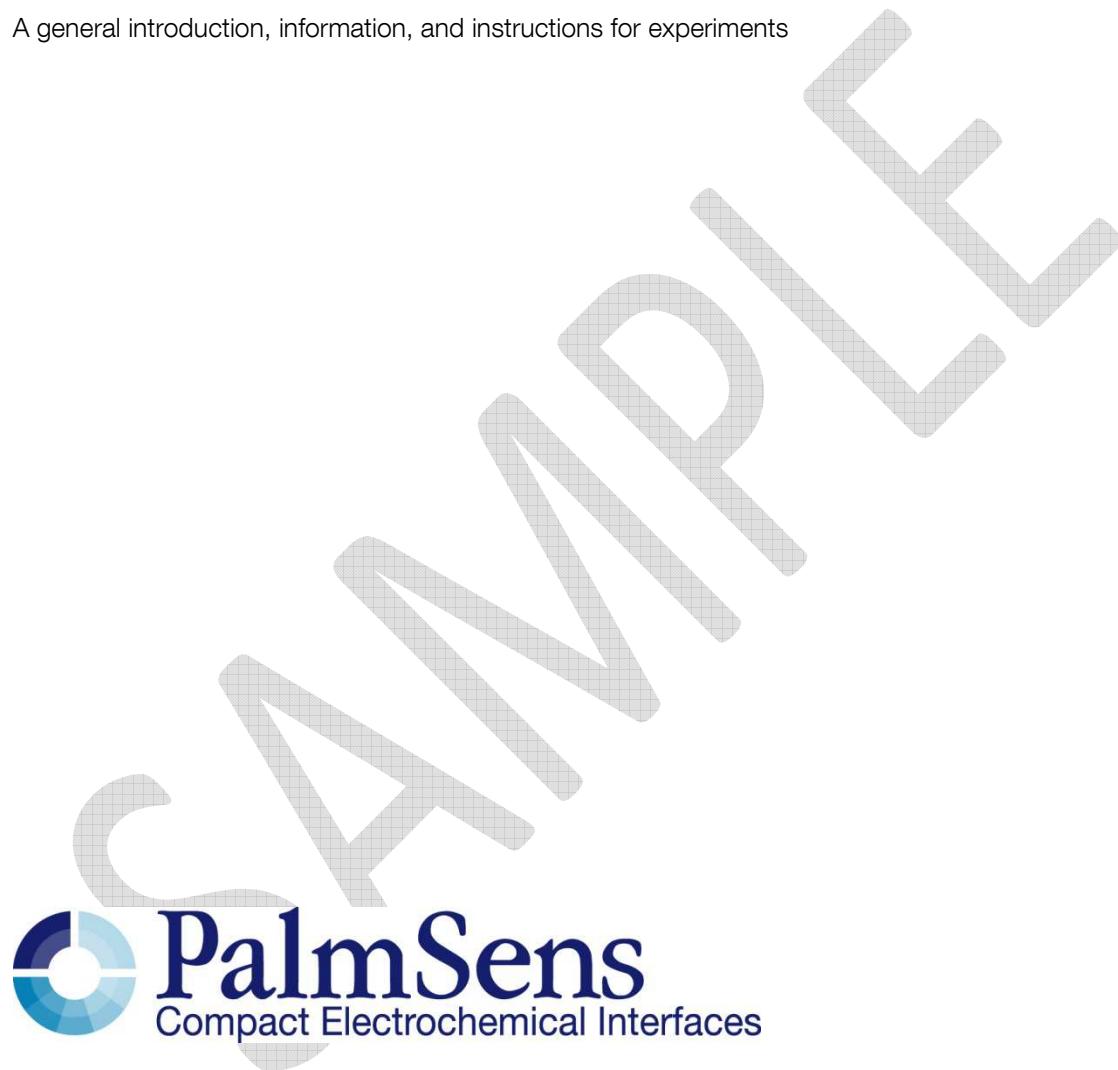


Teacher's Guide to the PalmSens Educational Kit

A general introduction, information, and instructions for experiments



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Contents

1	List of Symbols.....	1
2	Introduction	3
2.1	Why Electrochemistry?	3
2.2	Goals	3
2.3	Use.....	4
2.4	Literature	4
2.5	Required Knowledge	4
2.6	Content	4
2.6.1	Devices, Accessory and Consumable.....	4
2.6.2	This Guide	4
3	Potentiostats	6
3.1	What Is a Potentiostat Good for?.....	6
3.2	How Does a Potentiostat Work (in a nutshell)?.....	6
3.3	Electrodes	8
3.3.1	Working Electrode	8
3.3.2	Reference Electrode	9
3.3.3	Counter Electrode.....	10
3.3.4	Screen Printed and Film Electrodes	10
3.4	About Potentiostat's History	12
3.4.1	Troubleshooting.....	14
3.5	Conclusion of Chapter 3	15
4	Experiments	16
4.1	Copper and Nickel Deposition	17
4.1.1	Goals	17
4.1.2	Introduction	17
4.1.3	Experimental.....	19
4.1.4	Instructions	20
4.1.5	Results	23
4.2	The Cottrell Experiment and Diffusion Limitation	25
4.2.1	Goals	25
4.2.2	Introduction	25
4.2.3	Experimental.....	29
4.2.4	Instructions	30
4.2.5	Results	32
4.3	Cyclic Voltammetry – the Most Used Technique.....	35
4.3.1	Goals	35
4.3.2	Introduction	35
4.3.3	Experimental.....	42

4.3.4	Instructions	43
4.3.5	Results	47
4.4	Detection of Multiple Heavy Metals by Stripping Voltammetry	52
4.4.1	Goals	52
4.4.2	Introduction	52
4.4.3	Experimental	57
4.4.4	Instructions	58
4.4.5	Results	63
4.5	Detection of Glucose with a Self-Made Biosensor Based on Glucose Oxidase	65
4.5.1	Goals	65
4.5.2	Introduction	65
4.5.3	Experimental	71
4.5.4	Instructions	72
4.5.5	Results	75
4.6	Detection of Hydrogen Peroxide with Selfmade Prussian Blue Electrodes	77
4.6.1	Goals	77
4.6.2	Introduction	77
4.6.3	Experimental	81
4.6.4	Instructions	82
4.6.5	Results	84

2 Introduction

2.1 Why Electrochemistry?

Electrochemistry is a versatile discipline that has applications in various fields of research and industry. Corrosion research develops methods to save millions of Euro that are needed to remove the damage done by corrosion. Electrochemistry delivers the insights about the processes causing corrosion and is capable to investigate means against corrosion in hours instead of weeks. Although these experiments cannot replace the long term test under real conditions, they can provide the knowledge for a fast pre-selection of approaches that should be investigated more closely.

Another big field of electrochemistry is energy conversion. Batteries, especially rechargeable batteries, have been important for portable technologies for quite some time, but since the boom of the mobile internet the limitations of batteries have become the limitations of our electronic society. The electric car only makes sense if we have batteries with enough capacity to drive hundreds of kilometers without recharging. Either that or we need another electrochemical device, the fuel cell, to power the cars and reduce the request for fossil oils. In the same context solar panels