Team Results Document
WDsense

Wageningen University & Research + Technical University Delft

Team members:

David Feith
Erwin Hogeveen
Jochem Feith
Joël Vandervinnen
Nele Jarnot
Valérie Hogenes
Victor van der Doorn

Supervisor:

Dr. Johannes Holbein
Dr. Vittorio Saggiomo

Coaches:

Rio Pals

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SensUs

SensUs 2024
Acute Kidney Injury

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1. Abstract

Elevated creatinine levels are a critical biomarker of kidney disfunction and failure. In our designed biosensor we employ electrochemical sensing technology to provide real-time, continuous monitoring of creatinine levels in interstitial fluid. For our proposed sensor we use an enzyme-based approach in which the high specificity of the chosen enzyme for creatinine allows for accurate measurements.

Our biosensor consists of a glassy carbon electrode functionalized with the enzyme Creatinine deiminase. This protein reacts with Creatinine in which Ammonium is released as a byproduct that can interact with the electrode's polymer coating. This leads to a change in the conductivity on the electrode surface which is electrochemically measured. Gold nanoparticles deposited on the electrode's surface are incorporated to increase conductivity. By relating the change in conductivity to the amount of converted Ammonium, the creatinine concentration can be inferred.

Thus far, we have successfully functionalized the electrodes and we are working towards achieving more reliable measurements of creatinine levels. In the future, we hope our designed biosensor can enable timely medical intervention, ultimately improving patient outcomes and quality of life.

2. Biosensor

Molecular recognition

The WDsense creatinine biosensor consists of a glassy carbon electrode functionalized with the enzyme creatinine deiminase to convert creatinine to NH_4^+ and a polymer layer that interacts with the formed NH_4^+ via redox reactions. This interaction causes a change in current that can be measured using a potentiostat.

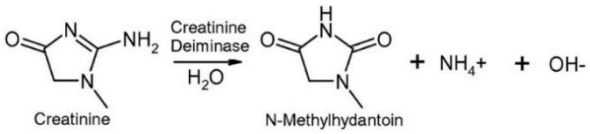

Figure 1 Creatinine Deiminase reaction (Do et al., 2018)

Creatinine deaminase is an enzyme that catalyses the reaction of creatinine to N-Methylhydantoin and NH_4^+. An enzyme-based sensor is chosen due to the high specificity of enzymes for their substrates, which allows for accurate detection. Creatinine, in particular, is challenging to detect using conventional, cost-effective methods,(Do et al., 2018) making enzyme-based sensing a valuable approach.

Another method that relies on enzymatic reactions to detect creatinine uses a combination of three different enzymes to eventually convert creatinine to the electrochemically detectable H_2O_2 (Killard & Smyth, 2000). The use of three enzymes consecutively, however, is complex and costly. Creatinine deiminase can convert creatinine into NH_4^+ which can be measured electrochemically after an additional step.
Polyaniline is a polymer of the aniline monomer. It is a conductive polymer that can be used to construct an ion-specific film. This modification is done by electrodeposition of polyaniline to the working electrode surface. Polyaniline has multiple redox states and can interact with NH_4^+ which causes a change in conductivity on the electrode surface (Farina et al., 2024).

The polymer is present on the surface after electrodeposition in the emeraldine salt (protonated half-oxidized) form. When the salt is submitted to a current of 0.2V and present in neutral pH it loses protonation and is present in the emeraldine base (EB) form Figure (2), right. This EB form forms a complex with NH_4^+ in solution (Equation (S.1) in the supplementary information), NH_4^+ will occupy empty protonation sites and form hydrogen bonds with the polymer. The formation of this complex causes an increase in oxidation current, that can be measured electrochemically. Because the NH_4^+ is oxidized and the current applied at the same time (Equation (S.2) in the supplementary information), the formed electrons and protons drive a simultaneous reduction of the base form of the polymer. This results in a protonated reduced form of the polymer (Leucoemeraldine Figure (2), left), that is subsequently oxidized again to the $PANI_{EB}$ form (Equation (S.3, S.4)) (Korent et al., 2022). The polymer is then able to form a complex with new NH_4^+ of the following sample.

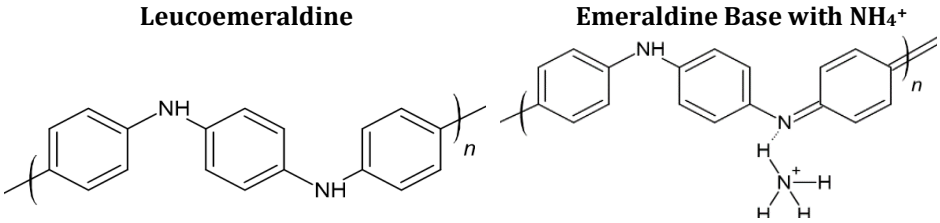

Figure 2: This figure shows the relevant redox states of poly-aniline, with on the left leucoemeraldine and on the right emeraldine base with NH_4^+

Physical transduction

The physical sensing principle of our biosensor is based on electrochemical detection using a functionalized Glassy Carbon Electrode (GCE). The detection process is currently achieved through cyclic voltammetry (CV) but the future intention is to apply chronoamperometry, in which the current response as a function of the applied voltage is measured over time to monitor redox reactions at the electrode surface.

