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**Electrodes
and
Electrokinetic Systems
for
Biotechnological Applications**

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During the course of the research underlying this thesis, Sara Nilsson was enrolled in Forum Scientium, a multidisciplinary doctoral program at Linköping University, Sweden

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Till Johan & Noah

Abstract

Research in bioelectronics studies biological systems and materials in combination with electronic interfaces for the development of devices, e.g., for medical applications, drug and toxicity tests, and biotechnology in general. Neural implants and pacemakers are examples of products developed from this area of research. Conducting polymers such as poly(3,4-ethylenedioxythiophene) (PEDOT) bridge biology and electronics with a combination of biocompatibility, flexibility, and capability to themselves undergo redox reactions. Electrokinetics, a related branch of science, describes the motion of fluids and particles caused by the application of an electric field, and includes various separation techniques such as gel electrophoresis. Applying an electric field in a sufficiently small diameter silica capillary can cause the liquid in the capillary to move. This phenomenon, referred to as electroosmosis, plays an important role in miniaturized microfluidic systems and can be used to drive flow in a so-called electroosmotic pump.

This thesis describes research at the interface between biology, chemistry and electronics. The first two papers probe the adsorption mechanism of poly-L-lysine, often used in biotechnological applications, onto hard materials such as metals (platinum) and metal oxides (indium tin oxide). By employing a gravimetric technique, quartz crystal microgravimetry with dissipation monitoring (QCM-D) combined with electrochemistry, we studied the process by which poly-L-lysine is deposited onto two different conducting substrates under anodic conditions. We found that indium tin oxide is more suitable than platinum for anodic electrodeposition of PLL, however, the exact film deposition mechanism is not fully understood. Paper 3 demonstrates the applicability of using conducting polymers, (e.g., PEDOT) instead of platinum as electrode material in gel electrophoresis. The last paper describes the fabrication and characterization of an electroosmotic pump consisting of a potassium silicate stationary phase in a fused silica capillary and the integration of the pump into a system for use, e.g., as a bioreactor.

Populärvetenskaplig sammanfattning

Biologi och elektronik har historiskt varit två separata forskningsområden som i nuläget till stor del även kombineras inom fältet bioelektronik. Ett viktigt exempel inom bioelektroniken är utvecklingen av pacemakern under 1950-talet. Andra exempel på applikationer är bl. a. biosensorer, neurologiska implantat och miniaturiserade analysystem, s.k. *Lab-On-a-Chip*. Inom bioelektronik syftar man till att studera och påverka biologiska system och material med elektroteknik. År 2000 gavs Nobelpriset i kemi till Heeger, MacDiarmid och Shirakawa för deras utveckling av elektriskt ledande polymerer samt möjligheten att öka dess ledningsförmåga genom en process som kallas dopning. Konjugerade polymerer såsom poly(3,4-ethylenedioxythiophene) (PEDOT) är viktiga verktyg för att binda samman biologi med elektronik genom deras flexibilitet, biokompatibilitet samt förmågan att själv genomgå oxiderings- och reduceringsreaktioner. För att ett material ska kunna implanteras i kroppen krävs att materialet i fråga inte förstör celler eller omkringliggande vävnad. Det får heller inte direkt stötas ut ur kroppen/vävnaden av immunförsvaret. Hårda material såsom metaller kan göras mer biokompatibla genom att beläggas av ett mjukt material. Därför är det väldigt viktigt att förstå hur biologiska molekyler interagerar med dessa material. Elektrokinetik är ett samlingsbegrepp av hur partiklar (elektrofores) och vätskor (elektroosmos) rör sig vid påläggandet av ett elektriskt fält. Gelelektrofores är en teknik som utnyttjar det förstnämnda fenomenet och som idag används flitigt inom områden såsom genetik, molekylärbiologi och rättsvetenskap. Tekniken separerar makromolekyler, (t.ex. proteiner eller DNA) baserat på dess laddning och storlek. Elektroosmos är rörelsen av en elektrolyt (vätska) som induceras av ett elektriskt fält när den befinner sig i t.ex. en glaskapillär med tillräckligt liten diameter. Elektroosmos spelar en central roll i *Lab-On-a-Chip* komponenter då fenomenet kan användas för att förflytta vätskor i systemet i en s.k. elektroosmotisk pump.

Denna avhandling behandlar interdisciplinär forskning mellan biologi, kemi och elektronik. I de två första artiklarna studerades deponering av "mjuka" biokompatibla material såsom poly-L-lysin på positivt laddade hårda material såsom metaller (platina), och metalloxider (indium tenn oxid). Genom att använda en teknik känslig för massändringar, *Quartz Crystal Microgravimetry with Dissipation Monitoring* (QCM-D), tillsammans med elektrokemi studerade vi det hastighetsbestämmande steget i depositionsmechanismen av poly-L-lysin på de två olika elektriskt ledande substraten. I den tredje artikeln demonstreras användning av den elektriskt ledande polymeren

PEDOT som elektrodmaterial i gelelektrofores istället för platina för att underlätta miniatyrering av system i framtiden. Den sista artikeln i denna avhandling redogör för tillverkningen samt karakteriseringen av en elektroosmotisk pump till mikrosystem samt integrationen av denna i ett modellsystem med syfte att efterlikna en bioreaktor.

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List of publications

Paper 1

Electrochemical quartz crystal microbalance study of polyelectrolyte film growth under anodic conditions

S. Nilsson, F. Björefors, N. D. Robinson

Applied Surface Science, **2013**, *280*, 783-790

Author contributions

Helped design experiments, performed all measurements, analyzed the data, wrote the first draft, and edited the manuscript.

Paper 2

On the anodic deposition of poly-L-lysine on indium tin oxide

S. Nilsson, N. D. Robinson

Manuscript

Author contributions

Conceived, designed, performed, and analyzed all experiments and wrote major parts of the manuscript.

Paper 3

Conducting Polymer Electrodes for Gel Electrophoresis

K. Bengtsson, S. Nilsson, N. D. Robinson

PLoS ONE, **2014**, *9*, e89416

Author contributions

Designed and performed the preliminary experiments. Edited the final manuscript.

Paper 4

Electroosmotic pumps using potassium silicate frits

S. Nilsson, P. G. Erlandsson, N. D. Robinson

Submitted

Author contributions

Conceived the method, fabricated devices for testing, performed and analyzed electrokinetic measurements, and wrote the manuscript together with the co-authors.

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Chapter 1 - Introduction

The integration of electronics into biological system opens exciting opportunities when the fundamental questions involved can be answered and the tremendous challenges overcome.¹ The pioneer in this area was an Italian physicist and medical doctor named Luigi Galvani, born in 1737 in Bologna. He paved the way for the new branch of science, bioelectronics, by using electricity in biology.² How do nerves control the muscles in our body? Maybe that was one of the questions occupying Galvani's mind? One can only speculate of course, but when sending electricity through the legs of dead frogs, and discovering twitching of the legs, Galvani was indeed entering new exciting scientific ground.

Today, the field of bioelectronics³ is of huge interest and the applications spun out from this emerging field of science span from, for instance, biological fuel cells^{4,5} all the way to the stimulation and recording of neuronal signals.^{6,7} One key aspect of bioelectronics is the fundamental understanding of the interactions at the interface between biological molecules,^{8,9} e.g., proteins, and hard materials like metals. This understanding is particularly important when discussing implants in the human body, e.g., the pacemaker or tissue engineering.¹⁰

In order for a foreign material to be implanted in the human body and not immediately rejected or damaged in some way, the materials exposed need to be *biocompatible*. This means that the material in question has to have the capability to coexist with human tissue without causing severe harm to it. For this to happen, one feature that is highly desirable is that the material should be chemically unreactive.¹¹

Moreover, for many applications it is important that the implant is robust and long-lived as they are supposed to function for a great deal of a person's life.

When dealing with the human body and all its associated complexity, it becomes clear that even though many successful implantable devices have been developed, large obstacles within certain areas of research still remain.^{12,13,14} Implanting a foreign object into the body can cause severe problems like, e.g., an undesired immune response, toxic effects and protein adsorption^{15,16} to materials which can cause clogging of the device or implant. Clogging or blockage can leave the implant unable to stimulate or receive signals/information from the tissue. For this reason, it is highly important to improve the suitability of materials for long-term *in-vivo* experiments.¹⁷

Lab-on-a-chip (LOC) or microfluidic devices belong to another research area within bioelectronics with high demands on biocompatibility. LOC technology aims to miniaturize chemical and biological analysis systems and potentially even entire labs.^{18,19} One purpose is to reach people in developing countries with, e.g., devices able to diagnose certain diseases. As infectious diseases that are easily diagnosed in the western world instead cause widespread death in many developing countries, LOCs and, specifically, point-of-care tests devices^{20,21} can fill an important need.²² Examples of existing point-of-care tests include, e.g., the blood glucose test and the home pregnancy test. In addition to point-of-care testing devices, LOCs have also a role to fill in applications such as drug discovery and toxicity testing.^{23,24} These are two areas which, at the moment, are very time-consuming and expensive. Usually it takes more than ten years for a new drug to reach the market. Scaling down and integrating several laboratory parts into one small single chip gives advantages such as smaller consumption of expensive chemicals and reagents, faster analysis, lower costs, portability, reduced time and also the possibility to process highly parallelizable devices allowing for analyzing a massive amount of samples at the same time.^{18,25} These advantages are probably most pronounced in the early phases of, e.g., drug development processes, when screening of thousands of candidate drugs can be tested in parallel. As these LOCs become more advanced, cells have been seeded in microbioreactors,²⁶ opening the possibility of using human cells in LOCs instead of animals for, e.g., testing of new medicines.

