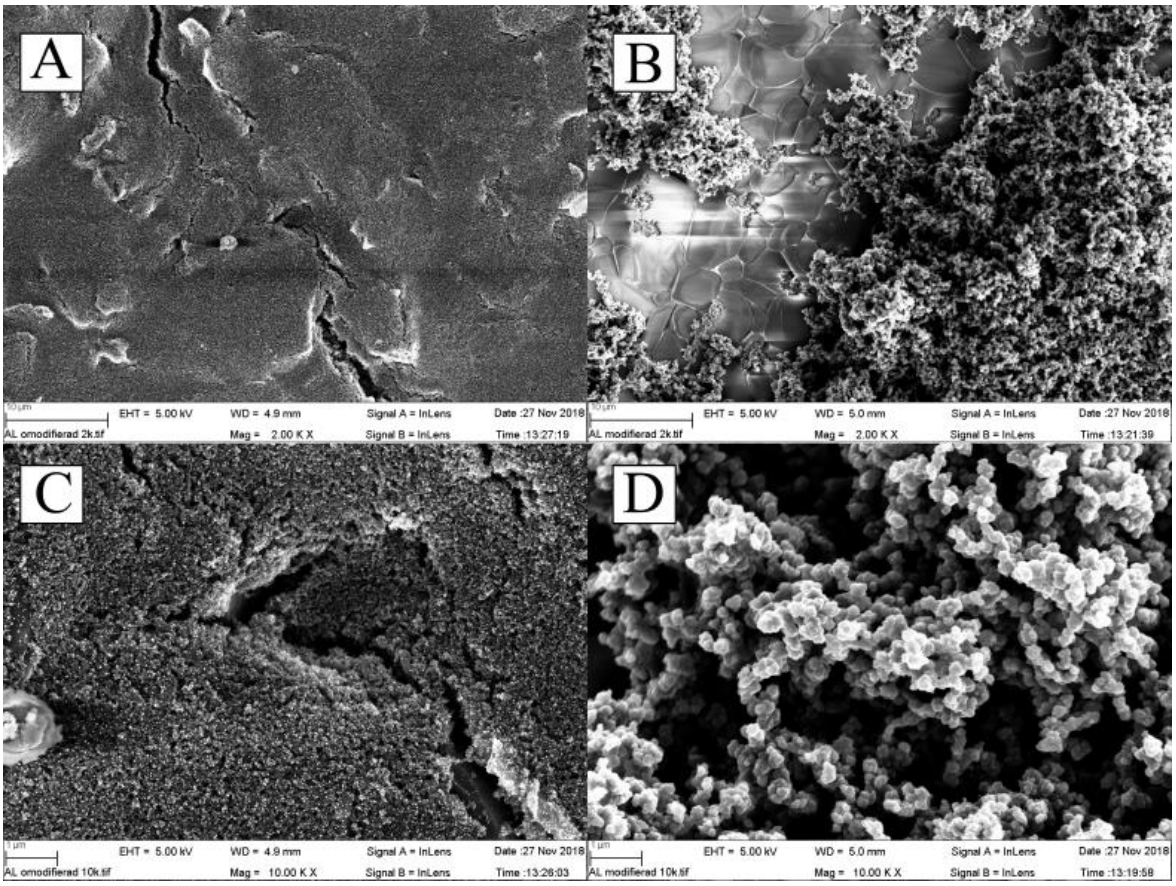


Figure 16, SEM picture of the SPCE-AuNP without and with modification. A: SPCE-AuNP at 2000 times magnification, B: modified SPCE-AuNP at 2000 times magnification, C: SPCE-AuNP at 10000 times magnification and D: modified SPCE-AuNP at 10000 times magnification.

4.4 Calibration with dummy cell

From the cyclic voltammogram of the dummy cell at different current ranges, the slopes and intersection of the linear regression can be seen in Table 7. The low R^2 values for the current rang ±1 µA and ±10 µA may be as the current was greater than the limits and therefore some flat lines can be found in these curves, as shown in Figure 17.

Table 7, results from calibration of dummy during 4 different current range. The slope, intersection and R^2 value corresponding to each of the ranges.

Current range (µA)	Slope	Intersection	R^2
±1	10.9	4.43	0.64
±10	1.56	2.39	0.83
±100	0.99	-0.84	0.99
±1000	0.99	-7.78	0.99

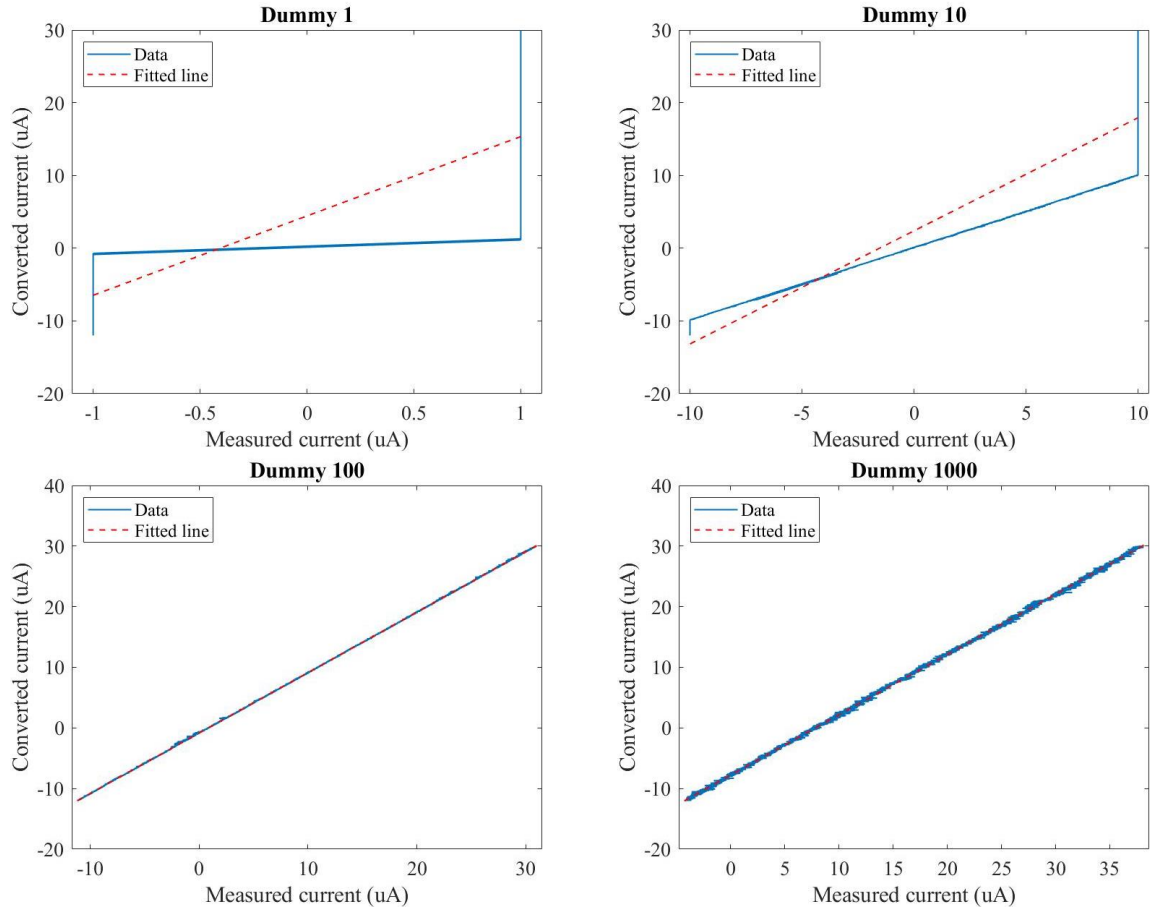


Figure 17, curves of the measured current compared to converted current from the calibration of Rodeostat via a dummy cell.