The functionalized GCE consists of several layers, each playing a vital role in the specific recognition and transduction of creatinine into a signal. The layers are reviewed in a top down manner, see Figure (3).

1. Embedded Creatinine Deiminase (CD): Creatinine is immobilized into a polymer via crosslinking bovine serum albumin (BSA) and glutaraldehyde (GA) by dropcasting it as a solution on top of the synthesized PANI layer.
2. Poly-aniline (PANI): On top of the Nafion layer PANI is synthesized. PANI is a redox-active polymer that plays a role in the sensor's electrochemical readout.
3. Nafion and Gold Nanoparticle (AuNP): A 2% wt. Nafion with AuNP solution is dropcasted or electrochemically deposited onto the GCE and dried to form a layer. Nafion is a cation exchanger and serves as a charge compensator during the polymerization of PANI (Ying-Tai Shih et al., 1999) and in addition, it serves as a ''glue'' for the subsequent layer. The AuNPs were added to the layer to improve the conductivity of the GCE.

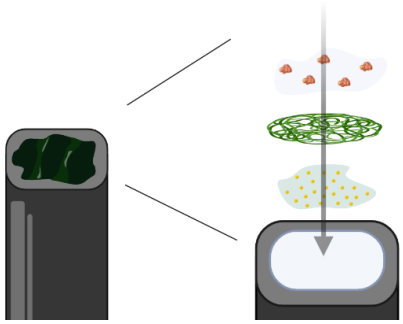

Figure 3: A graphical top-down overview of the functionalized layers on the Glassy Carbon Electrode.. The top layer consists of a mixture of GA and BSA, for the immobilization of the CD-enzyme. The second layer is composed of PANI layer, which is adhered to the electrode via the third layer, made of Nafion. Embedded in this layer are AuNPs.

For the reaction, a sample is loaded into the sensor in which the creatinine is converted into the N-methyl hydantoin and ammonium at the top layer of the functionalized GCE. As mentioned, the redox-active PANI undergoes oxidation and reduction in the presence of ammonium, which causes a conductivity change that can be measured by using a potentiostat via Chronoamperometric measurement. Because the peak area is proportional to the concentration, creatinine levels in the samples can be determined.

Cartridge technology

In our current setup, we do not have implemented a cartridge technology yet. To this day, we are still in the testing phase of our sensor. We are trying to improve and refine our general concept because it is not yet working the way it is supposed to. Currently, we apply what we call the "droplet method" (see Fig. 5). The functionalized working electrode and reference electrode are fixed approximately 0.5 cm apart from each other. In the gap between the electrodes, the analyte (70 µl) is placed by pipetting and held in place by the droplet's surface tension. The counter electrode is positioned inside the droplet. Consecutive measurements are performed by removing and adding a new analyte drop with a pipette.

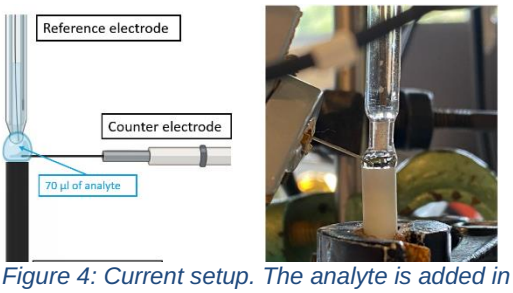

Figure 4: Current setup. The analyte is added in between the functionalized working electrode and the reference electrode. Surface tension holds the analyte droplet in place inside which the counter electrode is placed. Consecutive measurements are performed by placing and removing the droplet by pipetting.

This "droplet method" is a simplistic but effective approach for the current testing phase of our sensor. Nevertheless, in future applications, the setup with the analyte and electrodes should be fully shielded allowing reliable usage. The cartridge design we want to implement in the future is a PDMS-based microfluidics setup fabricated using the "Embedded SCAffold RemovinG Open Technology" (ESCARGOT) (Saggiomo et al., 2015). A measurement chamber will hold the analyte during measurement. In this chamber, reference and counter electrodes will be embedded. There will be an extra port for the working electrode such that it can be removed if polishing or functionalizing is required. A pressure pump will be used to flow the sample inside the measurement chamber. Incoming liquid will displace the old one out of the measurement chamber.

Reader instrument and user interaction

As a reading instrument, the PalmSense Emstat pico Development kit was chosen. This kit is used to run measuring attempts and read out data via the provided interface on which the final value of the creatinine concentration is based. PStrace Version 5.10 was used as the software that offers an easy-to-use analytical setup from which cyclic voltammetry measurements can be made. The measured data can be read out and followed live with this software or be read out in an Excel file or data frame. With the help of premade data set with known concentrations a calibration curve will be used as the main source of concentration identification.