Even though the advantages of LOCs are great and the optimism is constantly leading to new and enriched technology, there are of course disadvantages. Since it is a relatively new technology, there are parts that are not yet fully developed. When

scaling systems from macro- to microscale, the dominating physical and chemical effects change, and therefore the fundamental understanding of these systems are highly important in order to achieve a suitable design for the right purpose.²⁷

Aim and outline of thesis

This thesis deals with questions related to electrokinetic systems for biotechnological applications. Papers 1 and 2 are the results of studies concerning the deposition mechanism of the positively charged polyelectrolyte Poly-L-Lysine, PLL, onto first, platinum, Pt (Paper 1), and then indium tin oxide, ITO (Paper 2). We used a technique based on microgravimetry (QCM-D) in combination with electrochemistry and, despite many challenges, we managed to deposit PLL onto Pt (Paper 1) and also learn that Pt was etched under certain conditions. ITO does not catalyze water electrolysis to the same degree as Pt. As a result, even though the potentials applied exceeded that needed for water electrolysis, PLL was still deposited without the disturbance of electrolytically-formed bubbles, as described in Paper 2.

Paper 3 goes into the area of biomolecular and biochemical separation techniques. In, e.g., medical analysis and forensics etc., it is of great importance to be able to separate individual molecules from each other. Therefore, many techniques have been developed in this area. One frequently-used technique is gel electrophoresis,²⁸ in which macromolecules like DNA are placed in wells in a gel electrolyte connected to two electrodes on each side. When an electric field is applied over the gel, positively charged molecules migrate toward the negatively charged electrode and vice versa, and hence, molecules can be separated due their charge and relative size. Often, metal electrodes are used, but in this study we explore the possibility of using conducting polymers instead.

Paper 4 involves the production and characterization of electroosmotic pumps, using potassium silicate as the stationary phase in a fused silica capillary. Using this stationary phase, high resistance against pressure-driven flow is gained as well as a high EO flow. We also show integration of the pump together with a fluidic system printed from a 3D-printer, functioning as a model for a cell chamber. The long-term goal with this project is to develop highly-parallelizable microfluidic chips aiming at reducing the use of animal testing and making drug and toxicity testing faster and more reliable while saving both money and time.

Chapter 2 - Electrochemistry

Electrochemistry is a branch of chemistry dealing with chemical changes produced by electricity and, vice versa, the production of electricity by chemical reactions. This field of science spans more than 300 years and is credited to Alessandro Volta,²⁹ an Italian physicist, who invented the electric battery, paving the way for the development of electrochemistry. Central to this field is the study of electrochemical reactions, that is, chemical reactions that take place at an electrode and involves the transfer of charges across the electrode interface.

Electrochemical cell

A central aspect in electrochemistry is the study of processes taking place at the interface between an electronic conductor (electrode) and an ionic conductor (electrolyte). Valuable information regarding thermodynamic processes may be obtained by means of an electrochemical cell. Such a cell consists of three parts, namely an electrolyte, electrodes (immersed in the electrolyte), and conducting wires connecting the electrodes.³⁰ When an external power supply is connected to the electrodes and a potential is applied between them, the setup is called an electrolytic cell. The more positively electrode is where the oxidation occurs and referred to as anode, whilst the more negatively polarized electrode, referred to as the cathode, is where reduction reaction takes place. Electrons migrate via the conducting wire, and at the same time in the electrolyte, negatively charged species (anions) migrate towards the anode and positively charged species (cations) to the cathode.³¹ See Figure 1 for a simple example of a 2-electrode electrochemical cell.

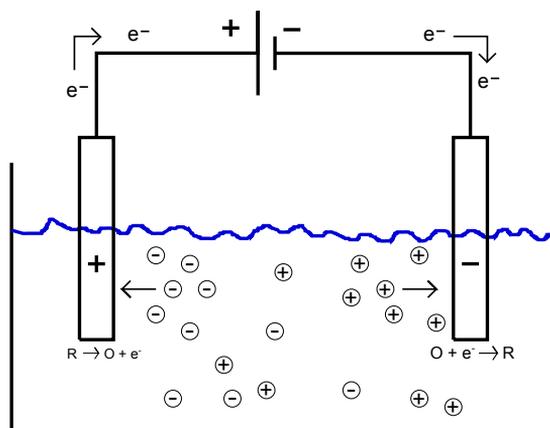
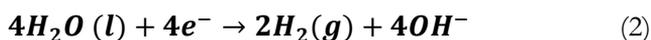


Figure 1. Simple sketch of an electrolytic cell. Anions (-) and cations (+) in the electrolyte are attracted to the anode (+) and the cathode (-), respectively, when an external potential is applied between the electrodes. Electrons flow from the positively charged electrode to the negatively charged electrode.³⁰

The situation in Figure 1 is true as long as the electrochemical reactions at the electrodes continue and, as a result, current is transported through the electrolyte via the ions.

Electrochemical reactions are often explained as two half-reactions, namely oxidation and reduction reactions, as a way of representing the overall redox reaction taking place in the cell. The half-reactions describe when a substance loses (oxidation) or gains (reduction) an electron.^{31,32} For example, water, $\text{H}_2\text{O} (l)$, is electrolyzed (decomposed) into oxygen gas, $\text{O}_2 (g)$ and hydrogen gas, $\text{H}_2 (g)$ via the following half-reactions:



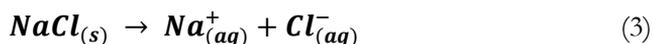
Electrodes

There are several kinds of electrode materials, e.g., metals, metal oxides and electrodes based on conjugated polymers (which will be discussed in more detail in Chapter 3). Metal electrodes are typically used when studying an electrochemical reaction that does not involve the electrode itself. Examples of common metal electrode materials are Pt and Au. The electrode has to be inert and robust in order not to participate in the reaction. However, even though, e.g., platinum is considered to be a robust electrode

material, this stability is limited. It can, if a high enough potential is applied (>1 V vs. Ag/AgCl) and oxygen is present in the electrolyte, form an oxide layer on the surface.³⁰ This process can even continue to oxide dissolution³³ and etching as described in Paper 1.

Electrolytes

An electrolyte is a solution of a substance that splits into two or more constituents when dissolved in a suitable solvent, e.g.,



The subscript (aq) stands for dissolved in water. The electrolyte acts as an ionically conductive medium. The conductivity of a solution is a measure of the electrolyte's capability to conduct electricity. Therefore, the concentration of dissolved salt, e.g., $\text{NaCl}_{(aq)}$ in water is an important factor when it comes to conductivity. In the work described in this thesis, only water-based electrolytes are used, as our goal is to produce biocompatible systems, but there are other options. There are electrolytes based on organic solvents, and also electrolytes in gel-form (Paper 3) or even solid ones. One important aspect to consider when choosing a suitable electrolyte is its electrochemical stability window. Within this window, the substances in the electrolyte do not undergo oxidation or reduction. Depending on the application and/or experiment, different kinds of electrolytes may be used. As described in reaction schemes 1 and 2 earlier, water electrolyzes and gives rise to oxygen bubbles at sufficient applied potential; > 1 V vs. Ag/AgCl (a reference electrode, more about it in the potentiostat section). The bubbles generated may disturb an experiment in, e.g., a microfluidic device and therefore care must be taken when designing a proper experiment.

Electric double layer

When a material is immersed in a fluid, an electrical double layer (EDL) is formed at the interface. As for any process in nature as a whole, electroneutrality is the norm, i.e., any local excess of positive or negative charge causes charged species to immediately rearrange until the local charge is balanced, see Figure 2. The same goes for an interface between a solid and a solution. If the solid material has a positive surface charge, the ions or molecules in closest proximity to its surface have to be of negative charge in order to balance the charge of the solid.³⁴ These ions or molecules are adsorbed onto the surface of the electrode due to chemical interactions and are said to

be in the inner Helmholtz plane (IHP). The second layer of ions or molecules in the outer Helmholtz plane (OHP) are associated to the surface by electrostatic interactions.³² See Figure 2 for a schematic view.