4.5 Concentration curves

By using the different solutions of known lactic acid content, concentration curves could be obtained.

4.5.1 SPCE with gold nanoparticles

From each of the electrodes a cyclic voltammogram for each of the concentrations could be obtained. This can be seen in Figure 18, where the local minimum between -1.1 V and -1.5 V is correlating to the lactic acid concentration. The peak current is decreasing with the increasing lactic acid concentration which is further evaluated to create concentration curves.

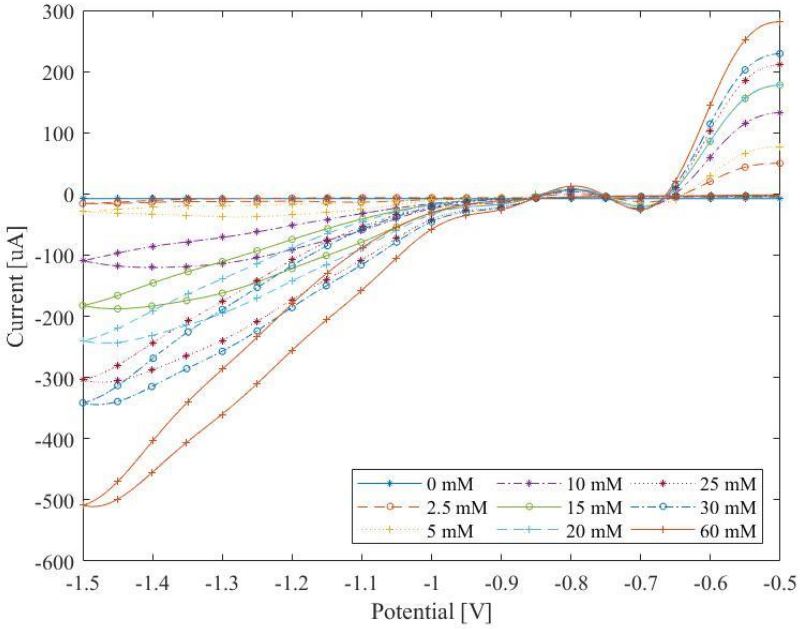


Figure 18, example of cyclic voltammogram measurement from one SPCE-AuNP with increasing concentration of lactic acid in 0.1 M KCl (tap water)

The linear regression obtained from concentration curves are displayed in Figure 19. Where there is a clear indication that the R^2 is decreasing in combination with an increase in the complexity of the solution used, with the exception of the artificial sweat measurement. This can be due to multiple parameters such as there being very low concentrations in the artificial sweat and only a few of the many analytes found in sweat and therefore being mostly distilled water.

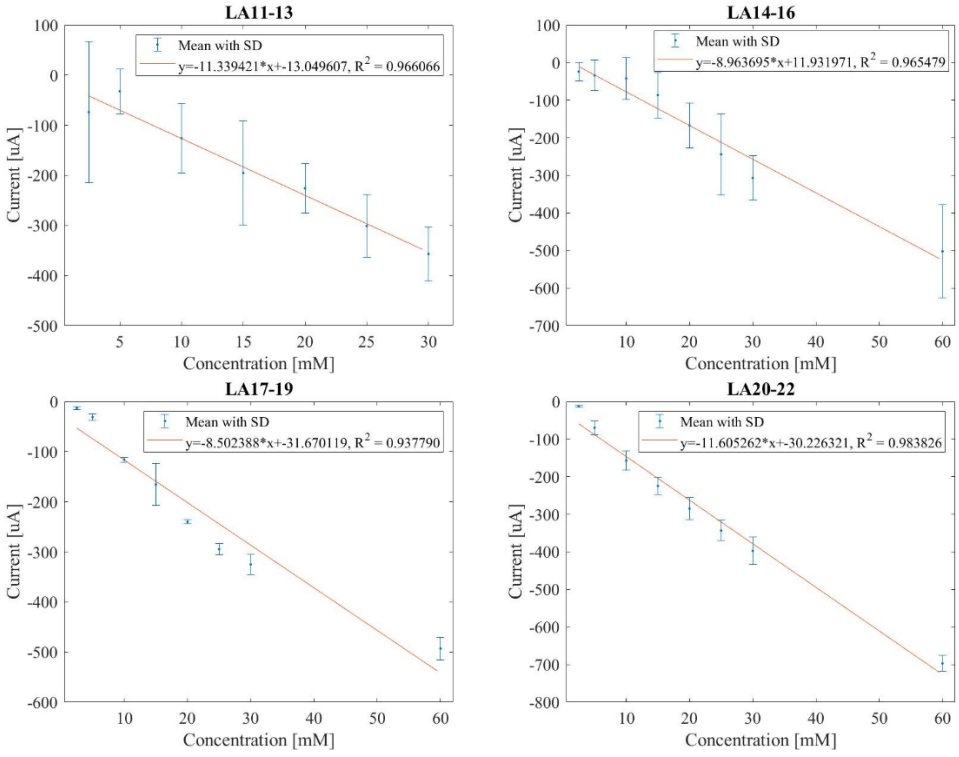


Figure 19, concentration curves obtained from measurement with different solutions. LA11-13: 0.1 M KCl in distilled water, LA14-16: 0.1M KCl in tap water, LA17-19: only tap water and LA20-22: artificial sweat.

Further comparison between the different solutions can be observed in Table 8, There are no significant differences between the different solutions, the slope from each concentration curve is within the same range and there are very small difference between the different R^2 values. However, the standard derivation is smaller for the more complex solutions.

Table 8, slope and intersection obtained from the different concentration curves using SPCE-AuNP.

Solution	Lactic acid range	Slope [µA/mM]	Intersection [µA]	R^2
0.1 M KCl in distilled water	2.5 - 30 mM	-11.34	-13.05	0.966
0.1 M KCl in tap water	2.5 - 60 mM	-8.96	-11.93	0.965
Tap water	2.5 - 60 mM	-8.50	-31.67	0.938
Artificial sweat	2.5 - 60 mM	-11.60	-31.23	0.984

4.5.2 SPCE with gold nanoparticles and modification

Because of the few available modified electrodes only one scan per concentration could be made, which increases the uncertainty of the concentration curve. In addition, the electrodes could not be reused so each measurement was conducted on a new electrode further increasing the error. With this in mind, the results from the concentration curve, shown in Figure 20, is similar to the result from the unmodified electrodes tested with artificial sweat.