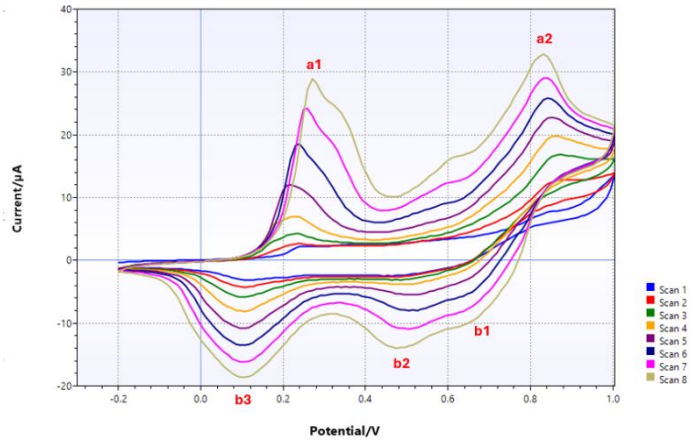

Figure 5: Cyclic voltammogram of PANI deposition, which shows the oxidation transitions, a1 and a2, and the reduction transitions b1, b2 and b3.

3. Technological feasibility

The process of the biosensor development can roughly be divided into two parts, the full functionalization of the working electrode (PANI and enzyme) and performing the measurements.

Firstly, in literature is found that the electrodeposition of the polymer on the electrode using CV displays distinct reduction and oxidation peaks in the cyclic voltammogram (Farina et al., 2024). These peaks show the oxidation and reduction of the polymer. In our experiments, those same distinct peaks also occur, (see Figure 5). This concludes that our PANI functionalization is successful. After electrodeposition, the polymer is present in the emeraldine salt form, which has a distinct green color. This can also be seen on our functionalized GCE, depicted in Figure (S.1) in the supplementary information.

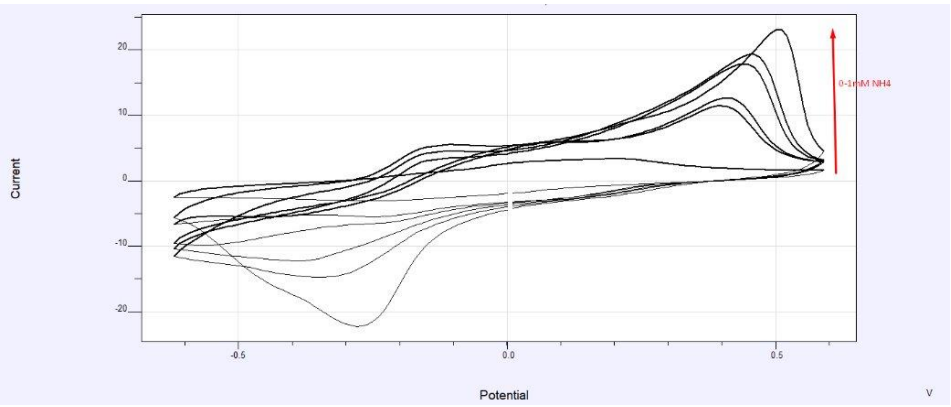

Figure 6: Cyclic Voltammogram of increasing NH_4^+ concentrations on the fully functionalized GCE. The concentrations are: 0 mM, 0.1 mM, 0.2 mM, 0.5 mM, 0.6 mM and 1 mM. The red arrow depicts the increase of the PANI oxidation peak in correlation to the increase of NH_4^+ concentrations.

After successfully functionalizing the GCE, we conducted measurements of pure NH_4^+ samples in PBS buffer (pH = 7.4) at varying concentrations; 0 mM, 0.1 mM, 0.2 mM, 0.5 mM, 0.6 mM, and 1 mM. This result, depicted in Figure (6), demonstrates a correlation between the increasing concentration of NH_4^+ and the oxidation peak of PANI (Red arrow). This trend confirms the sensor's sensitivity to NH_4^+. However, this is not where we expected the oxidation peak to occur, according to literature it should take place at 0.2 V (Farina et al., 2024).

We attempted to reproduce the NH_4^+ measurements under the same conditions, but unfortunately, we were unable to replicate the initial results. Despite following the same procedures, the expected correlation between NH_4+ concentration and the oxidation peak of PANI was not consistently observed, which indicates possible experimental inconsistencies. Different experimental parameters were tested to see if the desired result could be replicated, like adjusting the range of the scan, faster or slower scan rate and adjusting the thickness of the nafion layer. Eventually, it was concluded that it has to be an issue in the hardware. Namely the reference electrode. In the meantime experiments with creatinine continued some peaks were observed when conducting a cyclic voltammetry measurement, however, these results are still inconsistent and not reproducible. Recently a reduction peak was observed quite consistently in measurements with creatinine, in addition a relation was observed between concentration and position (potential) of the peak (see Figure 7 and 8), which is a promising result.

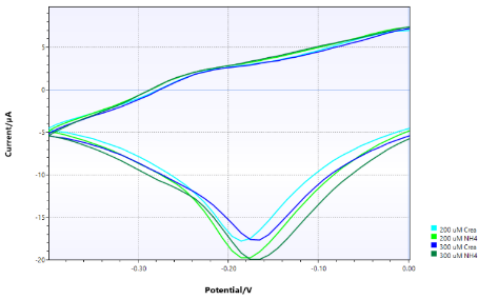

Figure 7: part of a Cyclic voltammogram showing reduction peaks for different concentrations NH_4^+ and creatinine, measured using a GCE working electrode with PANI deposition and immobilized creatinine deiminase.