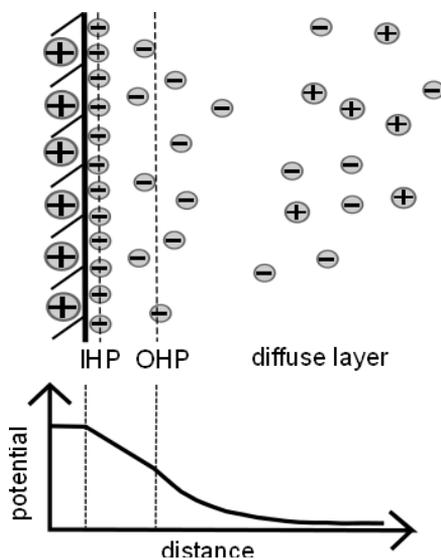


Figure 2. The Stern modified Gouy-Chapman model of the electrical double layer (EDL). The adsorbed layer of ions closest to the immobile ions on the electrode is called the inner Helmholtz plane (IHP) and the outer layer, which consists of more loosely bound ions is consequently referred to as the outer Helmholtz plane (OHP). Further from the metal electrode is the diffuse layer, for which the thickness depends on the concentration of the ions in solution. The inverse situation is true at the negatively-biased electrode.^{34,35}

There are many models of the EDL. The first model was developed by Herman von Helmholtz in the 19th century. He discovered that a charged metal immersed in an electrolyte attracts ions of the opposite charge and, moreover, that the structure of the double layer was equivalent to a capacitor consisting of two parallel plates. Figure 2 shows the model developed by Louis Georges Gouy and David Leonard Chapman in the early 1900s. They each introduced, independently, the idea of a diffuse layer. The diffuse layer consists of ions of both negative and positive charges which are only loosely associated to the charged electrode. The concentration of charge opposite that of the electrode is highest in the proximity to the electrode and, as seen in the upper panel in Figure 2, decreases with distance away from it. In contrast to Helmholtz, who did not take into account any layer other than that of the ions closest to the electrode surface and neglected the electrolyte concentration, Gouy and Chapman discovered

that the capacity of the EDL depends on both the potential applied and the concentration of the electrolyte.^{31,32,36} In many electrochemical experiments, the influence of a charging current due to the EDL needs to be considered. A charging current is the rearrangement of the EDL, i.e., changing the polarity of the electrodes gives rise to the migration of ions in the solution. The short-lived charging current is also referred to as a nonfaradaic current or EDL current.³⁷

The potentiostat

There are many methods for studying electrochemical reactions. One commonly used instrument for this is the potentiostat. It is an electronic device connected to three electrodes, the working electrode (WE), the reference electrode (RE) and the counter electrode (CE), see Figure 3. The reaction of interest takes place at the WE and the current through this electrode is measured while the potentiostat controls its potential relative to the RE. A RE is an electrode that has a stable and well-known potential and one commonly used is the silver/silver-chloride (Ag/AgCl) electrode. It consists of the silver metal (Ag) and its salt (AgCl), and functions as a redox electrode via the reactions between the Ag and AgCl. The third electrode that completes the electrochemical cell is the CE. Current is driven between the CE and the WE such that the desired potential between the WE and RE is achieved. The CE is usually made of a very inert material like platinum and does not take part in the electrochemical reaction of interest. However, solvent electrolysis often takes place at this electrode.

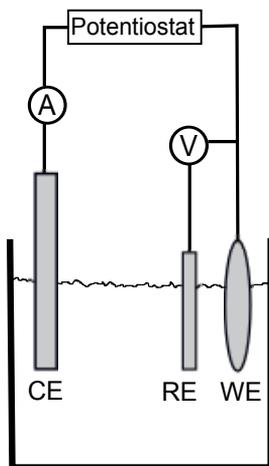


Figure 3. Sketch of a potentiostat connected to a three-electrode cell. The current (A) is driven between the working electrode (WE) and counter electrode (CE) such that the desired potential difference between the reference electrode (RE) and the working electrode (WE) is achieved.

The current flowing in the cell goes through the CE and therefore this electrode should have a larger area than the WE. In order to make sure that the potential difference set between the WE and the RE is correct, the current between the WE and CE is continuously adjusted. It is possible to connect the lead for the RE directly to the CE, thereby using a two-electrode system. When doing so, current is influenced by the electrochemical reactions occurring at both electrodes, and not, as for the three-electrode system, just the electrochemical reaction taking place at the interface between the WE and the electrolyte.³⁷

The potentiostat is a very powerful tool for studying electrochemical reactions and, in addition, can be combined with other instruments to obtain even more information about the processes at an electrode. In Papers 1 and 2, the potentiostat was combined with QCM, which will be further explained in Chapter 4.

Electrochemical techniques

There are several techniques for studying electrochemical processes employing a potentiostat. In this thesis, two techniques were used, one where a constant or pulsed potential was applied (Chronoamperometry) and one where the potential was slowly and continuously varied between two potentials (Cyclic voltammetry). These two techniques will therefore be discussed further in this section.

Chronoamperometry

Chronoamperometry (CA) is an electrochemical method where a potential is applied and the current vs. time response is monitored. A three-electrode system is used and the potential applied can either be constant or pulsed. See Figures 4 and 5 below.

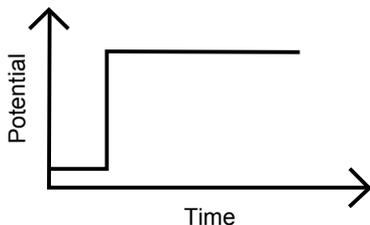


Figure 4. A graph describing a constant chronoamperometric method. First the open circuit potential (OCP) is measured. Thereafter, the potential of interest is applied.

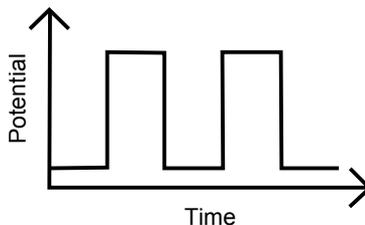


Figure 5. This graph describes a pulsed chronoamperometric method, varying the applied potential between two levels over time.

Upon increasing the applied potential from the open circuit potential (OCP), the first thing one sees in the resulting current plot, shown in Figure 6 is the charging current, which corresponds to the charging of the EDL as described earlier. The OCP is the potential of the WE with respect to RE when no external potential is applied. After a quick decrease in current, the curve declines at a much slower rate and what one sees there is the Faradaic current, i.e., current generated from the oxidation reactions at the interface between the electrolyte and WE. The Faradaic current is also present from the beginning of the experiment, but the charging current is much larger at the start. By performing a CA experiment, information about the capacity of the EDL as well as the concentration of the oxidized/reduced species may be obtained.³⁸

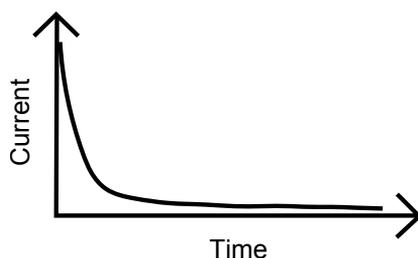


Figure 6. A typical curve from a chronoamperometric (CA) experiment in which the potential is increased from the open circuit potential (OCP) to a constant applied potential. The large current in the beginning of the curve corresponds to the charging of the electric double layer (EDL) and the current in the lower current region represents the Faradic current.

Pulsed CA (1-1.3 V vs. Ag/AgCl) and constant CA (1.3 V vs. Ag/AgCl) were used in Papers 1 and 2, respectively, together with the QCM. As we were primarily interested in the response from the QCM and how the applied potential influenced the deposition of PLL on Pt and ITO, we did not analyze the current in detail. However, we used the current when comparing the rate of water electrolysis to previous results obtained by van Tassel et al.^{39,40} This will be further discussed in Chapter 4.

Cyclic voltammetry

Cyclic voltammetry (CV) is a very common technique and probably one of the first to use when examining an electrochemical process. As in CA, a three-electrode system is used, but here, instead of using a pulsed or constant applied potential, a triangular waveform potential is applied, see Figure 7, and the current vs. potential is monitored.

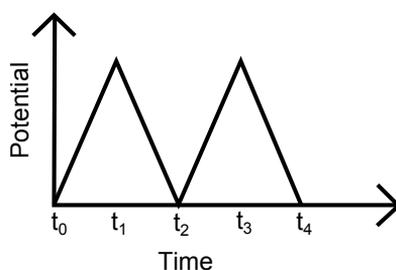


Figure 7. This graph describes the form of the applied potential in a cyclic voltammetric measurement where the potential is cycled between two vertex potentials in a triangular waveform over time.