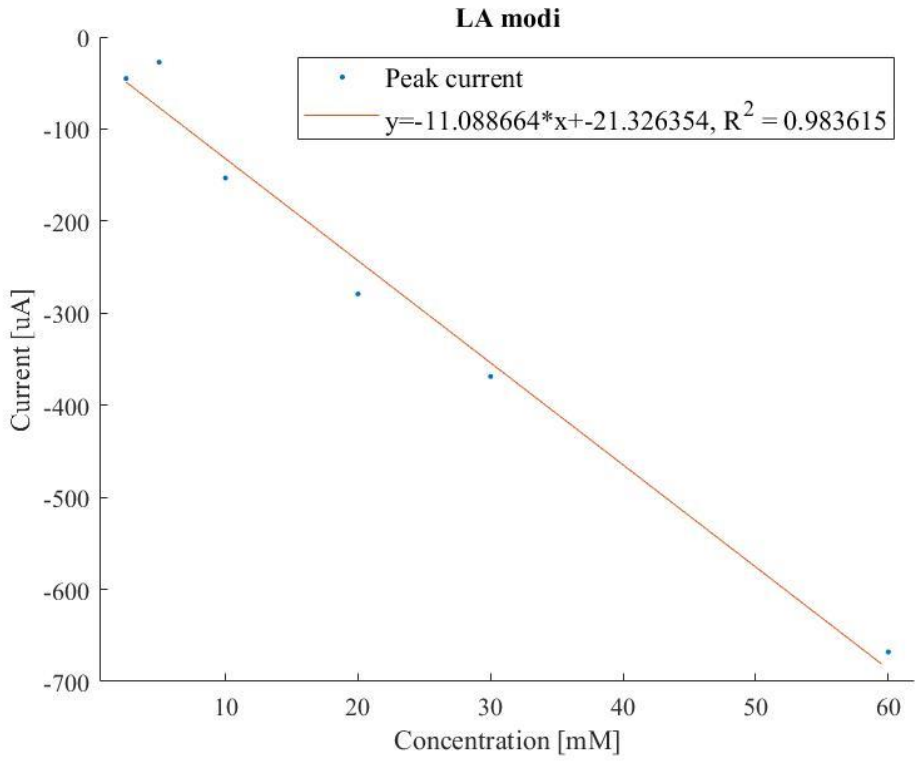


Figure 20, concentration curve with test of artificial sweat samples on modified SPCE-AuNP.

4.5.3 Sweat sensor

By scanning real sweat samples the lactate concentration could be evaluated. For the modified electrodes there was no reusability so different electrodes was used for each measurement (Blank, LI and HI) while the unmodified was reused for all three measurements. As seen in Figure 21, a difference can be observed between the samples taken during high intensity training compared to the one at low intensity training. Lower peak current (at -1.4 to -1.5 V) can be observed for the high intensity exercise compared to at low intensity.

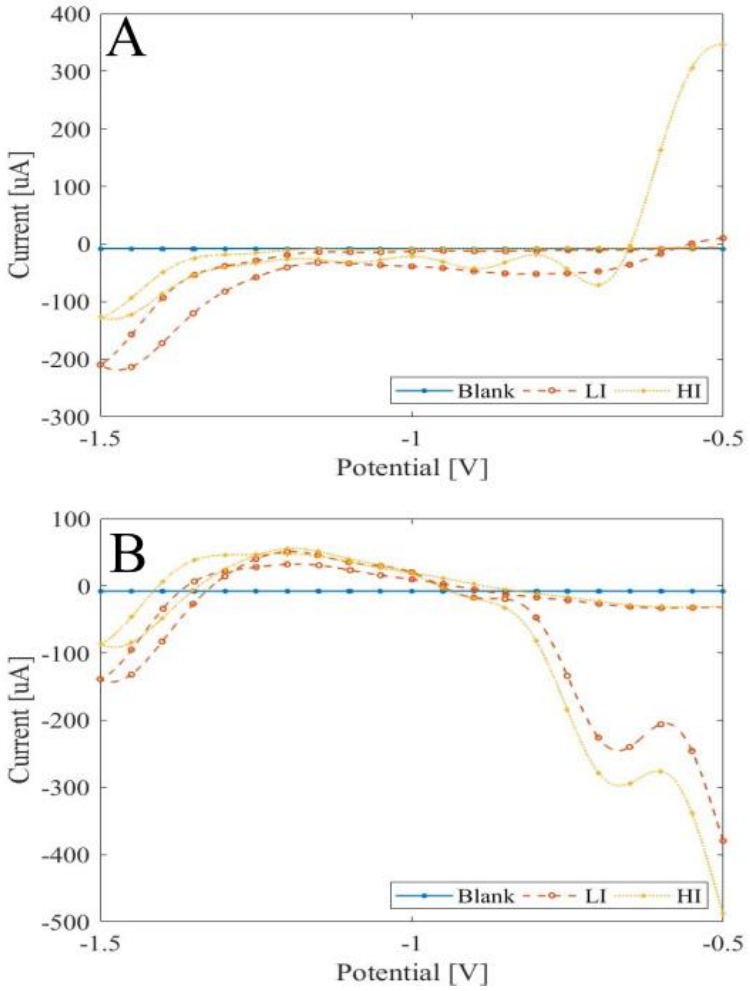


Figure 21, cyclic voltammogram of sweat samples on A: SPCE-AuNP and B: modified SPCE-AuNP.

Using the equation obtained by the concentration curves displayed in Table 8 and Figure 20, the lactate concentration can be evaluated. The results of this compared to the true value from the standard addition measurement can be observed in Figure 22.

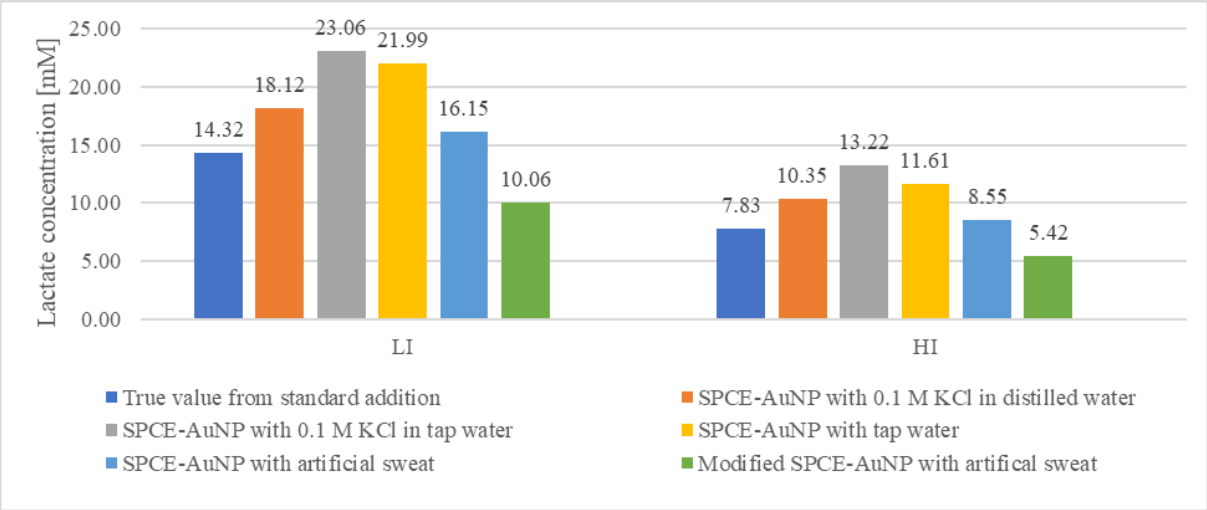


Figure 22, bar plot over the observed true value of lactate in sweat sample compared with the calculated samples.