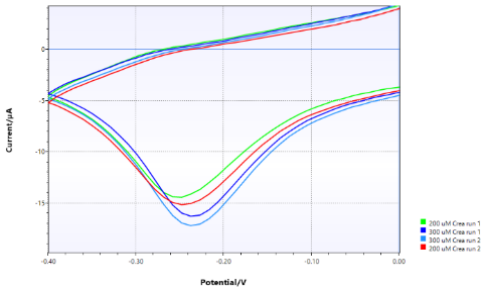

Figure 8: part of a Cyclic voltammogram showing reduction peaks for different concentrations creatinine, measured using a GCE working electrode with PANI deposition and immobilized creatinine deiminase.

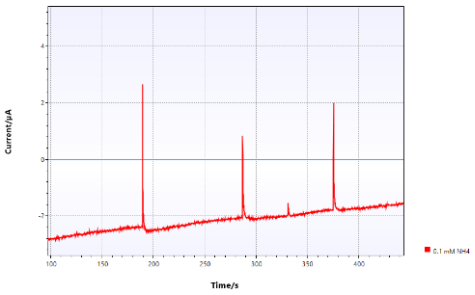

Figure 9: Chronoamperiometric measurements of 0.1 mM NH_4^+ over time in 1 µL to the 70 µL droplet.

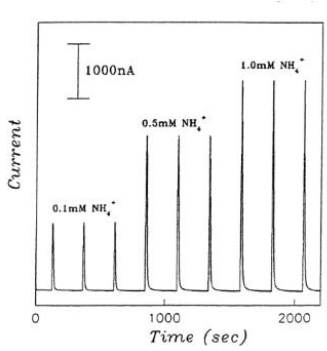

Figure 10: Chronoamperiometric measurements of 0.1 mM, 0.5 mM and 1 mM NH_4^+ using a continuous flow from (Ying-Tai Shih et al., 1999)

We also tried to conduct Chronoamperometric measurements by applying an oxidation voltage of 0.2 V. At this voltage PANI was fully converted into its emeraldine base form, which resulted into a baseline. When NH4+ was introduced, it occupied the free unprotonated nitrogen atoms, leading to its oxidation and the subsequent reduction of PANI back to the leucoemeraldine form. Due to the constant 0.2 V applied, PANI was then re-oxidized into its emeraldine form. This redox cycling generated a spike in the current measurement, which returned to baseline after the reaction was complete. The height or area under this spike was found to correlate with the concentration of the NH4+ analyte.

We attempted to replicate this approach by injecting small volumes of 0.1 mM NH_4^+ solutions in our 70 µL drop. While we did observe increases in current, as seen in Figure (9), the results were not as consistent as those reported in one of the studies, seen in Figure (10). We can conclude out of this that the sensor works, yet not consistent enough.

We have returned to the basics by thoroughly cleaning and restoring the Ag/AgCl reference electrode. Fortunately, this does not mean starting from scratch, as we've already performed bare electrode measurements that confirmed the functionality of the three-electrode system. Since we have previously achieved successful full functionalization, the upcoming testing period should be manageable.

Our plan is to first remeasure a range of NH4+ concentrations to determine if we can now obtain reproducible data. Once we confirm the consistency of these measurements, we will proceed to test creatinine samples, which will allow us to create accurate calibration curves. We do not anticipate significant challenges in testing with ISF. The use of creatinine deiminase ensures specificity for creatinine, which should minimize interference from other components. During our DTE experiments, we did not observe any unusual artifacts from other cationic ions present in ISF. With this, we are optimistic about successfully moving through this phase in the near future.

4. Originality

By the team captains

Continuous biosensing itself is a novel way of sensing, it opens up the possibility for monitoring biomarkers, offering the potential for real-time tracking, minimal invasiveness, and seamless integration into patients' daily lives. For our team this was a very exciting new challenge because of the future applications of such a biosensor.

Obviously this was also an added challenge with respect to the point of care biosensors that where developed all previous years. Mainly the timeframe of the challenged proved difficult. Lateral flow assays, that where the core of the method used by our teams in previous years where not feasible. So we were in uncharted territory. Particularly in an area where our university lacks expertise in electronics. To address this, we sought collaboration with motivated students from Delft, and although we found only one collaborator, his insights significantly broadened our perspective and helped us think beyond our usual scope.

Our biosensor concept is novel in the way that it combines enzymatic conversion of the biomarker, and the utilization of a conductive polymer for electrochemical sensing in complex media. Although the development of a fully working continuous biosensor was somewhat out of our league we are very proud of our concept and the progress we have made so far.

We could not have done it without the help of our supervisors, partners and coaches.

By the team's supervisor

This year's competition brought two major changes for the WUR team. First, as the test requirements were changed towards continuous monitoring, the lateral flow immunoassays that had been used successfully for the last two years by the WURk team had to be discarded. Secondly, the team in Wageningen decided to collaborate with students from the Technical University in Delft, thereby increasing the complexity of working together over two locations.

This year, the students decided to employ functionalised glassy carbon electrodes detecting a creatinine deaminase reaction. Earlier ideas on utilising screen-printed electrodes (SPEs) were discarded due to their limited re-usability. Notably, none of the two main supervisors (Hohlbein and Saggiomo) have much experience with the utilised technologies so the students had to find the required expertise elsewhere. Both the chair group "Biobased Chemistry & Technology Group" in Wageningen and the Rwei group in Delft played an important role in providing support in electrochemistry and conceptualisation of the sensor. Further contacts with the company PalmSens, which specialises in electrochemical instrumentation, enabled specific support and product discounts.