CV can provide information about the oxidation and reduction processes and the potentials at which they occur, but also other details of the electrochemical mechanism, e.g., the kinetics of a reaction, whether there is an intermediate in a certain reaction as well as whether the reaction is reversible.³⁸

Before starting a CV measurement, it is important to determine the experimental parameters. For instance, the choice of electrolyte, the material of the WE, and an appropriate scan rate are all important factors that need to be considered before starting a CV. If using an electrolyte based on an organic solvent, a wider range of potential can be set, which is due to the electrochemical stability window of the substance used as electrolyte. If the scan rate is set to a value that appears to be too fast, the outcome can be that some reactions cannot be tracked in the resulting current-potential curve. Figure 8 gives an example of a typical cyclic voltammogram:

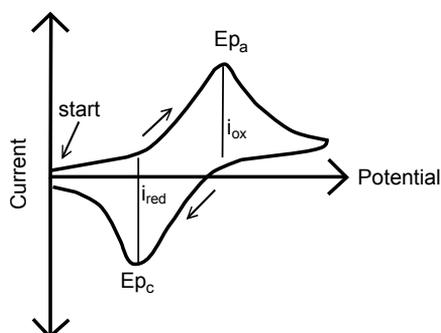


Figure 8. A general current vs. potential plot (voltammogram) from a cyclic voltammetry experiment. The peak current for the oxidation i_{ox} occurs at the potential E_{p_a} . The peak current in the reduction i_{red} is found at E_{p_c} .

From a standard cyclic voltammogram containing a redox couple, i.e., species that can be oxidized and reduced reversibly, one expects, at the start arrow in Figure 8, a small increase in the current due to the charging of the EDL (charging current). As the voltage is swept to higher potential values, Faradaic reactions start and the current increases more rapidly as the species being studied oxidizes. Then, at a certain potential (E_{p_a}), the current peaks. After this peak in the current, the current decreases as the species being oxidized is more or less consumed at the interface. In the reverse sweep, a reduction peak is obtained.³⁷ In this illustration, the redox reaction is reversible and the product oxidized in the forward sweep is reduced in the reverse sweep. If the oxidation and reduction peaks are similar in shape, the redox couple is

reversible. If there is no redox couple available in the electrolyte, one can obtain information about, e.g., a reversible oxidation taking place at a metallic surface which was the case for us in paper 1 where we oxidized Pt.

In Papers 1 and 2, CV was used in order to study the mechanism by which PLL was deposited onto either Pt or ITO.

Chapter 3 - Conjugated polymers

A polymer is a large molecule consisting of many repeated units, thereof the word polymer, originally derived from the Greek language, poly = many and mer = parts. Polymers exist in nature in the form of, e.g., DNA and other biomolecules, but they can also be synthetically produced, which is the case for polyethylene, which is used in plastic bags. Polymers as a class of material are used for a broad range of applications, and when Heeger, MacDiarmid and Shirakawa in year 2000 received the Nobel Prize for their discovery and development of electrically conjugated polymers,⁴¹ it opened the door to many more applications, e.g., sensors and actuators,⁴² self-healing materials^{43,44} and controlled drug delivery.⁴⁵

Structure and Properties

A polymer chain consisting of many repetitions of its monomer can, in its simplest form, be represented by polyethylene:

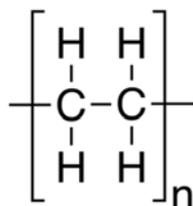


Figure 9. Chemical structure of polyethylene.

Polyethylene (Figure 9) is an insulator, meaning that it is not electrically conducting. The reason is that the carbon in the backbone of the polymer chain binds to four atoms, two hydrogens and two carbons, and is sp^3 -hybridized. Hybridization in this case refer to the interference of electron orbitals, and for the carbon in the polyethylene polymer chain, this result in four sigma-bonds, i.e., strong covalent

bonds.⁴⁶ For the case of conjugated polymers, each carbon in the backbone is sp^2 -hybridized, meaning that every carbon binds to three atoms (sigma-bonds) instead of four. See Figure 10, for example, showing the conjugated polymer polyacetylene. Each carbon has four valence electrons, three occupied in sigma-bonds whilst the fourth is left to a non-hybridized orbital oriented orthogonally to the carbon chain. These orbitals overlap along the backbone of the polymer and give rise to π -bonds in which electrons are delocalized. Due to the resulting extended π -system of alternating single and double bonds along the backbone, the polymer is referred to as “pi-conjugated”.⁴⁷

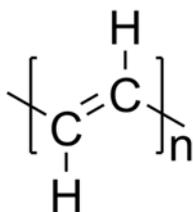
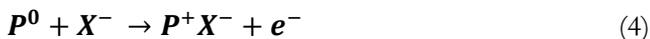


Figure 10. Chemical structure of polyacetylene.

Conjugated polymers are, due to the overlapping π -bonds, electrically conductive, as electrons are delocalized and can move along the polymer chain. As stated previously, this property makes the polymers very applicable in areas where, before, only inorganic materials, e.g., Si, were considered to be of use. The degree of the conductivity of conjugated polymers can be increased by “doping”. By doping the material, one can increase the conductivity several orders of magnitude. The polymer can either be n -doped or p -doped. The n refers to “negative” and here one adds an electron to the polymer. This type of doping is far less common than the latter, where p stands for “positive” and here an electron is withdrawn, leaving behind a positively charged “hole”.^{47,48} There are several ways to dope the polymer, i.e., chemical doping, electrochemical doping, and photo doping, however only electrochemical doping will be discussed in this thesis.⁴⁹

Upon doping a conjugated polymer, properties such as color,⁵⁰ wettability,⁵¹ and as mentioned, conductivity change, making these polymers suitable for applications such as electrochromic displays,⁵² solar cells,⁵³ and cathode materials for batteries.⁵⁴ Many polymers are industrially produced and relatively cheap. Also, the fact that many are organic and biocompatible makes them applicable in fields such as biology and medicine.^{55,56} Other advantages are that they are flexible and easy to process. In miniaturized systems, conjugated polymers are an attractive choice for electrode

materials, in contrast to metal electrodes. As the prefix mini tells, miniaturized systems, e.g., microfluidic devices for various applications, deal with small amounts of water, and here, the use of electroactive conjugated polymers comes well into place. Electrolysis is the dominant electrochemical reaction occurring in most cases as a result of driving a current through a water-based electrolyte. The electrolysis of water gives rise to oxygen and hydrogen gas, which can totally block the electrochemical circuit as the channels in the device are sufficiently small to be blocked by a gas bubble. Using conjugated polymers, and taking advantage of their electrochemical activity, the need of water electrolysis in order to maintain an electric field is to a great extent reduced. In the next section as well as in Chapter 5, there will be more information about this. The oxidation scheme of most of the conjugated polymers follows reaction scheme (4):



In (4), P^0 denotes the neutral undoped polymer, X^- an anion (e.g., Cl⁻), and e^- an electron. Via the reverse reaction, the polymer can be reduced. There are, however, limitations in the use of conjugated polymers. One example is when the polymer gets overoxidized.^{57,58} This means that the conjugation in the polymer chain breaks, and that process is irreversible, leaving an insulating film behind.

PEDOT and PSS

PEDOT, short for poly(3,4-ethylenedioxythiophene), belongs to a class of conjugated polymers called polythiophenes. As with most other conjugated polymers, polythiophenes have a low solubility in water and because of this, PEDOT was developed in order to achieve a polythiophene derivative, also processable in water. However, even though it turned out that PEDOT was insoluble in water⁵⁹ still it possessed some very interesting properties such as ease of synthesis⁶⁰ and high flexibility. PEDOT is a well-studied polymer and found applications in many areas including light emitting electrochemical cells⁶¹ and computer memories.⁶²

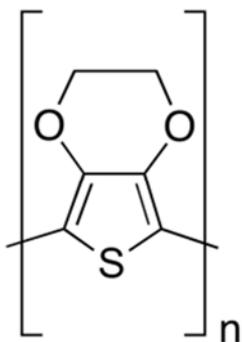


Figure 11. Chemical structure of PEDOT.

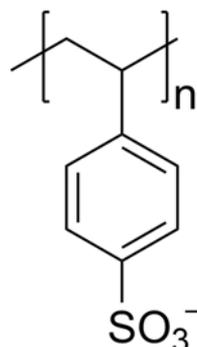


Figure 12. Chemical structure of PSS⁻.

Moreover, PEDOT is very stable in its *p*-doped state⁵⁹ and has a high conductivity (for a polymer) which can span quite a large range depending on the dopant (10^{-5} - 10^2 S/cm).^{63,64} By using polystyrene sulfonate (PSS⁻), a water-soluble polyelectrolyte, as counter-ion during polymerization into PEDOT:PSS, a solution-processed, aqueous form of PEDOT:PSS was developed. The structures of PEDOT and PSS⁻ are seen in Figures 11 and 12, respectively. The reaction scheme below (5) describes the oxidation (right arrow) and reduction (left arrow) of PEDOT together with the dopant/counter-ion PSS:



where PEDOT⁰ is the neutral form of PEDOT, M⁺ represents a metal cation (e.g., Na⁺) and PSS⁻ is the negatively charged counter ion. PEDOT⁺PSS⁻ denote the oxidized form of PEDOT doped with PSS⁻, and e⁻ represents an electron.