The least difference can be found between the true value and the value calculated from the test of artificial sweat on SPCE-AuNP. There is a 12.75 and 9.21 percent difference for the sample during low intensity and high intensity respectively. The non-modified electrodes showed in general a higher concentration than the true value while the modified showed a lower value. As discussed previously a too thick layer of polymer on the electrode can result in a negative effect of the sensing capability. The low value from the modified electrodes can be a result of a too thick layer of oPPy, not fully allowing the change of lactate to reach the electrode surface properly. More data is needed to be collected to get a more statistical foundation of the measurements.

5. Conclusion and future outlook

The aim of the project was to produce a non-enzymatic sensory device for lactate measurement in sweat, which was succeeded. As shown, there is a great possibility in measuring the lactate concentration of a sweat sample using the suggested method. However, there are some additional modification needed before a product can be produced and used for these types of measurement.

The transportation of the newly produced biofluid of interest to the sensors was investigated using a sweat collector within this project. A collector made of a less stiff material with a coating would possibly make the transport better and would be a contributed factor to possibly have a more continuous measurement. Because of the small sample volume of sweat needed for lactate concentration evaluation, a test protocol similar to those used for blood lactate measurements should be possible. This as the measurement with electrodes is both quick and easy.

The modification of the electrodes showed to underestimate the lactate value and could not be reused. Through a more elaborate study it would be possible to examine more monomers that could be used for the modification as well as finding the optimal conditions for production. However, using a more complex artificial sweat for calculation of concentration curves would give a more exact results of the estimated lactate level. A more complex solution would come closer to sweat and also take into consideration how the level amino acids and other organic compounds affects the scans. For a product the electrodes should be produced on a flexible surface to be able to put close to the produced sweat.

Lactate is sweat rate dependent there is a need for measuring the local sweat rate (LSR) at the site where the sweat is tested to further use lactate excretion rate (LER) as the measurement instead. There are multiple ways of doing this, one way would be to evaluate the Cl^- concentration. The relationship between Cl^- and sweat rate makes it possible to calculate the sweat rate from the concentration. The sweat rate can then be used for calculation of LER. The relationship between these parameters needs then to be evaluated further. Another possibility would be to use a complementary technique e.g. FT-IR for evaluating the content of lactate in sweat, to evaluate the dilution factor further. This by correlating the complementary technique with the suggested method. Other parameters that need to be evaluated further is how the response from the sensor is affected by the outer environment such as temperature and pH. These will be affected during exercise and should be taken into consideration when conducting a more complex model for sweat sensing. Further research is also needed to fully understand the correlation between the concentration of lactate in blood and sweat to be able to use this sensor for lactate threshold evaluation as there is both studies agreeing and disagreeing with this possible correlation. With that in mind there is a great possibility for further evaluation of non-enzymatic based sweat sensor with a quick response and accurate evaluation of sweat samples as shown in this report.

6. References

1. Jacobs I. Blood Lactate. Sports Medicine. 1986;3(1):10-25.
2. Nationalencyklopedin. ATP [Internet] n.d. [cited 2018 12-04]. Available from: https://www.ne.se/uppslagsverk/encyklopedi/l%C3%A5ng/atp.
3. Hardin CC, Knopp JA. Glycolysis, The Krebs Cycle, Gluconeogenesis and Electron Transport and Oxidative Phosphorylation. Biochemistry - Essential Concepts: Oxford University Press; 2013. p. 174-87.
4. Jamnick NA, Botella J, Pyne DB, Bishop DJ. Manipulating graded exercise test variables affects the validity of the lactate threshold and V˙O2peak. PLoS ONE. 2018;13(7):e0199794.
5. Svedahl K, MacIntosh BR. Anaerobic Threshold: The Concept and Methods of Measurement. Canadian Journal of Applied Physiology. 2003;28(2):299-323.
6. Harker M, Coulson H, Fairweather I, Taylor D, Daykin CA. Study of metabolite composition of eccrine sweat from healthy male and female human subjects by 1H NMR spectroscopy. Metabolomics. 2006;2(3):105-12.
7. Derbyshire PJ, Barr H, Davis F, Higson SPJ. Lactate in human sweat: a critical review of research to the present day. The Journal of Physiological Sciences. 2012;62(6):429-40.
8. Sato K, Kang WH, Saga K, Sato KT. Biology of sweat glands and their disorders. I. Normal sweat gland function. Journal of the American Academy of Dermatology. 1989;20(4):537-63.
9. Sonner Z, Wilder E, Heikenfeld J, Kasting G, Beyette F, Swaile D, et al. The microfluidics of the eccrine sweat gland, including biomarker partitioning, transport, and biosensing implications. Biomicrofluidics. 2015;9(3):031301.
10. Copeland RA. Enzymes: A Practical Introduction to Structure, Mechanism, and Data Analysis. New York / Chichester / Weinheim / Brisbane / Singapore / Toronto: John Wiley & sons, Inc; 2000.
11. Horemans F, Diliën H, Wagner P, Cleij TJ. Chapter 5 - MIP-based Sensor Platforms for Detection of Analytes in Nano- and Micromolar Range. In: Li S, Ge Y, Piletsky SA, Lunec J, editors. Molecularly Imprinted Sensors. Amsterdam: Elsevier; 2012. p. 91-124.
12. Kim D-M, Moon J-M, Lee W-C, Yoon J-H, Choi CS, Shim Y-B. A potentiometric non-enzymatic glucose sensor using a molecularly imprinted layer bonded on a conducting polymer. Biosensors and Bioelectronics. 2017;91:276-83.
13. Zaryanov NV, Nikitina VN, Karpova EV, Karyakina EE, Karyakin AA. Nonenzymatic Sensor for Lactate Detection in Human Sweat. Analytical Chemistry. 2017;89(21):11198-202.
14. Yarman A, Turner APF, Scheller FW. 6 - Electropolymers for (nano-)imprinted biomimetic biosensors. In: Honeychurch KC, editor. Nanosensors for Chemical and Biological Applications: Woodhead Publishing; 2014. p. 125-49.
15. Interest CEoB. Lactate (CHEBI:24996) [Internet] 2015 [cited 2018 10-11]. Available from: https://www.ebi.ac.uk/chebi/searchId.do?chebiId=CHEBI:24996.
16. Gladden LB. Lactate metabolism: a new paradigm for the third millennium. The Journal of Physiology. 2004;558(1):5-30.
17. Todd JJ. Lactate: valuable for physical performance and maintenance of brain function during exercise. Bioscience Horizons: The International Journal of Student Research. 2014;7:hzu001-hzu.
18. Da Poian AT, Castanho MARB. Regulation and Integration of Metabolism During Physical Activity. Integrative Human Biochemistry: A Textbook for Medical Biochemistry. New York, NY: Springer New York; 2015. p. 341-74.
19. Nationalencyklopedin. NAD [Internet] n.d. [cited 2018 10-11]. Available from: https://www.ne.se/uppslagsverk/encyklopedi/l%C3%A5ng/nad.
20. Mena-Bravo A, Luque de Castro MD. Sweat: A sample with limited present applications and promising future in metabolomics. Journal of Pharmaceutical and Biomedical Analysis. 2014;90:139-47.
21. Bruce Alberts AJ, Julian Lewis, Martin Raff, Keith Roberts, Peter Walter. Molecular Biology of the Cell. 5:ed ed: Garland Science; 2008.