We were delighted to learn that the team won the distribution testing event, highlighting the capabilities of the chosen approach and the effectiveness of the students in working together. With the planned improvement on handling small sample volumes with a flow injection system, the students will continue to work on the realtime capabilities and (semi) continuous monitoring of relevant creatinine concentrations.

Experiments and development were performed at the "Laboratory of Bionanotechnology" at WUR with support from lab members. Frequent meetings involving the team members and coaches/supervisors took place in offices provided by the WUR student challenges. Financial and practical support was successfully obtained provided by WUR Student Challenges, AFSG WUR, Fablab WUR, PalmSens, Unitron and the Dutch Kidney Foundation.

Johannes Hohlbein | *David Feith* | *Valérie Hogenes*

5. Translation potential

5.1 Diagnosis of kidney failure

A creatinine-based biosensor enables the monitoring of adequate kidney functioning. Along with urea concentration and volume output, creatinine is one of the biomarkers for renal activity. Kidney Failure can have a multitude of origins, but all point to injury of the kidney. In such an event, the kidney is unable to clear toxins such as urea and creatinine from the blood properly causing those toxins to accumulate. As such, any person developing kidney injury will show increased concentrations of these toxins. On the other hand, a decrease in urine produced is also an important factor in renal function analysis. This can point to a prerenal or glomerular injury. Thus, both creatinine clearance (CrCl) and glomerular filtration rate (GFR) are used diagnostically.

An important distinction is to be made between chronic and acute kidney injury. Chronic kidney injury develops slowly over time, where the kidney function deteriorates gradually over many years. The GFR decreases slowly and the CrCl increases slowly. Acute kidney injury, on the other hand, develops rapidly, typically within 7 days. In this case, a steep increase in CrCl and/or a steep decrease in GFR may be observed. This can occur, among other reasons, due to acute trauma leading to hypoperfusion of the *arteria renalis* or due to medicinal damage to the kidney tissue. Notable in this distinction is that acute kidney injury (AKI) occurs more in hospital settings, whereas chronic kidney disease (CGD) develops more at home (Kellum et al., 2017).

For the diagnosis of AKI, an index called "Kidney disease: improving global outcomes" or KDIGO is frequently used (see Figure (11)). This divides the patients into four stages, based on the severity of the symptoms (creatinine and urine output). Hospitals can use this KDIGO scale to appoint care. When a patient reaches stage 2, the treatment changes. For a baseline creatinine serum level, between 44-106 µmol/L is seen as a physiologically acceptable level (Mohabbati-Kalejahi et al., 2012) .

Stage[a]	Serum creatinine level	Urine output
Diagnosis	• Increase of ≥0.3 mg/dl (26.5 µmol/l) within 48 h, or • Increase of ≥1.5-fold above baseline, known or assumed to have occurred within 7 days	• <0.5 ml/kg/h for 6 h
1	• ≥1.5–1.9 times baseline, or • >0.3 mg/dl (26.5 µmol/l) increase from baseline	• <0.5 ml/kg/h for 6–12 h
2	• ≥2.0–2.9 times baseline	• <0.5 ml/kg/h for ≥12 h
3	• ≥3.0 times baseline, or • Increase of serum creatinine to ≥4.0 mg/dl (353.6 µmol/l), or • RRT or • In patients aged <18 years, a decrease in eGFR to <35 ml/min/1.73 m²	• <0.3 ml/kg/h for ≥24 h or • Anuria for ≥12 h

Figure 11: KDIGO scale of AKI diagnosis

According to a large meta-analysis, 1 in 5 hospitalized patients developed AKI in high-income countries (Lameire et al., 2013), although also numbers as high as 32% have been reported (Brown et al., 2016). Additionally, in a retrospective cohort study, it was found that approximately 1% of patients had evidence of subacute kidney injury, meaning an increase in Serum Creatinine, but developed over a longer period. When regarding AKI at a community-based level, multiple studies have shown that the incidence is increasing (Kellum et al., 2017) (Chawla et al., 2017). The incidence of AKI is often related to the prescription of nefrotoxic medications such as ADCE-inhibitors or NSAIDs, post-operative hypovolaemia, sepsis, and contrast media for imaging techniques. The mortality rate for AKI in high-income countries is somewhere between 10-20% (Hoste et al., 2018).

