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DEVELOPMENT OF A PRINTED ELECTROCHEMICAL BIOSENSOR

In the PrinLab Post-Processing Line

DEVELOPMENT OF A PRINTED ELECTROCHEMICAL BIOSENSOR

In the PrinLab Post-processing Line

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ABSTRACT

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Author: Késia Ranta
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Printed electronics is a rapidly evolving industry. The components of printed electronics are used to supplement conventional electronics since the printed components are light weight, cost effective, and if necessary stretchable and flexible. PrinLab, a development laboratory of Printed Intelligence is located at Oulu University of Applied Sciences. PrinLab has a wide range of manufacturing and research equipment for printed electronics.

The purpose of the thesis is to verify manufacturability of printed electrochemical biosensor with updated post-processing line of PrinLab. The biosensor should be able to be manufactured reproducibly. The updated post-processing line can improve manufacturability. The post-processing line is a device originally made for PrinLab by Omron Electronics Oy and Oamk. Post-processing line is capable of dispensing very small amounts of solutions onto the unwound roll by means of a Cartesian robotic arm. The equipment was upgraded in April 2021, which also allowed solutions to be dispensed onto a stopped roll or sheet placed in the work area.

The theoretical framework provides the reader with a background on the printing and research methods used in the process, as well as the materials used and their properties. In this study, the author used previously at PrinLab manufactured screen printed sensor bases to which the selected biomaterial was dispensed in the post-processing line. Glucose oxidase was chosen as the biomaterial because of its availability and reliability. The purpose was therefore to verify the manufacturability of the sensor with upgraded equipment and to test the functionality of the sensor using electrochemical methods. Various alignment tests were performed on the equipment using candy dye. As the post-processing line is a device designed and assembled separately for PrinLab needs, it does not come with an instruction manual, which is why the device commissioning training was given orally by Tuomas Lipponen, an automation engineer at Algol Technics who updated the device.

After training, the electrochemical biosensor fabrication process was verified by dispensing glucose oxidase on four sheets, each containing 20 finished sensor bases. After visual inspection and electrochemical tests, it could be concluded that this method succeeded in producing a functioning electrochemical biosensor.

Keywords: Printed Intelligence, Printed Electronics, Printed Sensor, Electrochemical Biosensor, Glucose Sensor

ABSTRACT

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Painettu elektroniikka on nopeaa tahtia kehittyvä teollisuuden ala. Painetun elektroniikan komponentteja käytetään täydentämään perinteistä elektroniikkaa, koska painamalla valmistetut komponentit ovat kevyitä, edullisia, tarvittaessa venyviä ja taipuvia. Oulun ammattikorkeakoulun tiloissa sijaitsee maailman pohjoisin painettavan älykkyyden kehityslaboratorio PrinLab. PrinLabissa on laaja valikoima erilaisia painettavan elektroniikan valmistukseen ja tutkimiseen käytettäviä laitteita, joista yksi on jälkikäsittelylinja.

Opinnäytetyön tarkoituksena on todentaa painetun elektrokemiallisen biosensorin valmistettavuus PrinLabin päivitetyllä jälkikäsittelylinjalla. Biosensori on kyettävä valmistamaan toistettavasti. Jälkikäsittelylinja on PrinLabiin tilaustyönä valmistettu laite, jolla pystytään annostelemaan hyvin pieniä määriä nesteitä auki kelatulle rainalle robottikäden avulla. Laitteeseen tehtiin päivitys huhtikuussa 2021, joka mahdollisti nesteiden annostelemisen myös pysähtyneelle rainalle tai työalueelle asetetulle arkille. Laitteen päivityksen on tarkoitus parantaa biosensorien valmistettavuutta.

Teoreettisessa viitekehyksessä taustoitetaan lukijalle prosessissa käytettyjä painanta- ja tutkimusmenetelmiä, sekä käytettyjä materiaaleja ja niiden ominaisuuksia. Työssä käytettiin aiemmin PrinLabissa arkille silkkipainettuja sensoripohjia, joihin valittu biomateriaali annosteltiin jälkikäsittelylinjalla. Biomateriaaliksi valittiin glukoosioksidi, sen saatavuuden ja toimintavarmuuden vuoksi. Tarkoituksena oli siis todentaa sensorin valmistettavuus päivitetyllä laitteistolla ja testata sensorin toimivuus elektrokemiallisia menetelmiä käyttäen. Laitteistolla suoritettiin erilaisia kohdistustestejä karamellivärjä käyttäen. Koska jälkikäsittelylinja on erikseen PrinLabin tarpeisiin suunniteltu ja koottu laite, ei sen mukana tule käyttöohjetta, tästä syystä laitteen käyttöönottokoulutus pidettiin suullisesti laitteen päivityksen hoitaneen Algol Technicsin automaatioinsinööri Tuomas Lipposen toimesta.

Koulutuksen jälkeen todennettiin elektrokemiallisen biosensorin valmistusprosessi annostelemalla glukoosioksidaasia neljälle arkille, joissa kussakin oli 20 kappaletta valmiita sensoripohjia. Visuaalisen tarkastelun ja elektrokemiallisten testien jälkeen voitiin todeta, että tällä menetelmällä onnistuttiin valmistamaan toimiva elektrokemiallinen biosensori.

Avainsanat: Painettava elektroniikka, painettava äly, painettu sensori, elektrokemiallinen biosensori, glukoosisensori

CONTENTS

ABBREVIATIONS

CAD	Computer aided design
Cartesian robot	A specific type of industrial robot that move on three axes — X, Y, and Z.
CE	Counter electrode
Conductor	A material that readily supports the flow of electric current
Current	The flow of electrons carrying electric charge
CV	Cyclic voltammetry
ECS	Electrochemical sensor
EMB	The Electro Magnetic Bellows
ERDF	European Regional Development Fund
Ethercat	Ethernet for Control Automation Technology network
Galvanic Cell	An electrochemical cell, also known as voltaic cell
Ginolis	A company providing innovative automation solutions for medical device and diagnostic industries. Also, a member of PrintoCent
GOx	Glucose oxidase
IC technology	Integrated Circuit technology
Insulator	A material that blocks the flow of electric current

IPA	Isopropanol
IUPAC	International Union of Pure and Applied Chemistry convention
mPa•s	Millipascal-second is a derived metric measurement unit of dynamic viscosity
Pa•s	Pascal-second
PBS	Phosphate buffered saline
PE	Printed electronics
PET	Polyethylene terephthalate, plastic substrate commonly used in printed electronics
PrinLab	Printed Electronics development laboratory in Oamk
PrintoCent	Industry cluster and pilot factory of printed intelligence
R&D	Research and development
RE	Reference electrode
Resistance	A material's resistance to the flow of electric current
RH	Relative humidity, a measure of the water vapor content of air
RSD	Relative standard deviation, also known as coefficient of variation
STD	Standard deviation, also known as σ (the Greek letter sigma)
SQL database	A database in SQL Server is made up of a collection of tables that stores a specific set of structured data
TCP/ IP	Transmission Control Protocol a communications standard

Viscosity	A quantity that describes a liquid's ability to resist flow, i.e., the unit of measurement for the internal friction of a fluid
Voltage	The force driving the flow of current
WE	Working electrode

1 INTRODUCTION

The purpose of the thesis is to verify manufacturability of printed electrochemical biosensor with updated post-processing line of PrinLab. The biosensor should be able to be manufactured reproducibly. The updated post-processing line can improve manufacturability.

The practical content of the thesis is testing the functionality of the post-processing line on the chosen sensor type and biomaterial, sensor manufacturability and manufacturing reproducibility and testing the function of the manufactured sensor.

The sensor to be manufactured will use a sensor base previously manufactured by PrinLab personnel using the screen printing method with semi-automatic Ekra E2 silkscreen printer.
This study utilized updated post-processing line of PrinLab, of which software update enabled dispensing the material also on a stopped roll or on a sheet that can be placed separately in the working area. In this study the biomaterial was dispensed to a sensor base sheet.

After attending an upgraded post-processing line commissioning training on April 15, 2021, the author performed various test prints on sheets of paper using candy dye to find out how accurately the robotic hand dispensed candy dye patterns on sheets of paper. The biomaterial was prepared and dispensed on a post-processing line onto four different sensor sheets and both amperometric and voltammetric electrochemical measurements on the biosensors were performed with a potentiostat.

Based on the electrochemical measurements, the functionality of the post-processing line in the manufacturing process of printed electrochemical biosensors was analyzed. According to the results, manufacturing a functional biosensor using the post-processing line succeeded.

2 THEORETICAL FRAMEWORK

2.1 Printed Intelligence

Printed intelligence also known as printed electronics (PE), is a technology based on conventional printing techniques as the means to manufacture electronics devices and components. The aim of printed intelligence is to make integrated electronic systems using printing technology to complete and add value to silicon-based IC technology which has been in use for 60 years. Printing is a simple process where the functional material can be directly printed as patterns onto the substrate. (1, 26) Substrates are usually stretchable, bendable and thin, plastic, fabric or fiber-based materials (2).

Printing is an additive manufacturing process, where instead of conventional etching, exactly the right amount of material is applied to the substrate. The method reduces material waste and costs in manufacturing processes and lightens end products. (2) Printed electronics is very similar with color printing in a conventional printing press. The only difference is the used inks. In printed electronics the inks have conducting, semiconducting, or dielectric properties that are electronic materials, not pigment as in conventional printing press. (1, 27-28)

Printed electronics is based on inks requiring a wide range of materials. In the manufacturing process the components of an electronic device are made by printing in additive fashion (1) and combining different technologies (3). In printed electronics, fluid functional materials create an ultrathin and flexible electronic device. Functional materials are printed in layers on a thin and flexible film. The functional film can be integrated in all sorts of devices and products. (4)

In printed electronics the functionality of a device is independent of substrate materials. Printed electronics offers an opportunity to manufacture flexible, large-area manufacturing, low-cost electronic products with less material waste and environmental pollution. (1, 38-39)

In addition to printing, coating is also calculated as fabrication methods in printed electronics. (1, 38-39) The layer thickness requirements as well as the properties of the printing ink have the most significant influence on the choice of the manufacturing method (2).

Printed intelligence is utilized in many different areas of technology, such as rapid diagnostics based on printing processes, in health technology and rapid tests for home use. Printed intelligence is also needed in the development of various sensors, which allows them to be integrated into devices better than traditional electronics. Printed intelligence is used in wearable applications, from heart rate measurement to acceleration, elongation or pressure measurements. Clothing with an integrated printed sensor that measure body functions are referred to as smart clothing. (2)

Printing techniques have been widely used for the manufacturing of biosensors. An established example of printed biosensor is disposable glucose test strips for the blood level of glucose measurement, that has already been on the market for several years. (5, 382)

2.1.1 Printed vs. conventional electronics

Compared to conventional electronics printed electronics are thin, flexible and transparent (4). Even though printing electronics is simpler and faster manufacturing technologies from conventional electronics, it cannot be replaced. With printed electronics it is possible to create large, lightweight, flexible and bendable advice that conventional silicon-based electronics is not able to do, but the performance of printed electronics is poorer. They cannot replace each other but rather complement each other. (1, 39-40)

Printed electronics is the low cost and low-end side of different applications. Switching times are long and the integration density is low in printed electronics. But when a high-end sophisticated system is needed conventional electronics are used. In conventional electronics switching times are extremely short and integration density is extremely high, but also the manufacturing costs are high and only rigid and small substrates can be used. (3)

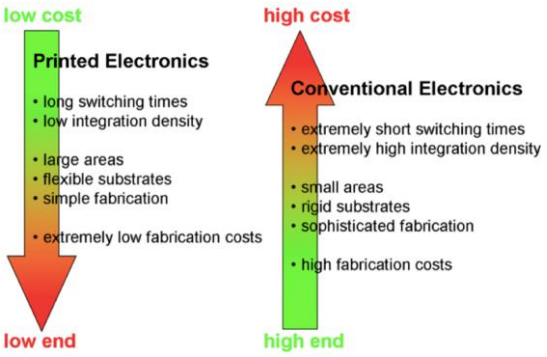

Figure 1 Printed vs. conventional electronics (3)

2.1.2 Benefits of printed electronics

The manufacturing methods of printed electronics are fast compared to the manufacturing of conventional silicon-based electronics. Printing enables mass production which can make the price of printed components much lower than components made by conventional methods. (3)

Printed electronics enable big surface area of the component, flexible and freely shaped and light weight substrates which brings a new perspective and multiple opportunities for designing. Manufacturing electronics by printing take advantage of existing technology that is well-known and proven. (3)

Printing is environmentally friendly because of the additive method that reduces material waste and there is no need to use the most toxic chemicals in the printing process (4).
Printed electronics enable manufacturing conventional electronics devices in a new way and create totally new inventions due to its thin, robust and stretchable shape (3).

2.2 Printing methods and equipment

This section describes the printing methods used in the manufacture of the biosensor that is the topic of the thesis, the equipment used, and the operating environment in which the manufacture was performed. There are several methods of manufacturing printed electronics, and they are selected on a case-by-case basis according to the intended use and requirements of the component to be manufactured.

2.2.1 Screen printing

Screen printing is simple, robust and mostly used in printed electronics due to the availability of commercial materials. The process has advanced because of the 2000-year long history. The screen printing method differs from other printing methods when the ink is squeezed through a stencil. Image elements and nonimage elements are located on the same surface plane, but the permeability is different between them. (5, 82)

The viscosity range of the printing ink is wide, ink viscosities are 10-100 times higher than inks used for doctor blading (6,12). The range of the inks is up to 70Pa•s. Low level viscosity inks cannot be used in screen printing, because the ink flows uncontrollably through the screen during the printing process (5, 86-87). For the above reason screen printing is best suited when a thick printing layer is required, and it is a suitable method for manufacturing of overlapping layers with accuracy of tens of μm (6, 41).

Screen printing enables print patterns on all kinds of surfaces and materials and produce thick layers more easily than other type of printing methods. A disadvantage of screen printing is its low resolution, which limits its use in printing fine structures and devices requiring accurate overlays. (1, 187) It is also relatively slow technique (5, 87). The most important issues in the screen printing process are the features of the screen, printing ink and its properties, suitable squeegee and the printing parameters (6, 12).

