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SCREEN-PRINTED ELECTROCHEMICAL PAPER SENSORS

Testing methods and performance evaluation

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ABSTRACT

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In this thesis the focus was on the measuring techniques of screen-printed electrochemical sensors. Screen-printing is one of the best-established methods of printed electronics and electrochemical sensors are commercially mass manufactured with it. Most of the sensors are made on substrates such as polymer or alumina. From environmental and economical point of view printing on paper is a desirable substrate to use for sensors. Paper also brings benefits to testing and modification of sensors. Testing methods and techniques of electrochemical sensors are necessary tools for use of sensors. Understanding the principle of the measurement methods and analytical thinking is required for using the sensors and understanding the data.

Extensive research of literature on the different methods for testing the functionality and quality of the printed sensors was done. The vast field of electrochemistry is described and the position of electrochemical sensors is illustrated. Different aspects of manufacturing of the sensors with screen-printing process are explained and the differences between paper and polymer substrates described. The best testing method and pre-treatment steps for the paper sensors is determined and the optimized method is used to compare the paper sensors to polymer sensors. The electrochemical methods used for the testing are cyclic voltammetry, chronoamperometry and variation of cyclic voltammetry, scan rate analysis.

A successful comparison of paper and polymer sensors was completed with cyclic voltammetry and amperometric methods. The scan rate measurement provided more information about the functionality and reversibility of the paper sensors. Electrochemical paper sensors work well, but the current values are lower and the reactions slower in comparison to polymer ones. Paper sensors bring other benefits to the measurements and can be developed to better operate. The improved testing methods can be used for paper and polymer sensors testing in research and development work to gain information on the fundamental reactions occurring on the surface of the electrodes.

Keywords:

electrochemistry, testing, sensors, screen-printing, cyclic voltammetry, amperometry, paper sensors, paper substrate, electrochemical sensor

PREFACE

I would like to thank my colleagues, the members of PrinLab at Oulu University of Applied Sciences, for their help and support during the writing of this thesis. Specifically, thanks to Harri for supervising this work, to Marja for inspirational and supportive conversations and to Tomi for practical tips, advising discussions and printing the sensors. Thanks to Anne Poutiainen for linguistic corrections and suggestions.

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1 INTRODUCTION

Electrochemistry is a vast field of chemistry dealing with reactions that produce measurable current. Electrochemical reactions are reduction and oxidation reactions where electrons are either received or donated. These chemical reactions involve electrons that can be measured. Electrochemical screen-printed sensors are small electrodes made with conductive, functional inks that can change the chemical reaction into a measurable electrical current. Basically, any aqueous substance that has molecules that ionizes, can be measured electrochemically.

Screen-printing is one of the most established manufacture methods of printed electronics. Screen-printed electrodes are traditionally printed on polymer films or alumina. The printed technologies also make it possible to manufacture the sensor onto more unconventional materials like stretchable or paper substrates. Especially paper is interesting as it is relatively cheap, friendlier to the environment and readily available. Electrochemical sensors can be modified into biosensors to detect some biological component. The paper gives beneficial surroundings for the biochemicals used in the modification. Paper can also be used to filter and spread the sample for added benefit.

Much of the use of electrochemical sensors is in measuring sensors. There are several different techniques that can be used to analyze or detect samples. The materials used in the manufacture, size and shape of the electrodes and the measuring technique have a big effect on the reliability and quality of the results. Potentiostats are used to measure the electrochemical reactions and the technique for measuring and analyzing the results must be carefully chosen.

In this thesis the focus is on developing a testing method for paper based screen-printed electrochemical sensors. The paper brings some challenges and changes to the manufacture and testing method in comparison to polymer-based sensors. The best measuring method for paper sensors is confirmed. Screen-printed electrochemical paper and polymer sensors are compared with cyclic voltammetry and amperometry which are common techniques for analysing electrochemical sensors. Scan rate measurements are made to give more information on the reversibility of the reduction-oxidation reactions happening on the surface of the electrodes.

2 ELECTROCHEMISTRY AND ELECTROCHEMICAL SENSORS

2.1 Electrochemistry as a field

Electrochemistry is a large field of chemistry that deals with interactions between chemical reactions and measurable electrical change. There are several different techniques and applications that utilize electrochemical reactions, like batteries, fuel cells, electrochromic displays, electroplating or analytical sensors. Corrosion, rusting of iron, is a very common occurrence of electrochemistry. Electrochemistry is a useful tool in research of chemical systems and can be used for example to detect changes in current or voltage in the system [1].

The name electrochemistry refers to the movement of electrons. Reactions where atom, ion or molecule either gains or loses electron or electrons are called oxidation-reduction (redox) reactions. When a molecule loses an electron, it oxidizes and when it gets one, it is reduced. The reduction takes place at a cathode and the oxidation at an anode [2]. There are several different techniques and variations used in electrochemical analysis. The techniques can be divided into categories based on what is controlled and what is measured. The relation of different techniques is presented in the table 1.

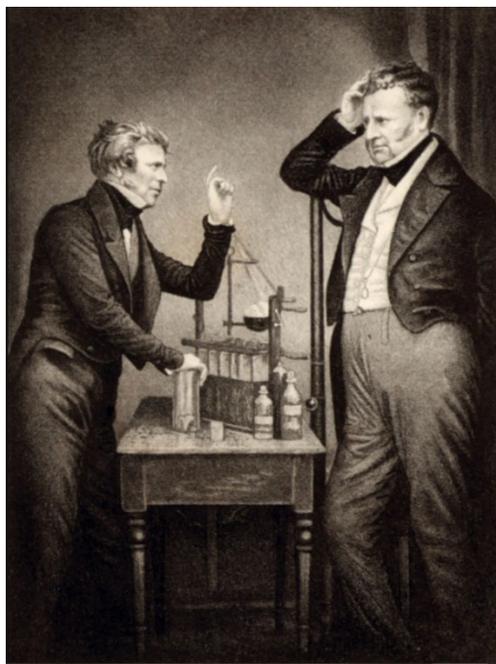
Table 1. Electrochemical techniques [3] The highlighted techniques are described in more detail in the later chapters. These highlighted techniques belong to the most used ones.

Controlled Potential	Potential Step	Amperometry	Chronoamperometry , Double Potential Step Chronoamperometry	
		Chronocoulometry, Double Potential Step Chronocoulometry		
		Sampled Current Voltammetry, Differential Pulse Voltammetry, Square Wave Voltammetry		
	Potential Sweep	Voltammetry	Stationary	Linear Scan Voltammetry
			Hydrodynamic	Cyclic Voltammetry
				Stirred solution/Flow Cell Rotating Disk Electrode, Rotating Ring-Disk Electrode
Anodic Stripping Voltammetry (Stationary/Hydrodynamic)				
Constant Potential	Bulk Electrolysis	Stirred Solution		
		Flow Electrolysis		
Controlled Current	Chronopotentiometry	Constant-Current		
		Linearly Increasing Current		
		Current Reversal		
		Cyclic		
	Coulometry	Coulometric Titrations		
	Electrolysis			
Controlled Charge	Charge Step	Coulostatic Methods		
Impedance Techniques	ac Voltammetry (ac Polarography)			
	Electrochemical Impedance Spectroscopy			

2.2 Short history of electrochemistry

Before it was called electrochemistry in around 1780 Luigi Galvani discovered that static energy could move frog legs. Later it was noticed that two different metals in a solution could do the same thing. Alessandro Giuseppe Antonio Anastasio Volta reasoned that the frog was an unnecessary part of the test setup. He developed a battery precursor called voltaic pile, where alternating zinc and silver plates wrapped in cloth saturated in salt solution generated current. William Nicholson and Sir Anthony Carlisle detected that with the voltaic pile they were able to decompose water into oxygen and hydrogen. This discovery of water hydrolysis led to a variety of research and development in the field of electricity and magnetism. Sir Humphry Davy did a lot of work on this field and discovered many elements in the process. Michael Faraday, who also worked as Davy's

assistant, is considered to be the father of electrochemistry, together with his student John F. Daniell, picture 1. Faraday created a lot of the basics in the field of electrochemistry and named many of the basic terms of electrochemistry like cation, cathode, anion, anode, electrode, electrolysis and electrochemistry itself. Daniell invented the electrochemical cell [4].



Picture 1. Michael Faraday and his student John Daniel working in the laboratory [5]

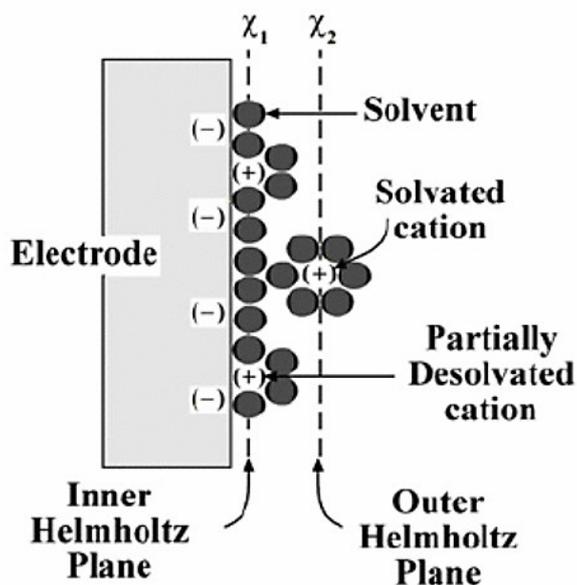
2.3 Electrochemical sensor

Sensor is a device that can transform physical phenomena into an electrical signal [6]. Chemical sensors have two parts: one part reacts to the analyte of interest, and the other transduces the chemical reaction into a numerical signal. Usually, the sensors do not act alone, but as an interface between the measured physical event and the computer. Sensors can transform phenomenon like temperature, pollution, saltiness of food, pressure or almost anything to data, that can be processed and analyzed [6].

In electrochemical sensors, the electrode is the transducer part of the sensor, and it converts chemical reactions into electrical, measurable data. Before electrochemical sensor in the 1960's the first chemical sensors were glass pH electrodes. In 1962 L. Clark Jr. demonstrated the first electrochemical glucose sensor based on amperometry. Since then, several different

electrochemical sensors have been developed to measure many significant analytes utilizing different electrochemical techniques, transduction principles and identification elements [7].

Some general electrochemical principles affect all measurements of electrochemical sensors. The sample is usually a liquid, and the concentration of the analyte is not necessarily the same in the entire sample. In the liquid there are three distinct layers close to the surface of the electrode. The interface at the electrode surface where the oxidation-reduction reaction occurs, between the molecules in the analyte and the immobilized electrode surface, is called the double layer [8]. Closest to the electrode is called the inner Helmholtz plane and is formed of adsorbed solvent molecules and some partially dissolved ions. The other layer is the outer Helmholtz plane and is formed by the fully dissolved ions, that adsorb to the surface by electrical charge, picture 2 [3]. This is the surface of the working electrode. Molecules drift toward the double layer in the diffusion layer by diffusion, migration, and convection. This movement of molecules, reagents or products is called mass transport. As other molecules move, others take their place. The distance to which this effect reaches is called the diffusion layer. Everything beyond that is called the bulk solution. However, the bulk solution starts at 0.1 mm distance from the surface of the electrode [8].



