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**Development of a Smart Bolus System for Multi-parameter
Monitoring of Bovine Welfare**

Thesis presented by

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LIST OF ACRONYMS

AC	Alternating Current.
ADC	Analog-to-Digital Converter.
AFE	Analog-Front-End.
AGC	Automatic Gain Control.
AI	Artificial Intelligence.
APP	Application.
CA	Chronoamperometry.
CE	Counter Electrode.
CMOS	Complementary Metal–Oxide–Semiconductor.
CV	Cyclic Voltammetry.
DAC	Digital-to-Analog Converter.
DC	Direct Current.
DI	Deionized.
DNA	Deoxyribonucleic Acid.
DPV	Differential Pulse Voltammetry.
DUT	Device Under Test.
ECG	Electrocardiography.
EIS	Electrochemical Impedance Spectroscopy.
ELISA	Enzyme-Linked Immunosorbent Assay.

FDA Food and Drug Administration (US).

FSM Finite-State-Machine.

FW Firmware.

GHG Greenhouse Gas.

GI Gastrointestinal.

GPIO General-purpose input/output.

GPS Global Positioning System.

GUI Graphical User Interface.

HAL Hardware Abstraction Layer.

HSI High-Speed Internal.

HW Hardware.

I2C Inter-Integrated Circuit.

IC Integrated Circuit.

IMU Inertial Measurement Unit.

IoT Internet of Things.

IP Ingress Protection.

IrOx Iridium Oxide.

ISF Interstitial Fluid.

ISM Ion Selective Membrane.

JTAG Joint Test Action Group.

LGA Land Grid Array.

Li-ion Lithium-Ion.

LiPo Lithium Polymer.

LOD Limit of Detection.

LoRa Long-Range.

LPWAN Low Power Wide Area Network.

LSB Least Significant Bit.

LSV Linear Sweep Voltammetry.

MCU Microcontroller Unit.

MedRadio Medical Device Radiocommunications Service.

ML Machine Learning.

MOSFET Metal–Oxide–Semiconductor Field-Effect Transistor.

NFC Near Field Communication.

NPG Nanoporous Gold.

NTC Negative Temperature Coefficient.

OCP Open Circuit Potential.

OpAmp Operational Amplifier.

PBS Phosphate Buffered Saline.

PCB Printed Circuit Board.

PEDOT Poly(3,4-ethylenedioxythiophene).

PLL Phase-Locked Loop.

POC Point-of-Care.

POM Polyoxymethylene.

PP Polypropylene.

PPG Photoplethysmography.

PPK2 Power Profiler Kit II.

PULSE Portable Unit for Lab-on-Site Electrochemistry.

qPCR Quantitative PCR.

RE Reference Electrode.

RF Radio Frequency.

RFID Radio-frequency Identification.

RNA Ribonucleic Acid.

RX Receiver.

SARA Subacute Ruminant Acidosis.

SD Standard Deviation.

SE Standard Error.

SEM Scanning Electron Microscope.

SNR Signal-to-noise Ratio.

SoTA State-of-The-Art.

SPE Screen-Printed Electrode.

SPI Serial Peripheral Interface.

SWD Serial Wire Debug.

SWV Square Wave Voltammetry.

TIA Trans-Impedance Amplifier.

TX Transmitter.

USART Universal Synchronous Asynchronous Receiver Transmitter.

USB Universal Serial Bus.

VFA Volatile Fatty Acid.

WE Working Electrode.

WLAN Wireless Local Area Network.

XOR Exclusive OR.

DECLARATION

This is to certify that the work I am submitting is my own and has not been submitted for another degree, either at University College Cork or elsewhere. All external references and sources are clearly acknowledged and identified within the contents. I have read and understood the regulations of University College Cork concerning plagiarism and intellectual property.

Ana Cláudia Ferreira

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ABSTRACT

The dairy industry is a growing sector of global agriculture, with a projected market value of US\$1,243 billion by 2028. However, its rapid growth brings challenges, including the need for sustainable practices, improved animal welfare, and enhanced productivity. Effective livestock health monitoring is at the core of these challenges, which is critical for ensuring animal well-being, minimising economic losses, and reducing environmental impacts. Despite advances in monitoring tools like wearable devices and smart boluses, current technologies are limited in providing comprehensive, real-time insights into bovine welfare. This research addressed that gap in currently available technologies, by developing a prototype PULSE (Portable Unit for Lab-on-Site Electrochemistry) system. This is a novel, multi-parametric embedded system, including electrochemical capabilities, for real-time monitoring in precision livestock farming. By designing and refining PULSE and its second-generation version, PULSE 2.0, this study explored advanced sensor technology and embedded systems for future facilitation of early disease detection.