The selected ink is applied over the screen. The rubber squeegee moves the ink through the screen to the substrate. The screen has a stencil that forms the desired pattern to be printed. Areas of the printing screen that do not want ink to permeate are filled with emulsion. The emulsion clogs the screen forming a non-printing surface through which the ink cannot transfer to the substrate. Often it is useful to use flood squeegee that prefills the screen mesh openings for the next print and retards the drying of the ink. After the printing process, the substrate is moved to the drying unit. (6, 14). Figure 2. illustrates the operating principles of a screen printing machine.

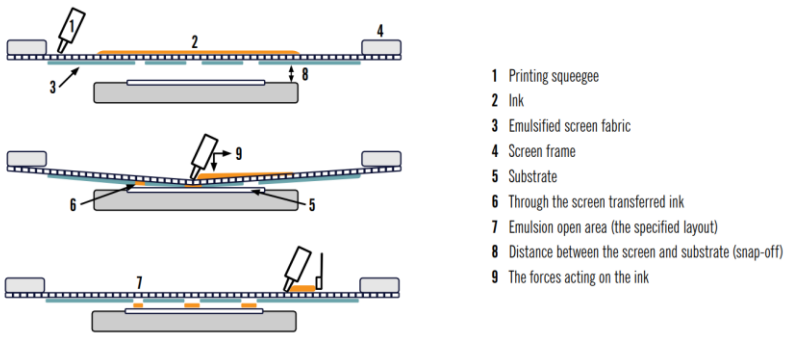

Figure 2 (6, 13)

Although printed electronics is a relatively new technology, the first heating in automotive section were manufactured in 1959 using screen printing technique with silver ink (5, 87-88). Screen printing has found applications in organic solar cells, transistors, sensors (1, 187) and, it is one of the most cost-effective ways of manufacturing biosensors (7).

2.2.2 **Dispensing**

Dispensing is a digitally controlled printing technique to precisely dispense a controlled quantity of fluid to substrate. Dispensing a non-contact printing technique enables to automatically dispense a certain amount of fluid into targeted area accurately. Because of the high accuracy of the printing method material deposition also to uneven surfaces and 3D substrates is possible. In printed electronics, conductive materials can be dispensed in precise volume and low quantities (8, 1). Dispensing is capable of forming relatively thick layers and the waste of material is minimal.

Printing layout is designed with Computer aided design (CAD) program that is translated into script file for printing. CAD tools are used to design printed electronics patterns because of highly accurate droplet placement on the target location. In addition, the layer information in CAD software can be utilized when patterns of different materials need to be dispensed to each layer. (8, 2-3)

Dispensing enables using highly viscous inks with high yield stress. The viscosity range for used inks is 1 – 1000000 mPa•s. (8, 24-25; 9) The ink viscosity should be more than 1000 mPa•s to achieve better electronics characteristics of printed patterns (8, 20) Ink viscosity often relates to the amount of functional material in the ink. The functionality of the electronics increases with the ink contents being more solid (8, 45). Additional advantage of using high viscosity inks is that substrate conditions affect less to printed patterns (8, 20).

The basic principle of dispensing printing is that printed ink is placed onto a reservoir from where it is pushed to valve body channel with air pressure. The valve is opened slightly to enable ink flow into the tip of the nozzle and then closed making the dispenser ready for use. Dispensing is made by air pressure and opening the valve that also controls dispensed ink quantity. Printing speed affects the printed quality. (9; 10) The used size of the nozzle inner diameter is directly related to line width or dot size (8, 21)

2.3 PrinLab

PrinLab, the world's northernmost development laboratory of printed intelligence is located in Oulu University of Applied Sciences at Linnanmaa campus. PrinLab started as an ERDF funded project in 2011 and was originally located at Kotkantie campus. PrinLab offers Printed electronics design, manufacturing, training and education as well as test services to support R&D work. PrinLab is also a part of the PrintoCent pilot factory. (11;12)

The purpose of the PrinLab is to include Printed electronics as a part of the engineering education, offer companies and end-users the environment to get familiar with Printed Intelligence. PrinLab offers “hands-on-ink” skills in different printing technologies and brings together different disciplines for development. (11;12)

PrinLab has a comprehensive range of facilities and equipment for small-scale printed Intelligence manufacturing for prototyping and testing. Laboratory has equipment for screen printing, two digital printing methods; inkjet printing and dispensing, a roll-to-roll machine with flexographic, gravure, rotary screen and hot embossing units that can be used individually or several units in one run. PrinLab also offers equipment for characterization and measurement such as optical microscopes, potentiostats, microplate reader, spectrophotometer, laboratory test chamber and surface topography measurement system. (11;12)

PrinLab crew has expertise especially in the printed and organic electronics functional material performance and printability testing, sourcing and testing of new type of materials for specific applications and characterization of materials. (11 & 12)

Figure 3 PrinLab (11)

2.4 Post-processing line

The post-processing line is an equipment, designed for dispensing of fluids on different substrates. The post-processing line is assembled from components available from Omron Oy. The device was originally made for PrinLab by Omron Electronics Oy and Oamk in 2016 and programmed by Omron. (13, 7) It is capable of dispensing very small amounts of solution to the printed sensors on the roll.

The device was updated in March 2021 and fine-tuned in April 2021 to improve manufacturability. The purpose of the software update was to reprogram the post-processing line. The software update enabled dispensing the material also on a stopped roll or on a sheet that can be placed separately in the working area.

The deployment testing started April 13, 2021, and commissioning training at the PrinLab April 14, 2021. Automation engineer Tuomas Lipponen from Algol Technics was responsible for the upgrade, fine-tuning and commissioning training. Algol Technics is a part of the Finnish Algol Group. (14)

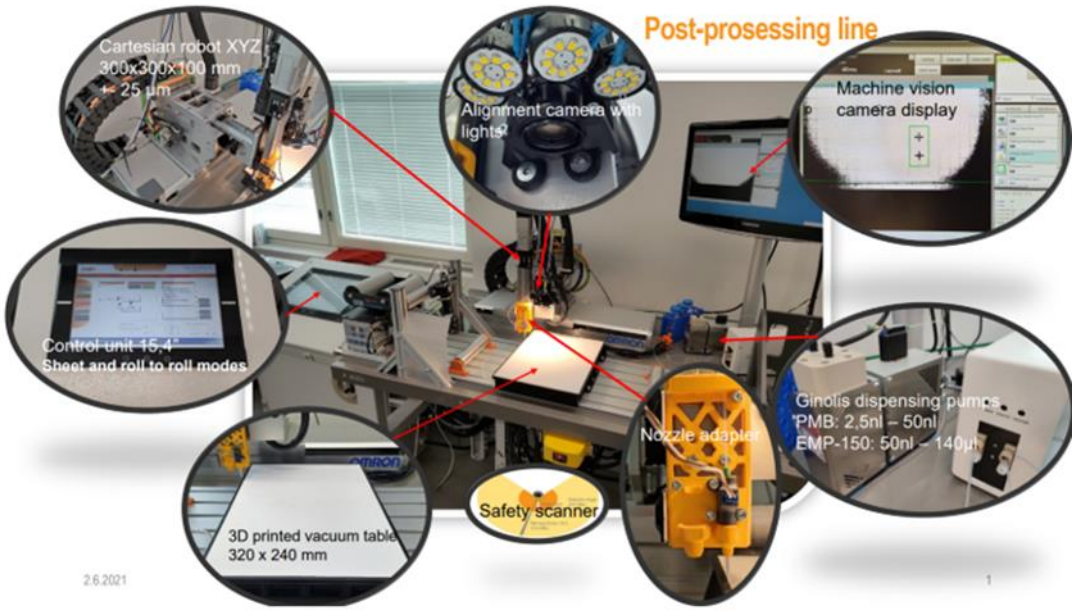

Figure 4 Post-processing line (11)

Description of post-processing line operation

The post-processing line dispenses very small amounts of solution on the printed sensors on the roll or a sheet. Optimal roll width is 80 mm. Ginolis EMB and PMB pumps are non-contact dispensing devices capable of dispensing accurately and repeatably small drop volumes from 2,5nl to 140µl volumes with patented pump technology. (11; 15). Ginolis pumps can be integrated to customized platforms, and they are manufactured in Finland with ISO 9001:2008 and ISO14001:2004 compliant quality systems. (16)

Ginolis EMB-150 pump

The Electro Magnetic Bellows (EMB) pump is an accurate liquid handling device for sub microliter dispensing. The deviation is under 0.5% over 500 nl and under 1.0% between 200 nl and 500 nl. The EMB technology can process all reagents including cells and beads. EMB technology is based on a metallic bellows driven by an electromagnetic actuator. EMB pump provides automatic tip clot detection based on a fluid path integrated pressure sensor, programmable dispensing speed, overshoot and delays. EMB 150 pump can dispense fluid from 50 nl to 140 µl with 1 nl resolution and relative standard deviation under 1%. The pump is capable of non-contact dispensing up to 40 drops per second depending on dose size and dispensing tip. The valve with input and output functions is opened for 250 microseconds. (16)

Figure 5 Ginolis EMB-150 integrated to post-processing line Photo Credit: Késia Ranta

Ginolis PMB pump

The Piezo Motor Bellows (PMB) pump has integrated pressure sensor for a leak, clog and air detection. Pressure stabilization during pre-dispensing is guaranteed with a pump pre-pressuring and automated monitoring. Highly accurate piezo motor with position encoder is connected to a bellows that contracts and expands with the motor. The bellows displaces a volume in a chamber that is equal to the dispensed or aspirated volume. The pump has a dispensing solenoid valve and ceramic tip for dispensing even volumes as low as nanoliters. The PMB pump displaces a volume of liquid defined, and the valve is opened for 100 microseconds to drop release. The pump is capable of non-contact dispensing up to 100 drops per second with drop volumes from 2,5nl to 50nl. (15) In this study, the Ginolis PMB pump was used.

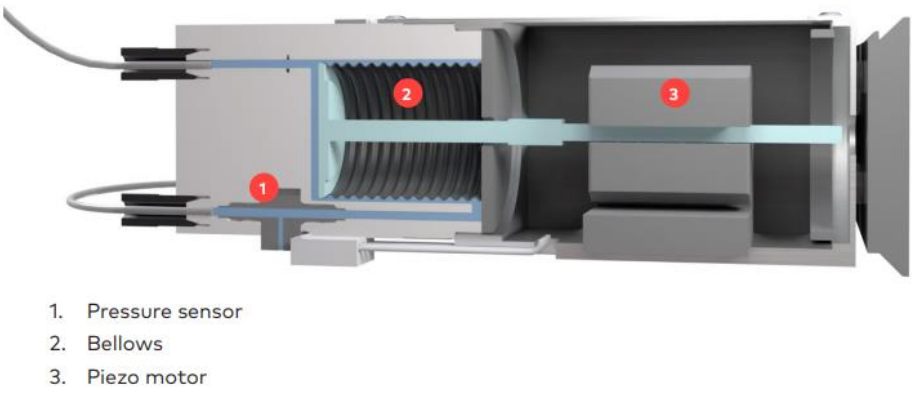

Figure 6 Cross-section of Ginolis PMB pump (15)

Figure 7 Ginolis PMB pump integrated to post-processing line. Photo Credit: Késia Ranta

The nozzle of the dispensing pump is attached to the arm of a Cartesian robot operating in the XYZ directions. The Z-axis moves the dispenser head vertically. The range of movement of the Cartesian robot is 300x300x100 mm, with an accuracy of ±25 microns. (17)

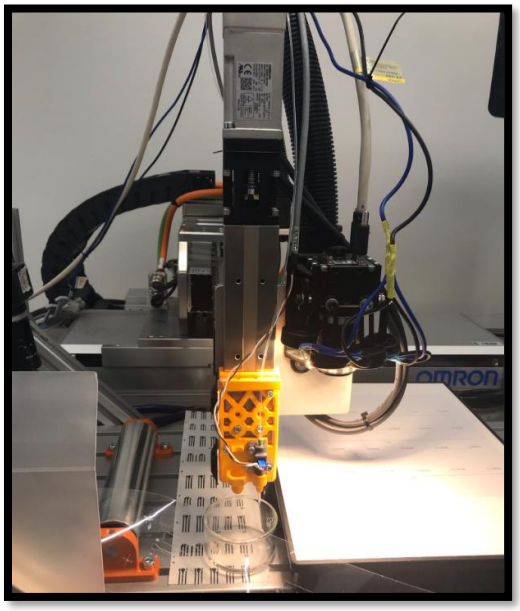

Figure 8 Cartesian robot arm of post-processing line. Photo Credit: Késia Ranta

The post-processing line includes an automatic vision registration system for alignment. The roll and the cartesian arm are controlled from the user interface. The substrate is aligned on the XY directions on the robot arm using the machine vision. The substrate alignment mark provides information to automation controller that corrects any mechanical positioning errors. (17)

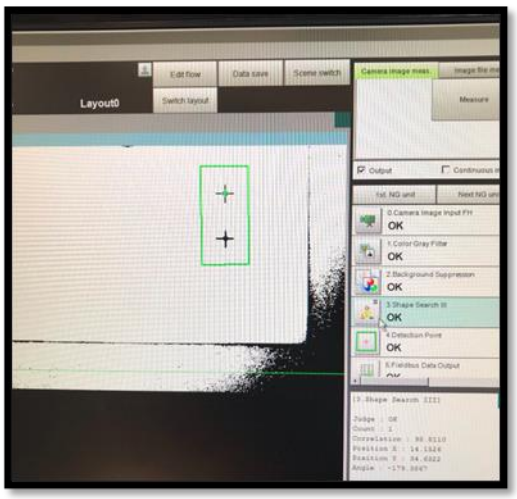

Figure 9 Alignment mark. Photo Credit: Késia Ranta

The roll is unwound with servo drives synchronized with the robot program cycle. This provides accurate information on the progress of the substrate after alignment with machine vision. A touch screen control unit (15,4”) is integrated in the machine automation controller. (17)