Picture 2. Electrical double layer, from Zoski [3]

At the surface of a working electrode, due to the oxidation or reduction reactions in the sample, electrons are generated or consumed. External power supply keeps the potential stable by compensating the movement of electrodes. Electrodes are pumped from and to a counter

electrode. The movement of electrodes is current, and it is always proportional with the number of reduction-oxidation reactions. The higher the rate of oxidation-reduction reactions the higher the current generated [2], [8].

Depending on the sample there can be several different reactions of different kind taking place at the same time in the solution. Reactions do not necessarily have any reduction-oxidation impact, and the analyte of interest can react in an undesirable way. Different reactions have individual reaction kinetics and can have an impact on the electrode surface at a different time. Sometimes reactions happen in chains and can have an effect on other reactions. Many things can happen especially in complex “real” samples that can interfere with the actual measurement of interest. In a laboratory test the sample is usually quite pure of interfering molecules [2], [8].

The potential of the electrode determines whether oxidation or reduction occurs. If the electron in the analyte detects that the energy level at the electrode surface is lower, it will favor it and the analyte will oxidize. On the other hand, the molecule can reduce if the potential is higher in the electrode. Electrodes move toward the lower potential [2], [8].

Final concept is that once the potential is set the current can only be measured. On the other hand, if the current is controlled, potential cannot be constant. The system always finds the lowest energy possible, and the change cannot be controlled without changing something. The rate and amount of the change can be measured [2], [8].

2.4 Most common uses of electrochemical sensors

Electrochemical sensors are used in a variety of applications ranging from healthcare, environment protection to food safety. In theory any analyte that has ions or can be formed into ions with a rapid reaction can be measured electrochemically. Especially the roll-to-roll screen-printing process has made it possible to mass manufacture high quality sensors for healthcare industry. Home testing of blood glucose is possible instead of measurements done by healthcare professionals. The printed electrochemical sensors are disposable, easy and affordable to use [9].

2.4.1 Biosensors

Biosensor is a device that has the capability to detect some biological analyte and a way to transduce the recognition to a measurable signal. In electrochemistry specific enzymes, like glucose oxidase for example, can be used to detect glucose. The electrochemical sensor can then transduce the biochemical reaction to electrical current. For each different analyte, the sensor must be treated with the specific biomolecule of recognition. The first-generation sensors measure the H_2O_2 produced by the enzyme. Second-generation sensors use a redox mediator that lowers the measurement potential, and the concentration of the analyte is related to the redox reactions of the mediator. The biocomponent in the third-generation sensors have the capability to generate the electron transfer directly to the electrode [10]. These descriptions apply mostly to sensors based on amperometric measurements. Biosensors based on amperometry are the largest group of these sensors, but not all amperometric sensors are biosensors and not all biosensors are based on amperometry.

The first electrochemical biosensors, measuring O_2 in blood by enzymatic reaction of glucose oxidase, were developed by Clark in 1956. Since many other applications have been developed to many different applications, but the glucose sensors remain to be the most successful one [10]. The glucose sensor for diabetes monitoring is a billion-dollar market [9]. These sensors comprise approximately 85% of the biosensors used in the world [11]. The market for biosensors is still rapidly growing; in the case of the glucose sensor in 2018 the revenue was USD 12.8 billion and estimated to reach USD 23.7 billion during 2022 [12].

3 MANUFACTURE OF SENSORS

Material plays a significant role in the fabrication of and usability of printed sensors. There are several printing substrates, inks and equipment to choose from. Sensors can be made by different printing methods, like gravure, screen-printing or inkjet printing. Gravure printing is a roll-to-roll printing method and inkjet is a flatbed method. Screen-printing can be made using both rotary and flatbed methods. In this work the focus is on the flatbed screen-printing method. Usually, the application, availability and budget determine what materials are used. The structure of the sensors is also chosen based on the application it is meant to be used for.

3.1 Substrate

In printed electronics the substrate is the platform where the printing is done, the base of the product. More commonly the substrates used in printed electronics are flexible, thin and have good temperature and solvent resistance. The substrate can be just a supportive inert material, or it can have some functionalities. Substrates can be made from varied materials and can have different properties. Materials can differ from each other for example in durability, stiffness, thickness, colour or hydrophilicity.

3.1.1 Common substrates used in printed electrochemical sensors

Originally more rigid substrates have been used as the platform for electrochemical sensors, like ceramic or silica [13]. The benefit of these is that the temperature and solvent resistance is very good. These materials are also completely inert and durable in usage [14]. Today sensors can be printed onto a wider variety of substrates (Picture 3), like polymer, stretchable materials, alumina or paper, giving more possibilities for the applications. These newer substrates are more flexible and often cheaper [13].

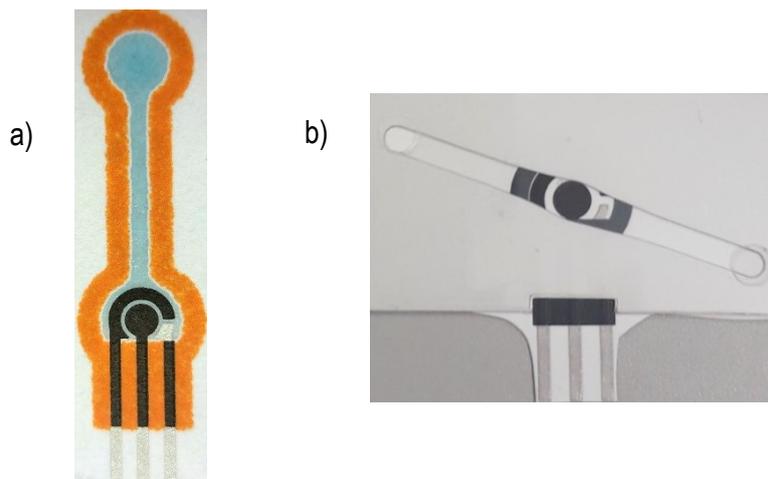


Picture 3. Different substrates used for printing electrochemical sensors: paper, and PET with different surface treatments and transparency. Photo Credit: Niina Tornainen

3.1.2 Paper as a substrate

Use of paper as a substrate in analytical sensors made with printed electronics has increased over the years. As a substrate paper can be more challenging, but it also has many benefits that cannot be achieved with more traditional substrates. One major benefit is the environmental impact; paper is much more environmentally friendly compared to polymer-based substrates. Many of the printed sensors used for analytical measurements are disposable even after a single usage and this makes it important to consider the environmental impact. Paper changes some aspects of the manufacturing and measuring in comparison to more solid substrates.

In addition to the environmental benefits, paper is light weight, easily bendable, cheap and a commonly available substrate. Smaller sample volumes can be used in the measurement and adding a sample is easy [15]. Some point-of-care applications have microfluidic structures, where the sample can travel through a channel to the detection zone (Picture 4). With paper, the sample can be passively transferred through the channel without a pump due to its natural capillary properties [16]. Many point-of-care assays include enzymes or other biological molecules. Drying and storage can be harmful for the activity for those. The complex inner structure of paper can help to keep the biocomponents active during drying and storage [17].



Picture 4. Microfluidic channel a) on a paper sensor printed directly on the paper substrate and microfluidic tape channel b) on top of a polymer-based sensor. Photo Credit: Niina Tornainen

3.2 Ink

Different inks are used for electrodes and other structures like channels. The inks used for electrodes are functional, but the ink used for channels is inert and insulating after curing.

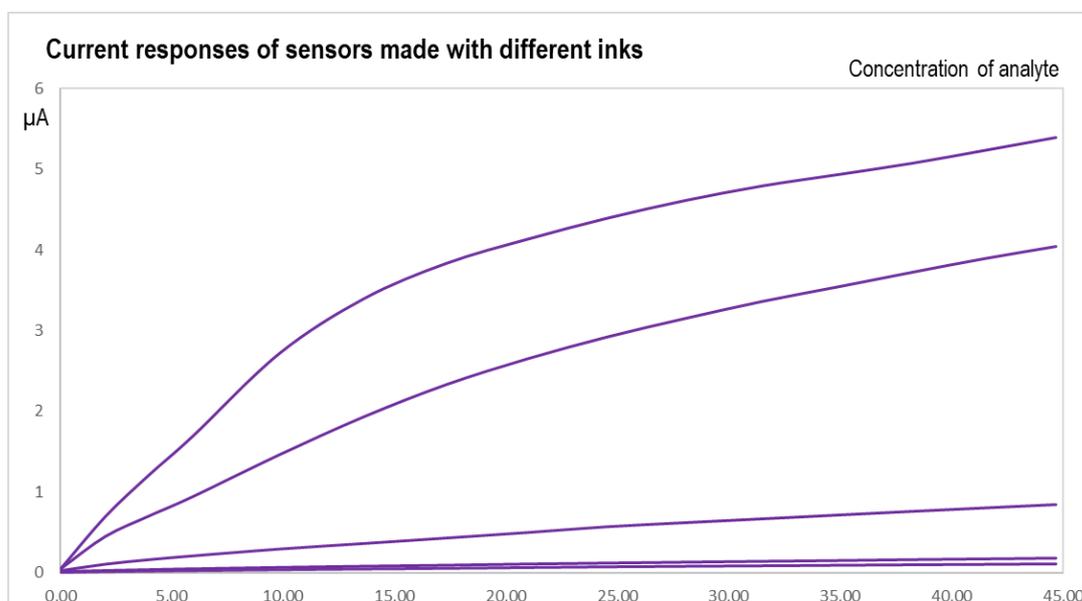
3.2.1 Electrodes

Similar inks can be used on paper as on other substrate materials. The porous and hydrophilic nature of paper might require some modification for the ink used. The most common ink used for electrochemical sensors are conductive metal or carbon-based inks. Common metals used in inks, in ascending order of conductivity, are copper, silver, gold and platinum. Graphite is one of the most used forms of carbon in electrochemical inks. Other forms of carbon used are for example carbon nanotubes, carbon black and graphene. In addition to the conductive material the other main components of the ink consist of liquid binder and solvent. The properties of the ink can be changed by differing the amount of binder and adding additives such as surfactants or stabilizers [18]. Carbon ink is often called carbon paste and therefore sensors made with such paste carbon paste electrode (CPE) [19].

The final use of the sensors and printing method affect the choice of ink. Silver is often used because it is relatively cheap, conducts well and is stable. Especially silver/silver chloride is a

common reference material for the reference electrode of an electrochemical sensor. Gold ink is used as counter and working electrode material especially if biomaterials like antibodies or enzymes are intended to be immobilized to the sensor. Gold is more expensive, but it conducts very well and does not oxidase. Carbon paste inks are extensively used because they are inexpensive and are optimal electrochemical properties for many applications [14].

In general terms, all different inks have different optimal voltage ranges for measurement, (picture 5). The ink composition and the size of the graphite particle can vary. Changes in the ink can cause the surface of the printed sensor to be smooth or rough. The more uneven the surface is the higher the surface area of the sensor is. This is not necessarily negative feature but can cause challenges if the roughness varies between sensors. Bigger surface area leads to higher currents in measurements but can hold more material to it. This can be beneficial when biomaterial is added, since more active component can exist in a smaller area. Higher amount of polymer makes it possible to more molecule to bind to it when using a technique called electropolymerization. With electropolymerization molecules and enzymes can be trapped on the surface of the sensor for example in a conductive polymer that binds to the polymer in the ink [20]. Inks from different manufacturers are different and it depends on the application which ink will give the best results [21].



Picture 5. Current response to changing analyte concentration with sensors made with different graphite inks. Sensitivity and linearity vary but bring benefits to a variety of applications.