are important to gain energy from a sustainable source, the sun. The electrochemistry of photovoltaics is providing important knowledge to improve solar panels and brings the idea to satisfy our energy demand with solar power within our reach. In analytical chemistry electrochemistry makes miniaturized devices possible that deliver a quantitative result in the ppb range. These devices are easy to handle point-of-care devices. Electronic components are very suitable for mass production and as a consequence these devices are affordable to a broad part of the population. The most known example of an electrochemical point-of-care device is the blood sugar test. Easy to handle, inexpensive, and improving a diabetes patient's quality of life drastically it is the most successful electroanalytical chemistry product up to today. The blood sugar sensor is based on an enzymatic reaction, that is it is a biosensor.

Gaining an insight into biological electrochemistry is a powerful tool for modern technology. Using enzymes in detection to create a very sensitive and selective sensor is just one of the many possibilities biomolecules offer to technology. Biofuel cells create energy out of glucose or lactate and can thus power an implanted diagnostic device inside a human body. Imitating the photosystem of plants leads to new developments in photovoltaics. DNA chips or protein chips allow a high density of recognition elements on an electronic circuit turning it into a lab on chip that can detect quantitative various viruses, bacteria, etc., with a single droplet of sample, e.g. human blood serum. In the last 10 years electrochemical impedance spectroscopy has been opening new possibilities in corrosion and coating research as well as opened the path to more label free methods, that is no chemicals need to be added and the analyte needs no modification to create an electrochemical signal.

These are all modern electrochemical applications and additionally classic electrochemistry applications are still essential like electrolysis or galvanization.

This very brief list of technologies, available because of knowledge in electrochemistry, shows how important it is to teach the basic concepts and applications of electrochemistry during the studies of chemistry, biochemistry, physics, biology, medicine, pharma, chemical engineering, and material sciences.

2.2 Goals

We believe that students should work with state of the art technology and learn to use as well as apply taught theories. PalmSens BV has always focused on small, portable, and economical devices. Our software was designed for users with different background knowledge, so also novice electrochemists can perform experiments without a long and complex introduction.

With this kit students will be able to learn how to operate a digital potentiostat, perform their own measurements, and process the results. The described theories, concepts, and applications should teach the basic concepts and real life applications.