5.2 Stakeholders desirability

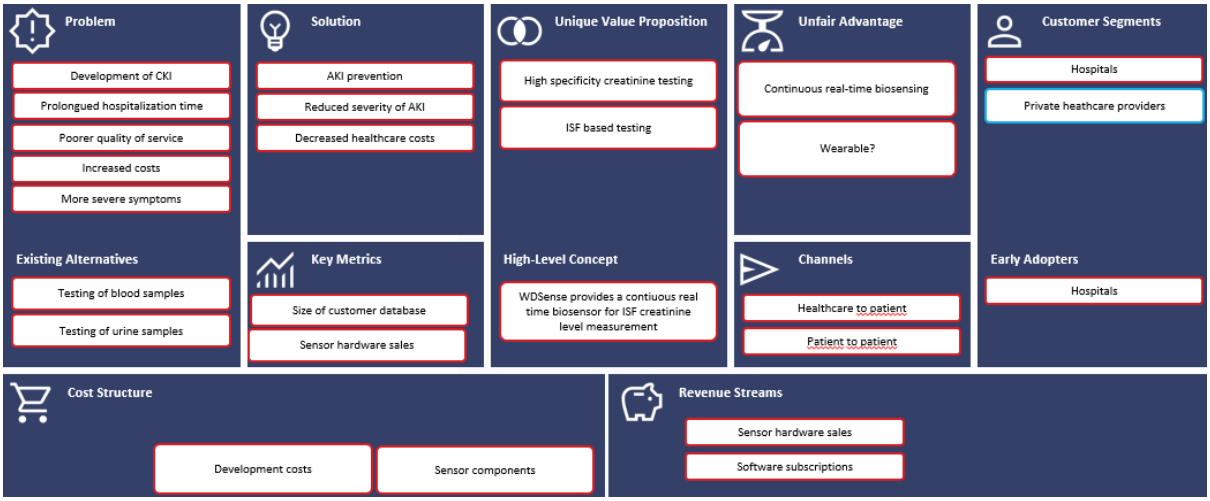

Figure 12: Business model canvas.

Various stakeholders may be identified within the AKI patient journey, each having a unique relationship with the product. In the first place, there is the patient suffering from AKI. It is in the patient's favor to be diagnosed as quickly as possible, as cheaply as possible, and as least invasive as possible. Furthermore, it is in the patient's interest to limit the side effects or aftermath of the illness to a minimum.

Secondly, there are the insurance companies, who primarily benefit from a reduction in costs from AKI. Insurance companies are strictly concerned with the financial side of healthcare, meaning that cost reduction is of their highest concern. This also includes the reduction of future costs by reducing the probability of chronic kidney disease and the need for return visits. Also associated with the insurance companies are the people who are subscribed to the insurance, as their insurance costs are related to the general healthcare costs.

Finally, there are the healthcare providers, chiefly among these are hospitals. They are primarily concerned with providing the optimal service to their customers: taking care of patients. A hospital aims to have the best possible healthcare, so the development of complications reduces their quality of service. Patients with AKI also take up more hospital beds which is a limited time-based resource. Longer hospitalization time means less available hospital beds for other patients. These problems might be reduced by a better treatment option for AKI, leading to decreased costs; by a method to reduce the severity of the developed AKI, leading to better service; or by a method to prevent AKI, leading to both a reduction in costs and improvement in service.

5.3 Business feasibility

The WDSense creatinine sensor can be regarded as existing of two components: the functionalized sensor with the creatinine deiminase protein embedded, and the cartridge containing among other things the counter and reference electrode and the potentiostat. The functionalized sensor has a low expected lifetime, see section 5.5.1., and as a result will need frequent replacement. This electrode needs constant re-functionalization which will be the main workload for a good product flow. This is a easy and repetitive task that is easily scalable.

The WDSense creatinine sensor also uses a Palmsens emstat pico potentiostat, which identifies Palmsens as a major partner. The potentiostat lifetime should be long and as such the supply chain should be manageable. Furthermore, Palmsens plays a pivotal role in controlling the quality of the potentiostats after a critical amount of cycles have been performed. Other important partner is the supplier of the electrodes and the supplier of the enzymes.

5.4 Financial viability

The WDSense creatinine biosensor may be deemed financially viable if the costs of purchase are equal to or lower than the opportunity costs of traditional treatment. In the case of equal costs, it may be assumed preferable to assign patients a WDSense creatinine sensor, as the quality of service is improved. For this financial assessment, we assume that every hospitalized patient, i.e. having to stay at least one night at the hospital, will be given a WDSense creatinine sensor as a precautionary measure. Because the development of AKI is in part a matter of statistics, this is the best method to make sure that every patient developing AKI is captured by the sensor. In other words, it makes the statistics easier, while presenting the most expensive scenario.

5.4.1 The costs of the WDSense creatinine sensor

The WDSense creatinine biosensor exists mainly of three components. A Palmsens Emstat pico potentiostat that measures the given potential; a working electrode functionalized with poly-aniline and containing the creatinine deiminase protein; a reference electrode; a counter electrode; and a casing to hold it all together. Each component has a unique cost and depreciation period associated with it, resulting in a unique cost per patient. These are all estimated and noted in the Table (1).

Table 1: Estimated costs of WDSense creatinine sensor components

	Initial cost	Depreciation period	Cost per patient
Emstat pico	€850	100.000 measurements	€4,90
Functionalized GCE	€30	24 measurements	€30
Counter electrode	€250	4000 measurements	€1,50
Ag/AgCl Reference electrode	€50	4000 measurements	€0,30
Outer casing	€5	4000 measurements	€0,03
Total			€36,73

In the Netherlands, the average hospitalization time is 4.5 days (Eurostat, 2023). If we assume that two sensors are used per patient, this would mean a measurement every two hours. This is of course a number that a customer may decide to alter. The used GCE must be replaced after these 24 measurements, but the rest of the material needs only replacement after about 1 year. The sum of €36,73 is the cost price. The expected sales price for this will be €50.