3.2.2 Channels

Channels or barrier layers can be made on paper by using different materials like wax or polymer. Channels can also be made by cutting the paper to a channel shape or changing the absorbent properties by embossing. What these materials have in common is that they all form a liquid-repellent and hydrophobic sections on the paper thus allowing the liquid to travel only where it is desired to travel. The channel material must also be inert and cannot react with the used analyte or buffers.

Wax barrier layer is done by applying a layer of wax onto the paper substrate to the areas that are desired to be hydrophobic. Then the wax is melted by heat, and it forms the barrier layers to the paper. Stencils or printing can be used to get the wax in the right areas [22]. Channels can also be made by soaking the whole paper in UV curable photoresist and masking the channel areas before curing, so the photoresist does not solidify under the mask [23]. There are other methods to make channels on paper, but here just a few to mention.

There are also screen-printable polymer inks available. With these inks the channels can be made similarly to the electrodes. Depending on the ink it can be either heat or UV-light curable. Screen-printing the channels will be discussed more in detail in the empirical section of the thesis.

3.3 Screen-printing

Technique of screen-printing has existed for a long time and is believed to have been developed in China during Song dynasty (960–1279 AD). Screen-printing nowadays is used in a variety of places, textile, paper, ceramics and electronics. Screen-printing is a popular method for printed electronics including electrochemical sensors. The method makes it easy to control the size, thickness and alignment of printed patterns. It is also a relatively reliable and fast method with good reproducibility. A variety of different materials can be used to print different functional layers for sensors [12].

In screen-printing ink is pushed through the openings in a mesh onto a substrate using pressure. The screen consists of a frame, mesh and an emulsion that covers the parts of the mesh that the printing is not wanted. Screen-printing is a very versatile printing technique. With silkscreen-printing

it is easy to manufacture and align layered structures. It is also a relatively fast and easy method. There are several factors that affect the quality of the print. Most critical components or issues in the screen-printing process are screen (mesh), squeegee, flood squeegee, printing parameters, ink and curing or drying of the ink [24].

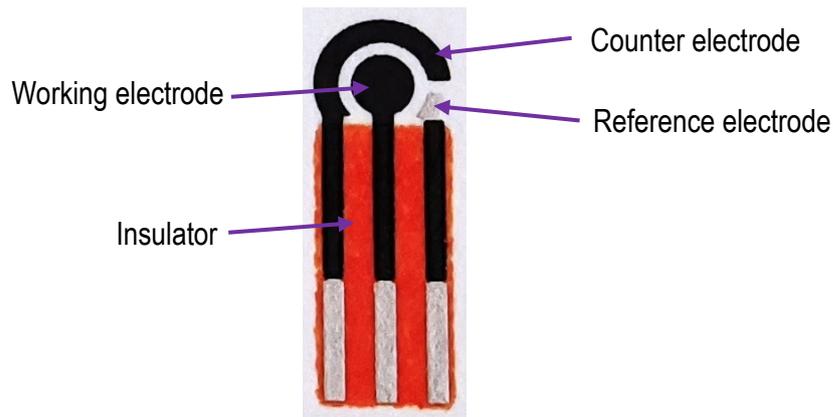
In the design phase of the screen, it is important to consider the diameter of the thread, the thickness of the mesh and the mesh opening area. The thickness of the emulsion together with the mesh mostly determine the layer thickness. In the printing process the screen tension is an important parameter to follow, if it changes it can cause distortions in the printed image [24].

Printing parameters need to be adjusted for each printing. The distance between squeegee, screen and snap-off distance need to be adjusted. The hardness of the squeegee needs to be chosen correctly for a print. The printing edge of the squeegee needs to be intact and sharp. If a flood blade is used, its distance from the screen needs to be adjusted. Printing distance is also a parameter that needs to be right for each printing, so the whole picture fits on the printed area [24].

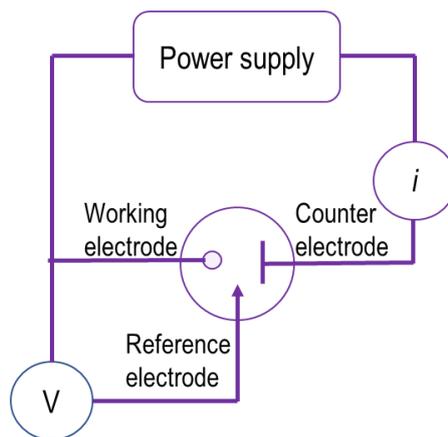
Screen-printing inks need to be mixed well before printing; the solvent may have separated during storage. The rheology of the ink can be tested to make sure the consistency is right. The curing temperature and time is different for different inks and needs to be adjusted so that the solvent evaporates [24].

3.4 The structure of the electrochemical sensors

The structure of the electrode must be adjusted for each application similarly like the choosing of the ink. The sensors used in research are usually more general and work for most kind of tests. When development or application goes further, more attention should be given to the size and position of the electrodes. Here the brief description of electrodes focuses on the common three electrode type sensor used in research and development applications. Three electrode sensors consist of working, reference and counter electrodes, Picture 5 and 6.



Picture 5. Paper based screen-printed electrochemical sensor with three electrodes. Height of the electrode is 20 mm and the width 7mm. Photo Credit: Niina Tornainen



Picture 6. Schematics of how the electrodes are connected to each other, adapted from Bard et al. [1]

3.4.1 Electrodes

Working electrode (WE), is sometimes also called indicator electrode. The recognizing and detection of analytes takes place at the working electrode. Increasing the size of the WE gives larger current values. The roughness of the surface in the working electrode can cause deviation in the results, especially in amperometric measurements. Counter electrode, (CE) sometimes called auxiliary electrode, is usually bigger than the working electrode so it does not limit the current flow. The current always moves between WE and CE, except in two electrode sensors where there is no CE. With the potentiostat the voltage between the CE and the working electrode (WE) can be

adjusted keeping the potential between WE and a reference electrode constant. Reference electrode (RE) should be as close to the WE as possible, so the resistance in the measurement solution is kept to a limit. RE should be stable and is usually made of material that is stable and has a known potential, like silver, gold or platinum [25]. The electrodes are insulated from each other by dielectric ink.

3.4.2 Mediating working electrode for biosensors

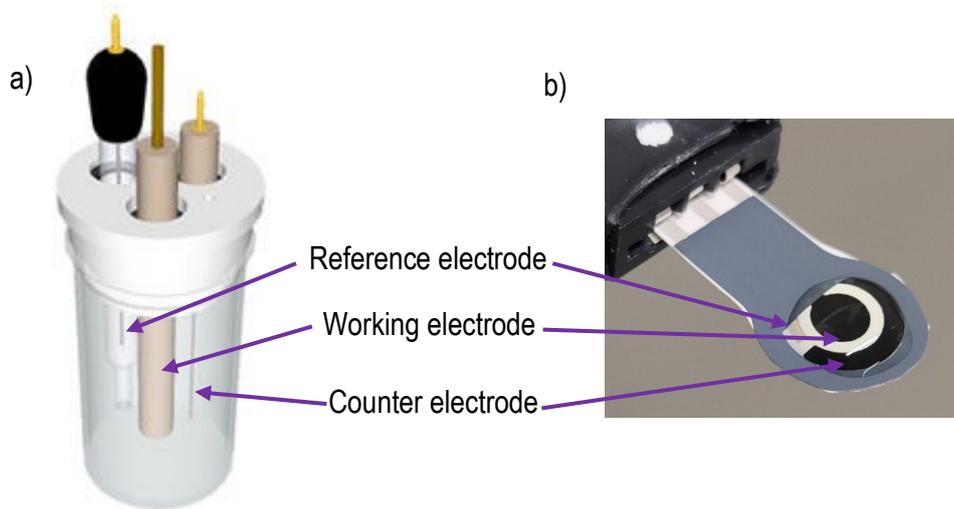
Commonly working electrodes are made of relatively cheap carbon and it has many different forms such as graphite paste, glossy carbon, carbon fibers also graphene. Often enzymes used in biosensor applications produce H_2O_2 molecule. H_2O_2 is capable of oxidizing metals that can be added to the electrodes using mediators to convert a chemical reaction into a measurable current. Without the mediating metal, like iron in Prussian blue, the potential used to recognize H_2O_2 is very high and can be harmful for the biomaterials [26].

There are several different ways a mediator can be added on the sensor. Some methods are simpler, and others require more expertise and extra steps. Solution of mediator can be drop casted on top of the WE or the mediator can be mixed into the graphite ink. Mediating can also be done via electro polymerization in which case it is covalently attached on the surface of the electrode [27].

In potentiometric sensors a solid, ion selective layer separating the surface of the electrode and the aqueous sample. This layer lets only specific ions to pass through. The ion-selective layers are added on top of the working electrode as a membrane [3].

4 TESTING METHODS OF SENSORS

The testing methods of electrochemical sensors are the same as in any other electrochemical setup. The scale is much smaller and the sensors are usually parallel in 2D -structure as seen in the picture 7, a and b, of a traditional and screen-printed measurement setup. Same principles apply to all electrochemical techniques and sensors. There are several different electrochemical methods that can be used for different analytes and applications. All the measurements are done with a device called a potentiostat, picture 8. There are several manufactures of potentiostats, like PalmSens BV and Metrohm AG.



Picture 7. a) Commercial, traditional 3D electrode setup in a container [28] and b) same sensor configuration on a 2D screen-printed electrode, sample drop connecting the electrodes Photo

Credit: Niina Torniainen.



Picture 8. PalmSens 8-channel MultiEmStat2 potentiostat. Photo Credit: Niina Torniainen

4.1 Most common methods

Cyclic voltammetry, amperometry, potentiometry and impedance are four of the most common electrochemical methods and they are described briefly in this chapter, mainly from the point of view of sensor testing. These methods can be used to analyse the quality and performance of an electrochemical sensor. Measurements are always done at the electrode-liquid interface. The methods can also be used to make qualitative and quantitative measurements of various analytes. Often sensors must be modified with post-treatment steps to make them selective for a specific analyte of interest. Some modifications are chemical, others biochemical and often a combination of these is required. If the electrochemical cell used in the method consumes energy from an outside source, it is called electrolytic. If electrical energy is produced in the process the method is galvanic [29].

When the current of reduction-oxidation reaction is measured, in controlled potential methods, the rate of the reaction can be determined according to the Faraday's first law of electrolysis (Equation 1). This fundamental law defines that the amount of measured current is directly proportional to the charge of the analyte. Simply said the amount of the analyte can be determined by the current change in faradaic process. In practice there are many other factors that affect the measured current, like the mass transport of electroactive species or the rate of the electron transfer in reactions. The slowest process is the step that determines the speed [29].

$$Q = nFN \quad (1)$$

Where Q is charge (C), n number of electrons moved in the reaction per mole, F is the Faraday constant (96487 C mol⁻¹) and N the moles of analyte (mol) [3].

There are several other different laws or formulas that often come up when electrochemical measurements are described. Some explain different phenomena in a particular method while others help understand the measured data. Some of the most common ones are presented here and are briefly described.

The correlation between the potential and the concentrations of an analyte's oxidized and reduced forms in a chemical cell can be described by the Nernst equation (equation 2). According to the

equation the potential of an ideal reversible, thermodynamical system can be calculated. In practice the Nernstian behaviour is often used to describe a perfect potential response [3].

$$E = E^\circ + \frac{2,3RT}{nF} \log \frac{C_O}{C_R} \quad (2)$$

Where E° is the standard potential of the redox reaction, R is the ideal gas constant ($8.314 \text{ J K}^{-1} \text{ mol}^{-1}$) and T is temperature in Kelvins [29].