PEDOT:PSS is an aqueous emulsion which is easily processed and cast into different forms by various techniques. Spin-coating is one of the easiest to use, but if more control over thickness and redox behavior of the polymer emulsion are desired, electropolymerization of PEDOT:PSS onto a suitable conductive substrate using CV is preferable.^{58,65} By using CV, information about the characteristics of the resulting films is obtained.

PEDOT:PSS as electrodes

In the section on electrodes in the previous chapter, electrochemically-active conjugated polymers were discussed as an alternative to metal electrodes.^{66,67} The

discussion continues here. In miniaturized electrokinetic systems that handle small volumes of liquid for, e.g., toxicity testing, the driving of a direct current between a pair of electrodes is necessary for sustaining electroosmotic (EO) flow, i.e., driving liquid through the system. The discussion about EO flow will be continued in Chapter 5. When applying a potential between a pair of electrodes in such a device, continual Faradaic reactions are required at the electrode-electrolyte interfaces to drive current through the system. If the electrolyte in question is water-based, water electrolysis is the overall electrochemical reaction and gives rise to oxidation products (at the anode) including H^+ (acid) and O_2 (gas), which may have a negative impact on the miniaturized system.⁶⁸ As mentioned before, the gas formed during the reactions can easily break the electrochemical circuit in a microfluidic device, while changes in pH can disturb the sample, for instance, proteins in their native state. If instead of metal electrodes, a pair of PEDOT:PSS-electrodes are employed, oxidation and reduction can occur within the polymer itself, and in turn eliminate issues related to metal electrodes, mainly water electrolysis which cause water consumption and pH changes.

Gel electrophoresis is a technique where charged molecules are separated due to their size or charge. The molecules to be separated are loaded onto a gel such as acrylamide or agarose, where the gel functions as an electrolyte. A power source connected to electrodes at opposite sides of the gel generates an electric field which causes the molecules to migrate in the gel. Depending on the size and charge of the molecule, the molecules move at different rates. Positively charged molecules will, e.g., move towards the cathode.²⁸

Commonly, metal electrodes like Pt are used in gel electrophoresis, but we wanted to test whether PEDOT:PSS could be an attractive choice considering the advantages explained above. Paper 3 describes how we tested this hypothesis in GE Healthcare's electrophoresis system PhastSystemTM.

Chapter 4 - Electrode coatings

Interactions between charged molecules and surfaces are found almost everywhere in nature. The interactions between, e.g., cells in our bodies and various molecules such as proteins constantly take place as a method of exchanging information. The adsorption of molecules onto surfaces usually occurs spontaneously and is very important as it plays a key role in a wide range of biological processes.¹⁶ Sometimes, however, this adsorption is undesired and needs to be avoided. Studies highlighting this include a study treating the correlation between structure and property of coated surfaces⁶⁹ and the effect the polymer architecture has on protein resistant surfaces.⁷⁰

When, e.g., implanting a foreign object, such as a medical device, into a body, the body's immune system reacts immediately and works to expel or encapsulate the foreign object. One route for the body to take is to make proteins that coat the foreign material, and shield it from the body while simultaneously reducing its sensitivity to whatever stimulation it was intended to detect (in the case of a sensor). One way to increase biocompatibility, i.e., a way to make the body "accept" this foreign object, is to coat the outside of the device with a material that the body recognizes.^{17,71} Common materials that the body accepts are silicone and Ti. The opposite situation, where increased protein adsorption onto a material is desired, may also be enhanced by coating the original material with another material that the body reacts strongly to.

Surface modifications

As mentioned in the introduction, the coating of hard materials that are unsuitable for long-term experiments with a biocompatible material is very important for many applications such as various devices for medical implants.^{12,14} Polyethylene glycol

(PEG) is an example of a material with properties that inhibit protein adsorption on the coated implant.⁷² Another common material for these types of applications is the polyelectrolyte poly-L-lysine (PLL), see the chemical structure in Figure 13. PLL belongs to a group of poly-amino-acids or polyelectrolytes and possesses a positive charge at pH 7 due to the protonated amino group.

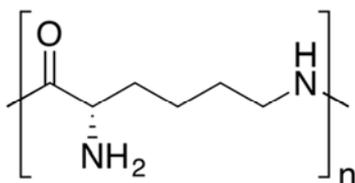


Figure 13. Chemical structure of Poly-L-lysine (PLL).

PLL is often used in order to make cells attach to the surface of tissue-culturing dishes and increases the number of binding sites on the surface.⁷³ Since the cell surface is negatively charged, the positive charge on PLL attracts it. It has also been shown that PLL grafted with certain other materials for medical implants can have antimicrobial effects.⁷⁴

Various coating strategies/techniques exist, but here the focus is on adsorption processes as in Papers 1 and 2. The general adsorption of a polyelectrolyte onto a solid surface of the opposite charge follows the characteristics as in Figure 14.

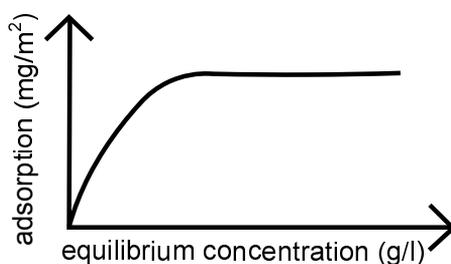


Figure 14. An example of adsorption (mg/m²) vs. concentration (g/l) curve for a polyelectrolyte onto an oppositely-charged solid surface. At low concentrations, there is a rapid increase in the adsorption, and at high, the adsorption is constant.

The adsorption is to great extent due to electrostatic interaction between a substrate and a polyelectrolyte. There is first a fast adsorption of electrolyte onto the surface

until the whole surface is covered with the adsorbed species. Then, the surface gets saturated, leaving room for no further adsorption and hence, the curve planes out and reaches a steady-state.^{75,76,77} The time for the surface to become saturated with a certain polyelectrolyte depends, of course, on many factors, e.g., the charge of the surface and the polymer, the concentration of the polymer,⁷⁸ the concentration of the electrolyte, pH, etc.

The requirements for the coating depend on the application. The thickness, density, charge and structure of the resulting coating are all important when designing the proper coating. One common way of producing a film of the desired charge, for instance, is the layer-by-layer (LbL) method.^{79,80,81} Here, layers of oppositely charged molecules are adsorbed on top of one another in several steps, resulting in a film consisting of at least two different adsorbents, see Figure 15. LbL assembly of polymer films can be done in a variety of ways including spin-coating and dip-coating.⁸²

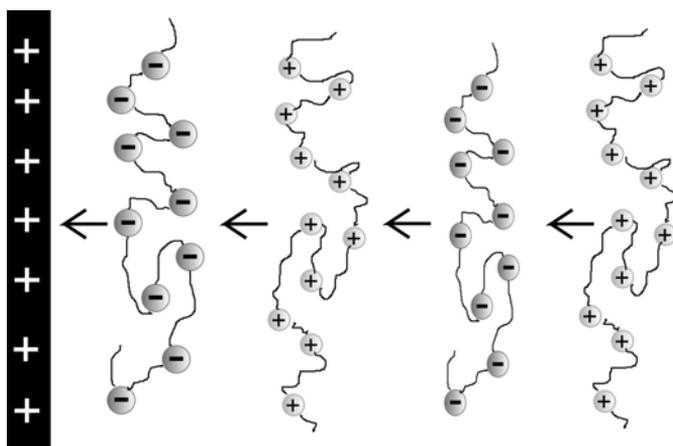


Figure 15. A schematic example of the Layer-by-Layer (LbL) assembly of polyelectrolyte (PE) films. Films containing PEs of alternating positive and negative charge can be fabricated via this method.

A discovery made in 2007 by Van Tassel et al. was that certain polyelectrolytes continuously adsorb over the time-scale of several hours when a positive electric potential is applied to the underlying substrate.³⁹ One advantage with this method, compared to, e.g., the LbL method, is that only one polyelectrolyte is needed, leading to single-component films achieved in one step. The scientists behind this study used

an optical technique called optical waveguide light-mode spectroscopy (OWLS), and indium tin oxide (ITO) as the conducting substrate upon which the polyelectrolytes were adsorbed. Factors such as salt concentration, pH and the effect of an electrical potential applied to the ITO were studied. They found that the adsorption could continue for several hours without apparent saturation. However, the exact mechanism is not yet fully understood. We chose to study the same process with a gravimetric technique, namely quartz crystal microgravimetry with dissipation monitoring (QCM-D) combined with electrochemistry (EQCM-D)⁸³ since QCM-D provides additional parameters of the resulting film. We tested the system with both Pt and ITO as substrates (Papers 1 and 2). More details about the QCM are found below, but first, a short description about different types of properties an adsorbed film may possess.