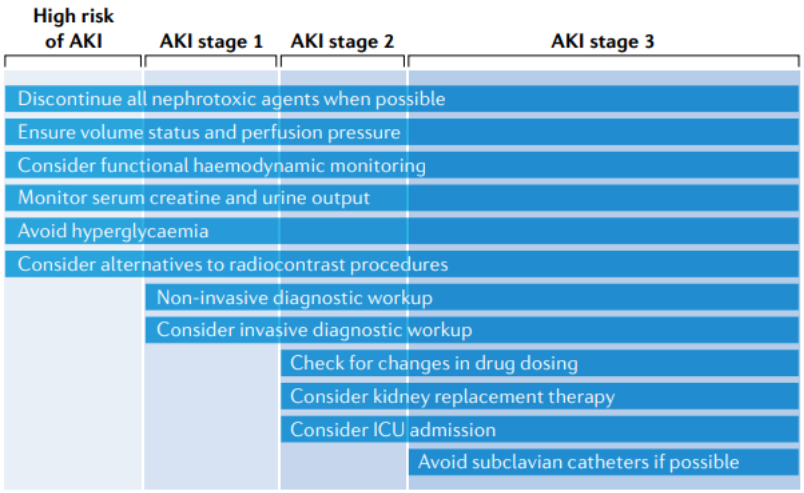

Figure 13: Overview of the medical treatment of AKI Patients

5.4.2 Costs of AKI patients

Hospitals base the assigned treatment of a patient based on the KDIGO scale. When a critical threshold has been reached, the treatment of a patient will change, such as avoiding nefrotoxic medicine. This is schematically shown in Figure (13) (Hoste et al., 2018)

As such, the treatment costs scale exponentially with AKI severity. Costs of treatment are a consequence of the unoccupied hospital beds, and the treatments themselves. On average, a stage 1 AKI patient has an increased hospitalization time of 5 days and costs the hospital approximately €3300. If the disease progresses to stage 2, the costs rise to approximately €11.500 due to potential ICU admissions. In the event of a stage 3 AKI, renal replacement therapy is applied, leading to a cost of approximately €57.400 on average (Rewa & Bagshaw, 2014). Taking into account the incidence rates, the total costs for a hospital may rise to €9521 per AKI patient, as can also be seen in Table (2) . When taking into account the incidence of AKI from patients, the expected AKI-related costs for any hospitalized patient is €1904,20 regardless of the condition.

Table 2: Patient based costs of AKI treatments (Brown et al., 2016)

	Treatment costs	Incidence (% of AKI patients)	Average cost per AKI patient
Stage 1	€3300	88,5	€2920
Stage 2	€11.500	6,8	€3903
Stage 3	€57.400	4,7	€2698
Total			€9521

5.4.3 Cost benefit analysis

For the cost benefit analysis, only the financial sides are taken into account. The quantification of a life's worth is a highly sensitive and complex matter outside the scope of this evaluation. It should be noted however that acute kidney failure increases mortality rates.
Early detection of AKI can contribute to the prevention, but most importantly a decreased severity of the symptoms. A consequence of the reduced severity is that it may not be needed to place a patient in an ICU and renal replacement therapy may not be needed. This has the potential to greatly reduce the costs of treatment. It may also be possible to better detect subclinical AKI cases. When sensing (semi)continuously, any rise in serum creatinine level may be detected, regardless of the baseline. Should a patient have a naturally good clearance (e.g. a Serum Creatinine of 45 µmol/L), a 2x increase would still be regarded in the physiological range. These cases, which normally would remain subclinical can be better visualized, but might be a reason to better monitor kidney function.
Taking into account that the WDSense creatinine sensor best serves its purpose when applied to any patient, the costs will be expressed on a per-patient level. Due to early detection, the costs per patient are expected to decrease. The exact decrease would need heavy clinical testing, so for this cost analysis, we would need a treatment cost reduction of 5.2% per AKI patient to break even. This would reduce the AKI-related costs by €190,- per hospitalized patient. The expected costs of assigning each hospitalized patient a WDSense biosensor are €100,- per patient, assuming two sensors are used per patient. This means that an effective cost reduction of about €90,- may be realized per patient when a WDSense creatinine biosensor is applied. On top of that, a reduced mortality rate and reduced return rates may be expected as well.

6. Team and support

6.1 Contributions of the team members

Valérie Hogenes | Team Captain
Member of: Innovation-, Lab- and PR-Team

Victor van der Doorn | Head of User Interface
Member of: Business-, and Innovation-Team

Joël Vandervinnen | Treasurer; Sponsor Relations
Member of: Business-, Innovation-, Lab- and PR-Team

David Feith | Head of Lab Team
Member of: Innovation-, Lab- and PR-Team

Jochem Feith | Head of Innovation Team
Member of: Business-, Innovation- and Lab-Team

Erwin Hogeveen | Secretary; Head of Business Team
Member of: Business- and Innovation -Team

Nele Jarnot | Head of PR-Team
Member of: Innovation- and PR-Team

6.2 People who supported us

BCT Chair Group and the Biobased Products Research group (Wageningen University & Research):
Pedro Mazaira Couce and Sotiri Mayrikis provided invaluable support in the field of electrochemistry, offering insights into both the technique and the interpretation of results. Their expertise significantly advanced our project, enabling us to better understand and apply electrochemical methods.