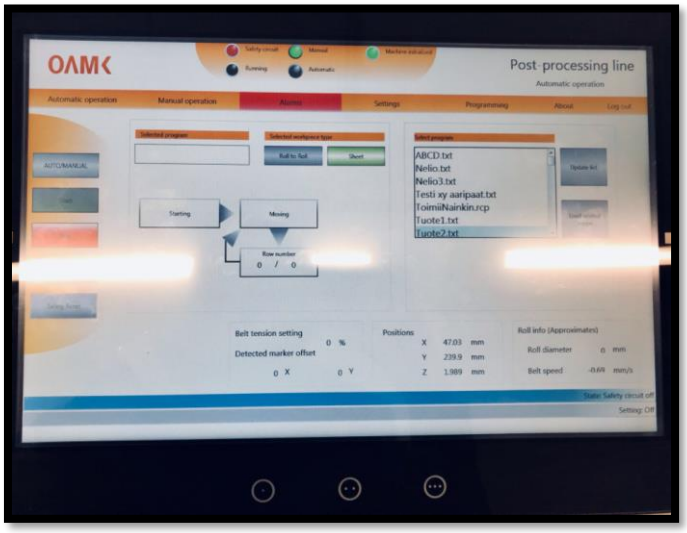

Figure 10 Touch screen control unit. Photo Credit: Késia Ranta

The control unit is used for machine operation related parameters and displays the machine status and measurement information. The devices under the machine automation controller operate on a common Ethercat (Ethernet for Control Automation Technology) network. The controller can also be connected to a TCP/ IP network and an SQL database. (17)

The operating range of the machine is monitored by a safety scanner that connects to the safety controller. The machine may only be operated when there is no movement in its area of operation. (17)

Figure 11 Safety scanner. Photo Credit: Késia Ranta

2.5 Electroanalytical techniques applied to biosensors

According to world's leading biosensor manufacturing company Zimmer & Peacock "Electrochemical biosensing is the study of the relationship between a voltage, current, resistance or capacitance, and an identifiable chemical/biological change" (7)

Electrochemistry is a field of physical chemistry that studies the relationship between electrical energy and chemical change (19); in other words, it is an electrical way of measuring biological and chemical events. (7) Electrochemical methods used in this study are based on the measurement of the current released in redox reactions (18).

Redox is a shorthand for reduction oxidation reaction, which is a chemical reaction in which one or more electrons are transferred from one atom to another. In oxidation one or more electrons are lost during a chemical reaction, which means that its oxidation number increases. When atom loses the negative charge of the electron, increasing the oxidation number. (20) In general oxidation is loss of electrons and reduction is gain of electrons (21).

In reduction, electrons are obtained. When an atom gains electrons, its oxidation number decreases due to the electron's negative charge. The oxidation number decreases when an atom gains an electron because it gains a negative charge. Consequently, the oxidation number is reduced in reduction. (20)

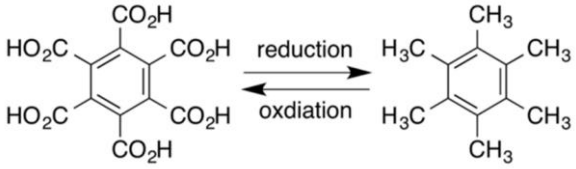

Figure 12 Redox reaction (21)

In addition to electroanalysis sensors and biosensors, electrochemistry can be applied to solar cells, batteries, fuel cells and capacitators together with studying corrosion and electrochemical synthesis. Electrochemistry is the science behind modern glucose sensors because it is cost efficient and easy to manufacture. It is one of the most direct ways of turning biochemical events into an electrical signal and it demands only limited sample preparation. However, glucose sensors have not always been based on electrochemical detection. The image by Zimmer and Peacock below shows the evolution of glucose sensors. (7)

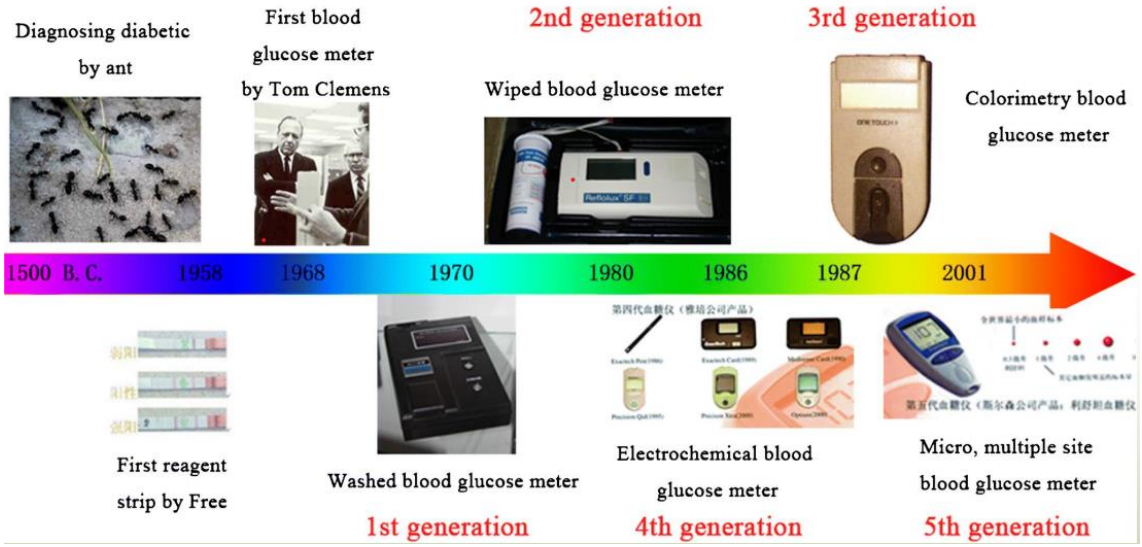

Figure 13 The evolution of glucose sensors (19)

2.6 **Cyclic voltammetry**

Cyclic voltammetry (CV) is an electrochemical method to investigate the reduction and oxidation processes of molecular species. (22, 321) It is the most used technique for collecting qualitative data on electrochemical reactions. In an electroanalytical study, cyclic voltammetry usually is the very first experiment performed. (8, 29) With cyclic voltammetry it is possible to acquire information about the reactions observed in the system and the potentials at which they occur. (18)

Cyclic voltammetry provides information on speed, stability, and reversibility of the sensor. Voltammetric system is composed of a potentiostat, a computer and a sensor composed of a reference electrode, a counter electrode and a working electrode. (18) The cyclic voltammetry tests are performed with a potentiostat controlling the voltage between the reference electrode and working electrode, while measuring the current through the counter electrode. (19) The potential of a working electrode is scanned linearly (8, 29) during classically defined triangular pulsed potential waveform. The applied potential is measured against the reference electrode, while the counter electrode closes the electrical circuit for the current to flow. (23, 18)

The potentiostat measures the current resulting from the applied potential during the potential sweep. (8, 29) The voltage is generated between the working electrode and reference electrode, and the current is measured between the working and counter electrodes. Electrochemically active

substances are oxidized and reduced to a certain potential which can be seen at as the peaks in the graph. The potential of the working electrode is changed back and forth at a certain voltage range and the resulting current is measured. (18) The resulting graph is termed a cyclic voltammogram which is a time-dependent function of several physical and chemical parameter. (8, 29)

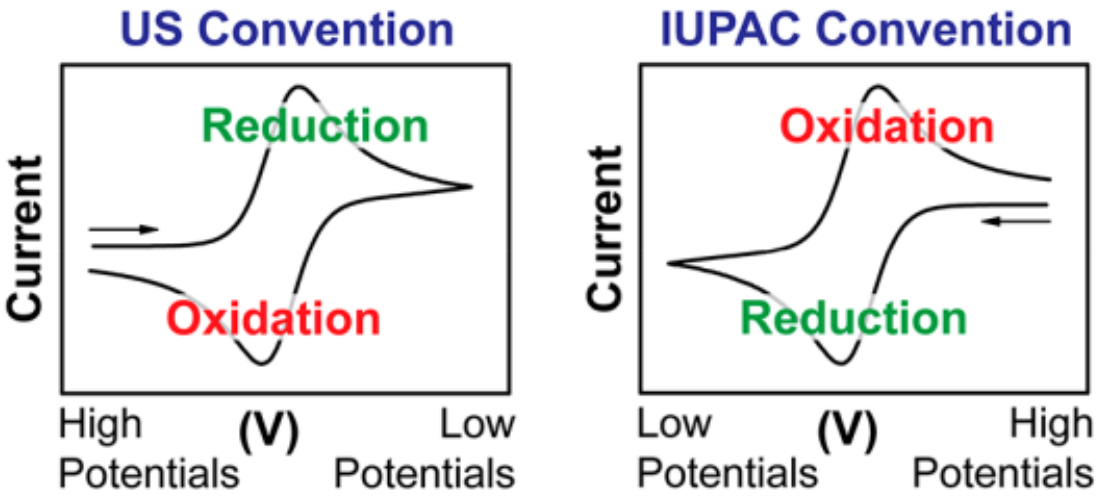

Figure 14. The two different conventions of cyclic voltammograms (24, 198))

As the figure 14 shows there are two conventions commonly used in cyclic voltammograms, US convention and IUPAC convention. In the US convention the anodic peak is negative, and the cathodic peak is positive, the x-axis runs from positive to negative values, indicating reduction left to right and oxidation from right to left. In the IUPAC convention the voltammogram is rotated by 180 degrees compared to the US convention. In the IUPAC convention the anodic peak is positive, and the cathodic peak is negative. The x-axis runs from negative to positive values, indicating reduction from right to left and oxidation from left to right. (24, 198)

2.7 **Amperometry**

Amperometry consists of recording the current as a function of time at a fixed potential. Amperometry provides priceless time resolution, even a sub millisecond event can be observed. (23, 18) It is based on the current resulting from redox reaction (18). The current generated from a redox reaction is directly proportional to the concentration of the analyte. Amperometric current measuring is clearly different from measuring in the galvanic cell, that does not require the application of a potential (25, 1)

Amperometry is an electroanalytical technique where the biosensing element is immobilized on the surface of the working electrode. (22, 313) During the measurement the potential is set at a sufficient level to oxidize or reduce the target analyte at the electrode surface (18). Potential of the working electrode is applied constant, and the resulting current is measured (22, 313) as a function of time. The current obtained is proportional to the concentration of the analyte (25, 2). Amperometry measures different concentrations of a known sample; consequently, it can be used for quantitative measurements (18)

Screen printed amperometric sensors have become promising analysis tools for minimal sample preparation and fast result readout. Amperometry has commonly been used to develop biosensors for direct evaluation of the concentration of the electro-active compounds. (25, 2-3)

Amperometric techniques enable analyses in different and unusual environments due to portability, low-cost instrumentation and speciation capability. The techniques have become more tempting and cost-effective due to the new sensing concepts that combine many technological innovations. The development has led to a new generation of sensors that are often used together with flow analysis. Some of them are used in pharmaceutical, clinical, industrial, environmental, and agricultural applications. (25, 1)

2.8 Printed electrochemical biosensor

This section describes the properties and operating principles of biosensors at a general level, as well as information about the structure and materials of the biosensor manufactured in this study.

2.8.1 Biosensor

A sensor is a device used to detect and transfer chemical measurands into an electrical signal.
A sensor that requires an external power source is called an active sensor. Self-generating or passive sensor can provide the output power without an excitation voltage. (5, 357, 387)

A biosensor is a compact analytical device able to monitor biomolecular interactions in real time (5, 382; 26, 2). A biosensor consists of two elements: a biological recognition system and a transducer

for selective bioanalysis. (27, 82) The term is a short for a biological sensor. The electronic component detects and transmits the signal, while the biological component acts as a sensor. (26, 3)

The biomaterial used in biological recognition may be an antibody, an enzyme or a nucleic acid. When the biomaterial reacts with the analyte, a biological response is formed, which is converted into an electrical signal. (26, 3) The electrical signal is transformed into a measurable electrical parameter like voltage or current (27, 37). A biosensor is a self-contained integrated device that can be integrated into bigger analytical systems as a receptor collecting information (28, 1).

Biosensors can measure the concentrations of a huge variety of substances, such as glucose, oxygen, lactate, hydrogen peroxide, pH, chloride, potassium, calcium, alcohol, uric acid, sodium, phosphate, nitrate, nitric oxide, ammonium, sulfate and ketone. (7)

2.8.2 Electrochemical sensor

Electrochemical sensor (ECS) converts chemical information into an electrical signal.
An electrochemical sensor consists of three electrodes: a working electrode (WE), a reference electrode (RE) and a counter electrode (CE). (5, 369) Electrochemical sensors are integrated with a recognition element and a signal transducer. The recognition element detects of the interest analyte, and the transducer converts a chemical signal to a signal that can be used to determine the analyte. (29, 114)

Glucose detection is of great significance for diagnostic purposes for diabetic patients. Diabetes is a metabolic disease which causes apoptosis and inflammation types of problems. Patients with diabetes have an abnormal glucose level which needs to be monitored regularly to avoid complications. Electrochemical methods are cost-effective, sensitive and fast methods for glucose detection. (30, 5)

Reference electrode

The function of the reference electrode (RE) is to produce a constant potential during the measurement. (26, 44) RE is the first layer printed on the sensor. No current passes through the reference electrode. (18) The material must be different from the material of the working electrode or the

counter electrode. Other requirements for the material are good conductivity and known reduction potential. Commonly used materials are gold, silver and platinum. (31 ,218)

Figure 15 First layer of electrochemical sensor (6, 43)

Working electrode

The working electrode (WE) is the main electrode where the electron transfer occurs (26, 43). Working electrode is the second layer printed on the sensor. The working electrode and the counter electrode may be of the same material. The material needs to be conductive and chemically inert such as carbon or graphite. Often a mediator is added to the surface of the working electrode. The function of the mediator is to improve electrochemical properties. (31, 219)

Counter electrode

The counter electrode (CE) is an electrode that closes the current circuit in the electrochemical cell. Electrical current flows across the counter electrode (5, 369) but it does not participate in the electrochemical reaction. Counter electrode is also known as the auxiliary electrode (AE). The surface area of the counter electrode must be larger than the working electrode in order not to limit the electrochemical reaction. Inert material such as platinum, gold or graphite is usually used in manufacturing of a counter electrode. (26, 44) CE is the second layer printed on the sensor (18).