Film properties

A viscoelastic film has both viscous and elastic properties. The first term, viscosity, refers to a material's resistance to flow. One example of a high viscosity material is honey. The second term, the elasticity, is the ability of a material to return to its original form after some kind of deformation. Water is an example of a material that has low viscoelasticity, while most polymer solutions are viscoelastic.

When adsorbing a material onto a surface, water may be trapped within the chains of the material, depending on several factors including the structure of the material (see Figure 16). The water itself contributes with a mass that needs to be considered when analyzing the data from a mass-sensitive technique such as QCM.

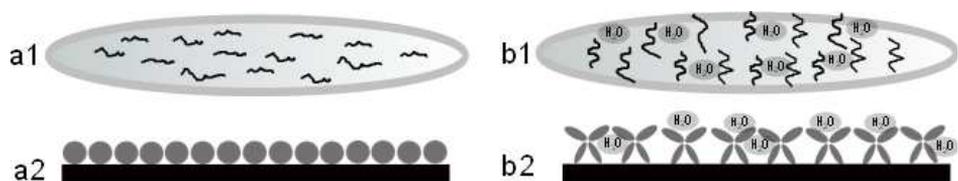


Figure 16. Schematic of different ways molecules can adsorb onto a surface. a1 and b1 correspond to the top-view, and a2 and b2 represent the side-view of a QCM-crystal. In a1, the molecules are oriented horizontally lying down, and as a consequence, little water will be coupled to them. In b1, molecules are standing up, allowing more water to couple to the adsorbed molecules.

The trapped water leads to an underestimation (according to the Sauerbrey relationship explained below) of the total mass (water plus adsorbed material) since

the total mass of the film will not be fully coupled to the oscillation of the crystal. Therefore, it is of great importance to investigate both the change in frequency and change in dissipation when studying adsorption in a water-based system.^{84,92} This is not as straightforward as for rigid films whose masses can be estimated using the Sauerbrey relationship (explained in the coming section). Instead, sophisticated models are needed and this often complicates the interpretation of the mechanisms studied.

QCM-D

QCM-D is a powerful gravimetric technique and acts as a very sensitive balance with the ability to measure changes in mass on a surface down to the order of ng/cm^2 .^{85,86} This high sensitivity makes the technique suitable for studying, e.g., adsorption mechanisms, structural changes of adsorbed species, and interactions between molecules.^{87,88} The heart of the QCM is the quartz crystal, which is piezoelectric. That means that the quartz is prone to accumulate electrical charge when mechanically stressed.

The QCM was first developed to function only in vacuum⁸⁹ but several years later was developed for use with liquid phases as well.^{83,90} The development of QCM for use in liquid environments made it possible to study biological systems,⁹¹ as cells and proteins do not behave under vacuum in the same manner they do in their native (wet) environment.

Principle of QCM

In the QCM, an AC-voltage applied between two electrodes on opposite sides of the quartz crystal causes the crystal, which also serves as the substrate, to oscillate. This oscillation is strongest at the crystal's fundamental resonance frequency and harmonics thereof. When molecules from an adjacent fluid deposit on the crystal (or electrode) surface, the resonance frequency (Δf), decreases as the effective mass of the crystal increases. If the adsorbed molecule is soft, i.e., viscous, dissipation (ΔD), changes. If the chemical or biological system being studied is reversible, that is, if the adsorbed molecules can be washed away and released from the surface, this is observed as an increase in Δf , see Figure 17 for an example. The active (exposed) area of the QCM crystal can be almost any material, but common materials are Au, Pt, ITO, and SiO_2 .^{85,92}

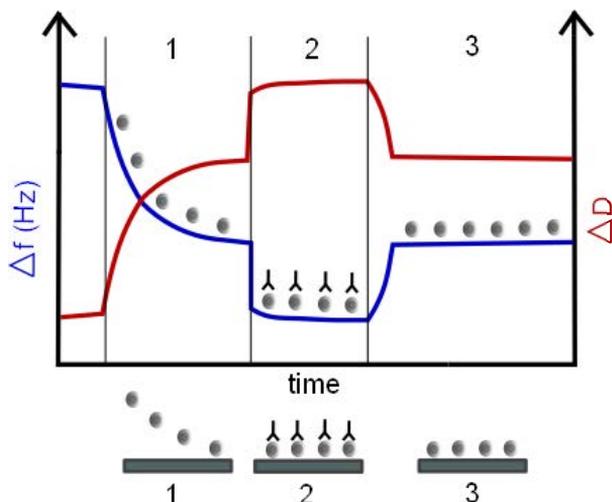


Figure 17. Sketch of the basic principle and resulting graph from the QCM-D. The blue line corresponds to the resonance frequency (Δf) and the red line corresponds to the change in dissipation (ΔD). 1) A molecule (grey circle) adsorbs onto a QCM-crystal. 2) A second molecule (inverted “Y”) binds to the molecule adsorbed in the first step. 3) The second molecule is washed away. Below the graph is the representative side-view of the QCM-crystal during the adsorption and subsequent removal of molecule number 2.

Films in vacuum / rigid films

In vacuum (and under certain conditions such as rigid films), the film’s mass (or thickness if the density is known) can be estimated and calculated directly using the Sauerbrey relationship:⁹³

$$\Delta m = -\frac{c\Delta f}{n} \quad (\text{eq. 1})$$

This equation is only valid if the resulting film is rigid, sufficiently thin (much thinner than the crystal itself) and evenly distributed on the surface. Δm is the change in mass, Δf is the change in resonance frequency, and c is a constant that depends on the crystal’s dimensions and mechanical properties. The crystals used in the work described here (5 MHz quartz crystal) have a constant of $17.7 \text{ ng}\cdot\text{Hz}^{-1}\cdot\text{cm}^{-2}$. n is the overtone (harmonic) number: 1, 3, 5, 7, 9, etc.

Films in liquid systems

It is quite straightforward to calculate the thickness of a film deposited in vacuum, because the instrument “feels” the entire film. However, in a system containing liquid,

the liquid may enter the deposited film and contribute to the resulting signal from the instrument. In this case, another factor is measured and monitored together with the change in frequency in real-time. That factor is the dissipation factor, D , and is defined as the energy loss per oscillation period divided by the total acoustic energy stored in the system. D corresponds to the viscoelasticity of the film deposited on the surface of the quartz crystal.

$$D = \frac{E_{lost}}{2nE_{stored}} \quad (\text{eq. 2})$$

The opportunity to study the fundamental frequency and the overtones provides extra information, e.g., viscosity and correct thickness of the film studied when combined with a viscoelastic model (Voigt model).⁸⁷

As mentioned earlier, the QCM can be combined with the potentiostat, allowing the study of electrochemical processes occurring at the sensor surface. In the first two papers, EQCM-D was employed in order to study the deposition of PLL on Pt and ITO, respectively, under the influence of an applied (DC) potential.

Chapter 5 - Miniaturized systems for biology

Today, if you are sick, you typically need to go to the doctor and, depending on the symptoms, maybe leave a blood- or urine sample. These samples are then sent away, often to a remote lab, for analysis. This process is time-consuming and expensive, and for people living in developing countries, getting the right help or treatment can be difficult or impossible. Often, the lack of sophisticated laboratories and/or medical doctors and other experts means medicine cannot be distributed, and in the worst case, patient death. Even medical treatments routinely administered in the western world, e.g., cures for infectious diseases, are not available in third-world countries in part because diagnosis is not available. This is why the technology of miniaturized analysis and diagnostic systems has the potential to dramatically improve many peoples' lives.

Point-of-care (POC) diagnostics²¹ are analytical devices designed to bring testing closer to the patient simply, quickly, and reliably. Microfluidics is a field involving the design and assembly of devices in which fluids are carried through small capillaries in order to perform these tasks.