The first-generation PULSE prototype represented a significant advancement in livestock health monitoring, incorporating multiple sensors, including temperature and acceleration sensors, as well as a selection of electrochemical sensors for measuring pH, nitrate, and nitrite. These parameters are important analytes that provide insights into the animal's health, while also having an indirect connection to livestock greenhouse gas emissions. The multi-parametric design of the device advances the state-of-the-art in bovine health monitoring and commercial potentiostat technologies by providing a fully customised platform capable of measuring both electrochemical and physical parameters. Tailored analog front-end circuitry was developed to accurately perform electrochemical techniques, including open-circuit potentiometry, chronoamperometry, and cyclic voltammetry. These techniques are widely used and enable rapid and sensitive detection of the target analytes. Validation experiments, which benchmarked the device's capabilities against standard laboratory benchtop instruments, demonstrated correlation coefficients exceeding 0.99 for pH, nitrate, and nitrite measurements. This strong agreement highlighted the device's accuracy in matching the measurements of

standard instruments. The PULSE enabled rapid pH detection via potentiometry, achieving a response time of 2 seconds. Additionally, the pH calibration curve followed a Nernstian behaviour with a slope of -60.43 mV/pH, establishing the developed system as a promising tool for point-of-care applications. This work highlighted the device's operational versatility and customisation potential, allowing tailored configurations to meet the specific requirements of sensors and applications. The PULSE device demonstrated its potential to enhance the speed and quality of measurements, and reduce associated costs and energy consumption.

The device was tested in a complex ruminal fluid environment, maintaining an accuracy of 98.9% for pH over a period exceeding 42 days, showcasing its robustness for real-world applications. While these results are promising, further investigation is required to fully characterise the long-term durability of the sensors in this environment. The system demonstrated a sensitivity of $1.92 \mu\text{A}/\text{ppm}/\text{cm}^2$ and a Limit of Detection (LOD) of 0.335 mM for nitrate, while for nitrite, it achieved a sensitivity of $6.32 \mu\text{A}/\text{ppm}/\text{cm}^2$ and a LOD of 0.854 mM. These results, along with the high correlation between the systems, highlighted the relevance and potential use of PULSE for animal health monitoring. Furthermore, the integration of both commercial and proprietary in-house sensors expanded its functionality as a point-of-care device for a wide range of monitoring applications.

Building on the first prototype, PULSE 2.0 enhanced functionality through miniaturisation, portability, and sensing capabilities. Designed for field deployment, PULSE 2.0 integrated Bluetooth 2.0 communication, a rechargeable lithium-ion battery, and a user-friendly Android application, enhancing its accessibility for agricultural settings. Its extended sensing parameters included sodium and potassium, selected as demonstrators due to their critical role in monitoring electrolyte balance in ruminal health. Investigation in ruminal fluid demonstrated high accuracy, including 99.88% for pH, 99% for sodium, and 99.02% for potassium. These results highlighted the novelty of PULSE 2.0 by differentiating it from the current state of the art through its tailored operational range, multiple-sensing capabilities, and integrated sensors. This established the PULSE 2.0 as a novel point-of-care system adapted specifically for real-time electrochemical analysis of key parameters in rumen fluid. The PULSE 2.0 system demonstrated its potential as a highly versatile sensor platform, which can be adapted in form factor and functionality for future portable or implantable devices. It is part of an overall advanced precision livestock monitoring system to enable data-driven decision-making solutions.

These advancements positioned PULSE 2.0 as a transformative device for continuous livestock health monitoring, improving disease management, herd

productivity, and environmental sustainability. In the future, its modular design could extend its application to human health, aquaculture, and industrial monitoring. This work represented a significant milestone in embedded systems for electrochemical sensing, highlighting its pioneering contributions to precision agriculture and multi-sensor technology.

INTRODUCTION

1.1 Problem Statement

The dairy industry is a growing sector with significant impacts on the environment, economy, human health, and diet-related issues [1, 2]. The escalating global demand for animal-derived products has highlighted the critical need for effective livestock health monitoring to improve dairy production efficiency [3]. Health issues in dairy cattle are often unpredictable, compromising animal welfare and, consequently, the quality of dairy products [4]. Therefore, producers require efficient and reliable systems for livestock health monitoring.

Effective cattle management involves data-driven decision-making to ensure animal well-being while optimising operational costs and minimising environmental impacts [5]. Achieving these objectives demands improved data collection and analytic tools integrated with health monitoring systems to support decision-making for herd management [6]. A robust health monitoring tool enables early detection of diseases or anomalies, facilitating timely interventions that can reduce costs and minimise livestock losses. The development of such a device also opens opportunities for decision-support systems to mitigate the economic consequences of diseases and enhance farm productivity.