Figure 16 Second layer of electrochemical sensor (6, 43)

Insulator

Insulator provides mechanical protection for the printed electrode by forming a protective wall around the working electrode. The purpose of the wall is to ensure that applied biomaterial stays within the working electrode. Insulator is the third layer printed on the sensor. (31, 220)

Figure 17 Third layer (6, 43)

2.8.3 Electrochemical biosensor

A biosensor which has an electrochemical transducer is an electrochemical biosensor (26, 42). A transducer is a device that converts a signal from one form to another (32, 14). The function of an electrochemical biosensor is based on a redox reaction on the electrode surface. In general, electrochemical biosensor measures the current produced from redox reaction. (26, 42; 33, 17-18) In practice the functioning is based on the measurement of the current which is produced of potential between working electrode (WE) and reference electrode (RE) which leads to oxidation or reduction of the electroactive species. (27, 37)

Biosensors are typically electrochemical where the receptor is immobilized on the working electrode. The reactions between the receptor and the analyte causes variations of the current measured between the CE and RE. The variations can determine the amount of an analyte in a certain test solution. (5, 382)

In addition to biological materials like enzymes, tissues, cells and specific ligands, electrochemical biosensors can also detect nonbiological matrixes. Through indirect monitoring inorganic substances such as heavy metals, cyanide and fluoride can also be analyzed. (28, 1-3)

Electrochemical biosensors are more cost-effective than other biosensors and they do not require complex sensor setup. Electrochemical biosensors offer extensive detection limits even with small volumes of analyte. (28, 3)

As a disadvantage of electrochemical biosensors can be mentioned that the surface architecture does not enable high sensitivity neither individual recognition of the response to a selected biochemical event which may cause the ionic strength and the pH of biofluids varying widely and thereby influence the behavior of biosensor. (28, 3)

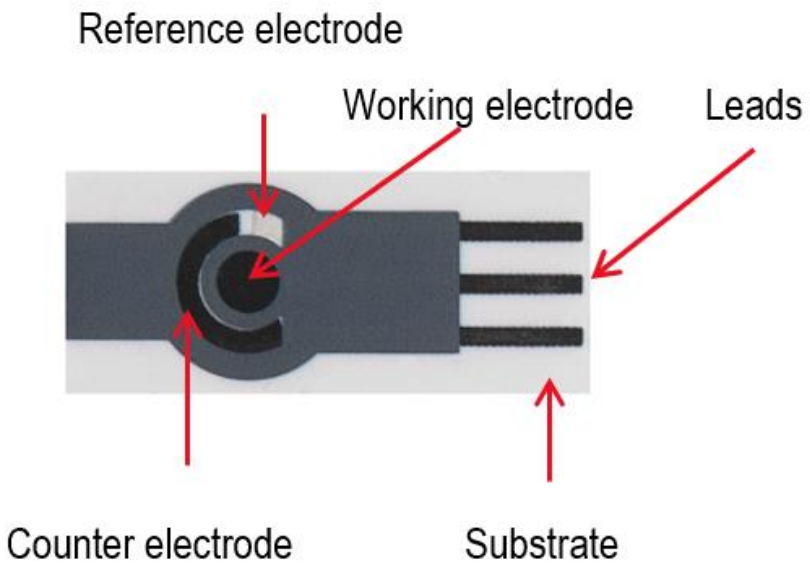

Figure 18 Electrochemical biosensor

2.8.4 Enzymatic biosensors

Biosensors can be divided based on the transduction process into different categories. In this thesis the focus is on electrochemical sensors, but the transduction can also be optical, piezoelectric, or thermal biosensors. Biosensors can also be classified according to the receptor type, such as enzymatic biosensors, microbial biosensors or immunosensors.

Enzymatic biosensor is an analytical device having an enzyme as a bioreceptor immobilized to transducer to produce a digital electronic or optical signal. The signal is directly proportional to the concentration of analyte in the sample. (26, 332).

Enzymes have the property of recognizing an analyte which enables usability for sensing applications. Disadvantages of free enzymes are weak stability and adaptability, also reuse is complicated. Enzymes are fragile by nature which leads to short life and early denaturation. (30, 2)

2.8.5 **Glucose oxidase**

Glucose oxidase (GOx) is an ideal enzyme for biosensor because of its high specificity, high stability, low cost, easy availability. (18; 32, 2) GOx is a dimeric protein with a small structure that is at the same time a very stable enzyme. The enzyme glucose oxidase plays an important role in nature because it is needed in honey formation (35, 22) and it is primarily produced by fungi and insects (34, 8).

Glucose oxidase outer shell is a hydrocarbon, and its active centre is located within the protein. (35, 22) In the glucose sensor, a sample of glucose oxidase initiating a redox reaction, i.e., glucose at various concentrations, is placed on top of the sensor strip. The electric current consists of negatively charged electrons moving in a redox reaction. (35, 23-24)

Glucose oxidase has widespread applications in pharmaceutical, medical, food and textile industries (34, 2), due to its ability to oxidize glucose and produce hydrogen peroxide. For many of these industries GOx is used in a biosensor. (34, 12)

Glucose sensor with GOx as biomaterial was presumably manufactured in 1962. Nowadays, GOx is widely used in the most common methods for blood glucose measuring. (34, 15)
In the glucose sensor, with the help of the GOx enzyme, glucose can be changed into a form from which a detectable change in current can be obtained as a result of a chemical reaction (18).

3 EXECUTION OF THE WORK

3.1 Testing the equipment

April 13, 2021

Repeatability tests were performed by dispensing the ink onto the same place of a paper sheet several times, holding the sheet in place between dispensations and removing and repositioning it. The author performed electrochemical tests, using a potentiometer and the PS-Trace program under the guidance of laboratory analyst Niina Torniainen.

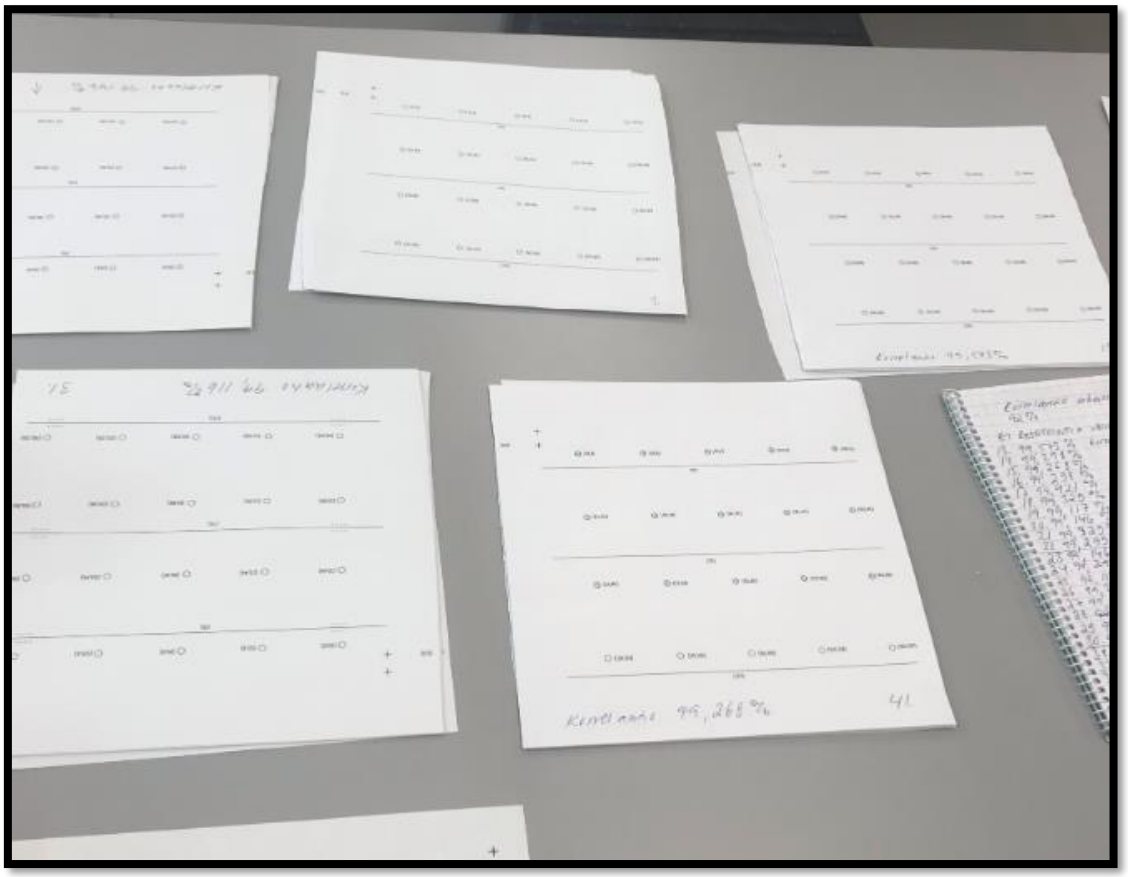

Figure 19 Test sheets. Photo Credit: Késia Ranta

April 14, 2021

The alignment accuracy of the post-processing line was tested by dispensing water-mixed caramel color on paper test sheets with different dispensing patterns.

A dot (1 mm) was dispensed to the center of the circles on the test sheets 1.-12. One test sheet has four rows of circles and five circles on each line. The device was reset after each test sheet was printed.

The results indicated that top three rows were visually successful for the middle stages of the circle on almost all sheets, but the dots on the bottom row were printed at the top right of the circle. The dispenser also made a line of about 5mm from the center of the alignment mark toward the bottom right of the sheet. In the top row, the first circle on the left was inadvertently left blank. Figure 20. illustrates the errors of the first test round.

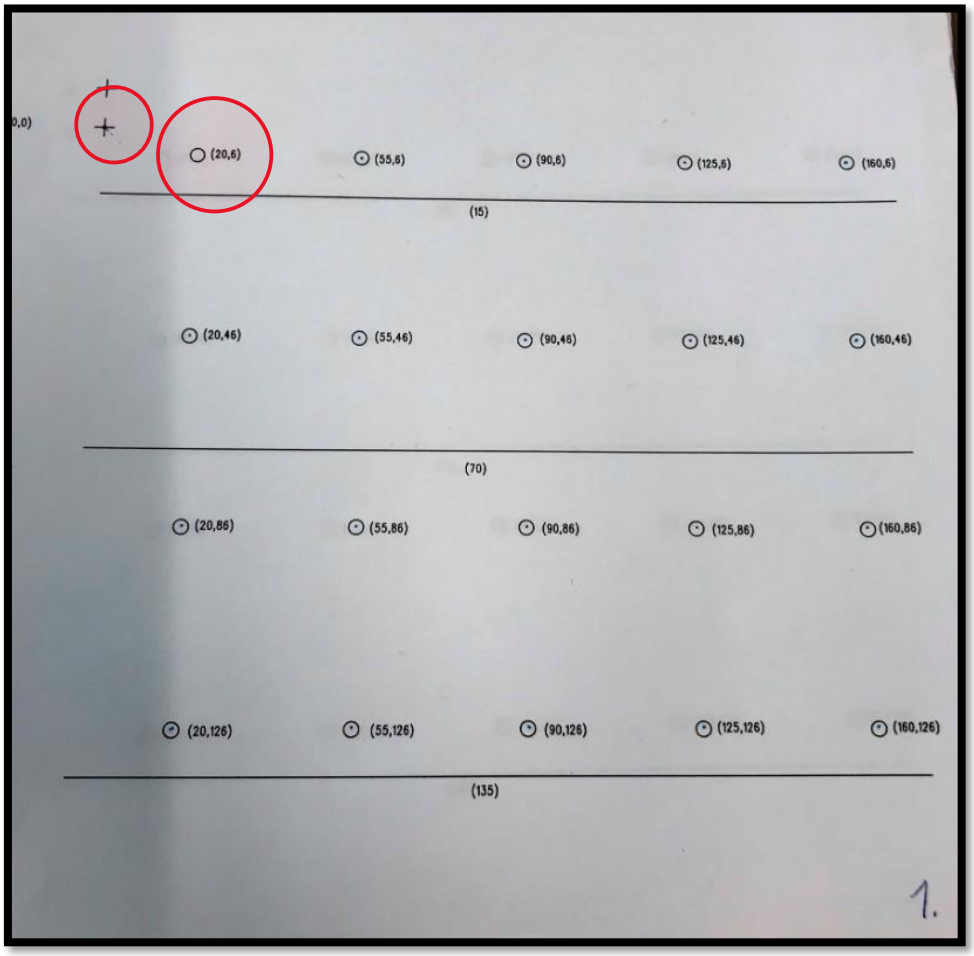

Figure 20 Alignment accuracy test, sheet 1. Photo Credit: Késia Ranta

When dispensing the caramel color onto the sheets 13-22 the device was not reset between test strip printing. The correlation coefficient of each alignment was recorded to determine whether the correlation coefficient had a relationship to the accuracy of the dispensing. From now on, the correlation coefficient of all test runs was recorded.

The results indicated that all rows failed to align. Again, the bottom row targeting was the most misplaced, the dots were printed at the top right of the circle and were hardly inside of the circle. The dispenser made a line of about 5mm from the center of the alignment mark toward the bottom right of the sheet. In the top row, the first circle on the left was inadvertently left blank.
Figure 21. illustrates the errors of the second test round.