We would like to teach how parameters influence your measurements and why, how a kinetic analysis can be performed, how samples can be quantified, the standard edition method, the calibration curve method, and the construction of a biosensor. Some of these goals will be reached with this version of the kit; some experiments are still in preparation. These will be delivered as soon as they are ready. For customers who already own a copy of the educational kit the documents for the new experiments will be free of charge.

3 Potentiostats

3.1 What Is a Potentiostat Good for?

A voltmeter does measure the potential difference between two points. To do so the circuit needs to be closed, but a very tiny current is usually flowing. This way you can see the potential difference between two points with almost no disturbance for the investigated system. Knowing the potential between two points is useful but manipulating potentials is even better.

This is what a power source does. In every household batteries are the most common example of DC power supplies: a power supply having constant non-alternating potentials. They are used for flashlights, mobile phones, clocks, etc. In electrochemical applications DC power supplies are used for galvanization (the deposition of metals on conducting materials, often other metals). Another well-known application is the electrolysis of compounds. A well-known industrial example is the chloralkali process where salt (NaCl) and water (H₂O) are split into chlorine (Cl₂), hydrogen (H₂), and sodium hydroxide (NaOH).

The disadvantage is that you cannot investigate a single electrode and thus a single event. The current flows through the anode (electrode where oxidation happens) and the cathode (electrode where reduction takes place). So both these electrodes influence the measured current and the current limiting process cannot be determined. This is especially an issue in analytical chemistry. An electrochemical analysis that can be used easily with a power supply is the electrogravimetry. An electrode's weight is determined and all the metal in a defined sample volume is reduced, which leads to the precipitation of the metal on the electrode. The weight is measured again and thus the amount of metal in the sample volume will be determined. Although this method works fine, it has some disadvantages. The process takes time, e.g. 30 min plus drying and weighting for copper, nickel or lead oxide. In the given potential window only the analyte metal should be reduced. Hazardous side products may be produced. The solution needs to be heated and stirred to decrease the concentration polarization and to make the conversion of the analyte complete. The metal layer must stick to the electrode properly, otherwise a precise weighting of the electrode is not possible. To use other electroanalytical methods a potentiostat is needed.

A potentiostat uses three electrodes and a feedback loop to control the potential and measure the current flowing at just one of these electrodes, the working electrode. The potential will be measured to a fixed reference point and thus a lot of information about the event happening at the working electrode can be gathered.

The potentiostat controls the potential of an electrode while measuring the current flowing through that electrode.

3.2 How Does a Potentiostat Work (in a nutshell)?

As mentioned before a potentiostat controls the potential of the working electrode and measures the current flowing through it.

Why not just two electrodes? One of the reasons is that we cannot measure the potential of the working electrode against a fixed point when we just have two electrodes. Imagine a two electrode system that consists of the already mentioned working electrode and the electrode, which potential should be our fixed reference point, the reference electrode. We apply a certain potential between these electrodes and an electrochemical reaction happens at the working electrode, but since the circuit needs to be closed and current needs to flow, a reaction that is inverse to the reaction at the working electrode must occur, that is if an oxidation occurs at the working electrode, a reduction must take place at the reference electrode. If a current flows at a constant potential, an electrochemical reaction must happen according to Faraday's law:

$$Q = n \cdot z \cdot F$$

3.1

This equation says that the charge Q flowing through an electrode is proportional to the amount n of a species that took or gave z electrons at the electrode. F is the Faraday constant and represents the charge of 1 mol electrons. The current I is the charge Q per time t flowing through the electrode:

Rough surfaces will lead to high currents due to the charging of the interface, which will be explained in chapter 4.2.2.2. Dirty surfaces can show artifacts in the measurements or can be the cause that not the full active surface has access to the electrolyte. So cleaning an electrode is an important step in the electrode preparation.

A blank electrode is often just the beginning of the preparation step. For many applications an electrode's surface needs a modification: To produce an ion selective electrode polymer films are put on electrodes, to produce a biosensors enzymes or other biomolecules, for example DNA, are immobilized at the electrode, to produce immunosensors parts of the immune system are immobilized at the electrode and also inorganic catalysts are used for electrode modification.

A very interesting working electrode is the mercury electrode. Due to its liquid state mercury is used as a droplet electrode. At the end of a tube a mercury droplet is formed and used for the corresponding experiment. To clean the electrode the droplet is enlarged until it drops and a new surface is prepared with clean mercury. This way clean and easy to reproduce surfaces are obtained, but due to its toxicity in most labs mercury is not common. In general modern electrochemistry avoids mercury, but some experiments deliver the best results with mercury, so that the mercury electrode is still used nowadays.