The global dairy industry is projected to reach a valuation of US\$1,243 billion by 2028 [7], highlighting the growing need for precise and comprehensive monitoring technologies. Studies demonstrated that continuous monitoring of cattle health enhanced farm decision-making and positively impacted environmental sustainability, economic efficiency, and human health [6, 8]. Real-time monitoring of key bovine health parameters facilitates early disease detection, helps prevent the spread of illness within herds, and mitigates related complications [9–11].

Advances in cattle health monitoring tools include wearable devices such as ear tags [12], leg braces [13], and collars [14, 15]. While these tools provide valuable data on parameters like motion and body temperature, they can not measure critical chemical and biochemical markers. To address this gap, smart boluses have been developed. Inserted into the cow's reticulum, these devices collect essential physiological data [16–18]. However, most existing smart boluses are primarily designed to monitor temperature and pH [19]. While these parameters are valuable, the narrow sensing capabilities restrict their broader applicability and underutilise the full potential of these devices within the animal. These limitations result from challenges such as device size constraints, power consumption, battery life, device control, and sensor integration.

1.2 Project Objectives

The main objective of this project is to develop a novel multi-parametric embedded system with customised Hardware (HW) and Firmware (FW), based on the characteristics of selected sensing capabilities. The system is validated using a range of electrochemical sensors applied to real-world biofluids.

The goals to obtain this objective include:

- **HW Development:** Design and build of innovative electronics suitable for integration into a smart bolus prototype, incorporating multiple sensors. The HW development aims to integrate a potentiometric and voltammetric circuit in a single device, with a dynamic operational range, multiple sensor integration, wireless communication, and precise signal control.
 - **Prototype Enhancement:** Miniaturise and optimise the prototype for portability and usability, adding Bluetooth communication and rechargeable capabilities.
- **FW Development:** Develop robust FW to control and manage sensors with high accuracy and reliability in harsh real-world conditions. The FW supports accurate electrochemical techniques for a novel, real-time, multi-parameter health monitoring system for livestock. The FW aims to enable simultaneous multi-sensing data collection, precise control of Digital-to-Analog Converter (DAC) signals, and accurate implementation of electrochemical techniques for reliable measurements.
- **Application (APP) Development:** Create a tailored, user-friendly APP for device control, real-time monitoring, and data visualisation, ensuring effective communication between the device and the end users.

- Development of Android APP for field applications and user accessibility.
- Performance Validation: Evaluate the innovative prototypes' accuracy, reliability, and performance using laboratory and real-world samples.
 - Benchmark the systems capabilities against the standard and advanced laboratory instruments for health-related parameter monitoring.
 - Investigate performance in rumen fluid to ensure accuracy and precision in complex biological media.

By achieving these goals, this thesis is creating new knowledge around the development and integration of a novel multi-parametric embedded technology for real-time health monitoring. This thesis expands knowledge that will support future data collection for Artificial Intelligence (AI)-driven decision support tools and early disease detection. For details on research excellence outputs related to the work developed in this thesis, refer to Appendix A.

1.3 Thesis Structure

This thesis is organised into six chapters. In this introductory chapter, a general overview of electrochemistry fundamentals and techniques is given and discussed, establishing a foundation for understanding the subsequent chapters. A literature review follows this in Chapter 2, which focuses on the biosensing parameters relevant to bovine health monitoring. The chapter highlights key analytes, including nitrate, nitrite, sodium, and potassium, as well as properties such as pH and temperature, emphasising their importance for animal health and productivity. In addition, it examines the latest cutting-edge advancements in emerging technologies for bovine health technology, identifying the limitations of existing tools, and positioning the research within the broader context of livestock health monitoring. This literature review aims to bridge the fields of biosensors and electronics, highlighting the need for a multi-parametric device.

Chapter 3 focuses on the development, characterisation, and validation of a novel fast-detection multi-parametric embedded system prototype named Portable Unit for Lab-on-Site Electrochemistry (PULSE). This chapter details the customised development of FW, HW, and APP, and evaluates the performance of electrochemical techniques by integrating selected sensors into the system. This device is benchmarked against a well-established benchtop potentiostat, achieving pH detection within 2 seconds with high accuracy and completing Chronoamperometry (CA) measurements in 20 seconds. Additionally, this chapter presents

a correlation analysis comparing the calibration curve datasets across all implemented electrochemical techniques from both systems, demonstrating a strong agreement between them.

Chapter 4 investigates the device’s performance in measuring key parameters for monitoring of bovine well-being, including nitrate, nitrite, pH, and temperature in fluids of varying complexity, including Phosphate Buffered Saline (PBS) solutions and artificial Interstitial Fluid (ISF). Additionally, this work evaluates the potential deployment of the device in real-world scenarios by testing pH in ruminal fluid. A comparison with a benchtop potentiostat reveals a lower Limit of Detection (LOD) and higher sensitivity when performing measurements with PULSE. The device demonstrates strong performance in ruminal fluid for pH detection, highlighting the need for further investigation into its behaviour in this challenging medium.

