AdUp Sense

Uppsala University

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1. Summary for the SensUs website

Typical procedures to measure analytes in patient's blood such as ELISA are expensive, lengthy and require specialized personnel. Thus, the need for cheap, reliable and simple to use devices in healthcare applications is driving the development of handheld biosensors.

Electrochemical immunosensors are currently quite trendy due to the potential for a simple, reliable method which can be adapted to a series of molecular recognition systems, ranging from antibodies to enzymes and even aptamers.

Briefly, the biorecognition element is immobilized on a conductive surface via covalent bonds, ensuring reproducibility and potentially reusability. Afterwards, a sample is placed on the surface and allowed to incubate for 20 minutes. The sensor is now ready for a measurement, which takes about 30 seconds. The readout is based on differential pulse voltammetry, which measures the current intensity as a function of the potential at the electrode interface.

While detailed mechanisms of this process are not completely understood, a combination of certain parameters including surface coverage and antibody complex surface charge is thought to affect the current intensity. Once calibrated, this signal can be used to infer the analyte concentration.

2. Biosensor System and Assay

Our system consists of graphene oxide (GO) coated screen-printed electrode (SPE) modified with a capture antibody specific for Humira. Surface modification, as well as antibody binding events, lead to changes in the electrochemical properties of the electrode surface which can be used to determine the analyte concentration.

2.1 Molecular recognition assay

Our biosensor uses a capture antibody, anti-adalimumab, as the molecular biorecognition element. In order to covalently immobilize the capture antibody, the graphene oxide covered working electrode is activated using EDC/NHS chemistry. EDC couples NHS to carboxylic acid groups in GO, resulting in an NHS-ester that can then react with primary amines to form a stable amide bond (figure 2.1.) [1][2]. Unreacted active sites are then quenched with the addition of 4 µl of 0.5% BSA. Following this, the electrode is ready to perform a measurement. An analyte sample is added onto the electrode surface and allowed to incubate for 30 minutes. The electrode is then rinsed with PBS solution. Since the capture antibody is specific for adalimumab, they are able to bind together, leading to changes in the current produced at the electrode which can then be related to the analyte concentration. Finally, a redox couple such as potassium ferrocyanide/ferricyanide is applied onto the electrode to enable a proper measurement.

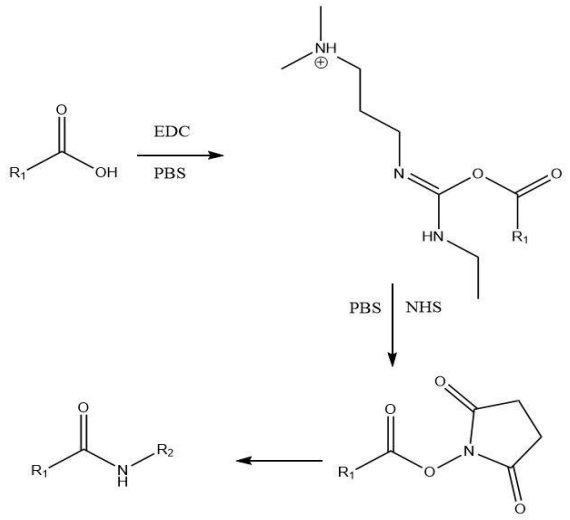

Figure 2.1. EDC/NHS chemistry. The image shows how EDC/NHS facilitates the covalent binding of an antibody to carboxylic acids.

2.2 Physical transduction

Our electrochemical immunosensor uses differential pulse voltammetry (DPV) for the readout and an equimolar mixture of the potassium ferricyanide/ferrocyanide redox couple, acting as a probe for signal generation. The current is measured as a function of time and potential between the indicator and reference electrode. As the potential is increased towards the redox potential of the probe, it starts undergoing redox and a current is hence produced up to a peak value. The current is subsequently brought into a diffusion-controlled state and as a result, the total current response takes its shape as a peak.

The immunocomplex restricts the surface availability for the redox probe, which lowers the number of redox events and therefore reduces the current signal. A summary of the principles of DPV and molecular interactions have been illustrated on figure 2.2.