The working electrode is the electrode where the investigated processes occur. It needs to be carefully prepared, so that the surface is reproducible and known.

3.3.2 Reference Electrode

The reference electrode should deliver a constant potential. A current flowing through an electrode leads to an electrochemical reaction that will change the composition of the electrode's environment and thus the potential. As a consequence there should be as little current as possible flowing through the reference electrode.

To establish and keep a constant potential an electrode of the second type is often chosen. Their potential usually depends indirectly on the concentration of a single anion. The electrodes of the first type are basically just metal surfaces in an electrolyte. According to Nernst's law (see equation 3.6) the potential is depending directly on the surrounding solution. An electrode of the second kind is usually a metal surrounded by a hardly soluble salt of itself. This electrode is then immersed into a solution containing the anion of that salt. Usually the anion has a high concentration to make sure that the metal salt is not dissolving fast and that small changes in the concentration have a low impact on the potential. A common reference electrode is the silver / silver chloride electrode (see Figure 3.3). A silver wire is coated with a film of silver chloride. This wire is immersed in a potassium chloride solution.

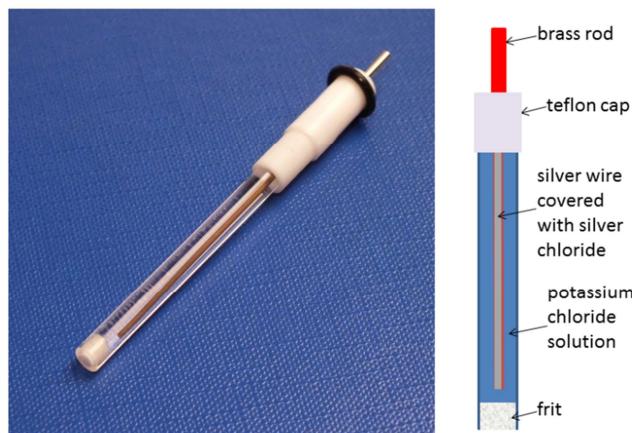


Figure 3.3: Photo and scheme of a Ag/AgCl/3 M KCl reference electrode

The potential of the silver wire depends on the silver ions dissolved in the potassium chloride solution.

$$E = E^{0'} + \frac{0.059 V}{z} \log \frac{[Ag^+]}{[Ag]} \quad 3.7$$

4.1 Copper and Nickel Deposition

4.1.1 Goals

Most students already know the basics of electrochemical potentials and how they are applied in batteries or electrolysis. This experiment is meant to facilitate a transition from what is taught in most schools and the slightly more advanced level of potentiostatic experiments. This experiment can be used to get to know the device or as a starting point in a series of lessons about electrochemistry. Because galvanization for depositing metals or other protective layers is a widely used technique in industry as well, some basic knowledge of this important process should be gained. The experiment and its discussion should teach:

- A general idea about corrosion protection
- How can the different standard potentials be exploited for metal deposition?
- How do electrodeposition paints work?
- How does electrogravimetry work?

4.1.2 Introduction

4.1.2.1 Corrosion research and corrosion protection

Corrosion is the destruction of materials by chemical reactions with the environment. It is a big cost factor. Replacement of corroded materials in buildings, bridges, or monuments and painting or galvanization of building elements is a huge investment into maintenance.

The study "Corrosion Costs and Preventive Strategies in the United States" showed that in 1998 corrosion cost the U.S. 276 billion\$. This is ca. 3.2 % of the US gross domestic product.¹ Painting the Eiffel tower costs ca. 4 million€ every 7 years.² Without a proper and closed layer of paint this monument would become unstable and break down.

As a consequence one of the most important fields in electrochemical research focuses on corrosion. A lot of companies fund academic research for investigating the mechanism of corrosive processes and for creating new methods for corrosion protection. Companies check paints and lubricants for their corrosion protection capabilities and develop new methods for creating coatings or creating non-corrosive environments for machinery, for example flooding the entire gears with oil.

From a chemical point of view protection by ordinary paints is easy to understand. The paint is usually a mixture of a polymer, color pigments, and various additives for adjusting the physical properties of the paint. For example these additives create smooth or rough surfaces or grant UV-light protection. The paint is usually dispersed or dissolved in some solvent (water or an organic solvent). The solvent evaporates after the paint has been applied and a polymer with the additives and pigments stays on the painted surface. The result is a physical barrier between the surface and its environment. This barrier protects the surface from corrosion. A crack in the layer of paint will grant access to the surface and corrosion may occur.