Microfluidics

Microfluidics is an interdisciplinary field of science which spans physics, (bio)chemistry, and material science. The potential applications within this field are numerous and include medical diagnostics⁹⁴ and DNA analysis,⁹⁵ making the advantages of flexible, easy-to-use, disposable so-called Lab On a Chip (LOC) devices obvious.⁹⁶ Microfluidics deals with the motion of fluids in small channels (micro or sub-micron scale), and when scaling down from a macro- to a microsystem, fluids start to behave differently. Due to the high surface-to-area ratio, surface effects such as

surface tension become much more important in microfluidics than in macro systems.^{18, 97}

Depending on the application, a LOC device comprises components such as microfluidic channels, pumps,^{98,99} cell chambers¹⁰⁰ and mixers.¹⁰¹ See Figure 18 for a simple sketch of a concept for a LOC. In the middle of the sketch, a cell chamber containing cells introduced by microcarriers is shown. To the left of that is a channel from a well pumping cell media to the cells and to the right, the outlet well for waste products. The small channels above and below the cell chamber represent wells from which substances can be introduced into the cell chamber by the individually-controlled sample channels. The light-blue squares underneath the wells represent PEDOT:PSS electrodes. Drug- and toxicity testing, each very time-consuming and expensive, would benefit tremendously in terms of efficiency if the tools used could be reduced in size, e.g., smaller quantities of the expensive potential medicines being tested would be required and the total time for analysis would be reduced significantly. By replacing animal models with (human) cells in microfluidic bioreactors, fewer animals would be sacrificed and more reliable (for humans) results would be obtained.¹⁰²

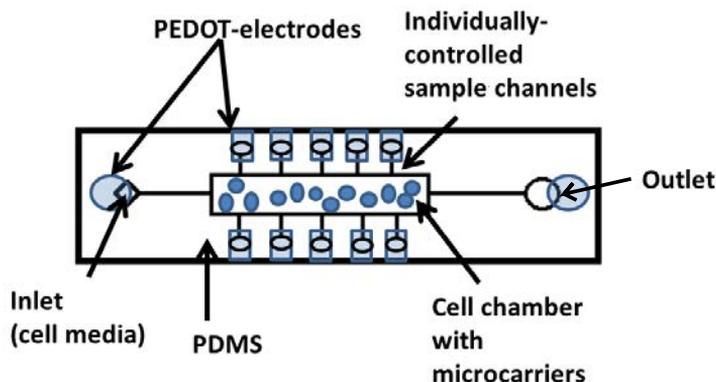


Figure 18. An example of how a Lab On a Chip (LOC) can be designed. The chip is built from polydimethylsiloxane (PDMS) and contains integrated PEDOT:PSS electrodes, a cell chamber and fluidic channels.

Reynolds number

One very important aspect in microfluidics is understanding the flow in the channels. The flow of fluid through a microfluidic channel can be realized by various means (by external, e.g., syringe pumps or peristaltic pumps, or on-chip EO pumps) and can be characterized by the dimensionless Reynold's number (R_e) which is defined as the ratio between the inertial forces to the viscous forces:

$$R_e = \frac{\rho U l}{\mu} \quad (\text{eq. 3})$$

Where ρ is the density of the fluid, U is the relative velocity of the fluid, l is the characteristic length of the system and, μ is the viscosity of the fluid. R_e indicates whether a fluid is flowing with a laminar ($R_e < 2300$), transient ($2300 < R_e < 4000$) or turbulent ($R_e > 4000$) flow regime.

Laminar flow profiles are typically generated in microfluidic systems, compared to flow in the macro scale, which often results in turbulent flow.

Depending on how fluid is driven through a microsystem, i.e., what type of pumping method is used, various types of flow will be generated, and two of the most common ones, Poiseuille flow and electroosmotic (plug) flow, will be discussed in the coming section.^{25,103}

Poiseuille flow

This type of flow is the most characterized flow type when it comes to microfluidics because it describes (within reason) the flow in the majority of situations in LOC devices, and is relatively easy to understand. Poiseuille flow describes laminar flow resulting from a difference in pressure between the two ends of the vessel. Hagen-Poiseuille flow is a specific example of Poiseuille flow describing the flow in channels with circular cross-sections.^{103,104} In such a circular vessel, which when small we call a capillary, the Poiseuille law¹⁰⁵ (eq. 4) states that the volumetric flow rate (Q) is directly proportional to the fourth power of the cylinders radius (r) and inversely proportional to the length (L) of the pipe and viscosity (μ) of the fluid being transported under a constant pressure difference (ΔP) according to:

$$\Delta P = \frac{8\mu L Q}{\pi r^4} \quad (\text{eq. 4})$$

This equation is valid under the assumption that the fluid is Newtonian (a fluid for which the viscosity stays unchanged even if the flow rate changes) and incompressible. Poiseuille flow has a parabolic flow profile as seen in Figure 19. The parabolic flow profile is induced in part by the no-slip boundary condition, which means that the velocity of the fluid adjacent to the wall of the capillary is zero.

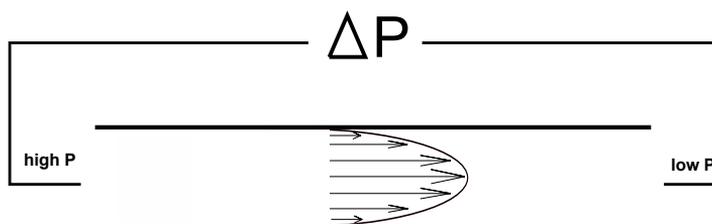


Figure 19. Flow profile for pressure-driven (Poiseuille) flow in a cylinder. ΔP denotes the pressure difference between the open ends of the cylinder.

The pumps associated with LOCs are often external, e.g., syringe pumps or peristaltic pumps, and as a consequence, associated with numerous and sometimes complicated tubing connections. One way to overcome these often troublesome connections is to integrate an EO pump directly into the chip. These pumps will be described in more detail in the following section.

Electroosmotic (EO) flow

Electroosmosis is the movement of fluid in a capillary under the influence of an electric field. This discovery is credited to Ferdinand Frederic Reuss, Moscow University. In 1809 he reported the motion of water through porous clay under the influence of an externally-applied electric field. The principle behind EO flow is the electrical double layer (EDL) on the charged surface of the capillary. The ions closest to the walls start to move toward the electrode of opposite charge when an electric field is applied. This induces viscous forces into the nearby liquid which, as a result, will be set into motion. See the schematic of the principle in Figure 20.

Since this flow stems from the electrical forces induced by the ions in the EDL, EO flow is most prominent in small channels (less than $\sim 100\text{-}200\ \mu\text{m}$ in diameter) and is therefore of great interest in microfluidic applications such as separation techniques and chemical analysis. In addition, the flow profile generated by EO flow has a uniform velocity profile (called plug flow)¹⁰⁶ outside the EDL region as exemplified in

the sketch in Figure 20. This starkly contrasts with pressure-driven Poiseuille flow, which exhibit a parabolic flow profile.¹⁰⁴

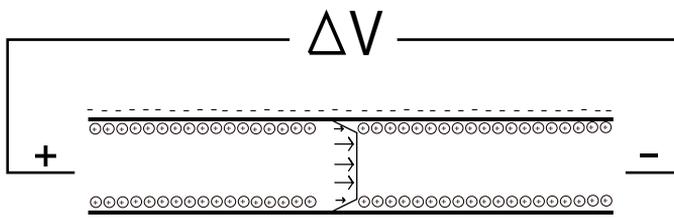


Figure 20. Example of a plug flow profile in a cylinder driven by an applied potential (V). The circles containing a + represent cations in the electric double layer (EDL).

As mentioned in Chapter 3, EO flow requires continual Faradaic reactions at the electrodes to maintain an electric field within the electrolyte. When the reaction is water electrolysis, products such as oxygen and hydrogen gas form bubbles that can disrupt the circuit in a microfluidic system. Simultaneously, the generation of protons (acid) and hydroxide ions (base) change the pH. Maintaining a constant pH is very important when dealing with cells and proteins as they may otherwise be damaged or denature.

Electroosmotic pumps

Electroosmotic pumps are an attractive choice for moving liquids through microfluidic systems. Some of the advantages of EO pumps are that they have no moving parts, are capable of generating high flow rates, and are relatively easy to integrate into LOC devices.¹⁰⁷ Moreover, EO-pumps are capable of generating high pressures and can be designed to resist undesired pressure-driven flow. As described above, they require a channel with a charged surface and small diameter (or a network of such channels as in the clay particles studied by Reuss), and an electrolytic fluid.

A simple way of fabricating these pumps is by using fused silica capillaries. A fraction of the silanol groups (Si-OH) on silica spontaneously deprotonate when in contact with an aqueous electrolyte, resulting in a negatively charged surface (Si-O^-).^{108,109} The negative surface attracts cations, resulting in an EDL at the inner wall of the capillary. The cations migrate toward the cathode when an electric field is applied, dragging the bulk of the liquid with them (see Figure 20). These kinds of pumps do not require

anything but electrical connectors, that is, no need for external fluidic connections in order to drive the liquid through the channels in the microfluidic device.¹⁰⁴

Fabricating devices entirely of small capillaries with sufficient surface charge is occasionally unfeasible, for example when using plastic materials or using low-resolution fabrication techniques like 3D printing. This limitation can be overcome by filling a channel that would otherwise not support EO flow with a porous material with appropriate surface charge. Potassium silicate is an example of such a material.¹¹⁰

Using potassium silicate, together with a mixture of formamide and water, to make a frit, results in an EO pump with high EO flow and resistance to pressure-driven flow. Details of this can be read about in Paper 4. Besides being a fast and robust process, fabricating these frits is relatively simple as the materials used are all commercially available.

Electrophoresis

This phenomenon was also discovered and reported by Reuss, a couple of years before he described EO flow. Electrophoresis describes the motion of charged particles relative to a fluid under the influence of an electric field. Electrophoresis is an electrokinetic phenomenon caused by the electrostatic forces that ions feel in an electric field.^{28,95} Electrophoresis is widely used in various separation techniques (gel electrophoresis is described in Chapters 1 and 3) in areas such as forensics and DNA sequencing. The velocity of a particle (v_{EP}) is proportional to the applied electrical field (E) and the electrophoretic mobility (μ_{EP}) according to:

$$v_{EP} = \mu_{EP} \cdot E \quad (\text{eq. 5})$$

μ_{EP} describes a particle's capability to move in the presence of an electric field.¹⁰⁴

The last section in this chapter briefly describes some fabrication techniques used during my graduate education together with various materials used for building model microfluidic systems.