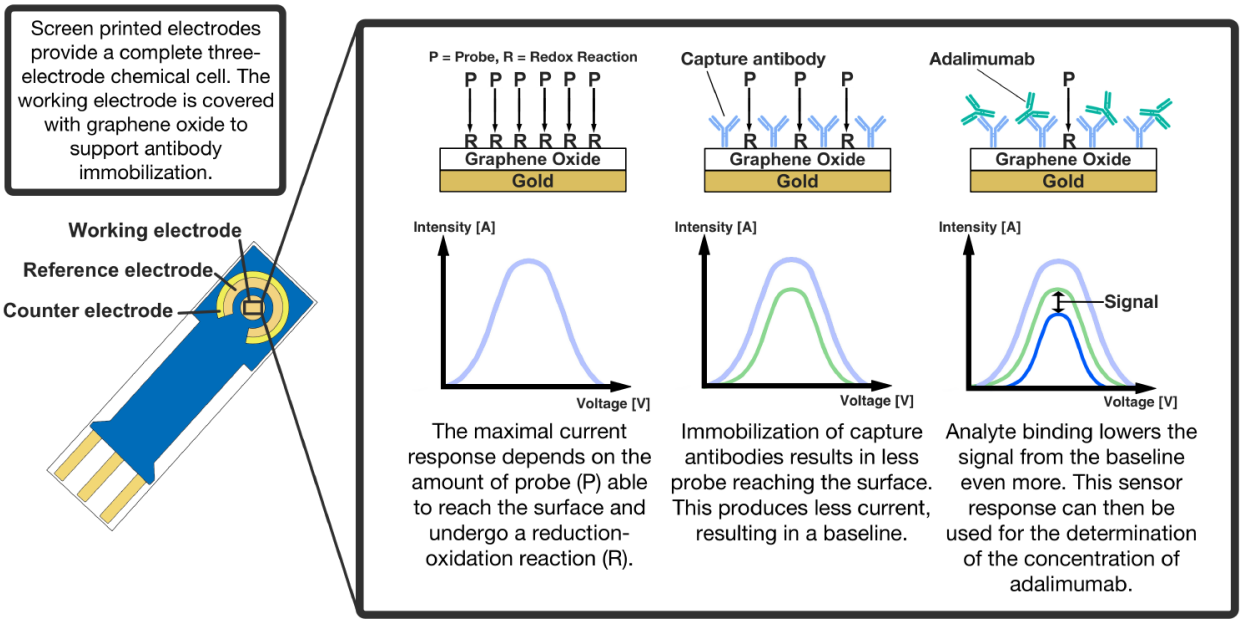

Figure 2.2 Principles of DPV. The figure illustrates the response in differential pulse voltammetry to change on the working electrode surface.

Measurements are performed after the immobilization procedure to achieve a baseline which is compared to the signal obtained after analyte incubation on the electrode.

Samples with known concentrations of the analyte are used to prepare a calibration curve which can then be used to quantify the amount of the analyte in an unknown sample.

2.3 Cartridge technology

Our screen-printed electrodes can be used as disposable. Incubation of the sample on the electrode is performed outside of the potentiostat. The sample is applied by the technician on the tabletop after which, the sample is incubated in a sealed container in 4 ºC. After sample incubation, the electrode is manually washed off with PBS into a waste container. The electrode is inserted into the adaptor on the potentiostat and quantification can be performed. After running the measurement, the electrode can simply be discarded into biological waste. Our electrode chips could potentially be regenerated and therefore, reused.

2.4. Reader instrument and user interaction

The size of our biosensor is 95mmx75mmx75mm making it compact. It consists of a sealed potentiostat and electrode chip. The only handling required by the user is related to the electrode chip described in section 2.3.

The output of the measurement can be viewed and analyzed with the PSTrace software (PalmSens). The data from known concentrations of the analyte can be used to externally produce a calibration curve which can then be used to infer the analyte concentrations of an unknown sample based on peak differences.

Additionally, we have developed an app which can measure the raw data in real-time during the measurement, using Bluetooth. The data obtained can be automatically converted to the concentration

of adalimumab in blood. The app provides graphs on current and past measurements, making the interpretation of the optimal drug usage straightforward to assess.

3. Novelty and creativity

3.1 Already available

An EmStat pico development kit was purchased from Palmsens Together with, screen-printed electrodes with a gold working electrode, a gold counter electrode and a silver/silver chloride reference electrode. Adalimumab was obtained from Biovision. An Elisa kit with anti-adalimumab was purchased from Bio-Rad. Graphene oxide was purchased from Graphene Supermarket. Ethanolamine, EDC, NHS, potassium hexacyanoferrate and human plasma were kindly provided by Merck. PBS and human plasma were kindly provided by the Immunology, Genetics and Pathology department at Uppsala University Biomedical Centre.