In controlled potential methods the Cottrell equation (equation 3) describes the current decay (diffusion) over time as the diffusion layer expands [29]. The change of the current is limited by the rate of the diffusion that can be described by the Fick's laws of diffusion (not presented here) [4][3].

$$i_D = nFAC_0 \times \sqrt{\frac{D_0}{\pi t}} \quad (3)$$

Where n is number of electrons, A the surface area of the electrode (cm^2), C_0 concentration in the beginning (mM), D_0 diffusion coefficient (cm^2/s) and t time (s) [29].

One useful equation in cyclic voltammetry data analyzing is the Randles-Sevcik equation (equation 4) which describes the correlation between the peak current and the square root of the scan rate. This is a useful way to analyze the reversibility of a sensor used in measurements based on redox reactions [30].

$$i_p = 0,4463nFAC \left(\sqrt{\frac{nFvD}{Rt}} \right) \quad (4)$$

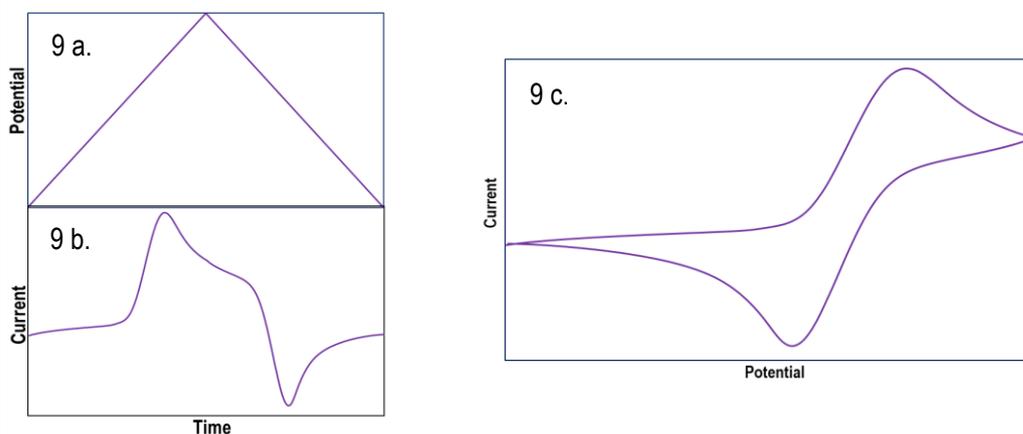
Where scan rate (V/s) is presented as v .

4.1.1 Cyclic Voltammetry

Cyclic voltammetry (CV) is a voltametric and potentiometric technique which scans through a potential range back and forth, while current is being measured. Cyclic voltammetry is a useful method to analyse the capabilities of an electrode at the solution electrode interface. With this

method the reduction and oxidation maximums, as well as the potential where those are, can be determined. This means that at certain potentials the electrons move the most toward the working electrode or away from it [29]. Molecules always choose the lowest energy level which is thermodynamically more favourable. Oxidation occurs when an electrode moves to a lower potential and leaves the original molecule, in reduction vice versa [31]. Any electroactive chemical components that can reduce or oxidase can also be examined with this technique. At certain potentials, the electron transfer increases as the diffusion layer gets bigger [29]. The measurement is done in an unstirred solution, so diffusion has time to reach its limits [3]. This measurement method can also reveal a lot about how well the electrodes functions in measurements [29].

In practice cyclic voltammetry is measurement of current as potential is changed between negative and positive voltage and back again in a certain time. Cyclic voltammetry is a potential sweep method that is run in cycles. The potential is applied in a triangular wave form against time. Normally the resulting voltammogram is presented as a current vs. potential curve. There are several variations of potentiometry that are very close to cyclic voltammetry, measured using different wave forms [4]. An example of a common waveform showing the change in the potential and the detected current change is presented in the pictures below. The cyclic voltammogram is usually presented in potential – current plot also presented below (Picture 9 a, b and c).



Picture 9 a. The waveform of a cyclic voltammogram, 9 b. current change over time and 9 c. the common way to present the current change over potential in a cyclic voltammogram.

More commonly, especially in research, three electrode sensors are used. Then most of the current moves between the working electrode (WE) and the counter electrode (CE) and does not affect the potential (V) between WE and the reference electrode (RE). In two electrode systems the current (i) moves though RE and according to Ohm's law (equation 5) can cause voltage-drop the size of

iR_s (R_s being the resistance of the solution). Generally, two electrode systems can be used if the potential stays below 2 mV. [3]

$$V=iR \quad (5)$$

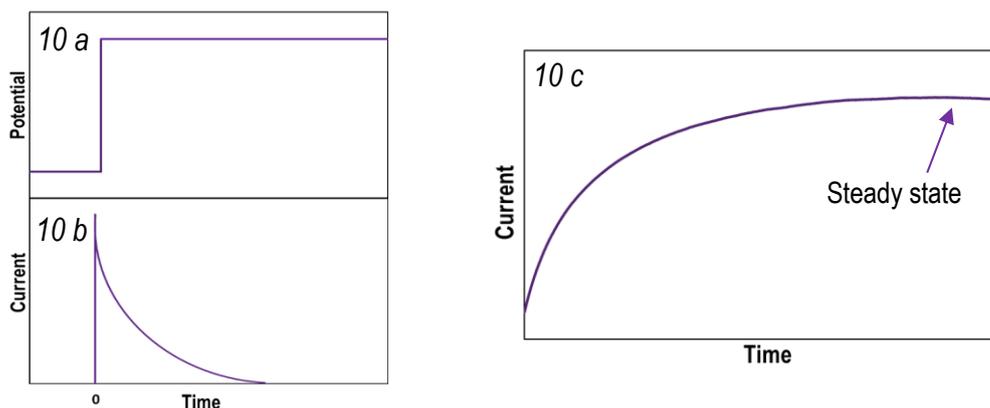
One modification of basic cyclic voltammetry measurement is the scan rate measurement. As described previously in equation 4, the Randles-sevcik equation the measured current is directly proportional to the concentration and increases as the square root of the used scan rate [29]. This can be utilized in CV – measurement, by increasing the scan rate and following the current [30]. With this method sensors capability to function in redox reactions can be evaluated.

4.1.2 Amperometry

In amperometric measurement the potential is fixed. The current is measured from the working electrode against time. A variation of amperometry where potential is changed in steps is called chronoamperometry [2]. The measurement potential is chosen so that the analyte of interest either reduces or oxidases, depending on the application. Usually, the potential is better to be as small as possible to ensure less unwanted, possible interfering ions and to influence the measured current. This will improve the specificity of the measurement. Suitable measurement current can be determined from the CV redox maximums [20]. The measurement potential can also be lowered by adding a mediator on the surface of the electrode. Mediators are small molecules or ions that have redox properties, but their reactions occur in a lower potential than without it. Using a lower potential can make the sensor more specific and keep the sensor surface cleaner. The usage of mediator is especially useful in biosensor measurements, which is a popular application of amperometry [27].

Similar rules and assumptions apply than in CV-measurement. Amperometric measurement can be measured in a stirred solution because time can be adjusted so that the reaction has time to reach its steady state (see picture 10 c.). At the steady state, the measured current does not change anymore and all the ions in the sample have reached the state determined by the measurement voltage [27]. Since the measured current is faradaic, it is proportional to the concentration of the analyte of interest [10]. The measured currents from different known concentrations of the sample can be made into a standard curve. Then unknown concentration can be compared to that, or the curve can just be used to evaluate how well the sensors work [27]. There are some different

variations of the most common waveform presented below in the picture 10 a and the resulting current response over time 10 b. For example, the potential can be pulsed and that can be helpful if the sample contains some component that is accumulating on the sensor's surface. The pulsed potential can also to reduce the baseline noise of the measurement. The common way to show the measured current response is presented in the picture 10 c where the steady state is pointed out [10].



Picture 10 a. The waveform of an amperometric measurement, 10 b. current change over time and 10 c. the common way to present the change in current over time in an amperogram.

4.1.3 Potentiometry

Potentiometry is the oldest way of electrochemical measurements. Sensors used in potentiometric measurements are often called ion-selective electrodes. It is used in clinical diagnostics, environmental analyses and in industrial processes. Many physiological measurements of ions in the blood are also based on potentiometry, like K^+ , Na^+ , Cu^{2+} , Mg^{2+} and Cl^- [29]. The potentiometric measurements are based on measuring the difference in potential between two electrodes (two half reactions of an electrochemical cell). One half of the cell is the working electrode, which reacts to the changes of the analyte, and the other is the reference electrode which is kept at a constant known potential. Unlike other electrochemical measurements potentiometric measurements are static, zero-current measurements [2].

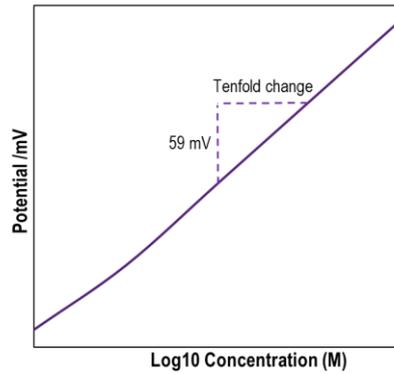
In potentiometric measurement the measured potential difference is logarithmically related to the concentration of the analyte in the sample as described in the Nernst equation (equation 2). The most common potentiometric measurement is the pH measurement. This is usually measured by comparing the potential difference on different sides of a glass membrane caused by different

concentrations of H_3O^+ -ions [32]. In traditional potentiometric measurement the electrodes are in different vials and the half reactions of the cell are combined with a salt bridge that allows the ions to move freely similarly to the glass membrane in pH electrode [2].

In screen-printed electrodes the ion selective membrane covers the working electrode. There is no liquid between the electrode surface and the ion selective membrane, so these sensors are also called solid state sensors. The membrane material is chosen so that only the selected ions reach the surface of the electrode and that makes it ion selective. The membrane consists of ionophore, polymer, plasticizer and additive. The ionophore is specific for the analyte, but the other components can be the same for several different type of sensors. Often a conductive polymer, like pyrrole, poly(3,4-ethylenedioxythiophene)-poly(styrene sulfonate) (PEDOT: PSS), polypyrrole (PPy), polyaniline or carbon nanotubes, can be added between the electrode and the ion selective membrane to enhance the ion-to-electrode conversion.[33].

Commonly used reference electrode is the silver/silver chloride. The biggest requirement for the reference is that is stable since the potential of the working electrode is compared to it. Ions and cations in the sample liquid can interfere with the potential or the reference or dissolve the actual reference material. The change in pH or the uneven distribution of ions and cations also interferes with the measurement [29].

The potentiometric measurement results are presented in a logarithmic scale, because the measured potential corresponds to the logarithm of the concentration change in the sample according to the Nernst equation (equation 2). Because of the log-scale even a 1mV change in the potential corresponds to a 4% difference in the concentration of the sample per ion. Therefore, potentiometric measurements are rarely more accurate than 0,1 mV. It can also be deduced from the Nernst equation that a tenfold change in concentration at 25°C corresponds to a change in potential of 59 mV. This is the case in an ideal Nernstian response of a monovalent ion, meaning that only one electron moves in the reaction at the electrode per molecule [29], presented in the picture 11.