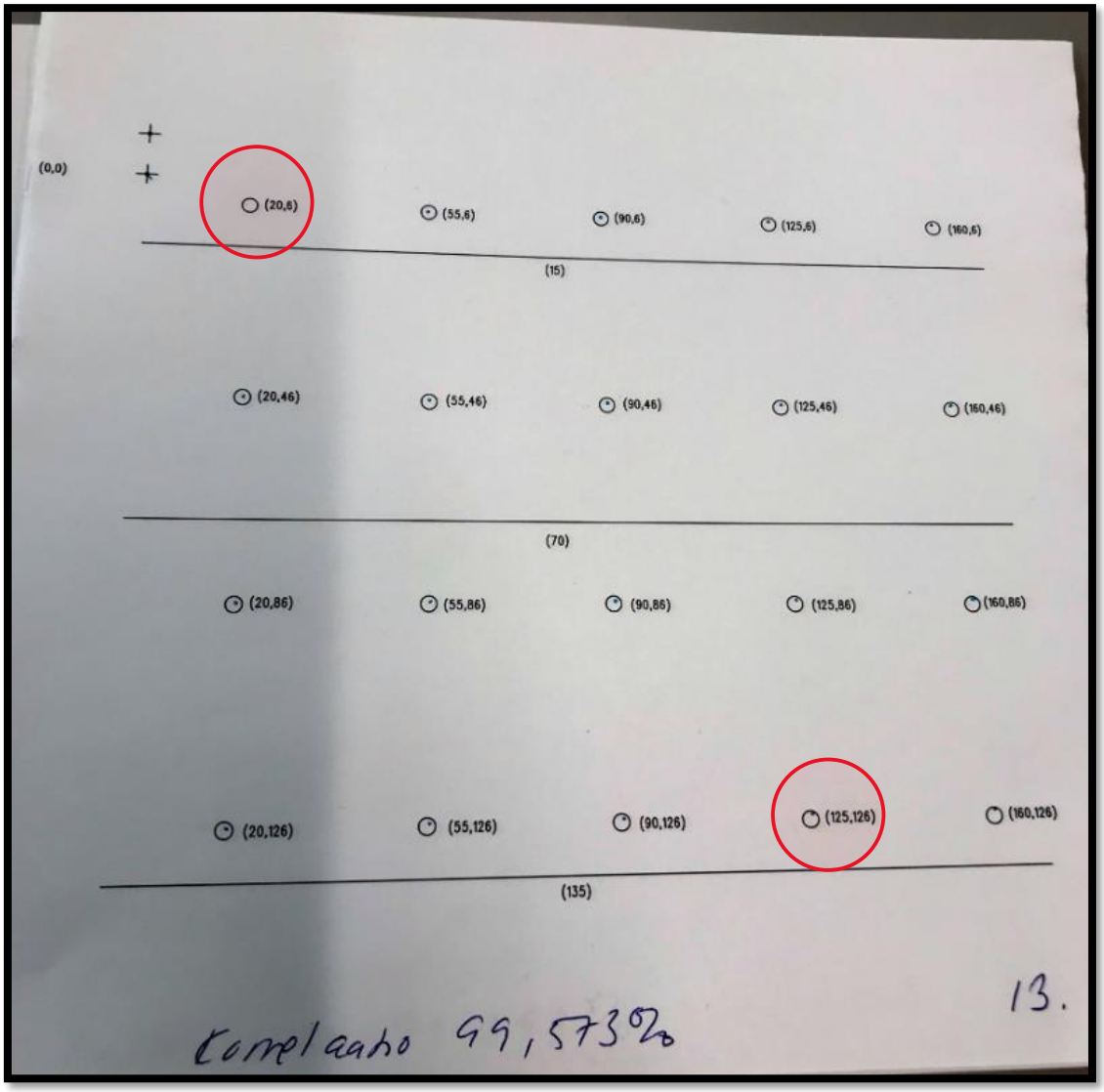

Figure 21 Alignment accuracy test, sheet 13. Photo Credit: Késia Ranta

Sheets 23.-30. were made in the same way as the previous ones, but the dispensation was started in the reverse order starting from the lower left corner, while the previous one was started from the upper left corner.

The results indicated that the hit accuracy with the set parameters was significantly better than the previous test rounds. In the top two rows, the dots visually hit very close to the center of the circle. The hit accuracy of the two lower rows was slightly lower. No circles were left empty and there was no extra line on the alignment mark. Figure 22. illustrates the errors of the third test round.

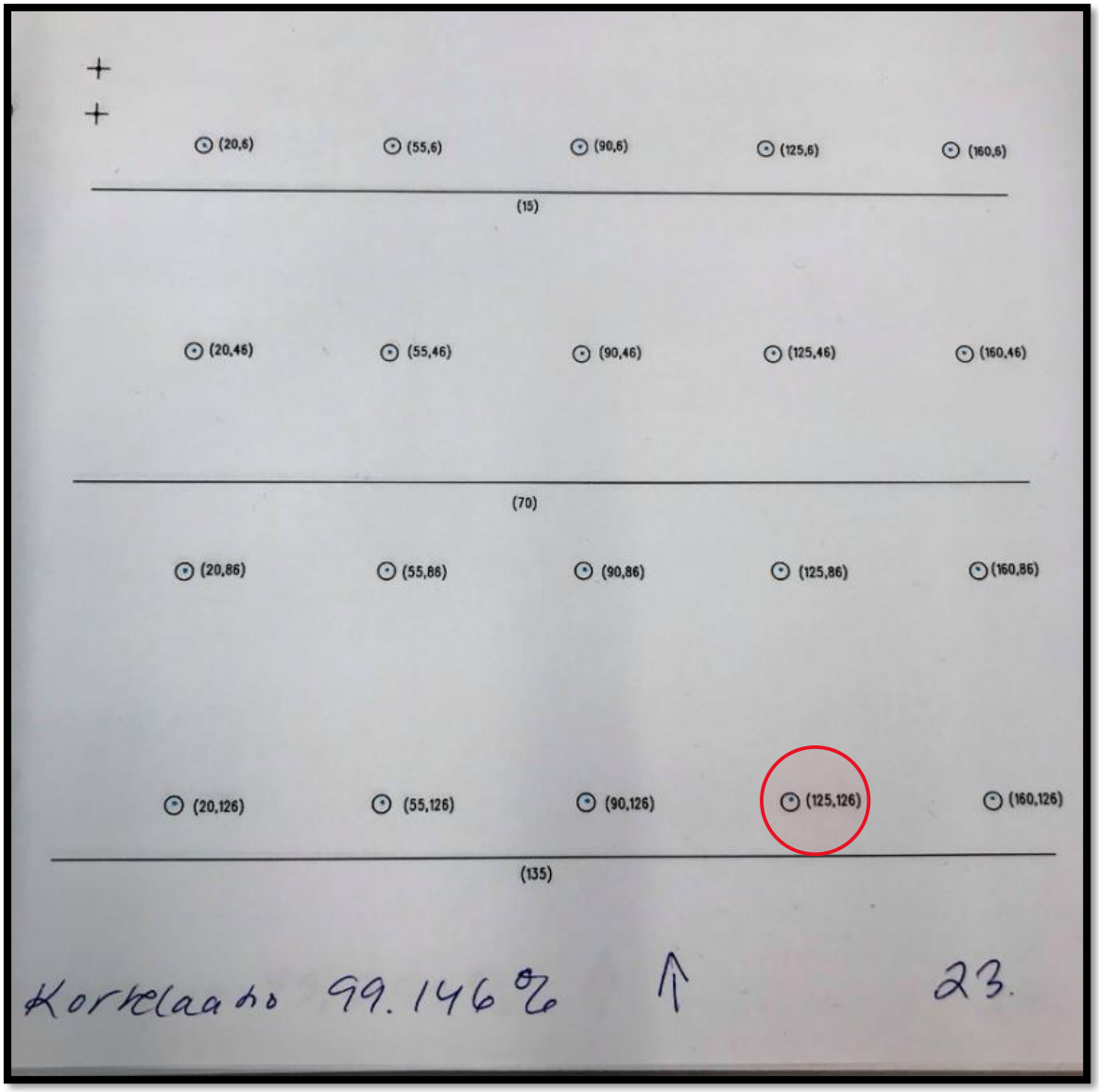

Figure 22 Alignment accuracy test, sheet 23. Photo Credit: Késia Ranta

Eight points were dispensed on both sides of the lines on the sheets 31.-40. so that four adjacent dots were printed on both sides of the line to see if the distance of the points to the line remained equal.

The results indicated that the dot rows in the bottom row were not equally far from the line. There was more space above the line between the dots and the line. While there was less space below the line between the dots and the line. The dots in the top three rows were all equally far from the line. Figure 23. illustrates the errors of the fourth test round.

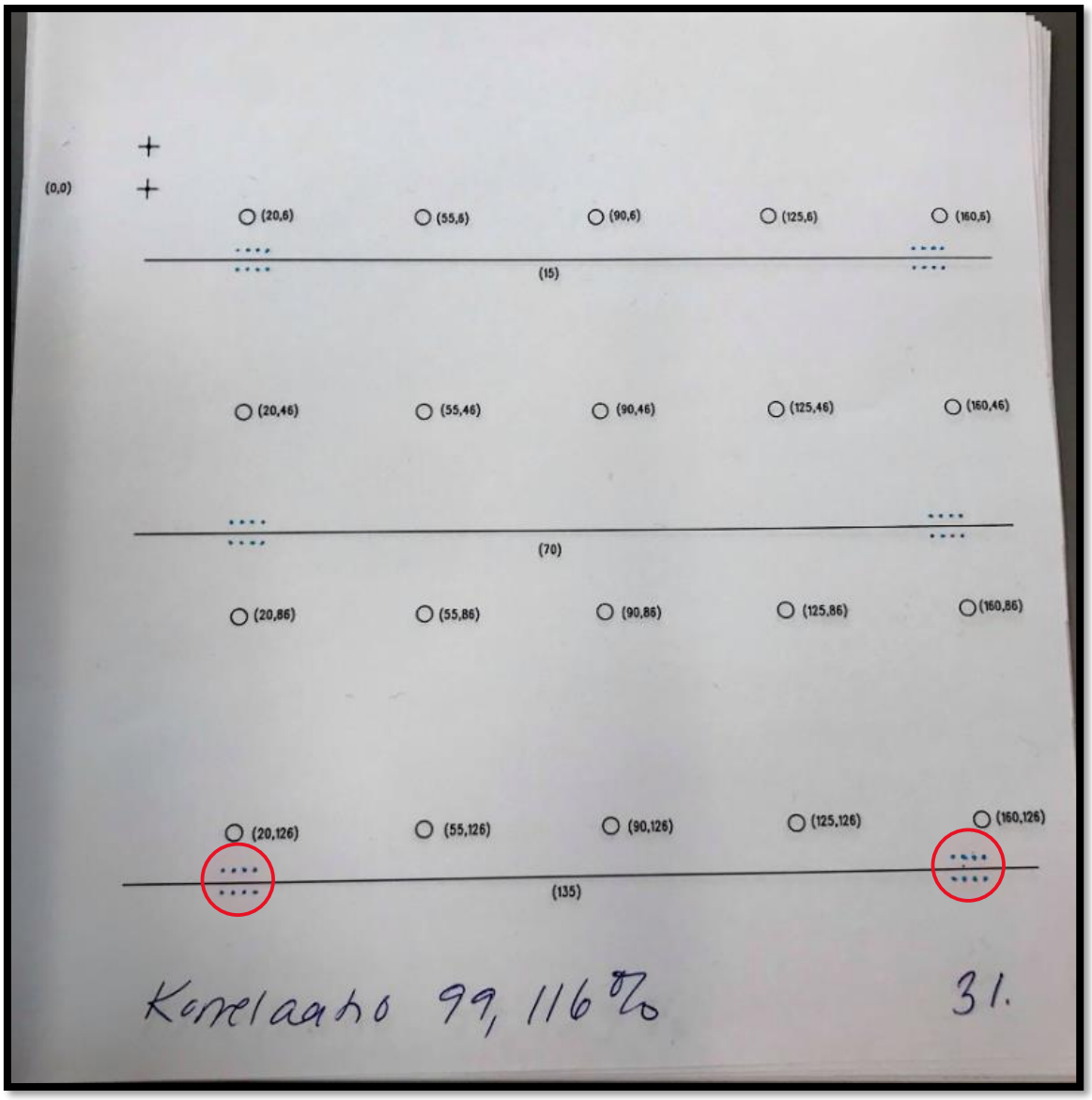

Figure 23 Alignment accuracy test, sheet 31. Photo Credit: Késia Ranta

So-called floral pattern was printed inside the circles on the sheets 41.-50. This made it possible to test whether the dispensed pattern remained within the boundaries of the circle. The circle of the sheet is of the same size as the working electrode of the biosensor.

The results indicated that the floral patterns in the top three rows were printed inside the circles as desired. Nothing was printed on the bottom line and the circles were inadvertently left blank. Figure 24. illustrates the errors of the last test round.