22. Baker LB. Sweating Rate and Sweat Sodium Concentration in Athletes: A Review of Methodology and Intra/Interindividual Variability. Sports Medicine. 2017;47(1):111-28.
23. Buono MJ, Lee NVL, Miller PW. The relationship between exercise intensity and the sweat lactate excretion rate. The Journal of Physiological Sciences. 2010;60(2):103-7.
24. Taylor NA, Machado-Moreira CA. Regional variations in transepidermal water loss, eccrine sweat gland density, sweat secretion rates and electrolyte composition in resting and exercising humans. Extreme Physiology & Medicine. 2013;2(1):4.
25. Hamouti N, Coso JD, Estevez E, Mora-Rodriguez R. Dehydration and sodium deficit during indoor practice in elite European male team players. European Journal of Sport Science. 2010;10(5):329-36.
26. Ring M, Lohmueller C, Rauh M, Eskofier BM, editors. On sweat analysis for quantitative estimation of dehydration during physical exercise. 2015 37th Annual International Conference of the IEEE Engineering in Medicine and Biology Society (EMBC); 2015 25-29 Aug. 2015.
27. Buono MJ, Ball KD, Kolkhorst FW. Sodium ion concentration vs. sweat rate relationship in humans. Journal of Applied Physiology. 2007;103(3):990-4.
28. Sakharov D, Shkurnikov M, Vagin M, I Yashina E, A Karyakin A, G Tonevitsky A. Relationship between Lactate Concentrations in Active Muscle Sweat and Whole Blood2010. 83-5 p.
29. Gengchen Liu MA, Bunyamin Sahin, Samuel E. Snelgrove, Jeffrey Edwards, Axel Mellinger, and Tolga Kaya. Real-time sweat analysis via alternating current conductivity of artificial and human sweat. Applied Physics Letters. 2015;106(13):133702.
30. Elgrishi N, Rountree KJ, McCarthy BD, Rountree ES, Eisenhart TT, Dempsey JL. A Practical Beginner's Guide to Cyclic Voltammetry. Journal of Chemical Education. 2018;95(2):197-206.
31. Heinze J. Cyclic Voltammetry—"Electrochemical Spectroscopy". New Analytical Methods (25). Angewandte Chemie International Edition in English. 1984;23(11):831-47.
32. Kissinger PT, Heineman WR. Cyclic voltammetry. Journal of Chemical Education. 1983;60(9):702.
33. Pereira Silva Neves MM, González-García MB, Bobes-Limenes P, Pérez-Junquera A, Hernández-Santos D, Vidal-Iglesias FJ, et al. A non-enzymatic ethanol sensor based on a nanostructured catalytic disposable electrode. Analytical Methods. 2017;9(35):5108-14.
34. Martín-Yerga D, Costa Rama E, Costa García A. Electrochemical Study and Determination of Electroactive Species with Screen-Printed Electrodes. Journal of Chemical Education. 2016;93(7):1270-6.
35. Cai X, Yan J, Chu H, Wu M, Tu Y. An exercise degree monitoring biosensor based on electrochemiluminescent detection of lactate in sweat. Sensors and Actuators B: Chemical. 2010;143(2):655-9.
36. Jia W, Bandodkar AJ, Valdés-Ramírez G, Windmiller JR, Yang Z, Ramírez J, et al. Electrochemical Tattoo Biosensors for Real-Time Noninvasive Lactate Monitoring in Human Perspiration. Analytical Chemistry. 2013;85(14):6553-60.
37. Garcia SO, Ulyanova YV, Figueroa-Teran R, Bhatt KH, Singhal S, Atanassov P. Wearable Sensor System Powered by a Biofuel Cell for Detection of Lactate Levels in Sweat. ECS journal of solid state science and technology : JSS. 2016;5(8):M3075-M81.
38. Gao W, Emaminejad S, Nyein HYY, Challa S, Chen K, Peck A, et al. Fully integrated wearable sensor arrays for multiplexed in situ perspiration analysis. Nature. 2016;529:509.
39. Anastasova S, Crewther B, Bembnowicz P, Curto V, Ip HMD, Rosa B, et al. A wearable multisensing patch for continuous sweat monitoring. Biosensors and Bioelectronics. 2017;93:139-45.
40. Dieffenderfer J, Wilkins M, Hood C, Beppler E, Daniele MA, Bozkurt A, editors. Towards a sweat-based wireless and wearable electrochemical sensor. 2016 IEEE SENSORS; 2016 Oct. 30 2016-Nov. 3 2016.
41. Kudo H, Enomoto K, Jingushi Y, Ieee. Flow Injection System for Real-time Monitoring of Lactic Acid at the Outer Surface of the Skin. New York: Ieee; 2016.
42. Rathee K, Dhull V, Dhull R, Singh S. Biosensors based on electrochemical lactate detection: A comprehensive review. Biochemistry and Biophysics Reports. 2016;5:35-54.