3. 2 New developments

Graphene and graphene-related materials have been the subject of increasing attention from the scientific community in recent years due to their interesting properties, particularly with respect to good conductive properties. However, graphene itself has several undesirable characteristics. Due to its hydrophobicity, it is difficult to solubilize graphene or make it adhere to certain surfaces. Furthermore, the lack of any functional groups and graphene's general inertness complicate protein bioconjugation approaches without resorting to harsh conditions or the use of hazardous chemicals [3][4][5][6][7].

At the other end of the spectrum, graphene oxide, produced by oxidation of graphene sheets possesses interesting functionalities such as carboxylic acids, can be dispersed in aqueous solution and displays better adherence. Unfortunately, the conductive properties of GO are inferior to that of graphene due to its imperfect structure. Thus, deciding which graphene-related material to use can become quite a challenge [8].

Recently, several research groups reported a way to harness the rich functionalities of graphene oxide while circumventing its poor conductive properties by coating another surface with improved conductivity, such as glassy carbon, with GO [9][10][11].

When designing our biosensor, we took inspiration from these approaches. We covered a gold working electrode with graphene oxide to facilitate the immobilization of a capture antibody for adalimumab on the screen-printed electrode. Our method does not require a secondary antibody to amplify the signal, thus providing a label-free technique which in principle at least can be adapted to any antibody-antigen interaction.

4. Analytical Performance

Our biosensor requires minimal sample handling. Before running a measurement, the 4 µl of sample is applied on the electrode surface and allowed 30 minutes to incubate. The next step involves rinsing

the electrode with a PBS solution, followed by the addition of 50 µl of probe solution. The sensor is now ready to perform a measurement, which takes approximately 30 seconds.

Sample volume required	4 µl
Incubation time	30 minutes
Measurement duration	30 seconds

Cyclic voltammetry was employed to validate antibody immobilization. As antibody is covalently attached to the surface, a portion of the available surface is not available for the probe to undergo a redox event. As a direct consequence, less current is produced as can be seen by the depression of the curve in figure 4.1.

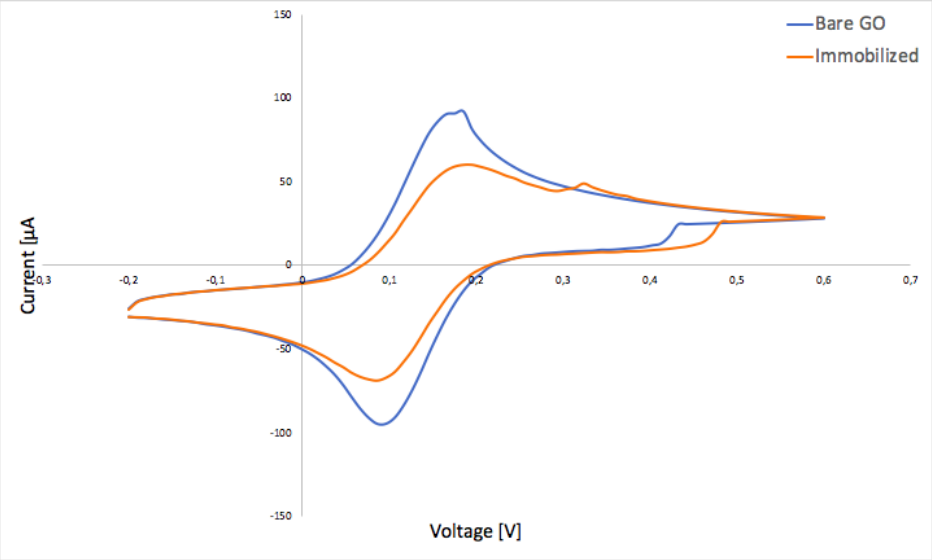

Figure 4.1. Validation of antibody immobilization. Depression of the immobilized curve from the bare GO indicates that there is surface coverage.

Upon confirming the presence of anti-adalimumab on the electrode surface, samples with different concentrations of Adalimumab in PBS were tested (figure 4.2).