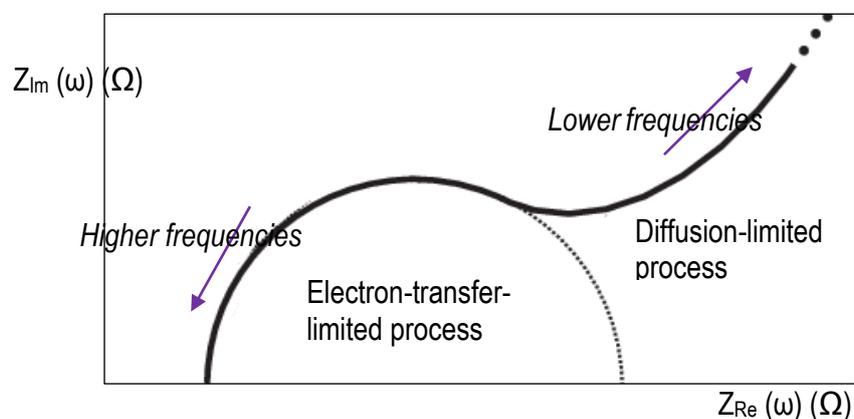


Picture 11. The Ideal Nernstian response of a monovalent ion at 25°C

4.1.4 Impedance spectroscopy

With impedance spectroscopy electrochemical reaction rates can be analysed. The reaction rates are affected by resistors, inductors and capacitors, in practice mostly by electron transfer and diffusion [29]. In impedance spectroscopy measurements low-amplitude AC voltage (with ω frequency) is added to the sensor creating a disturbed potential difference between working and reference electrodes. The resulting faradic impedance (Z) consist of a real part (Z_{Re}) and an imaginary part (Z_{Im}). Data of measurements done with screen-printed electrode are usually plotted to the imaginary part as a function of the real part in a Nyquits plot (Picture 12) [34].

This method of electrochemical measurements is used in affinity studies of biomolecules like antibodies and DNA. Impedance measurements can tell for example, a lot about how the attachment of antibodies change on the surface of the electrode. This is a useful method for immunosensors. The process of analysing the results is slightly more complex than with the other methods mentioned [29].



Picture 12. Nyquits plot for a typical sensor, adapted from [29]

5 EMPIRICAL PART

In the empirical part there is a short description of the manufacture of the electrochemical sensors by screen-printing. Then preparations and pre-treatment of sensors for electrochemical measurements. In the measurement section first includes the optimization of the paper sensor method, and then cyclic voltammetry and amperometric measurements with paper and polymer sensors. Finally, calculations, comparisons and analyzes of measurement results are presented.

5.1 Screen-printing of electrodes

Screen-printing was done in PrinLab, printed electronics lab, at the Oulu University of Applied Sciences. The screen-printing unit EKRA E2 was used for the printing and TERMAK TS8000 drying oven for curing the inks after printing. The sensors were printed on two different substrates: paper (qualitative filter paper, Grade 1, by Whatman®) and polymer (Kernowprint by Kernow Coatings).

5.1.1 Preparations

Before printing the paper and polymer substrates were cut to sheets sized 2,5 cm x 22,5 cm. The paper was taped onto a piece of polymer film as backing to ensure good attachment of the substrate onto the stone of the vacuum table and give support for the paper substrate. Without the backing the paper can get stuck on the underside of the printing screen and there is also a possibility that the inks would go through the paper. Between layers it is important to check that the paper is still well attached to the supporting polymer layer and there are no gaps between them. The polymer film was wiped with TechniCloth® dry nonwoven cleanroom wipers dipped in iso-propanol. This removes possible dirt and grease from the surface that can hinder the attachment of the inks.

5.1.2 Printing process

Normal preparations were made for the screen-printer and the accessories needed. There is a number of things and setting to be done before each printing process. The accessories are checked and installed, and different settings and adjustments are done to ensure the best printing quality possible. The list of things to check and parameters to check are presented in the table below

(Table 2). The printing method was optimized in previous work by Tuhkala et al. 2018 [24] and this method was used in the printing process of the sensors used in this work. The process is mostly the same for paper and polymer substrate; the biggest difference is the printing order of the insulator. With paper substrate the insulator is printed first and with polymer as the last layer.

Table 2. checklist to be done before printing

Select squeegee rubber and type
Select squeegee size
Check the condition of the squeegee with a stereomicroscope
Using a stencil, measure the tension of the screen 1,2,3,4 and 5
Adjust the distance between the screen and the platform
Adjust the height of the flood bar
Adjust the squeegee pressure
Set thickness of the substrate
Set printing area
Set the printing and flood bar
Clean the printing substrates
Check that alignment cameras are active

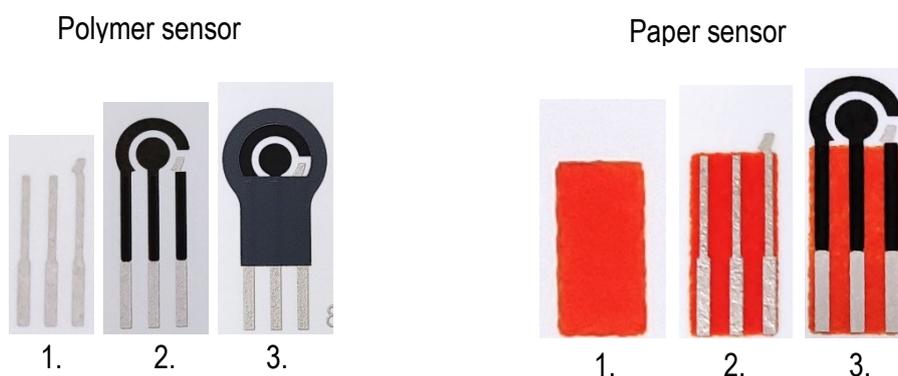
5.1.2.1 Channel/insulator

With paper substrate the insulator is printed in four layers, wet to wet, using two squeegees. This ensures that there is enough dielectric ink to go through the paper, so the measuring liquid does not short circuit the electrodes. The insulator ink can also be used to make channel structures leading the sample onto the sensor. The insulator is printed first so the other layers do not interfere the adsorption of the dielectric ink. Polymer-based and lacquer-based inks are available. Curing can be done in an oven or with UV light according to the manufacturer's instructions.

With polymer substrate the insulating dielectric ink is printed last. Since the sample does not absorb to the substrate, the insulator only needs to separate the electrodes from the upside. The ink used for printing on the polymer was Gwent Group: polymer dielectric D22070423P5 and for paper substrate Inkron printed dielectric IPD-326.

5.1.2.2 Electrodes

Electrodes were printed with same inks in similar methods onto paper and polymer substrates; first the Silver/SilverChloride and then the electrochemical carbon sensor ink. The silver ink used for the electrodes was SunChemical Ag/AgCl paste C2130809D5 with the 60/40 ratio of the silvers. The Carbon ink was also from SunChemical, Carbon sensor paste C2030519P4. The layouts of the electrodes and the printing order is presented in the picture 13 below. For these sensors, the area of the working electrode is 0.07548 cm².

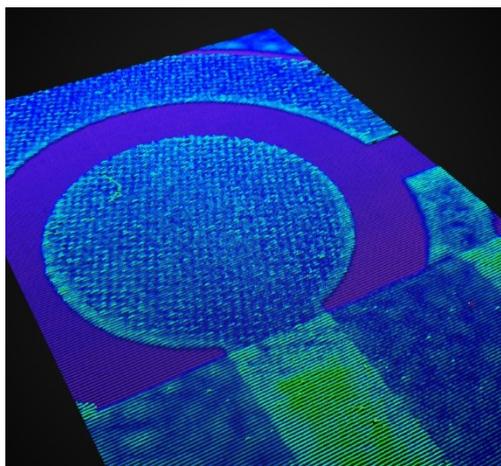


Picture 13. The printed layers of polymer and paper sensors with the printing order.

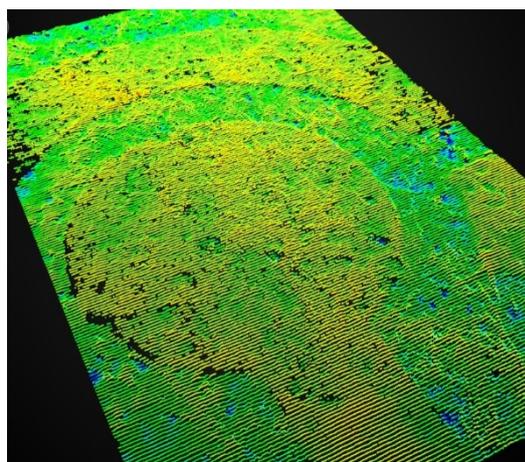
Photo Credit: Niina Torniainen

The surface structure of the electrodes was also scanned with a FocalSpec LCI-400 3D line confocal sensor. In the 3D -pictures (Picture 14) the surface of the sensor printed on polymer and paper are visible. The polymer sensor has clearly visible edges and the electrodes are easily detectable. There is a slight pattern of the printing screen visible on the working electrode. The paper sensor is difficult to distinguish from the paper substrate. The structure of the paper is clearly visible through the printed sensor. This makes it difficult to evaluate how much of the ink is adsorbed into the paper.

Polymer sensor



Paper sensor



Picture 14. 3D -surface structures from the surface of the polymer and paper sensors.

5.1.3 Curing

Curing of the inks was done according to the manufacturer's instructions. The time and temperature vary depending on the inks. During the curing process the solvent evaporates, and the ink becomes solid. The inks used in printed electronics usually remain quite flexible after curing. The conductive inks like Ag/AgCl and carbon inks in these sensors became conductive during the curing process.

5.1.4 Storage

Ready, printed electrodes should be handled with care. Scratches or dirt can adversely affect the quality of the sensors. It is a good practice to place a soft paper between each printed sheet when they are stored in a stack. The silver on the sensors can oxidise quite easily, so the best way to protect the silver is to store the sensors in a vacuum-sealed packs. A good practice is also to protect the sensors from unnecessary electricity and light sources. Well stored sensors can be used after years. These sensors were packaged in vacuum-sealed packs unless they were used shortly after.

5.2 Pre-treatment of sensors

Before measuring the sensors, different pre-treatment techniques were tested. The purpose of the pre-treatment is to ensure the sample liquid does not change the electrode area or spread

differently on the electrodes. The height of the liquid layer on top of the electrodes can affect the measured current. Usually, the lower the layer the higher the current.

5.2.1 Taping

The liquid movement and volume were controlled by using an electrically inert Kapton tape. The tape was placed on upside or underside of the electrodes, and on both sides. The tape can isolate the liquid movement to either side of the electrodes. The tape also gives some support for the easily bendable paper sensor.

5.2.2 Cutting

The size of the electrode area can be determined by cutting the substrate to a suitable size. In general, there is no need for the area of the sensors to be much bigger than the printed area. With paper substrate the liquid spreads easily to the area it is given and adsorbs into the substrate. With more hydrophobic polymer substrates the spreading of the liquid depends on the surface energy of the substrate and the surface tension of the liquid.

The sensors were cut by laser cutter or by scissors. Depending on the substrate material and needed accuracy of the shape, different cutting methods can be used. Paper was easier to cut to the desired size and shape than the polymer substrate.

5.2.3 Adding recognizing agent or biomaterial

If the sensors are made into biosensors the recognizing biomaterial is added to the working electrode as a pre-treatment step. There are several different methods and instructions how the materials can be added depending on the analyte, measuring technique and the mass manufacturing. The biomaterials can be added by screen-printing as a separate layer or mixed with the carbon inks. Often the biomaterial is dispensed or pipetted as drops on the working electrodes surface. Different surface treatments or added materials can enhance the attachment or functions of the biomaterials.