Here already electrochemistry can help improve the protection layer. Electrodeposition paints are paints that are made insoluble by an electrochemical reaction. A conducting work piece is dipped into the paint bath and a potential is applied. The created reaction close to the work piece's surface will make the paint insoluble and thus a layer of paint precipitates on the metal surface. All conducting parts will be covered with the paint, even usually hard to reach places. If the paint forms an insulating layer, the reaction will stop itself by blocking the conducting surface completely; only the needed amount of paint will be used. These paints have different mechanisms. The most common one is neutralizing charges. The polymers in the paints have some charged functional groups. These groups make them water soluble. These groups can be deprotonated acids or protonated bases. By way of illustration we assume a polymer with deprotonated carboxylic groups (COO^-) is used. Due to its negative charge it will migrate towards the positive charged electrode, the anode. The potential at the anode is high enough for water to split as follows:

¹ Gerhardus H. Koch, Michiel P.H. Brongers, Neil G. Thompson, Y. Paul Virmani and Joe H. Payer.

Corrosion Costs and Preventive Strategies in the United States – report by CC Technologies

Laboratories, Inc. to Federal Highway Administration (FHWA), September 2001

² <http://www.toureiffel.paris/en/everything-about-the-tower/themed-files/97.html>

4.1.5 Results

This chapter should give the instructor an idea of what the data are expected to look like. Furthermore we will briefly name some sources for deviations from the theoretical expectations.

The experiments were performed with Staples paper clips (product number 19284).

The setup with the set might look like this:



Figure 4.3: setup of the experiment

The results of this experiment basically consist of different appearances of the paper clip after the different depositions. A clear difference between the uncoated paper clip, the copper covered and the nickel or nickel copper alloy covered parts should be visible.

If during the copper deposition the solution turns green or yellow, iron is released from the paper clip. It seems the potential is too high. A deep red rust (iron oxide) also indicates the release of iron. The observed currents should be negative after the capacitive current has decayed.

The potential of the paper clip's iron is sufficient to deposit copper, so in many cases a deposition just by dipping will be observed. The potential control will lead to thicker layers of copper. These grow faster and less regular than the thin layer deposited by the open circuit potential of the iron. The thicker layers appear less shiny because they are rougher. In industrial application an additive to grow thick smooth layers of copper is often added. The paper clip is expected to look like this after deposition:



Figure 4.4: steel paper clip covered with copper at the right part

The paper clip can be replaced with a not insulated metal wire. The results can be influenced by different factors, but the deposition should work with any conducting material.

The zinc solution will have some solid zinc hydroxide inside. This is normal and no issue for the deposition. The less noble zinc will not deposit on the copper without potential control. The result is expected to look like this:



Figure 4.5: steel paper clip covered with zinc for half of the copper part

4.2 The Cottrell Experiment and Diffusion Limitation

4.2.1 Goals

Electrochemically the Cottrell Experiment is very easy. It is easy to perform and the electrochemical process is easy to understand. However, the physical observations during the experiment are important for many electrochemical techniques and experiments. The importance of mass transport, especially diffusion, as limitation for a flowing current and the additional current flowing due to charging of the electrochemical double layer are basic principles and influence almost every electrochemical experiment. The experiment and its discussion should teach:

- What is capacitive current and what is its influence?
- How does mass transport limit the current during electrochemical experiments?
- What is the Cottrell equation?
- How can a Cottrell experiment be used for analysis?

4.2.2 Introduction

4.2.2.1 The Cottrell Experiment

Simple experiments often lead to intensive theoretical observations, when performed under real conditions. Suddenly temperature, pressure, etc. influence the outcome of the experiment. The Cottrell experiment is one of the most basic potential control experiments and fortunately well understood. The basic idea is to keep an electrode at a potential that won't lead to any reaction at the electrode and when a stable state is reached a potential step is made that will lead to a chemical reaction.

In detail this means the potential E of the electrode is before the potential step below the formal potential E^0 of an electrochemical active species. This species is present in the solution surrounding the electrode. The potential before the potential step is named E_1 in Figure 4.6. In the following pictures and explanations it is assumed that the species is in its reduced form (Red). The concentration of Red is c^*_{Red} everywhere before the potential step, meanwhile the concentration of the oxidized form c^*_{Ox} is 0.

The potential step is raising the potential so that

$$E_2 > E^0$$

4.2

results. According to the Nernst equation (see equation 3.6) the ratio Ox/Red will become bigger. Red is consumed by the electrode by accepting an electron, that is Red is oxidized, and the oxidized form (Ox) is produced (see Figure 4.6). If E_2 is significantly higher than E^0 , c_{Red} is 0 at the electrode surface and all Red close to the electrode is consumed.