43. Piletsky S, Piletsky S, Chianella I. Chapter 14 - MIP-based Sensors. In: Li S, Ge Y, Piletsky SA, Lunec J, editors. Molecularly Imprinted Sensors. Amsterdam: Elsevier; 2012. p. 339-54.
44. Cormack PAG, Elorza AZ. Molecularly imprinted polymers: synthesis and characterisation. Journal of Chromatography B. 2004;804(1):173-82.
45. Kim D-M, Shim Y-B. Disposable Amperometric Glycated Hemoglobin Sensor for the Finger Prick Blood Test. Analytical Chemistry. 2013;85(13):6536-43.
46. Panasyuk T, Campo Dall'Orto∗ V, Marrazza G, El'skaya A, Piletsky∗∗ S, Rezzano I, et al. Molecular Imprinted Polymers Prepared by Electropolymerization of Ni-(Protoporphyrin IX). Anal Lett. 1998;31(11):1809-24.
47. Chuang S-W, Rick J, Chou T-C. Electrochemical characterisation of a conductive polymer molecularly imprinted with an Amadori compound. Biosensors and Bioelectronics. 2009;24(10):3170-3.
48. Mazouz Z, Rahali S, Fourati N, Zerrouki C, Aloui N, Seydou M, et al. Highly Selective Polypyrrole MIP-Based Gravimetric and Electrochemical Sensors for Picomolar Detection of Glyphosate. Sensors (Basel, Switzerland). 2017;17(11):2586.
49. Shiigi H, Kishimoto M, Yakabe H, Deore B, Nagaoka T. Highly Selective Molecularly Imprinted Overoxidized Polypyrrole Colloids: One-Step Preparation Technique. Analytical Sciences. 2002;18(1):41-4.
50. Law K-Y. Definitions for Hydrophilicity, Hydrophobicity, and Superhydrophobicity: Getting the Basics Right. The Journal of Physical Chemistry Letters. 2014;5(4):686-8.
51. Clayden J, Greeves N, Warren SG. Organic Chemistry: Oxford University Press; 2012.
52. Goldstein JI, Newbury DE, Michael JR, Ritchie NWM, Scott JHJ, Joy DC. Image Formation. In: Goldstein JI, Newbury DE, Michael JR, Ritchie NWM, Scott JHJ, Joy DC, editors. Scanning Electron Microscopy and X-Ray Microanalysis. New York, NY: Springer New York; 2018. p. 93-110.
53. Harris DC. Standard Addition. Quantitaive Chemical Analysis. 8 ed. New York: W. H. Freeman and Company; 2010. p. 106-8.
54. IO Rodeo. Rodeostat: open source potentiostat [Internet] Pasadena, CA: n.d. [cited 2018 09-20]. Available from: https://sites.google.com/iorodeo.com/potentiostat.
55. IO Rodeo. Cheapstat calibration with the potentiostat dummy cell [Internet] Pasadena, CA: n.d. [cited 2018 09-20]. Available from: https://docs.google.com/document/d/1F5Oa70H8IFfAvSGPDbv7mq4cR4ibNvTUuXRl6WSJk7I/edit#heading=h.9lo4uvui3ebd.
56. Harvey CJ, LeBouf RF, Stefaniak AB. Formulation and stability of a novel artificial human sweat under conditions of storage and use. Toxicology in Vitro. 2010;24(6):1790-6.
57. Dickson W. IO Rodeo2018 2018-10-4. [cited 2018 2018-09ö26]. Available from: https://blog.iorodeo.com/rodeostat-software/.

Appendix

A – Contract with participants

Information regarding study, *Development of a Non-enzymatic, Non-invasive Sensor for Detection of Lactate in Sweat*.

Background and aim

During physical exercise the body needs to produce more energy. If there is a shortage of oxygen for the muscles the body needs to find an alternative route for energy production. When there is no oxygen the process is called anaerobe and when the muscles start to work anaerobe, lactate starts to accumulate. Lactate is a biomarker for fatigue in muscles.

For many athletes it is important to know at what intensity the anaerobic threshold is, i.e. the highest intensity where the oxygen can account for the energy requirement. Lactate is measured in blood during increasing exercise intensity to evaluate at which intensity it starts to accumulate. Taking blood sample may be a discomfort for the athlete as well as shifting their focus during training. Some research has been conducted in sweat sensing where sweat is used instead of blood for measurement of these kinds.

Most non-invasive sweat sensor is based on degrading of lactate with enzymes. For endurance sport, such as marathon's, an enzyme-based sensor can't measurer for the total performance time without losing effect. Due to the increasing interest in non-invasive measuring methods this work aim to create the base for a non-enzymatic and non-invasive sensor for lactate detection. This by evaluating sensory alternatives and how it reacts to a complex fluid like sweat. Therefore, the aim of the project is to produce a sensory device, using a non-enzymatic solution to obtain quantitative measurement of lactate from sweat.

Study implementation and sampling

The study wants to follow you during your ordinary training routine and during same prerequisites of lactate threshold measurement as you are used to. The main difference is the use of an additional sampling during these occasions depending on the test protocol. Additional information from health survey as well as weight in connection with exercise will be collected.

The study aims to test two different concepts. The first being a sweat collector to easily collect sweat that is accumulated during physical exercise. This will be tested by attaching the sweat collector to you during a standard exercise session. Your performance in heart rate, exercise intensity, observed physical changes during testing will be evaluated.

The second is collection of sweat during similar prerequisites as lactate threshold testing. The sweat will be collected and used for detection of lactate by the produced sensor. The data obtained during lactate threshold tests will be compared to the data obtained from your sweat samples to be able to see if there is an indication of correlation between your blood and sweat lactate values. All information will be handled in a safe manner.

After documentation of your samples, they will be discarded and not traceable back to you.

Follow up after the studies implementation

You will be able to take part of all the results obtained by your samples.

Risks

There is no risk associated with sweat sampling for you both in short and long time.

Advantages of being part of the study

Due to your patriation in this study you are part of the research for a possible future sweat sensor. The results of the study may be used for further work within the area and provide a greater understanding to how sweat and blood correlate.

Data management and privacy

The data collected during this study will be processed and documented. Your name and personal information will be changed to a code during data processing for your security. The key to the code is only available for the responsible person of the study. If results from the study is published the results will be coded. Your information will be handled according to GDPR.

Compensation

Participation in the study is not compensated.

Voluntary

Your participant in the study is voluntary and you can cancel your participation without any explanation.

Additional information

For additional information regarding the study the following person can be contacted

Annie Ljungh
Responsible for the project

Phone:

Email:

The undersigned confirms that the active has read and understood the document as well as approves the terms in the document

I voluntarily take responsibility for all known and unknown risks, even if they are caused by negligence.

I will voluntarily and truthfully answer questions concerning my health and medical history that may affect activity or cause health risks.

I agree that my results are filed and may be used in research which results in articles published in local and international press.

I hereby voluntarily take responsibility for my practice and hereby revoke the persons working with this project, the Department of Physics and Chalmers University of Technology from responsibility for my safety and health. I hereby disclaim my right to claim compensation, damages or legal liability for bodily or material damage or loss even if damage is caused by gross negligence or misconduct.

I have read this disclaimer and fully understand its content. I understand that I give up my legal rights by signing the agreement and I do this with free will without compulsion from others

Signature_____
_____Date:_____

Clarification of signature:_____
_____

Minor (Age under 18)

Guardian signature_____
_____Date:_____

Clarification of signature:_____
_____

Health survey

Name:_____

Day of birth (YYYYMMDD):_____ Age:_____

Phone number:_____ Email:_____

Sport:_____ Comment: _____

How would you define your fitness level?_____

Have you interrupted training / competition in the last two to three weeks? **YES NO**

If yes, why:

_____

Do you have/ have had any injury that you believe may affect your performance? **YES NO**

If yes, which:

_____

Do you have /have had any respiratory or cardiovascular disease (e.g. asthma)? **YES NO**

If yes, what/ when:

_____

Do you have /have had any other known disease? **YES NO**

If yes, what/ when:

_____

Do you use any prescription drugs? **YES NO**

If yes, state which:

_____

How long time have you been healthy since your last cold?

_____

Have you trained and trained at high intensity since the cold?

_____

If you use any nicotine products, when did you last use that?

_____

If you drink any beverages contain caffeine, when did you last consume that?

_____

Do you feel fully healthy for a maximum physiological test? **YES NO**

If no, why:

_____

Is there any additional information regarding your health?