If paper is used as the substrate the biomaterial can be added from the unprinted underside of the electrodes. Since it is difficult to stop the liquid sample from absorbing to the paper substrate, it was decided to utilize the benefits of the paper. It is a known fact that the biomaterials like enzymes and proteins stay more active for a longer period of time when dried onto a piece of paper. The paper also helps to spread the material to cover the whole working electrode.

When the biomaterial is added on top of the carbon layer it often stays as a quite high drop and does not spread easily to cover the electrode. Some additives can be added to help the spreading and make the drying process easier for the biomaterials. Removing static electricity or rising the air humidity can also help with the spreading. However, it is more difficult than with paper and the biomaterials are not as protected.

5.3 Measuring

Based on the skills accumulated in the past from polymer-based sensor measurements, some assumptions can be made about paper sensor measurements. Not all measurement methods are possible with paper, but the target is to have a good enough method for testing paper sensors quality and using them in analytical analysis and development of assays.

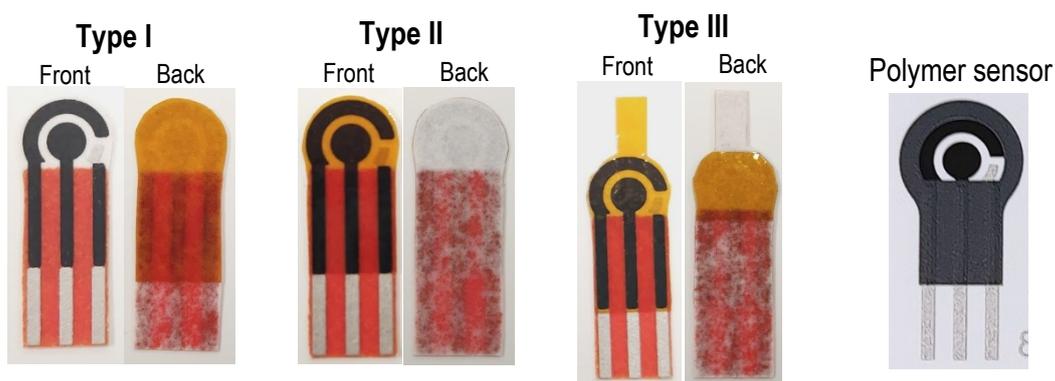
All measurements were done using PalmSens 8 channel MultiEmStat2 potentiostat. The measurement sample solution was Potassium ferricyanide ($K_3Fe(CN)_6$) in 0,1 M Potassium chloride (KCl), Phosphate-buffered saline (PBS). Potassium ferricyanide has an iron atom that can oxidize and then reduce, it is used commonly in electrochemical measurements as a test solution. Potassium chloride is added to ensure the sample solution contacts electricity well during measurements. Phosphate-buffered saline is not usually necessary in tests without any biomolecule. It helps keep the pH constant and favourable to most biomolecules. With just the sensor base water could also be used, but PBS does not interfere with the measurements either.

Cyclic Voltammetry (CV) was used as the test measurement. The cycle was run three times between -0,5 mV and 0,5 mV, with scan rate of 0,1 V/s and potential step of 0,005 V. PalmSens MultiTrace 4.4 software was used to adjust the measurement parameters. Results were compared with PalmSens PSTrace software. Both software's are available at PalmSens website <https://www.palmsens.com/software/>.

5.3.1 Determining the measurement volume

Three different type (I, II and III) tapings were done to the paper sensors and the suitable measurement volume was determined by adding a volume of sample that covered all electrodes. Picture 15, types of taping I-III. CV -scan was executed to ensure there was a sufficient amount of liquid for a good repeatable measurement.

For type I the suitable volume was determined to be 15 μl . The sample absorbed onto the paper substrate between the electrodes and more sample had to be added to cover the electrodes. For type II 6 μl was enough sample. The sensor was turned upside down with the connector so the sample could be added from above. This type did not need any manual spreading of the sample onto the electrodes, the paper spreads the sample easily. For type III the sample addition was also easy and 5 μl was a suitable volume. As comparison a polymer sensor was also used. A volume of sample to cover the electrodes was 40 μl .



Picture 15. Type I tape on the underside of the electrode. Type II tape on the upside of the electrodes. Type III tape on both sides of the electrode, Polymer sensor as comparison.

Photo Credit: Niina Tornainen

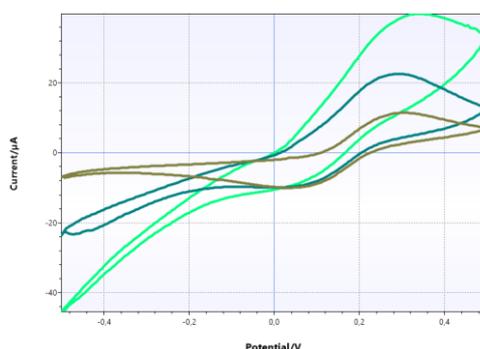
5.3.2 Measurement conditions

The air humidity can affect the spreading of the sample. Higher air humidity lowers the surface tension of a liquid drop and the sample spreads easier. The surface energy of a plastic substrate could be risen with a plasma treatment to make the drop spread easier. However, there is a risk that this kind of treatment damages the electrodes and will thus be unsuitable for

biosensors. With paper sensors the sample is not affected so much from changes on air humidity. Air temperature can also affect the sample, but the not much in small volumes. With biomaterials some reactions may benefit from heat higher than normal room temperature. For these test measurements the air humidity was 8,8 % RH and temperature 21 °C.

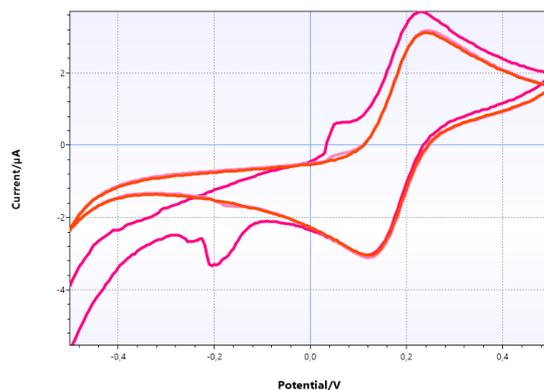
5.3.3 Measurement side of the sensor

The different types of taping the sensor were compared with CV -measurement. From the CV -diagrams it can be determined that the type I where the tape is on the backside causes more variations between parallel sensors, picture 16. The sample liquid touches the electrodes partially from both sides and the drop can fall underneath the sensor.



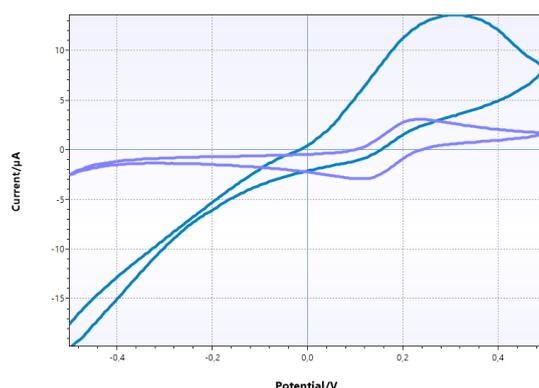
Picture 16. CV -curves of three parallel sensors of type I

When the tape is only on the upside of the electrode type II there is hardly any variation between parallel sensors. The sample liquid does bend the electrodes slightly, but it does not seem to cause significant disturbance. In one of the parallel sensors there is visible some disturbance that can be caused by some dirt or imperfection on the electrodes or the bending of the electrode. It can be seen as some extra oxidation and reduction peaks outside of the expected peak area and it affects the oxidation current and potential. It is a good practice to always have at least three parallel sensors to make sure the measurements are stable and repeatable. The shape of the CV -curves with type II is also good, oxidation and reduction peaks clear and of similar size, picture 17.



Picture 17. CV -curves of three parallel sensors of type II

The type III sensors are slow to assemble and not so convenient to use either. Still the measurement works and could be used if there is a need to attach the sensor to a structure where it is embedded from both sides. In these tests the parallel sensors (Picture 18) did not give similar results and that indicates that there might be some difficulties for the liquid to spread evenly to cover all the sensors.



Picture 18. CV -curves of three parallel sensors of type III

Based on these tests it was determined that the rest of the measurements would be done with the type II sensors, tape only on the top side of the sensors. This makes it easy to add the sample, since it spreads onto the sensors by the capillary forces in the paper. It also gives the most repeatable measurement results.

5.3.4 Cyclic voltammetry -measurement with optimized setup

Based on the optimized measuring volume, side and type, picture 19. The stability and ability of the screen-printed electrochemical paper electrodes was compared to the more common polymer-

based sensors. With paper sensors the sample volume was increased slightly to ensure the electrodes stay moist even if some evaporation occurs during the measurement.



Picture 19. Paper sensor with the optimized measuring setup.

5.3.4.1 Scan rate

Scan rate measurements were done to acquire more information how well the sensors perform in red-ox measurements. The sample used in all of the CV-measurement was 1 mM $K_3Fe(CN)_6$ in 0,1 M KCl. The rate of the scan should not affect the red-ox potential, but the measured current should rise in correlation to the scan rate. CV-cycle was done five times increasing the scan rate with each cycle 0.05, 0.1, 0.15, 0.2 and 0.25 V/s between -0,5 mV and 0,5 mV with potential step of 0,005 V.

Parallel measurements were done with five sensors. The current and potential at reduction and oxidation maximums were recorded for each scan rate. Same measurements were done for paper and polymer-based sensors. The measurement volume for paper sensors was 8 μ l and for polymer 40 μ l. The sample volume for polymer sensors must be high enough for the sample drop to cover all the electrodes completely. The surface energy of the substrate effects how wide does the drop spread on the substrate.

5.3.5 Amperometric measurements

Amperometric measurements were done with paper and polymer sensors using the same measurement volumes as in the CV-scans. The potential for the measurements was set to 50 mV, which is close to the reduction maximum of the sensors based on the CV-measurements. At the reduction peak voltage the iron in the $K_3Fe(CN)_6$ reduces from Fe^{III} to Fe^{II} . The measurement

potential should be chosen so that it is past the top of the peak rather than before, so the analyte has reduced or oxidised completely. The currents were measured for 100 s with six different concentrations of $\text{K}_3\text{Fe}(\text{CN})_6$ in 0,1 M KCl: 0, 1, 2, 5, 10 and 15 mM using three parallel sensors for each concentration. The currents at the end of the measurement are plotted to concentration versus current plot, the increase in current should be linear in correlation to the rise in the sample concentration.