BNT Chair Group (Wageningen University & Research):
Anton van Bunschoten and Vittorio Saggiomo, along with the entire chair group, were key in our project's success by granting us access to their laboratory and materials. Anton van Bunschoten, in particular, played a crucial role in ordering the necessary materials for our work.

Fablab (Wageningen University & Research):
Paul Toxopeus supported us by assisting us with printing our team clothing, ensuring we had the proper attire for our project.

Student Challenges (Wageningen University & Research):
Rio Pals, along with the entire Student Challenges team, supported us throughout the competition by providing a workspace and creating opportunities for us to connect with others, for example, the Dutch Innovation Days.

SenseWurk (Wageningen University & Research):
Marrit Bosch, from last year's team, played a pivotal role in getting our group and project off the ground, laying the foundation of our work, and guiding us where needed. Her support was valuable in setting us on the right path.

The Rwei Group (Technical University Delft):
Alina Rwei and Lena Fasching assisted in the conceptualization of the sensor, providing essential guidance that helped shape the idea of our project. Their contributions were key to our innovation process.

6.3 Sponsors and partners

Student Challenges (Wageningen University & Research):
Supports WUR teams financially and with guidance, which participate in student competitions.

Agrotechnology & Food Science Group (Wageningen University & Research):
AFSG focuses on research and education in areas related to agriculture, food sciences, and environmental sustainability. They provide support for various research projects.

PalmSens:
Specializes in electrochemical instrumentation and software, offering a range of products including potentiostats, electrodes, and sensors. They supported us with electrochemical knowledge and offering discounts on their products.

Unitron:
Unitron is known for their expertise in metrology and precision measurement offering a range of products and solutions for measuring and process optimization. They supported us in the early stages of the project by providing essential guidance on how to begin and key considerations to keep in mind throughout our work.

The Dutch Kidney Foundation:
This organization is dedicated to improving kidney health and supporting kidney disease research. We had the opportunity to interview Jasper Boomker, Program Manager, who provided us with pivotal information about the work they do and how they look on the research based on the topic of this year's SensUs Competition. This insight was crucial for understanding their impact.

DropSens;
The Company DropSens specializes in the design and manufacturing of screen-printed electrodes. In the beginning of the testing phase DropSens sponsored us a couple free SPE's.

WDsense would also like to thank everyone who granted us the opportunity to interview them and share their valuable insights on this project. It helped us greatly to understand different point of views on the societal impactful disease.

7. Final Remarks

We are truly grateful for the opportunity to participate in this competition. Balancing this challenge with our regular study programs has been demanding, but we're all pleased with the time and effort we've been able to invest. Over the past few months, our group has grown close—we began as a mix of familiar faces and new acquaintances, but we now proudly consider ourselves a group of friends with shared memories that will last a lifetime.

As you may have noticed, we deliberately chose a challenging path. Electrochemical sensing, a field not covered in our study programs, presented significant difficulties. However, rather than seeing this as a drawback, we embraced it as a challenge. Along the way, we have learned a great deal from professionals and through our own research.

We are proud to say that we are confident in our ability to present a functioning sensor for measuring creatinine concentrations in ISF within the next three weeks. This journey has not only been about technical achievement but also about personal and collective growth, and we look forward to what lies ahead**.**

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9. Supporting information

$$PANI_{EB} + NH_4^+ \rightarrow PANI_{EB} * NH_4^+ \quad (1)$$

$$2NH_4^+ \rightarrow N_2 + 8H^+ + 6e^- \quad \text{OX} \quad (2)$$

$$PANI_{EB} + 2H^+ + 2e^- \rightarrow PANI_{LE} \quad \text{RED} \quad (3)$$

$$PANI_{LE} \rightarrow PANI_{EB} + 2H^+ + 2e^- \quad \text{OX} \quad (4)$$

Equations: S.1) The occupation of empty proton sites on poly-aniline emeraldine base by ammonia. S.2) The oxidation reaction of ammonia into nitrogen gas at 0.2 V. S.3) The reduction of the emeraldine base state to the leucoemeraldine state. S.4) The oxidation of the leucoemeraldine state to the emeraldine base state at 0.2 V.

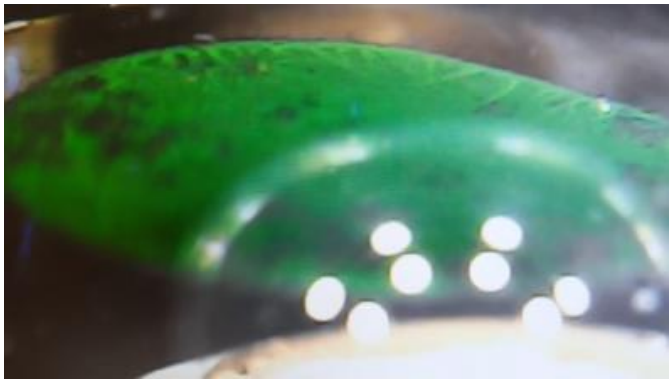

Figure S.1: A close up image taken of our GCE by a camera to show the successful functionalization of the PANI polymer in emeraldine salt state, which has its green distinct colour.