_____

I conduct the test(s) at my own risk:

Signature_____
_____Date:_____

Clarification of signature:_____
_____

Minor (Age under 18)

Guardian signature_____
_____Date:_____

Clarification of signature:_____
_____

B – Design of sweat collector

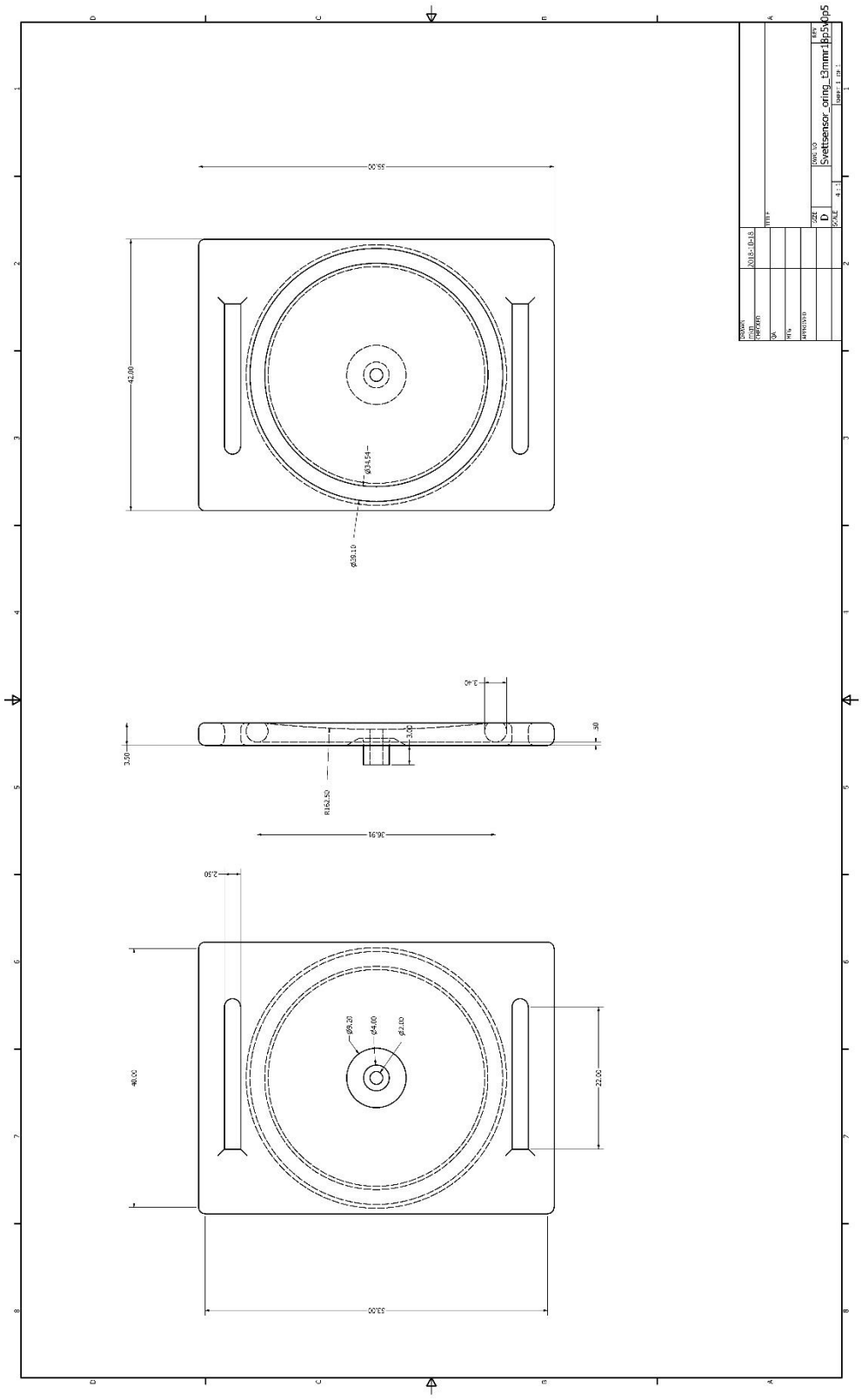


Figure B1, design of sweat collector with place for an O-ring.

C - Protocols

C.1 Set-up of Rodeostat before testing

For set-up of the Rodeostat before testing according to the blog provided by IO Rodeo (57)

- Connect the Rodeostat to the associated USB-cable.
- Open and run the Serialport-bridge software.
- Open the Rodeostat app webpage.
- Go to the device connection page.
- Connect Rodeostat to the computer.
- Activate the connect to Serialport-bridge (Drop window of USB ports should fall down)
- Select the USB port associated with the Rodeostat
    To find out what it is called, go to the Serialport- bridge software and go to the serial port tab. Plug/unplug your Rodeostat. The device will appear and the Rodeostat is associated with it.
- Connect by activating the open serial port switch. If the Rodeostat is connected the device connection tab will appear. Here the device information of firmware and software will appear.
- The Rodeostat is ready for testing.
- After testing, disconnect from the device connection page and carefully disconnect the Rodeostat from the computer.

C.2 Calibration of Rodeostat

In line with the information provided by IO Rodeo google docs(55) the Rodeostat was calibrated using a dummy cell and cyclic voltammetry. The dummy cell has a 50 kΩ precision resistor.

Cyclic voltammetry parameters

- Start: -0.6 V
- Stop: 1.5 V
- Slope: 0.1 V/sec
- Sample rate 100 Hz
- Cycles: 1

Method

- Run the cyclic voltammetry according to the instruction given in *C.1 Set-up of Rodeostat* before testing
- Choose the current range 1, 10, 100 or 1000 µA
- Connect the dummy cell with the associated cable to the Rodeostat
- Move over to the test & parameters tab
- Select the test that are of interest and fill in the desired parameters
- Go to the Data acquisition tab
- Press run test to start test
    During the test a progress bar will be shown, when the test is done the data will be shown on the screen
- Save the data from the testing including the pictures.
- Convert the voltage applied (given in the second column) to current using Ohm's Law (I=V/R).
- Make sure that the units are correlating to the current obtain from the data.
- Plot the converted current against the obtained current
- Get the slope and intersect values

- This equation can be used to convert the obtained current to the correct current for further testing.

Repeat this for each of the current ranges

D – Data processing

D.1 Data processing of calibration with dummy in MATLAB

```
% Collect data
dummy_1 = 'dummytest_1_ver_1.txt'; % Raw data from test with dummy +-1uA range
dummy_10 = 'dummytest_10_ver_1.txt'; % Raw data from test with dummy +-10uA range
dummy_100 = 'dummytest_100_ver_1.txt'; % Raw data from test with dummy +-100uA range
dummy_1000 = 'dummytest_1000_ver_1.txt'; % Raw data from test with dummy +-1000uA range

data = readtable(dummy_1); %Choose which dummy to do the calculations from

% Create vectors from the data
t = data{:,1}; % time of test (s)
E = data{:,2}; % Voltage applied (V)
i = data{:,3}; % Measured current (uA)

% Convert the applied voltage to current
R = 50 * 10^3; % Resistant in dummy (ohm)
i_converted = (E / R) * 10^6; % Converted current from voltage (uA)

% Create the fitted line between the measured current and the converted current
[p,s]=polyfit(i,i_converted,1); % gives the slope and intersection
slope = p(1)
intersection = p(2)
x=[min(i) max(i)];
y_fit= slope*x +intersection t;

% Calculation of R squared
r_sqr = power(corr2(i,i_converted),2)
```