6 CALCULATING AND ANALYZING RESULTS

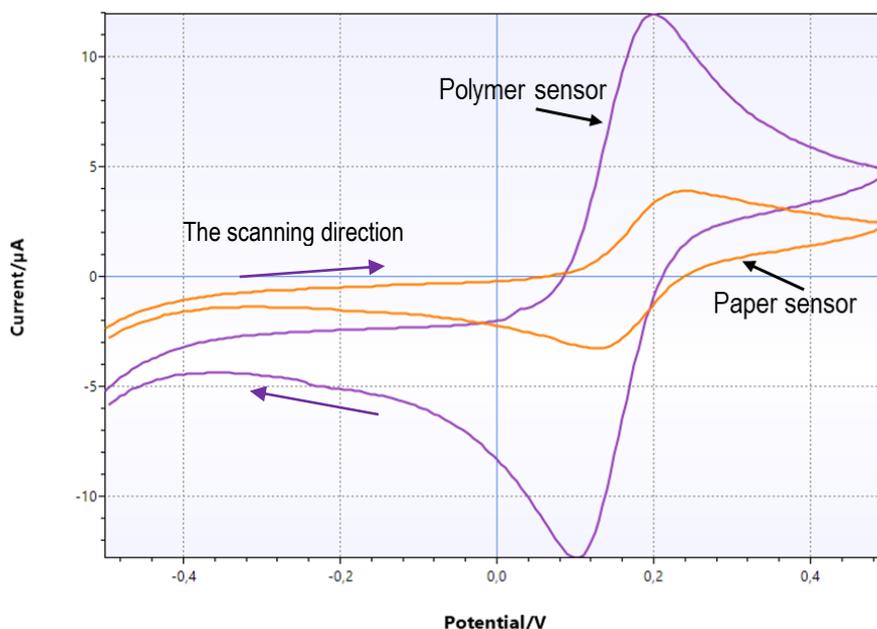
The measurement data from the cyclic voltammetry and amperometric data was transferred to Microsoft Excel. The oxidation and reduction peak voltages and currents from the CV measurements were read with the PalmSens PStTrace program before transferring to Excel. In Excel calculations and comparisons of the measurement data was done.

6.1 Results from cyclic voltammetry

The cyclic voltammograms from paper and polymer based sensors look quite different. First the overall shape of the voltammograms was evaluated and then the scan rate comparisons were made similarly to comparisons made by Uludag et al. [30]. Several factors about the reversibility and stability can be seen from the scan rate measurements.

In the picture of the CV curves of polymer and paper sensors (Picture 20.) the polymer sensor has higher current response in comparison to the paper sensors. In both, the oxidation and reduction peaks are clearly visible and there are only one of each as expected. The shapes of the peaks are also sharper with the polymer sensor. The lower current response of the paper sensor can be explained with the paper material interfering with the current by mechanical blockage.

Table 3. shows the numerical values of the peak current and potentials. The peak potential separation ΔE was also calculated in the table. For ideal Nernstian response of a sensor, the ΔE should be $59 \text{ mV} / n$ (one electron). Since the ΔE for both type of sensor is greater than that it indicates the electron transfer reactions at the surface of the electrode are slower than ideal [29]. The widening of the CV-curve can also indicate that the rate of the mass transport is getting bigger than the charge transfer process and higher potentials are required to achieve the same charge transfer. If the difference gets too big the reverse peak, reduction in this case, starts to disappear as the scan rate increases [3]. When both charge transfer and mass transport processes are present the sensor is called quasi-reversible [29]. Most likely neither of the sensor are completely reversible, but the scan rate measurements can disclose more about the functionality of the sensors.



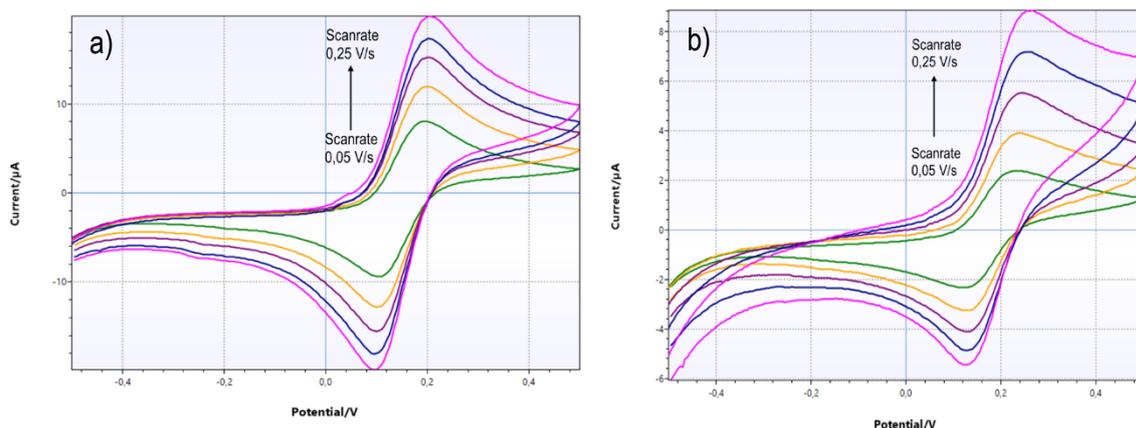
Picture 20. Cyclic Voltammetric curves of polymer and paper sensors.

Table 3. Potential and current values of CV-curves of polymer and paper sensors and the peak separation ΔE

	Peak	Potential /V	Current/ μA	ΔE / mV
Polymer Sensor	Oxidation	0,200	13,23	
	Reduction	0,105	-13,73	95
Paper Sensor	Oxidation	0,240	3,83	
	Reduction	0,135	-3,22	105

6.2 Results from scan rate measurements

The CV curves from the scan rate measurements show that the measured current increases as the scan rate gets faster from 0.05 V/s to 0.25 V/s, as seen in the picture 21, a and b. This is to be expected according to the Randles-Sevcik equation. As a side note the current also increases if the sample analyte concentration increases. The average numerical values, of the oxidation and reduction peak, current and potential, for five parallel sensors for each cycle are presented in the table 4. The peak potential separations, ΔE , are also presented in the table. For polymer sensor the ΔE increases from 88 mV to 103 mV \rightarrow 15 mV and for the paper sensor similarly from 105 mV to 133 mV \rightarrow 28 mV. As previously mentioned, for ideal sensor the ΔE is 59 mV. In scan rate measurements different aspects of the curve are evaluated to ensure the reversibility of the sensor.



Picture 21. Cyclic voltammograms from varying scan rates of a) polymer and b) paper sensor

Table 4. Numerical values, of the oxidation (forward) and reduction (reverse) peak, current and potential for each scan rate and the peak potential separation ΔE .

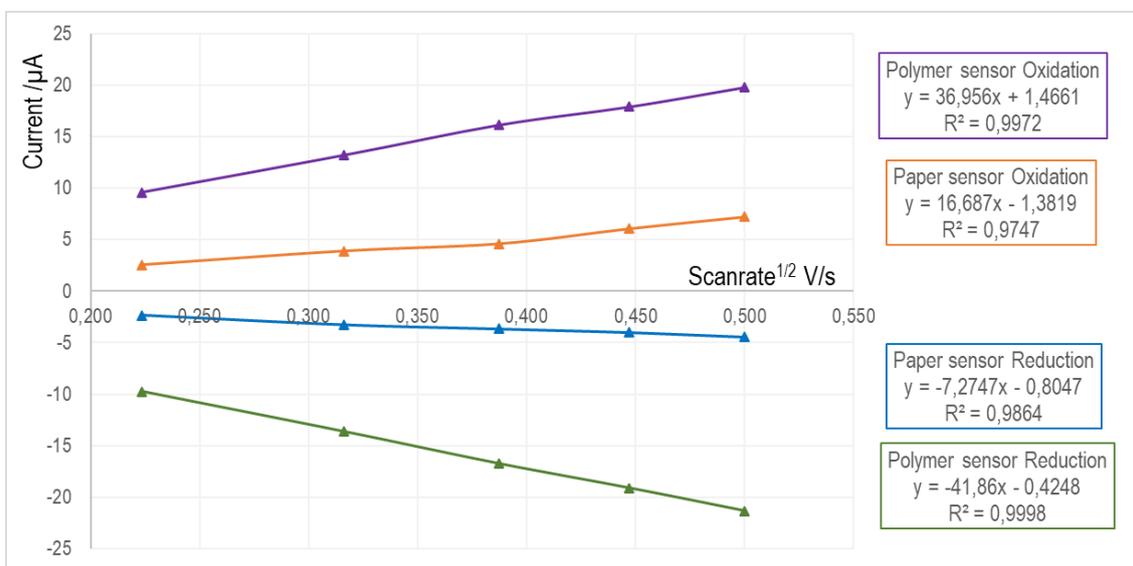
Polymer Sensor/ Peak	Potential/ V	Current/ μA	$\Delta E/ \text{mV}$	Scan rate / V/s	Paper Sensor/ Peak	Potential/ V	Current/ μA	$\Delta E/ \text{mV}$
Ox	0,195	9,6	88	0,05	Ox	0,230	2,5	105
Red	0,107	-9,8			Red	0,125	-2,3	
Ox	0,200	13,2	95	0,1	Ox	0,243	3,9	113
Red	0,105	-13,6			Red	0,130	-3,2	
Ox	0,200	16,1	98	0,15	Ox	0,245	4,6	113
Red	0,102	-16,7			Red	0,132	-3,7	
Ox	0,200	17,9	98	0,2	Ox	0,253	6,1	128
Red	0,102	-19,1			Red	0,125	-4,0	
Ox	0,202	19,8	103	0,25	Ox	0,258	7,2	133
Red	0,098	-21,3			Red	0,125	-4,4	

Evaluations about the functionality of the sensors can be made with guidelines similarly to normal CV measurements. The ΔE is bigger with paper sensors in comparison to polymer sensors and for both it gets bigger as the scan rate increases. This indicates that the process is not totally reversible, but most likely quasi-reversible. This means that the electron transfer processes at the surface of the electrode are limiting, and all the red-ox reactions do not have enough time to occur. Some of them do occur and therefore the sensors are not completely irreversible [3]. If the scan rate increases, eventually the electron transfer does not happen, and the reverse peak disappears. In practice the sensors with larger ΔE are slower.

Especially with the paper sensors it is visible that the current of reverse peak is smaller than the forward peak. It would be ideal for the peaks to be and stay of a similar size. This also indicates

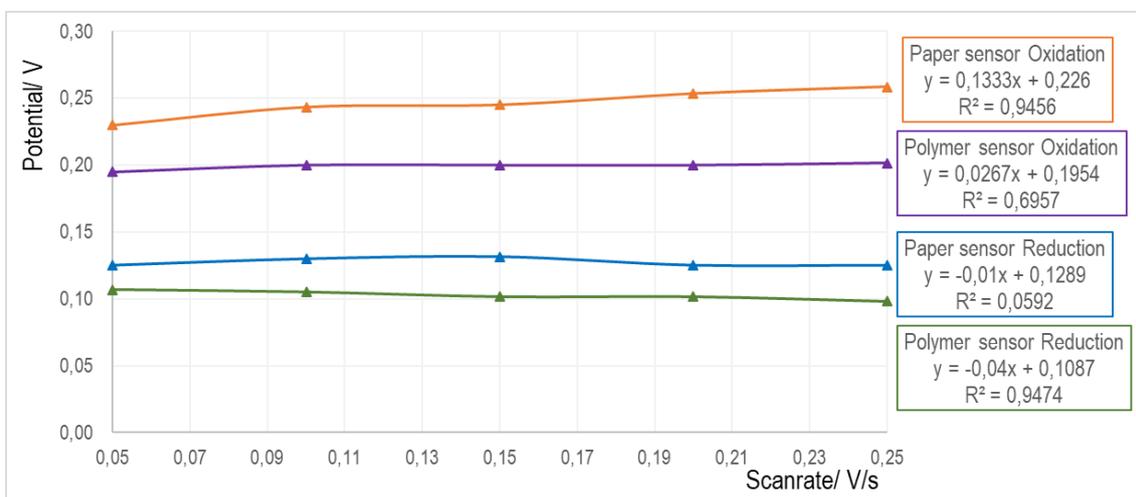
that especially the paper sensors are not completely reversible, and the reactions happen slower. The reverse peak with these scan rates is clearly visible, so there is relative reversibility.