Fabrication of microfluidic devices and fluidic channels

Microfluidic channels can be prepared by a variety of techniques, where photolithography and soft lithography are common. In soft lithography, stamps or molds are made from a master, replicating a pattern or a structure, such as a network of fluidic channels. Compared to direct photolithography, soft lithography is cheaper,

as it requires expensive clean-room facilities only for creating the master, which can be re-used to make dozens if not hundreds of devices. While photolithography still holds the number one position when it comes to fabricating components for the electronics industry, soft lithography is a technique well-suited for applications within biotechnology.¹¹¹

3D printing

As mentioned above, fluidic channels can be prepared in a variety of ways. One method is to print the desired system, including reservoirs for buffers etc., by using a 3D printer.^{112,113,114} This technique gives the opportunity to, in a quick and relatively simple way, fabricate 3D printed structures with enough complexity in order to continue the development of prototypes within, e.g., biomedical research.¹¹⁵ Since the 3D printing technique is still young (the commercial 3D printer has been around for about twenty years) challenges such as surface roughness and alignment still remain. Furthermore, the demands on the material used increase as the technique is used for applications such as biology, for instance, the printing of tissues of various kinds such as bone and vascular grafts.^{116,117}

In 3D printing, also known as rapid prototyping or additive manufacturing, a model from, e.g., a computer aided design (CAD) program is translated into a suitable format for the 3D printer to read and execute the actual print.¹¹⁸

A syringe-based 3D printer can pattern a wide variety of materials including sugars and polymers. An example of such a printer is shown in Figure 21. Depending on the application, the best suited material is chosen based on, e.g., viscosity, biocompatibility, and, in the case of sacrificial templates, how easily and thoroughly the material can be removed. Sacrificial templates are used as a temporary mechanical support for another material such as PDMS (described in the coming section). PEG is a good sacrificial material and has the advantages of being inexpensive and water soluble. However, PEG that can easily be extruded through a syringe tip at room temperature does not possess enough mechanical stability to act as a temporary support material. Our group has previously demonstrated how PEG together with a plasticizer creates a material that can be printed at room-temperature and used as a sacrificial support.¹¹⁹

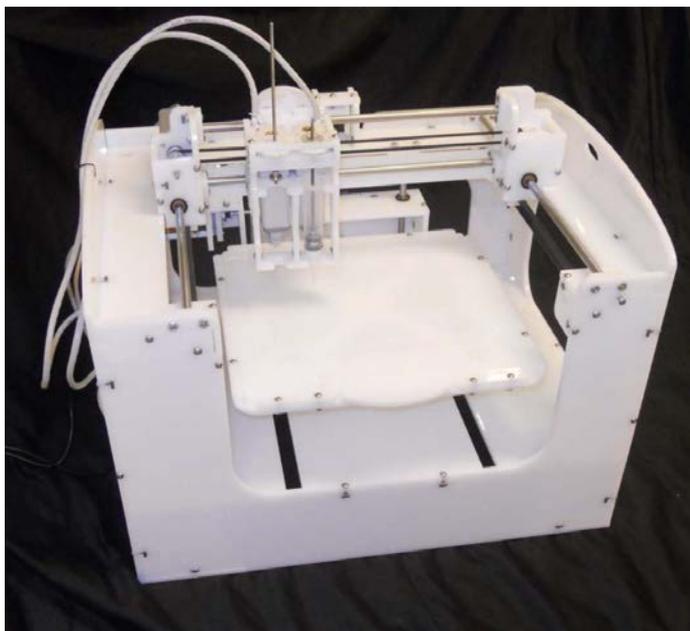


Figure 21. Photograph of a syringe-based 3D printer (Fab@home Model 3, Seraph Robotics™) from the lab of Surface Physics and Chemistry division in Linköping.

Polydimethylsiloxane

Polydimethylsiloxane (PDMS) is frequently used for microfluidic devices fabricated using soft lithography. PDMS is an elastomer, a polymer with both elastic and viscous properties, and its inherent characteristics including biocompatibility, transparency and gas permeability.¹²⁰ These properties are very desirable for biotechnological applications. When preparing a device, PDMS is poured over a template and then allowed to cure (polymerize). The template is then removed, either by lifting off the PDMS (traditional soft lithography), or by dissolving the sacrificial template.

PDMS has a hydrophobic surface after polymerization (curing). Using traditional soft lithography techniques, the surface of PDMS can be hydrophilicized by plasma-oxidation before annealing it to a substrate. Plasma-oxidation induces silanol groups on the surface, creating the desired hydrophilic surface. In microfluidic channels for biotechnological applications, a hydrophilic surface is important to allow the surface to be wet by the polar solvent. Additionally, by introducing the silanol groups to the PDMS-channels, EO flow is supported.¹²¹ The durability of the introduction of the hydrophilic charges on the PDMS surface varies a lot depending on the storage

condition of the prepared device. Placing the device in liquid immediately after oxidizing the surface helps maintain the introduced charges a significantly longer time (>100 hours) than placing the device in open air (reduced hydrophilicity after ~10 min).¹²²

In Paper 4, besides using PEG as sacrificial material, we also combined EO pumps and the use of PDMS when evaluating the fabricated EO pump in a microfluidic device.

Chapter 6 - Summary of papers and concluding remarks

In Paper 1, we aimed to study the mechanism of anodic deposition of PLL onto Pt employing the gravimetric technique QCM-D together with electrochemistry. However, since Pt strongly catalyzes water electrolysis, the results of the measurements were challenging to interpret. In addition to gas bubbles that negatively influenced the measurements, the oxidation also etched the conducting substrate. Compared to previous studies of PLL adsorption onto ITO, we observed water electrolysis on Pt to occur at a rate more than 100 times faster. However, despite these challenges, we managed to deposit PLL films onto Pt, and by the simultaneous measurement of several harmonics in the QCM-D, both before any PLL was deposited and after (when the crystal was dry), we could apply the Sauerbrey relationship to separate the effects of Pt removal and determine the actual amount of PLL anodically deposited.

Paper 2 continues the story of the first paper. Here, we used a metal oxide, ITO, instead of Pt as the conducting substrate while adsorbing PLL. We managed to deposit PLL on ITO without the unwanted oxidation products observed when we used Pt in the first paper. Moreover, the results obtained from the electrochemical methods CA and CV, contributed with additional information regarding the deposition mechanism. The main finding in this paper however, is that ITO seems to offer a unique surface for the study of anodic polyelectrolyte deposition, which means that the results obtained using, for example, OWLS, may not reflect those found using more biocompatible conducting materials.

Paper 3 describes an evaluation of the conducting polymer blend PEDOT:PSS as electrode material to replace the traditionally used Pt in gel electrophoresis using the

commercial available PhastSystem™. We showed that PEDOT:PSS can replace Pt-electrodes in SDS-PAGE separations without further modification to the standard protocol.

In Paper 4 we describe the production and characterization of potassium silicate as a stationary phase in fused silica capillaries functioning as electroosmotic pumps. We evaluated a recipe for making potassium silicate frits by varying the amount of water. The EO mobility, EO flow rate, and resistance to pressure-driven flow (R_{hyd}) in the final frits were measured. We found that by employing a potassium silicate frit as a stationary phase, high EO flow and resistance to pressure-driven flow were obtained. We also successfully integrated the pump into a PDMS-device representing a microfluidic chip with a cell chamber.

The relatively novel field of bioelectronics and use of electrokinetics in biotechnological applications is indeed inspiring as it offers many possibilities for miniaturizing diagnostic tools for purposes such as reducing the use of animals for testing. These tools may also include microfluidic devices with analytical and diagnostic capabilities as well as, e.g., screening for substances in the early phases of drug development. Requirements for these kinds of devices include robustness and reproducibility. Implanting the device increases the demands and requirements tremendously in terms of biocompatibility. These implants, produced from biocompatible materials, still lack important properties such as the control of, e.g., protein resistance. The possibility of coating the biomaterial in question with a more suitable material has been explored extensively, but many questions still remain. The use of conjugated polymers in medical applications has gained a lot of researchers attention recently, and the results in this thesis (Paper 3) demonstrate the use of PEDOT:PSS as an electrode material for gel electrophoresis. In the last paper in this thesis, the fabrication of a microfluidic system with an EO pump using soft lithography together with a 3D printer was demonstrated. By applying a potential to a pair of Pt-electrodes, an electric field induces the flow in the pump due to the generated EDL. Replacing the Pt with PEDOT:PSS electrodes would be a very interesting next step.

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