D.2 Data analysis of CV

```
% Housekeeping
clear all
clc
% ! Don’t forget to change test_name, txt and data_name
test_name = '-.xls';
txt= {'0 mM','2.5 mM','5 mM','10 mM','15 mM','20 mM','25 mM','30 mM','60 mM'}; %Name the
samples

data_name = ["LA_0mM_SPCE_AuNP_22.txt"; "LA_2.5mM_SPCE_AuNP_22.txt";
"LA_5mM_SPCE_AuNP_22.txt";
          "LA_10mM_SPCE_AuNP_22.txt"; "LA_15mM_SPCE_AuNP_22.txt";
"LA_20mM_SPCE_AuNP_22.txt";
          "LA_25mM_SPCE_AuNP_22.txt"; "LA_30mM_SPCE_AuNP_22.txt";
"LA_60mM_SPCE_AuNP_22.txt"];
         % ! Current range 1 (r = 1), 10 (r = 2), 100 (r = 3), 1000 (r = 4)
r = 4;

%
[a,b]=size(data_name);
for n = 1:a
   data = readtable(data_name(n));
   t_raw(:,n) = data{:,1};
```

```
    E_raw(:,n) = data{:,2};
    i_raw(:,n) = data{:,3};
end

%Background subtraction
[a,b] = size (i_raw) ;
for f = 1:b
    Background = i_raw(:,1);
    i(:,f) = i_raw(:,f)- Background;
end

[current_range] = xlsread('Results.xlsx','Calibration');
i_adj_matrix = [];
for n = 1:b
    c = 1;
    for m = 1:a
    slope = current_range(r,2);
    intersept = current_range(r,3);
    i_2 = i(c,n);

    i_adj=slope*i_2 + intersept;
    i_adj_matrix(c,n) = i_adj;
    c = c+1;
    end
end

% Creating a filter
d1 =  designfilt('lowpassiir','FilterOrder',12, ...
    'HalfPowerFrequency',0.005,'DesignMethod','butter');

% Plot data and find peaks
x_data = [];
y_data = [];

for k = 1:1:b
x_data = E_raw(:,k);
y_data = filtfilt(d1,i_adj_matrix(:,k));
line = {'-*','--o',':+','-.*','-o','--+',':*','-.o','-+','--',':','-.'};
plot(x_data,y_data,line{k},'MarkerIndices',1:100:a,'MarkerSize',3)
hold on
    if k ~= 1 %
    [PKS_max,LOCS_max]= findpeaks(y_data); % finds local max
    peak_header_max = {'i_max (uA)','E (V)'};
    peak_values_max = [PKS_max, E_raw(LOCS_max)];
    peak_max = [peak_header_max;num2cell(peak_values_max)];
    xlswrite(test_name,peak_max,k,'A1')

    [PKS_min,LOCS_min]= findpeaks((-1*y_data)); % finds local min
    peak_header_min = {'i_min (uA)','E (V)'};
    peak_values_min = [(-1*PKS_min), E_raw(LOCS_min)];
    peak_min = [peak_header_min;num2cell(peak_values_min)];
    xlswrite(test_name,peak_min,k,'C1')
    end
end
```

```
legend (txt,'Location','southeast','NumColumns',3)
set(gca, 'FontName', 'Times New Roman')
xlabel('Potential [V]')
ylabel('Current [uA]')
```

D.3 Data analysis for creating concentration curves

```
% Housekeeping commands
clear all
clc

% ! Dont forget to decide sheet, concentration unit and peak of interest
% d = 2 => peak current max, 3 => peak potential max
%     4 => peak current min, 5 => peak potential min
Sheet = 'LA';
c_unit = {'Concentration [mM]'};
d=2;

numData = xlsread('Results.xlsx',Sheet);
[a,b] = size (numData);

% Calculate mean and standard derivation
Mean=[];
S_D=[];
conc = numData(:,1);
Mean = numData(:,2);


for n = 1:b
   c=1;
   for m=1:3:a
      if n==1
         conc(c,1) = mean(numData(m:m+2,n));
      else
         response(c,1:3) = numData(m:m+2,2);
         Mean(c,n)  = nanmean(numData(m:(m+2),n));
         S_D(c,n) = nanstd(numData(m:(m+2),n));
      end
      c = c+1;
   end
end

[p,tbl,stats] = anova1(response);
figure
%Create the fitted curve from mean
md1 = fitlm(conc,Mean,'linear');
x = min(conc):max(conc);
y_fit = md1.Coefficients{2,1}*x + md1.Coefficients{1,1};

%plot the data, slope and error
errorbar(conc,Mean(:,d),S_D(:,d),'.');

hold on
```

```
xlim([min(conc)-2 (max(conc)+2)])
plot(conc,Mean,'.',x,y_fit)
title(Sheet)
xlabel(c_unit)
ylabel('Current [uA]')
legend ({'Peak current',sprintf('y=%f*x+%f, R^2 =
%f',md1.Coefficients{2,1},md1.Coefficients{1,1},md1.Rsquared.Ordinary)},'Location','northeast','fon
tsize',11)
set(gca, 'FontName', 'Times New Roman')
```

D.4 Data analysis for standard addition

```
% Housekeeping commands
clear all
clc

% ! Don't forget to decide sheet, concentration unit and peak of interest
% d = 2 => peak current max, 3 => peak potential max
%     4 => peak current min, 5 => peak potential min
Sheet = 'LI';

% % Known parameters, dilution taken into consideration
V0 = 50/10;
V = 500;
S_in = 1000/10;

numData = xlsread('Standard_addition.xlsx',Sheet);
[a,b] = size (numData);

% Calculate mean and standard derivation
Vs=[];
i_sx=[];
y_axis =[];
x_axis =[];

Vs = numData(:,1);
i_sx = numData(:,2:4);

% Calculate the x and y axis

for n = 1:b
   for m = 1:a
   if n==1
     x_axis(m,n) = (S_in*(Vs(m,1)/10/V0));
   else
     y_axis(m,(n-1)) = (i_sx(m,(n-1))*(V/V0));
   end
   end
end

for n = 1:a
          Mean(n,1)  = nanmean(y_axis(n,1:3));
          S_D(n,1) = nanstd(y_axis(n,1:3));
end
```

```
%
[p,tbl,stats] = anova1(y_axis);
figure

%Create the fitted curve from the means
md1 = fitlm(x_axis,Mean,'linear');
x = min(x_axis):max(x_axis);
y_fit = md1.Coefficients{2,1}*x + md1.Coefficients{1,1};

unkown =  (md1.Coefficients{2,1})/md1.Coefficients{1,1}*1000 %dilution taken into consideration

% plotting the data, slope and error
errorbar(x_axis,Mean,S_D,'.');

hold on
xlim([min(x_axis)-20 (max(x_axis)+20)])
plot(x,y_fit)
title(Sheet)
xlabel('S_i*(V_s/V_0)')
ylabel('I_(_s_+_x_)*(V/V_0)')
legend ({'Mean with SD',sprintf('y=%f*x+%f, R^2 =
%f',md1.Coefficients{2,1},md1.Coefficients{1,1},md1.Rsquared.Ordinary)},'Location','northeast','fon
tsize',11)
set(gca, 'FontName', 'Times New Roman')
```