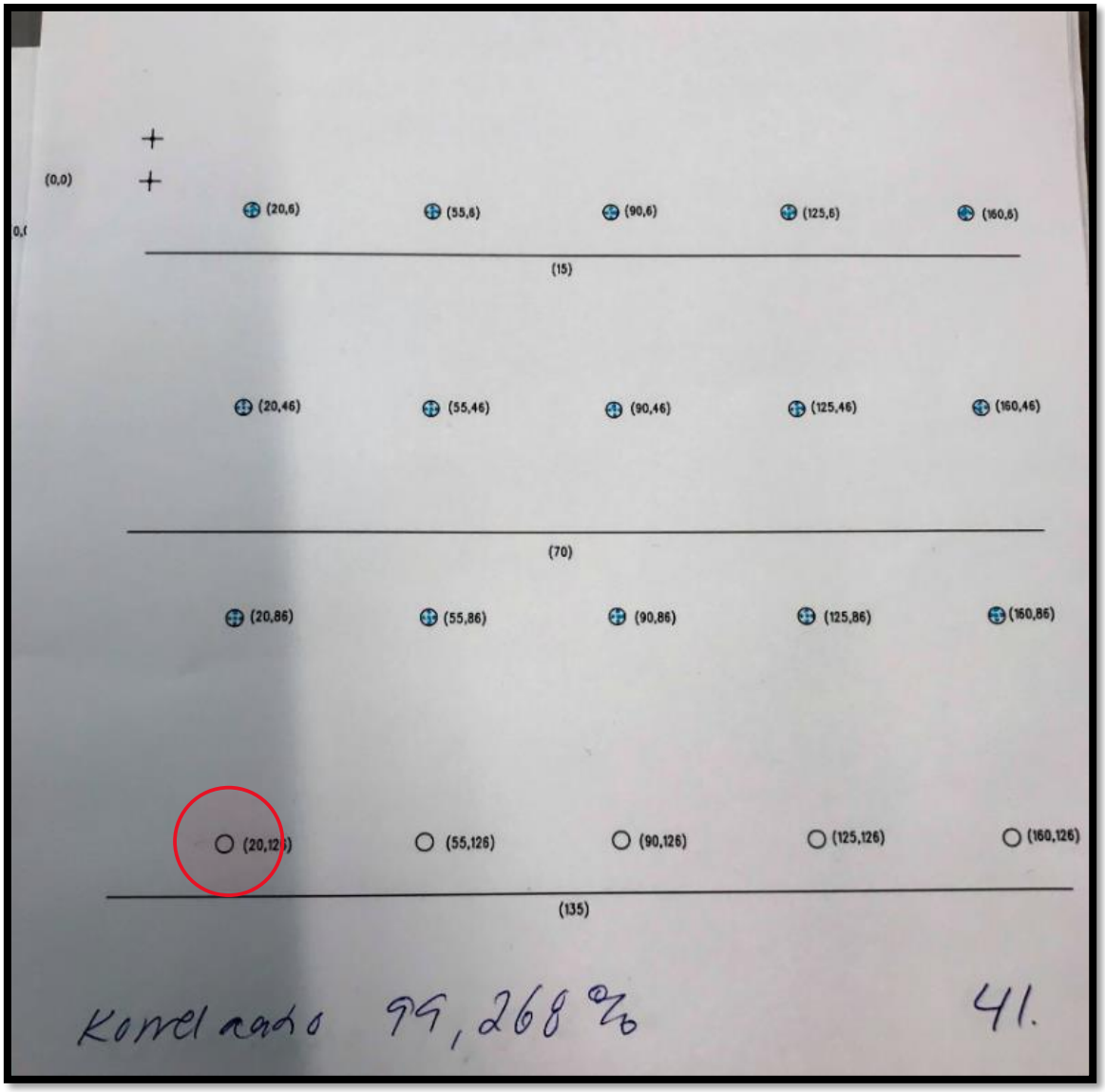

Figure 24 Alignment accuracy test, sheet 41. Photo Credit: Késia Ranta

3.2 Preparing the manufacturing and testing

April 15, 2021

The biomaterial used in the study was prepared (glucose enzyme).

3U/sensor=500nl/sensor

3% IPA ->6U/µl

->5x100nl/sensor

= 500microliters ->6U/microliters

The concentration:

13mg GOx

15µl IPA ->3%

485µl Buffer, PBS

Glucose solutions were prepared to test the functionality of the sensor's concentrations of solutions:

0 mM

0,25 mM

0,5 mM

1 mM

1,25 mM

1,75 mM

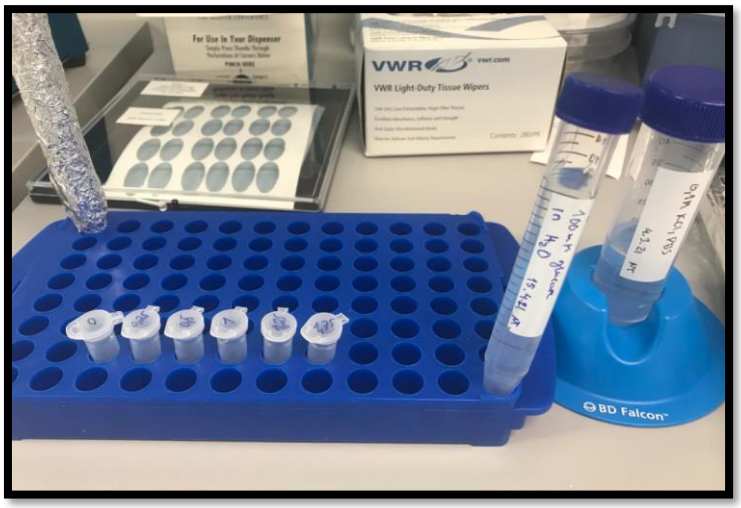

Figure 25 Different solutions of glucose. Photo Credit: Késia Ranta

3.3 Manufacturing and testing

April 16, 2021

At the beginning of the electrochemical measurements at 11:25, the temperature at the measurement point was 22,2 °C and the air humidity 18,6% RH. At the end on the measurements at 14:55, the air temperature was 22,1 °C and the air humidity was 21,2% RH.

Biosensor sheets

The biosensor consists of a screen printed electrochemical enzyme activated sensor. The three-electrode sensor platform was screen printed on a thin PET substrate. Silver/silver chloride (Ag/AgCl) is used as the reference material and in the contacts of the sensor. Counter and working electrode are printed with Prussian blue (PB) mediated carbon. Dielectric ink was used to insulate the structure.

Materials and fabrication parameters of the sensor base sheet with code P1169:

The inks used for the sensor base:

- first layer: reference electrode Ag/AgCl layer: Sunchemical (Gwent) C2130809D5
- second layer: counter and working electrodes: Prussian blue mediated carbon ink: C2070424P2
- third layer: Insulator: D2070423P5

Printing parameters:

-Print speed 75 mm/s
-Print pressure 0,9 bar
-Snap off 1,9/2,2 (Ag/AgCl)
-Squeegee: trailing edge 75 shoreA
-Drying temp 80 C, time 15/30 min (Insulator)

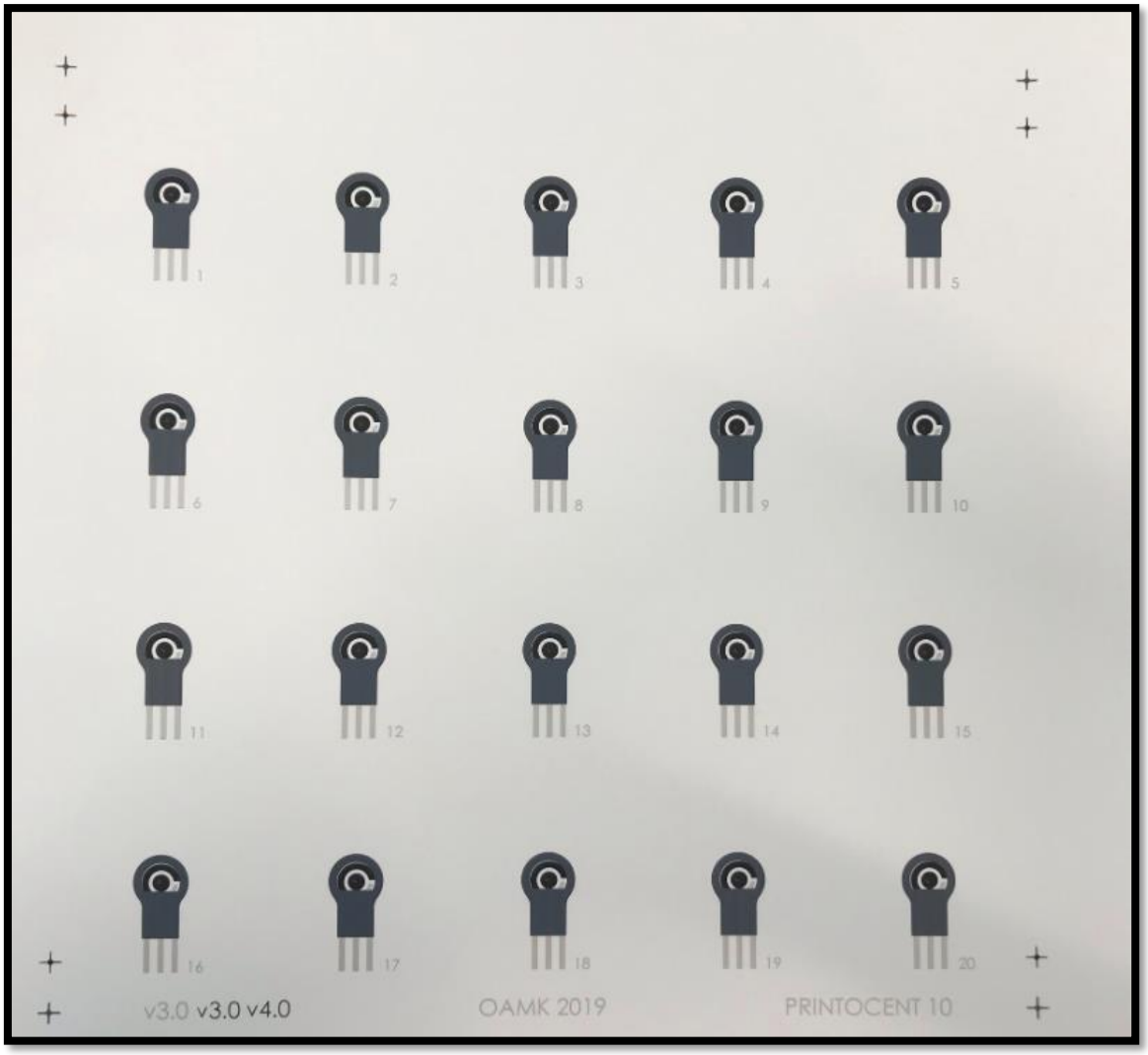

Figure 26 A sheet of screen printed biosensor bases. Photo Credit: Késia Ranta

The glucose oxidase mixture was dispensed on a total of four sheets, each containing 20 previously printed biosensor bases. The glucose oxidase concentration per sensor was 3U / 5 x 100nl. The glucose oxidase was dispensed on the surface of the working electrode of each sensor base.

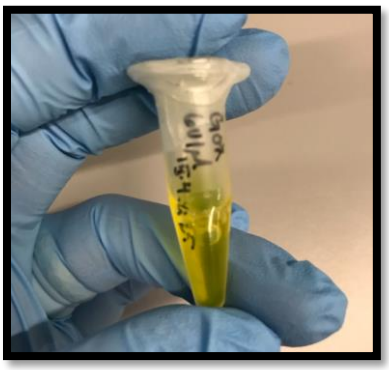

Figure 27 Glucose oxidase. Photo Credit: Késia Ranta

Printing parameters

Prime:
- Speed (nl/s): 4000
- Count (0-100): 20

Aspirate Front:
- Volume (nl) 50000
- Speed (nl/s) 8000

Aspirate Back:
- Volume (nl) 50000
- Speed (nl) 25000

Pressurize:
- Pressure (0-1.0 bar) 0.2

Dispense:
- Volume (nl) 50000
- Valve time (μs) 1000
- Drop count 1
- Delay (ms) 200
- Speed (nl/s) 5000

3.4 Electrochemical functionality verification

Electrochemical functionality verification is performed by pipetting a solution of glucose in PBS buffer to cover all the electrodes of the sensor and run cyclic voltammetry measurement with a potentiostat.

The functionality of the sensor is tested by measuring and analyzing the voltage, shape and current of the redox spikes, difference between voltages, relative standard deviation of the values and measuring the calibration curve (31, 220-221)

Measurements were performed with a PalmSens MultiEmStat potentiostat, which is a multi-channel potentiostat with 8 independent EmStat potentiostats. The potentiostat is connected and controlled by MultiTrace software that controls the channels. The potentiostat has 8 current ranges: 1nA to 10mA / 100 mA with a resolution of 1pA at the lowest current range and can be automatically selected to the optimal current range. (36)

Figure 28 PalmSens' PSTrace program connected to MultiEmStat potentiostat. Photo Credit: Késia Ranta

The sensors were cut off from the base and the sensor was placed in the connector so that the graphite wires of the sensor hit the pins of the connector. Then pipetted 30 µl of the sample onto the sensor so that all electrodes were covered with a drop of liquid. The measurement started when the autosave location was confirmed. (18; 36)

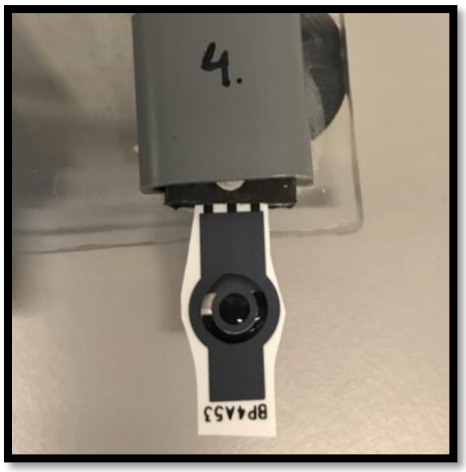

Figure 29 The sensors are cut off from the base and the sensor is placed in the connector. Photo Credit: Késia Ranta

Figure 30 Sensors placed in the connector. Photo Credit: Késia Ranta

3.5 Analyzing cyclic voltammograms

In this study, the data was analyzed with PalmSens' PSTrace program. From all cyclic voltammograms (CV) run, a third “scan” was selected, and the current and voltage values of the oxidation and reduction peaks were retrieved.

The values were entered into Excel for processing and the potential difference (ΔE) between the oxidation and reduction peaks was calculated. The potential difference was 220 mV.
The graphs of the different sensors were compared with each other.

0,26V-0,04V=0,22V

ΔE=220mV

The value of the reduction peak voltage (potential) of the glucose sensor (40mV) was set as the measurement voltage for amperometric measurements.

CV measurements were performed with two sensors at 0 concentration (and 0.1KCl, PBS) to get information of the sensor performance. The PalmSens MultiEmStat potentiostat was used to cycle potentials between 0.5V to -0,5V. Three measurements were performed at each solution strength. The average of the last five seconds was selected, then the average of all three.

Control lot was pipetted by hand, 50 microliters of sample + 50 microliters of PBS 1 microliter pipette per sensor.