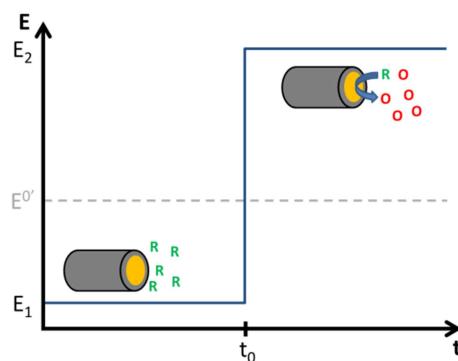


Figure 4.6: schematic potential step of a Cottrell experiment

The measured current after the potential step is a result of Red's oxidation to Ox. The current will flow as long as Red is consumed. Before the potential step the lack of potential as driving force was limiting the reaction and by increasing the potential it is no longer the limiting factor for this reaction. Since all molecules of Red reaching the electrode are consumed immediately, the mass transfer of Red towards the electrode becomes the limiting factor. The species Red is completely depleted in front of the electrode but in a distance x_1 from the electrode surface the concentration is still c^*_{Red} (see Figure 4.7a).

4.3 Cyclic Voltammetry – the Most Used Technique

4.3.1 Goals

Moving from passive potentiometric experiments to potentiostatic experiments by controlling the potential was an important development. However, the step that followed towards potentiodynamic experiments may have been even more important for modern electrochemistry. Potentiodynamic experiments made it easy to collect all the data needed for a plot of current I versus potential E . These plots are called a voltammogram and the technique used for measuring is called voltammetry. In a short period of time the cyclic voltammetry (CV) provides a lot of information and allows kinetic investigations. It is by far the most used technique by PalmSens customers. Experienced electrochemists read quite some information from the shape of a CV. The experiment and its discussion should teach:

- What is a cyclic voltammetry?
- Why does a cyclic voltammogram show a specific shape?
- What does the cyclic voltammogram say about reversibility?
- How can a cyclic voltammogram help to distinguish adsorbed and free diffusing species?
- How can a cyclic voltammogram be used to characterize catalysts?

4.3.2 Introduction

4.3.2.1 What is a Cyclic Voltammogram?

During a cyclic voltammogram the potential is controlled and the current is measured. The potential is linear increasing or decreasing. The change of the potential per time is the scan rate v . As can be seen from the mathematical definition (see equation 4.12) this is the slope of the linear potential.

$$v = \frac{\partial E}{\partial t} \quad 4.12$$

At the start the potential is usually in a region where no electrochemical reaction is occurring. The linear sweep of the potential is usually chosen in such a way that the potential crosses the formal potential of the investigated species (see Figure 4.16). After reaching a set potential the slope of the linear potential is inverted, that is a decreasing becomes an increasing potential and vice versa. This potential is called the vertex potential. One cycle is finished when the potential reaches the starting potential again. It is possible to repeat this process several times. The intention behind multiple cycles is often to observe the stability of a system. Modern software usually offers the option to choose two vertex potentials and a start potential, that is the potential sweeps between the two vertex potentials and starts at a potential between these two.

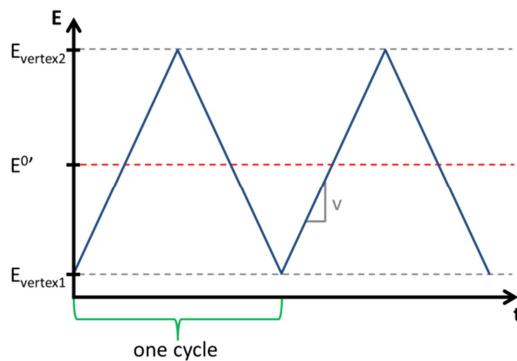


Figure 4.16: potential vs time during a cyclic voltammetry with indicated vertex potentials (E_{vertex}), formal potential of the investigated species (E^0'), and scan rate (v)

The prediction of the current signal has to take a few influences into consideration. Sweeping the potential will change the composition of the redox active species in front of the working electrode according to the Nernst equation (equation 3.6, p. 7). This change is done by oxidation or reduction and leads to a current flowing. If there would be no diffusion limitation, the measured current would have a sigmoidal shape (see Figure 4.17), but in real systems there will be diffusion limitation. If a species is

4.4 Detection of Multiple Heavy Metals by Stripping Voltammetry

4.4.1 Goals

For many lab technicians quantitative analysis is an everyday task. Monitoring processes, product quality, etc. are common tasks for electrochemical analysis.

With raising awareness of pollution monitoring heavy metals in water reservoirs, pipes, rivers, etc. is getting more and more important. Heavy metals are very suitable for electrochemical detection and very low concentrations can be measured. Quantification limits in the range of parts per trillion (ppt) have been demonstrated. The experiment and its discussion should teach:

- Why care about heavy metals?
- How does stripping voltammetry work?
- How can multiple analytes be detected in one experiment?
- How to operate the analytical mode of PStrace / PStouch?