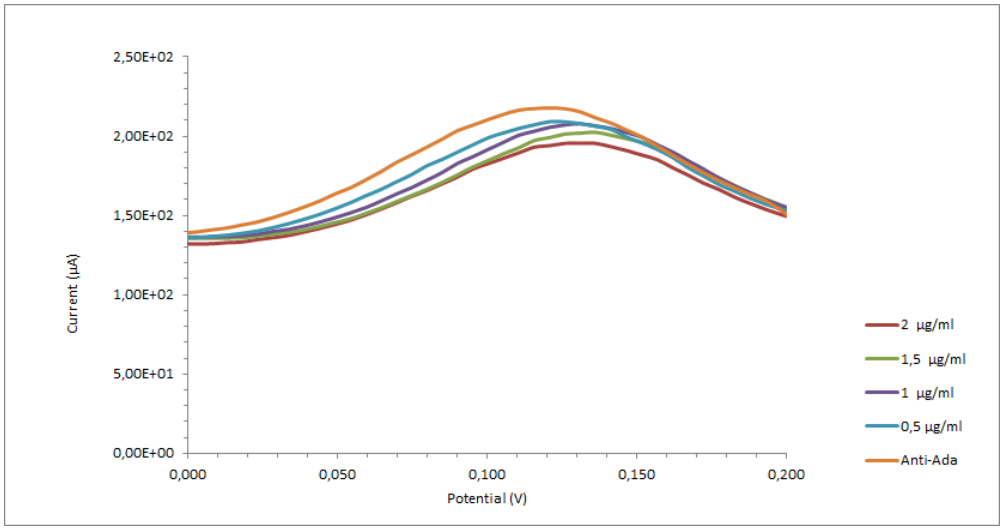

Figure 4.2. Differential Pulse voltammetry in response to Adalimumab. The analyte concentration ranged from 0.5 to 2 µg/ml in a buffer.

The results show decreasing peaks with increasing analyte concentration. The sample measurements also have a lower peak with respect to the electrode containing only the immobilized capture antibody (orange). This is expected, as the surface is covered with immunocomplex and less redox couple can pass over to the electrode surface.

The data was used to plot a calibration curve shown in figure 4.2. The results show a linear trend and the R^2 is 0.93.

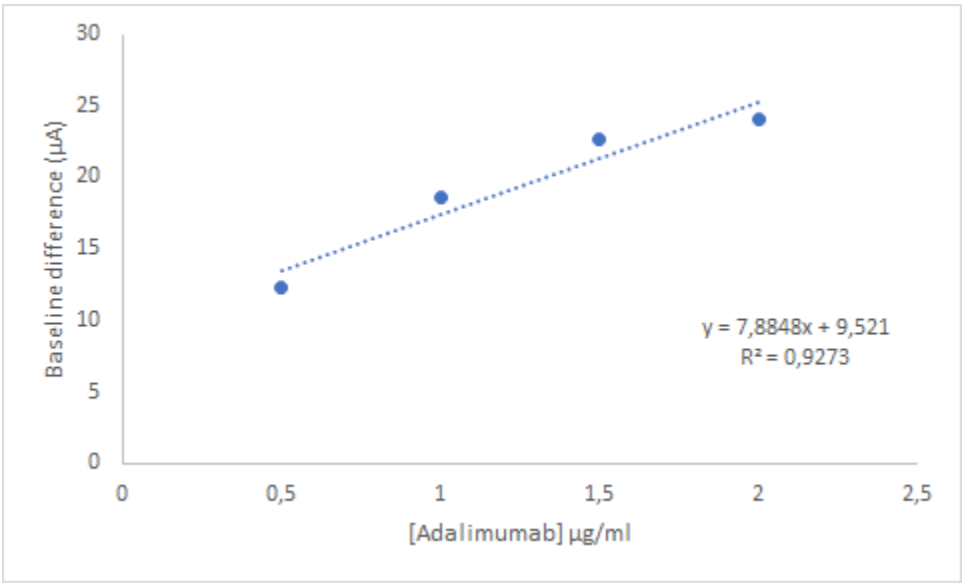

Figure 4.2. Standard calibration of Adalimumab concentration in a buffer. The values are derived from the baseline difference in DPV and concentration is shown in µg/ml.

The sensor is being optimized to be suitable for plasma measurements. This can be a challenge as plasma proteins can cause interference to the measurement, drowning the signal. Our preliminary

results show that it is hard to differentiate the antibody signal from the background noise from plasma, an issue that we hope to solve before the competition.

5. Translation potential

Rheumatoid arthritis is a disease that needs continuous monitoring, especially since the patient's immune system can form antibodies against the drug, resulting in regular visits to the doctor in order to receive optimal treatment. Furthermore, the current methods used for analyzing the drug concentration in the blood are time-consuming, expensive and require laboratory support for handling, analyzing and interpreting.