Parameters of cyclic voltammetry measurements

Current range 1mA
-E begin -0,5V
-E vertex1 0,5V
-E vertex2 -0,5V
-E step 0,005V
-Scan rate 0,1V/s
-Number of scans 3

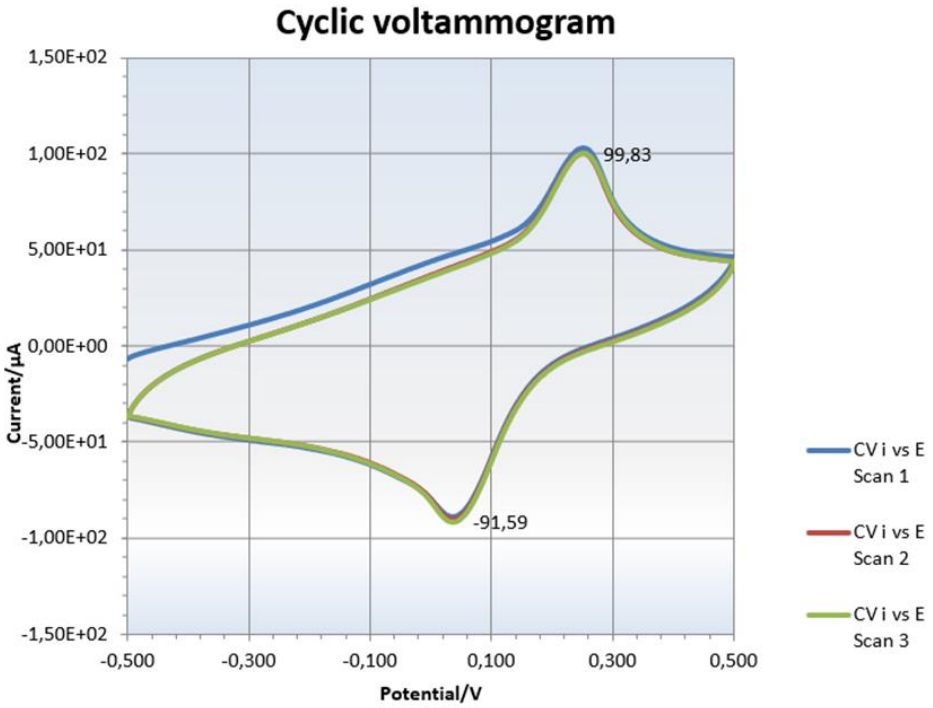

Figure 31 Cyclic voltammogram of tested sensors

3.6 **Analyzing the results of amperometric measurements**

The amperometric run graphs were opened in the PalmSens PS-Trace software window, and the data was transferred to Excel. The graphs were averaged over the last five seconds and the means, standard deviations and relative standard deviations were calculated from the means of the parallel measurements. A straight line was drawn from the means and the corresponding sample concentrations. A straight-line equation and a correlation coefficient were obtained for the line. (36)

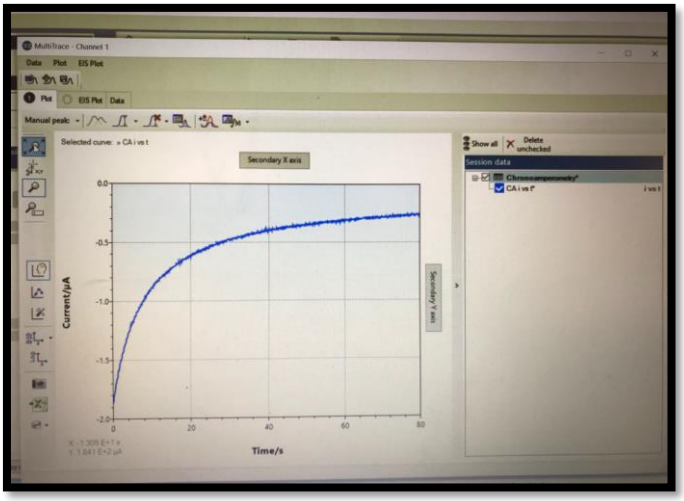

Figure 32 The run graphs are opened in the PalmSens PS-Trace software window. Photo Credit: Késia Ranta

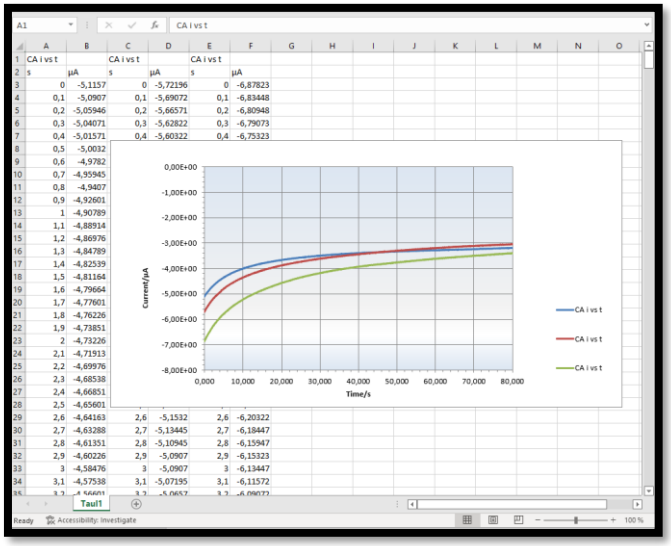

Figure 33 The data is transferred to Excel

3.6.1 Standard curve

In analytical chemistry, a standard curve, also known as a calibration curve, is a common method for determining the concentration of a substance and a way to account for the influencing factors that could change the analytical signal. The standard curve indicates the analytical signal that changes with concentration of analyte. The concentrations must be in the working range of the used technique. Analyzing each of the standards will produce a series of readings. A plot of response versus concentration will create a linear relationship. Operating in the linear response range the plot is supposed to be a straight line; deviations from this straight line indicate the precision of the result. External factors like temperature and pressure may affect the response as well as the condition of the analyte and any impurities. Standard curve creates a custom relationship, which considers the above factors. (37)

A standard curve of the averages of the known concentrations is drawn in the figure 34.
Glucose concentrations (mM) are shown on the x-axis and current (V) on the y-axis. Trendline, equation (y=1,7346x – 0,3686), correlation coefficient (R^2 = 0,9873) and error bars are added to the graph. Trendline shows the overall direction of the data and error bars are graphical representations of data variability showing the precision of a measurement.

The results of the measurements are roughly visible and can be interpreted based on this data. An increase in the negative value of the current relative to the increase in glucose concentrations indicates the performance of the manufactured glucose sensors. Most of the sensors tested provide the expected measurement data. The standard curve is not completely straight compared to the trendline and there are also error bars in the image. These aspects are next examined in more detail and become familiar with the measurements using standard deviation and relative standard deviation.

3.6.2 Standard deviation and relative standard deviation

A standard deviation (σ = the Greek letter sigma or STD) is a measure of how precise the average is. It indicates how dispersed the data is in relation to the mean. Low σ value means that the data is clustered closely around the mean and thus more reliable. High σ value indicates that the data

is more scattered and less reliable. Standard deviation is calculated as the square root of the variance.

Relative standard deviation (RSD% or coefficient of variation) is a measure of dispersion of frequency distribution. RSD% indicates how the different numbers in a particular data set are dispersed around the mean. RSD% is also called percentage relative standard deviation formula because it shows the dispersing on data in percentage. RSD% is calculated as the ratio of standard deviation to the mean for a set of numbers by dividing the standard deviation of a data set by the average of the data set multiplied by hundred. In general, a lower relative standard deviation means better reward for the asset. There is no specific value for a relative standard deviation that is considered as a good value but the more precise the data, the smaller the RSD%. (38)

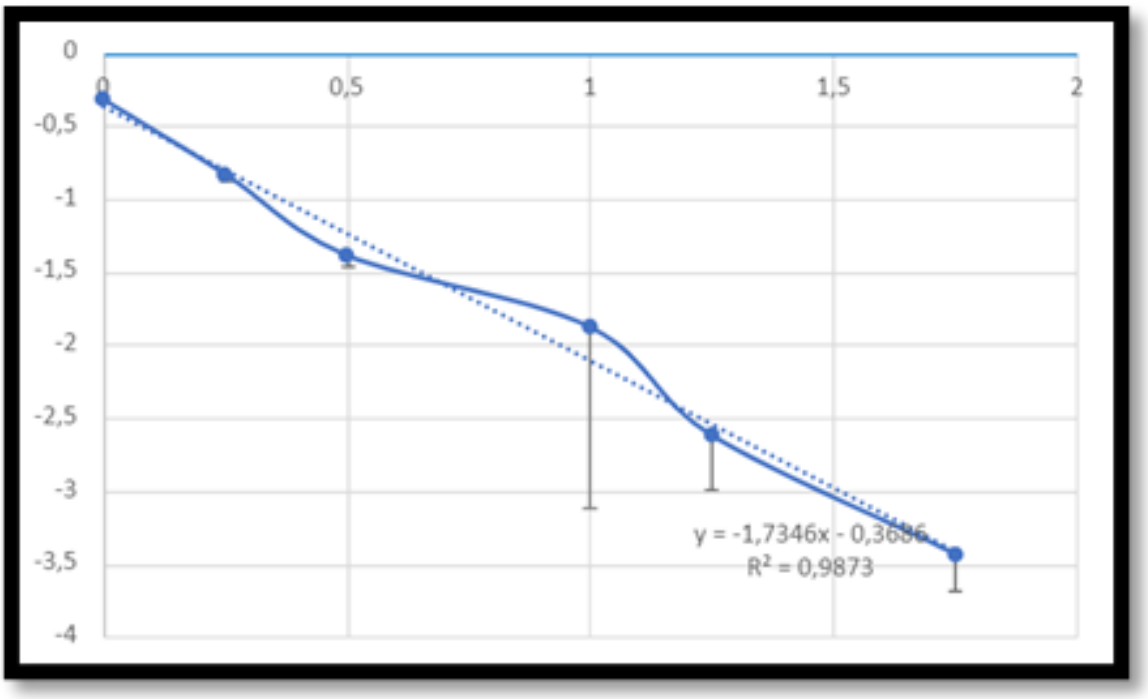

Figure 34 A standard curve of the averages of the known concentrations

4 RESULTS

4.1 The results of electrochemical measurements

In Table 1. The standard deviation (STD) and relative standard deviation (RSD%) are at an acceptable level for all other concentrations, except for a concentration of 1 mM in which the relative standard deviation is about 66% thus indicating that there is a significant difference between the measurements. If we look at the last amperometric graph (the green line in figure 35) at a concentration of 1mM, it differs from all the others. This signifies that either the amperometric measurement of that sensor is not successful or there is something wrong with the sensor itself.

The 1mM solution worked in other measurements so there was no fault in the solution. Based on the visual inspection, there was biomaterial on the working electrodes of all the sensors, so there is no reason to suspect a malfunction in the operation of the post-processing line either. It is possible that the error occurred during manual pipetting and that the sample may have become contaminated. An incorrect measurement result can also be caused by the sensor not being properly inserted into the connector.

As this one measurement is clearly not in line with the other measurements, it is reasonable to delete that measurement and look at the standard deviation and relative standard deviation without the value of this measurement, in which case the result is in line with the other results. Without this measurement, the standard deviation is 0.124 and the relative standard deviation is -5.28%. From now on, the measurement results will be processed without the failed measurement.

Table 1

Conc.	0mM	0,25mM	0,5mM	1mM	1,25mM	1,75mM
Average	-0,31571	-0,82638	-1,38493	-1,87596	-2,61829	-3,42984
STD	0,022357	0,041058	0,076916	1,23664	0,362093	0,246615
RSD%	-7,08159	-4,96849	-5,55383	-65,9202	-13,8293	-7,19027

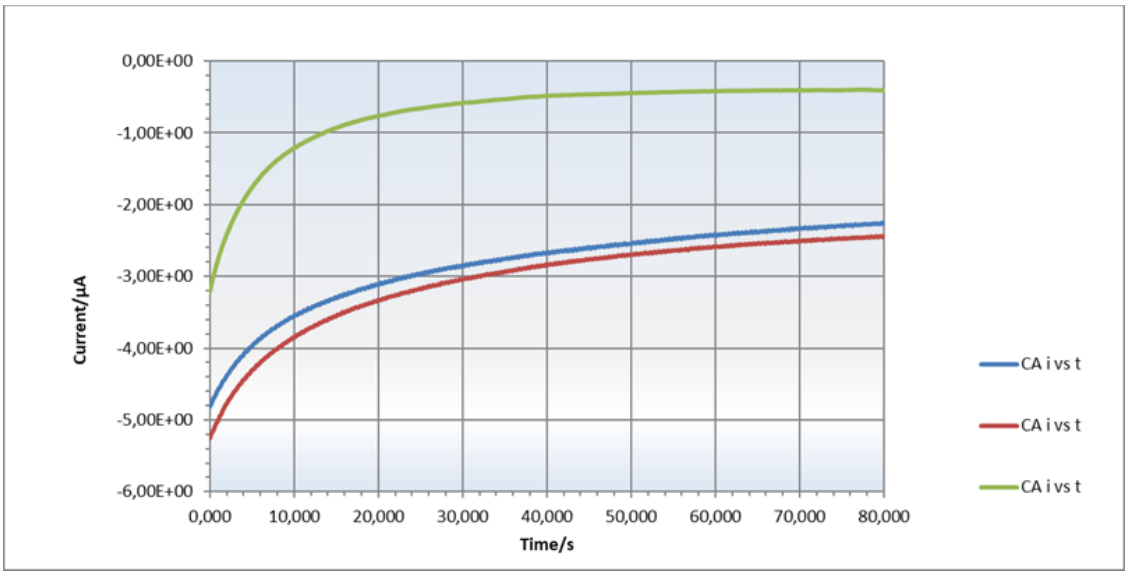

Figure 35 Amperogram with faulty sensor

When looking at the results, the negative number in the averages of the last five seconds of the measurements increases with increasing concentration. The relative standard deviation is less than 10 in other measurements, except for the fifth measurement, i.e., at a concentration of 1.25 mM. The graph in Figure 36. shows how significant the deviation is. The first (blue) graph differs slightly from the other two graphs. The average of the last 5 seconds of the first measurement was -2.20518, while the averages of the latter measurements were -2.76905 and -2.88065. The difference between the latter measurements is 0.11161, while the difference between the first measurement and the second largest reading, the last measurement, is 0.67547. This thus explains the reading of the relative standard deviation, which differs from the others.