4.4.2 Introduction

4.4.2.1 Why Care about Heavy Metals?

For a number of decades pollution has been a widely known issue. There is an increased public interest in unhealthy or outright toxic substances in resources we use for creating everyday goods or even consume ourselves, for example water, meat, milk, vegetables. Because of this research focused on these topics brings a lot of funding opportunities. Spoiled water is the cause of many illnesses and other health issues. Monitoring drinking water is an important task to keep people healthy. While for surface water bacteria are the main threat, in almost all urban areas water is consumed after flowing through pipelines. This or the direct pollution of ground water can lead to heavy metals in drinking water. Many heavy metals are biologically active and cause illnesses. A well-known example is lead poisoning.

Originally the term heavy metals referred to metals with a higher density than iron, but nowadays the term heavy metals is used in general as a term for all metals that are toxic for nature.

The majority of heavy metals released into the food chain and water is due to human intervention. Most heavy metals are present in the earth's crust, but mining is bringing them to the surface. Acid rain caused by air pollution or by other exposed minerals dissolves the metals from the ore and carries it into rivers and plants. Sometimes wastes containing heavy metal are disposed directly into rivers or oceans. All this had quite some impact on humans. The metal is not removed from the food chain but stays in it. And since the industrialization the heavy metal pollution has heavily increased. Many examples of the negative consequences of exposure to heavy metals are known. Mining waste containing cadmium was disposed in a Japanese river in 1910. As a result people eating the rice grown with river water got soft bones and suffered from kidney failures. The Mad Hatter from the book *Alice in Wonderland* goes back to the expression "mad as a hatter". The expression most likely derived from the negative effect of mercury used during manufacturing felt hats. Women drinking mercury stained water, due to mining wastes, gave birth to mentally disabled children. Lead is known to be poisonous since the times of ancient Greece and Rome. However, for 2013 the World Health Organization still estimated 143,000 deaths due to lead poisoning. Arsenic is also a rather common but highly toxic element. In ancient times only slaves would mine arsenic, because it was a sure death penalty. In 2014 thousands of people in Asia died due to arsenic poisoned ground water.

Monitoring the heavy metal content of food and water is important to prevent many deaths and identify the sources of poisoning.

4.4.2.2 Multiple Species Analysis with Square Wave Voltammetry

If an analytical process has become standard procedure and is widely used in laboratories or handheld devices, the time efficiency becomes an important cost factor. Every improvement of the measuring time, that is shorter times, will lead to lower costs for the process itself. Parallel analysis of multiple analytes is a good way to save time. In many biosensing devices like DNA-chips or other lab-on-a-chip devices different bio recognition elements are immobilized on very small electrodes and each electrode will start its own process to identify one analyte. This way only one sample needs to be applied for multiple analytes, but a device capable of measuring multiple electrodes is necessary. Multiplexers, which are switching a single potentiostat between different electrodes, are an economical solution. However, it is still one potentiostat and only one electrode can be measured at the same time. This means there will be

4.5 Detection of Glucose with a Self-Made Biosensor Based on Glucose Oxidase

4.5.1 Goals

Most electrochemical devices with low current (< 1 A) and potential ranges (< 10 V) are used for analytical methods. For this reason several experiments concerning the detection of different analytes are included in this kit. For many lab technicians quantitative analysis is an everyday task. Monitoring processes, product quality, etc. are tasks that are performed very often. New methods or improvement of the old ones promise cheaper or faster execution of the analysis.

Biosensors exploit biomolecules for sensing purposes. They have a high selectivity and sensitivity. They can be built for various illnesses or other analytes. Though biomolecules are often difficult to handle and need special care, the number of developed biosensors is increasing. The experiment and its discussion should teach:

- What is a biosensor?
- What are the basic principles?
- How can a biosensor be made?
- Why detect glucose?

4.5.2 Introduction

4.5.2.1 What is a Biosensor?

A biosensor uses a biological recognition element to convert a chemical signal into an electrical one. This very general concept is illustrated in Figure 4.46. This conversion can be achieved in many different ways. Biological recognition elements can be enzymes or parts of immune systems. An enzyme is a biological catalyst. It speeds up reactions or makes them possible under given conditions; additionally enzymes are very selective for certain reactions and only convert a specific substance or group of substances. The immune system of a complex organism like a mammal produces antibodies as a reaction to biological material from outside the body. Antibodies bind to this biological material specifically. The transducer element of a biosensor converts the reaction at the recognition element into an electrical signal. A very common method is using an electrode as a transducer. As introduced in chapter 4.3.2.3 p.40 a free diffusing redox mediator is able to transport electrons from a redox enzyme to the electrode, thus forming the transducer together with the electrode.