A small and simple biosensor requiring minimal handling expertise, such as the one we are developing, can provide a solution to how the tests are run today.

5.1. Business model canvas

<table>
<tr><th>Key partners</th><th>Key activities</th><th>Value Proposition</th><th>Customer relationships</th><th>Customer segament</th></tr>
<tr><td rowspan="2">• SensUs Competition
• Financial partners
• Uppsala University</td><td>•Research and design
•Production (Outer shell)
•Assembly
•Packaging and delivery
•Customer feedback</td><td rowspan="2">The device is a product that will allow the user to monitor the concentration of adalimumab (drug) in a short amount of time. The device is highly accurate and reliable. It is self-contained device, i.e. portable. No additional power source is needed.</td><td>The customer relationships will be built through:
• Automated service
• Community outreaches</td><td rowspan="2">The customer segment, mainly are:
• Hospitals
• Private clinics
• Diagnostic labs
• Individuals.</td></tr>
<tr><td>Key resources
•Intellect: Team members
•Financial: Sponsors
•Mentors and coordinators</td><td>Channels
The value proposition will be presented to the customers by:
•Communication
•Delivery
•General awareness campaign
•Internet</td></tr>
<tr><th colspan="3">Cost structure</th><th colspan="2">Revenue streams</th></tr>
<tr><td colspan="3">The entire project is value-driven as this is medical equipment.
Key activities are R&D, assembly and packaging, which will take most of capital.</td><td colspan="2">•Consumable parts
•Lending, leasing or renting.
•Licencing.
•Application Service.</td></tr>
</table>

5.2. Stakeholder desirability

Rheumatoid arthritis affects nearly one percent of the global population. According to the market report (MarketWatch, 2019), there should be a 9.7% CAGR in the global electrochemical biosensor market during 2014-2022. Research claims that by 2022, this market is estimated to reach up to a value of 23.7 billion dollars.

The value proposition of the project is based on interviews with patients and doctors. According to the customer segment (since it is a niche segment), hospitals, clinics and individuals were contacted in order to document customer pains, gains and jobs.

Customer Profile:

- Customer Jobs: monitoring drug concentrations accurately and quickly.
- Customer Pains: time-consuming, maintenance, dependent on laboratory and biomedical analysists.
- Customer Gains: easy interpretation of results and measurements; less expert knowledge requested; time-saving; less maintenance and labour work needed.

Value proposition map:

- Product and service: AdUpSense sensor will help the patient to monitor their drug levels with the help of a mobile application, and update their respective doctors, just in case for a consult.
- Pain relievers: Minimal assistance needed (Digital assistant provided in the application), Low setup or installation of the device, User-friendly/easy to operate, Monitoring and reminders through the application, detailed notes and analysis from physicians.
- Gain Creators: Application makes the results of analysis available in a shorter time. Product is less expensive than other tests, highly accurate, direct communication between doctors and patients, time-saving and also wireless.

Keeping these aspects in mind a strategic fit was achieved within which the sensor was developed to satisfy all the customer needs and increase their gains and relieve the pains.

5.3. Financial Feasibility

The total initial investment (considering overhead cost) is 900,000 Euros. The material and hardware required for the production of one biosensor costs as follows:

Potentiostat: 750 euros; Electrodes (replaceable): 3 euros; Application development and service: 10 euros/unit

The total Production cost of one biosensor: 763 euros.

The sales price of the sensor is set at 930 euros.

Assuming the rate to be 7% and taking the initial sales target to be 1000 sensors per year,

Net present value= 394,009 euros

Payback period= 4.71 years.

Internal Rate of Return (IRR)= 16%

Thus, calculations show that this is a long-term project with a good rate of return.

The product will be sold by the team's device representatives by contacting all the hospitals and doctors, private clinics and labs. The revenue is mostly generated by application services and consumable parts (electrodes). Routine maintenance of device and assistance will also generate a periodic revenue, however, to gain traction this service may be given as free for the first two years.

The business plan is developed for five years. The first year is focused on launching the product in the Scandinavian market, next two years in Europe and the fourth- and the fifth-year plan is primarily made with the launch and establishing the product in the western market. The strategy is bifurcated

into several parts because each market has its own set of laws and regulations which should be followed.