Table 2

Conc.	0mM	0,25mM	0,5mM	1mM	1,25mM	1,75mM
Average	-0,31571	-0,82638	-1,38493	-2,35254	-2,61829	-3,42984
STD	0,022357	0,041058	0,076916	0,124189	0,362093	0,246615
RSD%	-7,08159	-4,96849	-5,55383	-5,27895	-13,8293	-7,19027

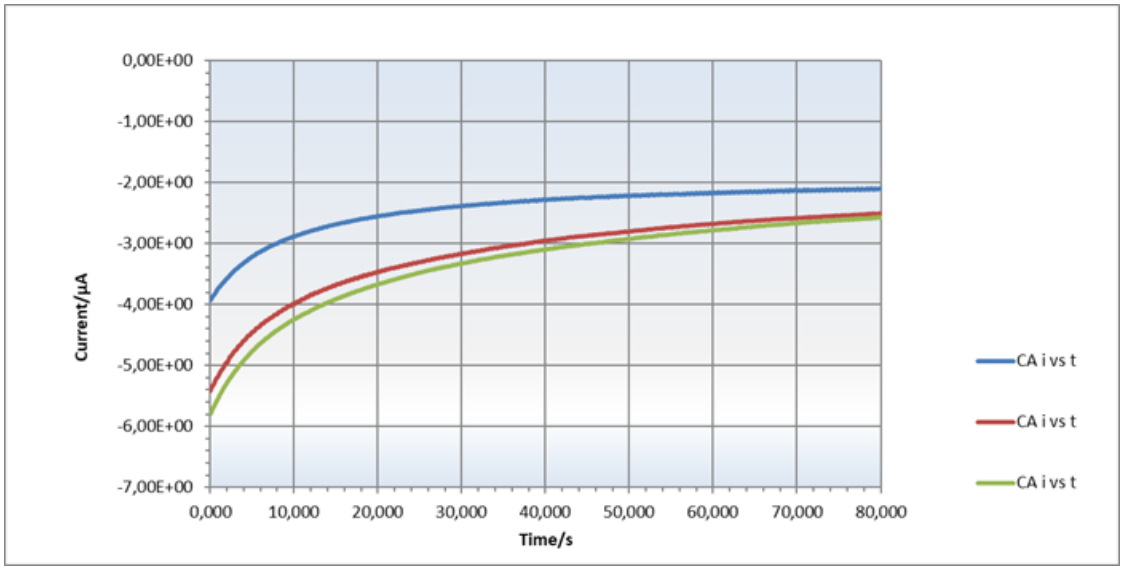

Figure 36 Amperogram without faulty sensor

4.2 Visual inspection of the results

The results of the manufacturing process were viewed in many ways. Prior to electrochemical measurements, a visual inspection was performed prior to removal of the sensor sheet from the vacuum plate to visually detect that the Cartesian robotic arm had successfully dispensed the bio-material droplets near the center of the working electrode of the sheet's sensor bases.

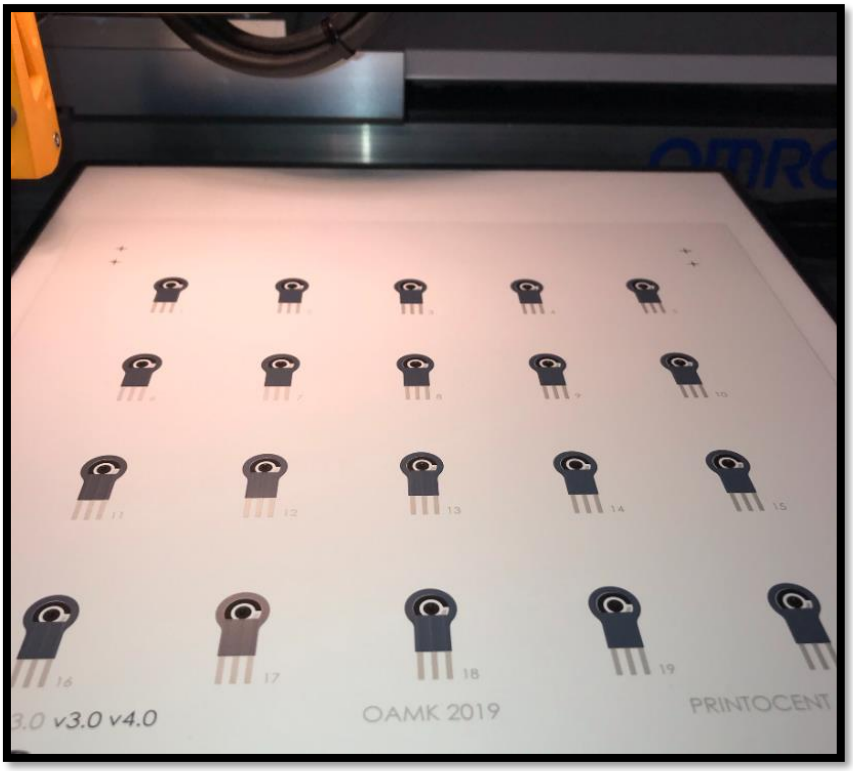

Figure 37 Sensor sheet on the post-processing line vacuum plate after dispensing. Photo Credit: Késia Ranta

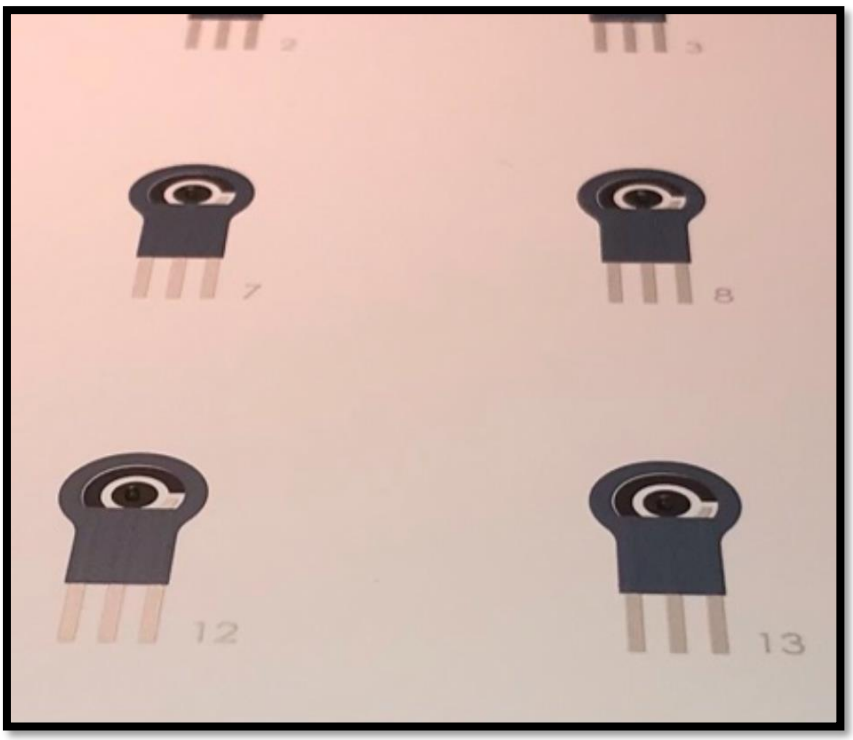

Figure 38 Dispensed drops on working electrodes. Photo Credit: Késia Ranta

Once the biomaterial was immobilized on the working electrode, its placement could be viewed with a magnifying glass. The close-up also showed better the different layers of the sensor ~~better~~. Reference electrode Ag / AgCl layer, counter and working electrodes Prussian blue mediated carbon ink and the insulator layer. The surface and location of the glucose enzyme shown on the surface of the working electrode. Since dispensing is a highly accurate printing method, the droplet of glucose enzyme could have been centered more correctly in the middle of the working electrode. The aim of the thesis was to manufacture a printed electrochemical glucose sensor, with the electrochemical performance as a top priority. However, this should be considered in future studies. Especially in mass production using the roll-to-roll method, such inaccuracy in alignment would not be acceptable.

Figure 39 Close-up of biomaterial immobilized on a working electrode. Photo Credit: Késia Ranta

4.3 Comments on the operation of the post-processing-line

The author found that the Cartesian robot did not function in all respects as desired. During the test runs it turned out that the robot arm did not move at all on the Z-axis, i.e., when adjusting the height, the height of the dispenser did not change at all. Despite this defect, the tests were able to be performed after manually adjusting the level at which the drop remained at the desired location. When the dispenser head was too low, the drop bounced back up to the tip of the dispenser and as the robot arm moved in the X and Y directions and the drop left with it, thus spreading on the sheet in an undesired manner. This problem disappeared when the tip was raised higher.

The author also found that the post-processing line did not work in the desired manner if the sheet was not placed directly. In the optimal situation, the robot's machine vision uses alignment marks to detect the position of the sheet, even if it is mounted at an angle or at the edge of the suction surface. When dispensing the sensors, sheets 1,2 and 4 were visually very good and all the printed sensors were usable. For the third sheet, the angle was too large, i.e., the machine vision did not function properly with respect to the alignment marks and the biomaterial was dispensed only on the surface of the working sensor of 8 sensors and the remaining 16 biomaterial was dispensed outside the working electrodes of the sensors. The manufacturer of the device should try to correct this feature in the future or, in the case of a feature of the device, to try to modify the shape or placement of the alignment marks in the future in order to make the device work optimally. The deficiencies observed in the operation of the post-processing line have since been corrected.

5 FURTHER STUDIES

The study carried out was the first of its kind with the updated features of the post-processing line, which made it possible to dispense the solution onto the sheet with the Cartesian robot arm. The aim of the study was to manufacture a biosensor by dispensing the selected biomaterial onto sensor bases produced by screen printing. Four sheets of twenty sensors were manufactured and their functionality was tested using electrochemical methods. The research provides a good basis for conducting a further study. In the future, sensors could be produced in larger quantities and the electrochemical measurement results could be compared with the results of this study in order to obtain better results on how uniform the quality of the biosensors produced on the post-processing line are.

Two dispensing pumps, Ginolis EMB and PMB pumps were integrated into the post-processing line, which enabled the dispensing of different drop volumes. In this study, the Ginolis PMB pump was used. It is capable of dispensing up to 100 drops per second with drop volumes from 2,5nl to 50nl. Sensors could be manufactured by using EMB 150 pump that can dispense 40 drops per second with drop volumes from 50 nl to 140 µl, and then compare the possible quality differences of the sensors dispensed with these two different pumps. (15)

An interesting topic for a follow-up study would be to manufacture a noninvasive biosensor with the post-processing line. Noninvasive sensing means there is no need to inject the body with a needle to collect body fluids such as blood, serum or cerebrospinal fluid. Sweat, saliva and tears can be used as target analytes in noninvasive biosensors. Noninvasive sample collection causes less stress to the patient and enables more frequent measurements. The best example of a noninvasive biosensor is an alcohol biosensor with a hydrogen peroxide-based electrode utilizing immobilized alcohol oxidase. (26, 38)

Sweat is an exquisite biofluid for noninvasive sensing because it contains different types of ions and, compounds such as lactate, glucose and ammonia which can offer plenty of information about a person's health. Depending on human physiological conditions the composition of the sweat may vary, which can be a sign of dehydration, food and dietary salt uptake, drug abuse or pathological diseases such as cystic fibrosis, osteoporosis and bone mineral loss. (26, 40; 39, 834)

As previously reported, glucose oxidase is a relatively easy biomaterial in terms of shelf life, usability, and reliability. Therefore, it would be interesting to test, for example, the fabrication of a lactate sensor on the post-processing line.

Lactate is an excellent biomarker for tissue oxygenation used for assessing physical performance. For evaluating athlete's performance in high strength activities and endurance, lactate is considered as a key component. L-lactate is a product of anaerobic metabolism of glucose in muscles. Sweat lactate has a positive correlation to the exercise intensity. During high-intensity exercise, lactate is produced faster than the body can absorb it and it begins to accumulate in the body, which is called the lactate threshold. (40) The imbalance of the lactate production may cause lactic acidosis and the increase of lactate production can lead to hemorrhagic chock or pulmonary embolism (41, 1)

6 REFLECTION

The author performed a more detailed analysis of the results only after the measurements were performed in the laboratory, as the author was in PrinLab in Oulu for a week. In retrospect, the results should have been carefully analyzed immediately after the measurements and at least one other similar measurement should have been performed to better ensure the reliability of the biosensors manufactured in the post-processing line.

Through testing and analysis, it was proven that the updated post-processing line improved the manufacturability of the biosensors and the reproducibility of the manufacturing process but it also revealed the potential for errors in the process, which requires process optimization to improve quality.

Optimizing the production process should ensure and minimize the variability of different work phases and reduce waste. For example, errors in alignment - the aim is to eliminate inaccuracy, reduce the number of rejects and disturbances. The updated post-processing line improves the performance of the entire process, i.e., productivity, and reduces costs caused by production fluctuations and waste. In connection with the further testing of reducing the amount of biomaterial, it would be a good idea to carry out optimization of the production process.

To an increasing extent, sustainable development, ecology and reducing the consumption of natural resources are emphasized in the manufacture of products. Faster development of printed electronics would certainly be one way to minimize energy consumption through production methods and material choices. However, perhaps the best way to influence the minimization of energy consumption would be to change people's consumption habits and attitudes but while waiting for that, it is better to improve the current manufacturing methods and carbon footprint even a small step at a time.

The significance and usability of the thesis was valuated thus creating a good basis for further research, the development of various sensors and the development possibilities of the post-processing line.

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APPENDICES

Cyclic voltammetry excel appendix 1

Amperometry; glucose concentration 0mM excel appendix 2

Amperometry: glucose concentration 0,25mM excel appendix 3

Amperometry; glucose concentration 0,5mM excel appendix 4

Amperometry; glucose concentration 1mM excel appendix 5

Amperometry; glucose concentration 1,25mM excel appendix 6

Amperometry; glucose concentration 1,75mM excel appendix 7