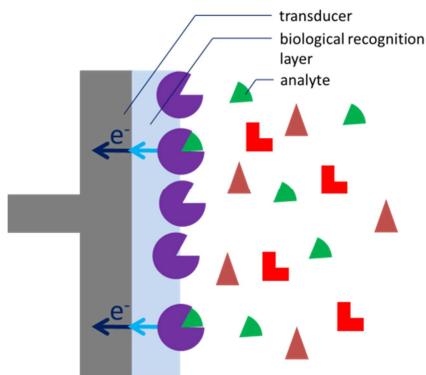


Figure 4.46: Scheme of a generic biosensor

Although the free diffusing mediator in combination with the free diffusing enzyme works in small scales, they are not suitable for a commercialized version of a biosensor. The mediator and enzyme will be washed away and the sensor can only be used once and only in small defined volumes. A better system is an immobilized biological recognition element. If a mediator is necessary, it should also be immobilized. The immobilization of a biological recognition element needs to be performed in a way that the enzyme, antibody, etc. keeps its activity. If the protein loses its structure due to the immobilization or if the way of the substrate from the bulk to the enzyme is blocked, the sensor will not work. Depending on the sensing system the movement of the mediator, products of the enzymatic reactions as well as the counter ions, has to be considered during a biosensor design as well.

4.6 Detection of Hydrogen Peroxide with Selfmade Prussian Blue Electrodes

4.6.1 Goals

Most electrochemical devices with low current ranges (< 1 A) and potential ranges (< 10 V) are used for analytical methods. For this reason several experiments concerning the detection of different analytes are included in this kit. For many lab technicians quantitative analysis is an everyday task. There is always a demand for new methods or improvement of old ones because less time or less costs for a test to monitor processes or product quality will save a big amount of money. Catalyzed reactions are often important for these kinds of sensors, to make them selective and to make their power consumption low. The experiment and its discussion should teach:

- What is Prussian Blue?
- Why are catalysts used for sensors?
- How to deposit Prussian Blue?
- What is a calibration curve?
- How to make a calibration curve?
- How to use a calibration curve for quantification?

4.6.2 Introduction

4.6.2.1 Why detect Hydrogen Peroxide?

Most likely hydrogen peroxide (H_2O_2) is best known for its use in hair bleach. It is the simplest peroxide (a compound with an oxygen-oxygen single bond). It is also a strong oxidizer. Due to its oxidizing properties, hydrogen peroxide is often used as a bleach or cleaning agent. It is used for bleaching hair, fabrics as well as teeth, disinfection of food packaging, medical equipment or to remove fungus. The artificial word "active oxygen" in advertisements for detergents usually indicates hydrogen peroxide mixtures. In many drugstores hydrogen peroxide solutions are available for removing blood stains or similar stains.

The oxidizing capacity of hydrogen peroxide is so strong that it is considered a highly reactive oxygen species. Concentrated hydrogen peroxide, or "high-test peroxide", is therefore used as a propellant in rocketry.

In fuel cells hydrogen peroxide is an unwanted side product. To achieve a high energy yield oxygen should be reduced directly to water consuming 4 electrons. If the reaction stops at hydrogen peroxide, only 2 electrons are accepted.

Organisms also produce hydrogen peroxide naturally as a by-product of the oxidative metabolism. A well-known enzymatic reaction is the oxidation of glucose by glucose oxidase. The produced hydrogen peroxide can be used to detect the glucose indirectly. Oxidases use the normally present oxygen as an electron acceptor and thus produce hydrogen peroxide after oxidizing their substrate. Consequently, nearly all living things (specifically, all obligate and facultative aerobes) possess enzymes known as catalase peroxidases, which harmlessly and catalytically decompose low concentrations of hydrogen peroxide to water and oxygen.

Accordingly hydrogen peroxide detection is useful for fuel cell catalyst research, for controlling the amount of disinfectants used, for waste water control and as basis for a biosensor with a hydrogen peroxide producing enzyme.

Hydrogen peroxide has two disadvantages: Its high oxidative power makes it unstable and corrosive. Because of these characteristics it qualifies as a propellant, but it also can lead to an explosion of high concentrated hydrogen peroxide. And hydrogen peroxide can also damage the skin. Its corrosive influence can be easily identified by white spots on your skin accompanied by a burning or stinging feeling. Washing the skin with a lot of water is advised. After a short period of time the pain should disappear, but the white spots may remain for a few days. The instability is an issue as well, despite the fact that many hydrogen peroxide solutions have additives to stabilize the peroxide, hydrogen peroxide solutions should be stored cold and dark, otherwise the peroxide might decompose fast. This has the advantage that hydrogen peroxide can easily be removed from any solution by boiling it for several minutes.