5.4. Business Feasibility

The key resources needed to make this plan feasible is intellect/expertise and financial support. The team members have sufficient knowledge and expertise to back this project and carry it forward. The manufacturing of potentiostat and screen-printed electrodes is mainly outsourced thus, the key activities include assembly, research and development, design and packaging. As the project scales up in five years according to the business plan, recruiting of right talent will also be essential for success. This will be done by our reliable mentors (also a key resource).

Our partners provide us with the initial platform needed by AdUp Sense to launch into the market. Uppsala University provided the space and resources needed for the design and the development of the sensor. Financial partners such as Merck provide capital and other resources that are needed to launch into the market. And finally, SensUs competition is the platform form where the campaign and marketing/promotion of the device will start. The scaling up of this project is spread over five years and major markets such as Europe and West have different requirements, which will be developed according to the needs of customers and expert's advice.

6. Team and support

6.1 Contributions of team members

Kedar is one of the team captains and his contributions range from keeping track of deadlines, helping out in the lab and technical design. Without him, we would forget to submit reports, attend meetings and fail to organize ourselves.

Luís is the second team captain, and his main contributions include literature research, working in the lab and overseeing the general scientific development of the biosensor.

Without Triinu's diligence and work ethic, we wouldn't have anything resembling a biosensor. When experiments failed, she was already a step ahead, looking for solutions and new ideas to explore. Triinu also participated actively in managing social media accounts.

Likewise, Michael's passion and lighthearted manner made all the hard work that much easier. His main contributions are literature research, lab work and bothering the right people for advice with our sensor development.

Esther and Adarsh's contributions are mostly related to the business plan of our biosensor. Adarsh also helped with several technical aspects of our biosensor design and Esther contacted doctors for insight on the current treatments for rheumatoid arthritis. They helped us customize our biosensor for the right market and made our business plan a reality.

Weam and Fawad's contributions are mostly related to the app development and its integration with the biosensor hardware, as well as general technical support. Without them, we would still be wondering how to turn the EmStat on and to connect it properly, let alone set up the Bluetooth.

Securing sponsorship from Merck would not have been possible without Therese, whose professionalism is beyond reproach. She also contributed to our team's visibility on social media and other technical aspects. Most of all, she never failed to make us look good on any occasion.

Anna contributed with literature research and lab work, the AdUpSense logo design and other technical aspects of our project, as well as our team's visibility on social media.

Duc's enthusiasm ranges from contagious to mildly annoying, but all agree that without him we would still be wondering what the graphs in the PStrace software meant. His knowledge of electrochemistry aside, he also contributed to literature research and lab work.

Spandana contributed mostly with literature research, lab work and biosensor design and in making this whole endeavour much more fun.

6.2 People who have given support

Our project wouldn't have been possible without the support provided by several people, and we hope we aren't forgetting anyone.

Professor Leif Nyhom's knowledge of electrochemistry was invaluable in our biosensor design. Without his input, our development process would have suffered.

We would like to thank professors Gunnar Johansson and Helena Danielsson for their insight into antibody-antibody interactions. They helped us understand some of the flaws in our design

Dr. Lars Gedda's input on our ELISA experiment helped us improve our experiments. Thanks to him, we managed to validate our capture antibody.

Javier Cruz for the advice, insight and input he gave us as well as all the assistance he provided.

We would like to thank Ehsan for is assistance in the biology lab. Without him, we wouldn't be able to find our way. Sorry for making you come to the lab on the weekends.

Finally, we would like to thank our project supervisors, Masood, Atena and Gemma, who accompanied us throughout these 9 months, for their support, criticism and most of all for being there.

6.3 Sponsors

We would like to acknowledge the kind assistance and professionalism of both Merck and PalmSens.

During our project, we contacted Merck, who kindly agreed to provide us with most of the chemical we needed for the development of our biosensor.

We first contacted PalmSens during the meet the partners' event. They provided us with the potentiostat we needed to perform our measurements and optimize the process as a whole. Our screen-printed electrodes were also purchased from PalmSens, and the adapters for the latter were generously included with them. We would like to leave an additional note of appreciation to PalmSens for their lightning-fast deliveries.

Finally, we would like to extend our appreciation to Uppsala University for the funding as well as the space provided.

7. Final Remarks

We thank the SensUs Organization for creating a platform where young scientists and engineers have an opportunity to conduct a research project related to a real-life problem. It has been a tremendous learning experience and the skills developed during this competition will benefit us throughout our career.

8. References

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