Two other assumptions can be made from scan rate (v) measurements. The measured current as the function of the $v^{1/2}$ is independent of v and that the potential at the peak maximum is also independent from v [29], [3], [30]. In the picture 22 the currents are presented as the function of $v^{1/2}$ for paper and polymer sensors. For both type of sensors, the lines are quite linear, but with polymer sensor they are slightly better. This indicates that there is quite good reversibility for both with these scan rates even though the ΔE was quite large. From Randles-Sevcik -equation diffusion coefficient can also be calculated to indicate the best working sensor [30], but it is only reliable for close to ideal reversible sensors.



Picture 22. The currents as the function of square root of scan rate for paper and polymer sensors.

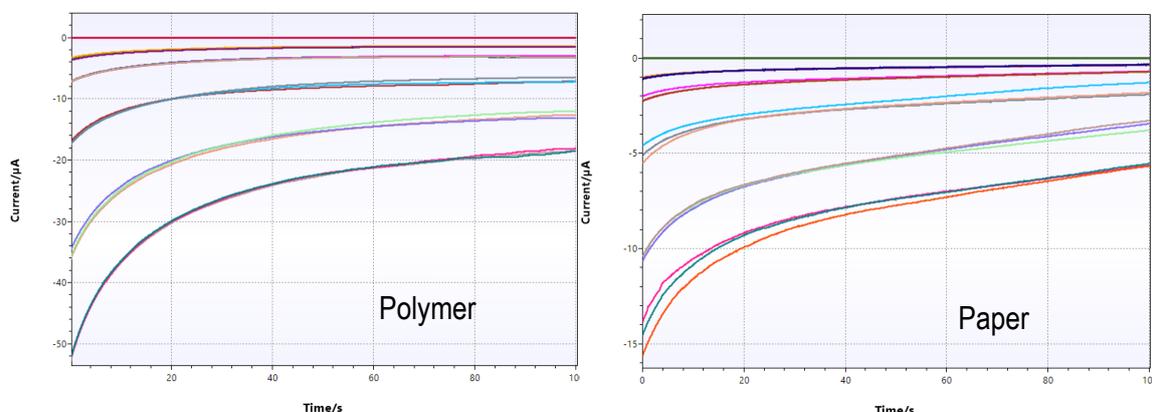
In picture 23 the potentials at the current maximums are compared at each scan rate. There is hardly any difference between the two types of sensors, but the biggest dependence of the scan rate is with the paper sensor oxidation. This was not so visible from the CV curves; the bigger changes seemed to be on the reduction side of the paper sensors.



Picture 23. The dependence of potential on the scan rate.

6.3 Results from amperometric measurements

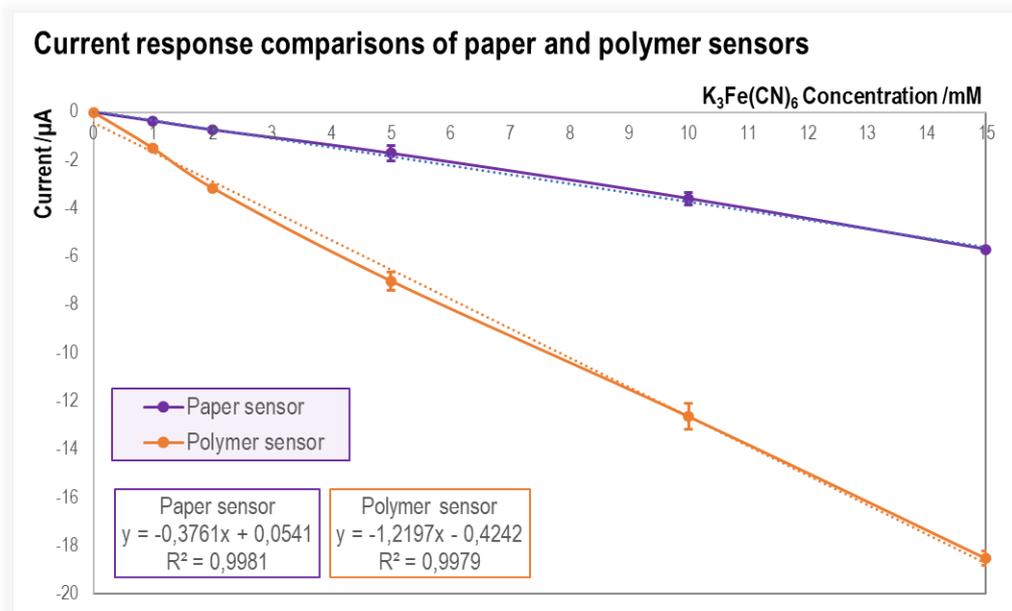
From the amperometric measurements (picture 24) the average current from 5 seconds at the end of the measurement for each concentration of analyte was read. There was slightly more deviation with the paper sensors than with the polymer sensors. The polymer sensors also reached the steady state earlier than the paper sensors. The paper sensors could have had longer measuring time than the used 100 s, especially with the higher concentrations. All the measured concentrations are clearly separated.



Picture 24. Measured current values from amperometric measurements with polymer and paper sensors, $K_3Fe(CN)_6$ concentrations of 1, 2, 5, 10 and 15 mM.

The average current values from the end of each curve were plotted against the used $K_3Fe(CN)_6$ concentrations in picture 25. Error bars indicate the deviation between parallel sensors at each concentration. It can be clearly seen that the measured currents for the paper sensor are about third of the current measured from the polymer sensors and the slope is much smaller. Both are very linear at these concentrations and the deviation between parallel sensors is small.

The smaller current values of the paper sensors were expected based on the CV measurements. Since the measurement is done from the backside of the sensor, inside the paper there is quite a lot of mechanical disturbance at the paper most likely interferes with the reactions at the sensors surface. It is impossible to see what the surface of the electrode looks like inside the paper. Because the smaller slope the paper sensor is not as good as the polymer sensors to detect small changes in the analyte concentration.



Picture 25. The measured current of paper and polymer sensor as the function of $K_3Fe(CN)_6$ - concentration.

7 CONCLUSIONS

The aim of this thesis was to gain a better understanding on how the electrochemical paper sensors should be tested. In the beginning the idea was to study literature on electrochemistry and electrochemical sensors for better methods for testing the functionality and quality of the printed sensors. In addition to those, deeper understanding of the measurement methods and data handling, what happens on the sensor and how does it affect the results, was to be gained. Prior to this work I already had experience with electrochemistry and screen-printed sensors when working at the Oulu University of Applied Sciences, printed electronics laboratory, PrinLab. This experience was valuable information that made it possible to focus more on the background effects of the phenomena of electrochemistry.

7.1 Theoretical section

Firstly, a lot of effort was invested in finding good, reliable and useful sources for the work. There is a lot of work done in this field of science. Some articles focus on the manufacture of the sensors such as methods, materials or layouts. In other scientific publications the focus is more on the testing or modification of the sensors for some specific usage. The challenge of utilizing scientific articles is that many of them are too specific and do not explain how and why some measured differences occur. It is often difficult to know if the analysed result only applies to a specific case or can they be generalized to other types of sensors, too. Mostly older articles discuss the fundamentals and theory of the methods. Moreover, books on electrochemistry were extremely useful sources of information.

In the theoretical section there were three focus areas: electrochemistry/ electrochemical sensors, manufacture of sensors and testing of sensors. Electrochemistry is a vast area of analytical and physical chemistry. It can be used in so many different applications that only small area of it could be discussed in this work. Electrochemistry is not used only in research, but in it has many practical applications and natural embodiments. At some point of the work, it was challenging to keep the focus on the electrochemical sensors.

Often it is the case that in electrochemistry the more traditional experimentation setups with rod-type electrodes are used. Especially there are hardly any books that discuss the printed sensors and the testing of them. The reactions of the printed electrochemical sensors occur on the surface of the electrodes and the scale of the sensors are a fraction of the traditional sensors. Frequently, it is not evident if everything works in identical manner on the printed sensors. Where the printed sensors are discussed the functionality of the sensors often changes with small modifications on the size of surface structure of the sensors. Based on how many variables and materials there are in the manufacture only a small amount of all the possible changes to the functionality can be controlled, but the quality of the sensor-base does not always guarantee the measuring works.

The most common electrochemical sensor measuring techniques were described from a practical viewpoint. There are not too many good references that would describe how to measure and use the sensors with different techniques. Many descriptions of the methods are very technical and describe the reactions through formulas and theories. It is important to understand the theory; it helps with understanding the effects and results, but it would be beneficial to have more practical how-to examples available. The examples that are available mostly show the ideally working systems or require to buy the exact sensor and measuring setup to receive similar results.

In the end, better understanding of the measurement methods was gained and can be applied to practice. Only so much can be done by small changes in the layout. Changing the substrate to a smoother one or one with different electrostatic features can have a big influence. A considerable effect to the final quality can be made with the inks used in the printing. There is no best sensor that would work for everything. The materials and layouts must be chosen for each specific application and the user must know or test what works best for them.

7.2 Experimental section

In the experimental section some basic assumptions could be made, but verification for the testing method was made. The paper and polymer sensors were compared successfully. It was assumed in the beginning that the polymer sensors would perform better. However, the paper sensors seemed to work very well, too. Mostly the difference is in the resistance of the electrodes. The paper understandably adds mechanical obstruction for the ions to move on the electrodes. This is reflected in the measurement results with paper sensors always having smaller current values in

comparison to polymer sensors. The reactions on the papers sensors also seem to take place more slowly than on the polymer. For this reason, paper sensors should be given longer measurement time.

The paper sensors are still easier to use than polymer sensors. The modification to biosensors is easy since the paper spreads the added material to cover the whole working electrode. This can be tricky with polymer sensors and the drying process can be more harmful for the enzymes used. Similar benefits are in the measurement too. The aqueous sample can be difficult to spread on the hydrophobic graphite surface of the electrodes, but with paper the sample covers the electrodes if the sample volume is sufficient. Paper sensors also require smaller volume of sample.

7.3 Future prospects

Printed electronics is developing fast and new materials emerge constantly. More emphasis is put on the environmental aspects and paper as substrate will become more interesting. There is a lot of research and development to be done to get the paper to work as well as the other substrate materials. There are some paper materials available that hardly adsorb liquid at all, but then you lose some of the other benefits of the paper. Some other printing inks could also work better with the paper substrate. The viscosity of the ink and the absorbent qualities of the paper need to work together well. The ink used for insulation should be well absorbed into the paper. It should go through the paper but not spread uncontrollably. Conversely, the inks used in the electrodes should not adsorb excessively and should compensate for the uneven surface of the substrate.

The paper sensors already work quite well. Some modifications to the testing methods could be done to give the paper sensors better chance to reach their best. Because the reactions run a little slower, the measurement time can be extended. In this case it is important to make sure the sample does not dry on the electrodes. Better conductivity of the electrodes would make the sensors faster, this could be achieved with different inks, like gold or platinum. In amperometric measurements the constant potential added could be increased so the electron transfer processes would happen faster. Increasing the potential too much can cause other unwanted reactions to happen. Paper sensors are good for developing and research purposes, but not yet as reliable as polymer sensors.

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