In Chapter 5, the research focuses on the development of a compact, portable, Point-of-Care (POC) prototype named PULSE 2.0. This device miniaturises the first prototype and incorporates wireless communication through Bluetooth 2.0 and a rechargeable Lithium-Ion (Li-ion) battery. This chapter investigates this prototype’s capabilities for multi-parameter sensing in ruminal fluid, including the measurement of pH, sodium, and potassium. The results assess the accuracy of the prototype in ruminal complex fluid, providing insights into its performance and highlighting areas for further development towards a fully integrated smart bolus system. The performance of PULSE 2.0 is benchmarked against standard laboratory devices, showcasing its high accuracy and strong potential for use in animal health monitoring and electrolyte balance assessment.

Finally, Chapter 6 summarizes the key findings of this thesis, highlighting the research contributions, and suggesting recommendations for future work.

1.4 Theoretical Fundamentals of Electrochemistry

Over the years, many definitions of a sensor have been proposed, typically describing it as a device or component that generates an output signal in response to a physical, chemical, or biochemical input of interest [20]. For the presented work, the term sensor is understood as a component capable of sensing a certain parameter and enabling its quantification through the measured outputs. Sensors have been widely explored across the world for a variety of applications, such as drug delivery [21, 22], food safety [23, 24], healthcare [25, 26], and aquaculture [27, 28], among others. This thesis focuses on the development of a multi-parametric system that integrates electrochemical sensors with advanced instrumentation to enhance bovine health monitoring. Wearable devices have been developed to

monitor behaviours and actions [29, 30]. Nowadays, these sensors are used across sectors such as healthcare [31, 32], security [33], and environment monitoring [34], among others [35, 36].

A comprehensive understanding of electrochemistry is crucial for advancing this work and related research fields. Electrochemistry is the study of the relationship between electrical energy and chemical reactions [37]. Therefore, electrochemical sensors utilise transducers to convert the analyte recognition event into an electrical signal that is proportional to its concentration [38]. In this context, the focus is primarily on electron transfer occurring at the interface between the electrode and the solution, driven by the application of a potential [39]. The electrical signal generated by the electrochemical reaction - whether in the form of voltage, current, or impedance - determines the classification of electrochemical sensors into potentiometric (voltage-based), amperometric or voltammetric (current-based), and impedimetric (impedance-based) types [40]. Thus, the transducers used are selected according to the target analyte [41].

The conventional electrochemical cell contains three electrodes: (1) the Working Electrode (WE), (2) the auxiliary or Counter Electrode (CE), and (3) the Reference Electrode (RE) [42]. Typically, the electrodes are connected to a device known as a potentiostat. The capacity to detect analytes depends on the materials, the surface modifications, and the dimensions of the electrodes to enhance sensitivity and selectivity to the analyte of interest [38]. This thesis validated the developed embedded system using a variety of electrochemical sensors applied to real-world biofluids, demonstrating its capability for comprehensive health assessment. Therefore, this chapter introduces the relevant theoretical fundamentals of electrochemistry.

1.4.1 Electrochemical Cell and Experiment Setup

As mentioned in Section 1.4, the most common electrochemical cell setup consists of three electrodes [43]. However, configurations of two electrodes or more than three also exist [44]. Even though the two-electrode configuration is the simplest, it also suffers some drawbacks in the form of control and stability of the potential at the electrodes [45]. This configuration works in low current density situations, where the current flow at the CE can be neglected. The three-electrode configuration, illustrated in Figure 1.1, solved this issue and is thereby the most popular setup. Immersed in an electrochemical solution, each electrode serves a specific purpose. Typically, the WE is where the reaction of interest occurs, as it directly interacts with the analyte. The CE acts as a source or sink of electrons to balance the current flowing through the WE, with its potential adjusted accord-

ingly. The RE provides a stable reference potential to ensure precise and accurate measurement at the WE. The solution in which these electrodes are immersed may contain the specific analyte being studied or serve as a medium for analysing the WE's behaviour. These electrodes are subsequently linked to a potentiostat, a device designed and configured to execute electrochemical techniques, which will be elaborated in more detail later in this section. To mitigate electrical background noise, the electrochemical cell can be positioned within a Faraday cage. Constructed from conductive materials, the Faraday cage facilitates the dissipation of electrical interference to the outer surface, effectively shielding the electrolytic cell from substantial electromagnetic noise. Lastly, the user interface is most effectively implemented as a Graphical User Interface (GUI), designed to operate on a computer or smartphone with the appropriate software. Each implementation is tailored to ensure usability, connectivity, and compatibility with device-specific requirements.

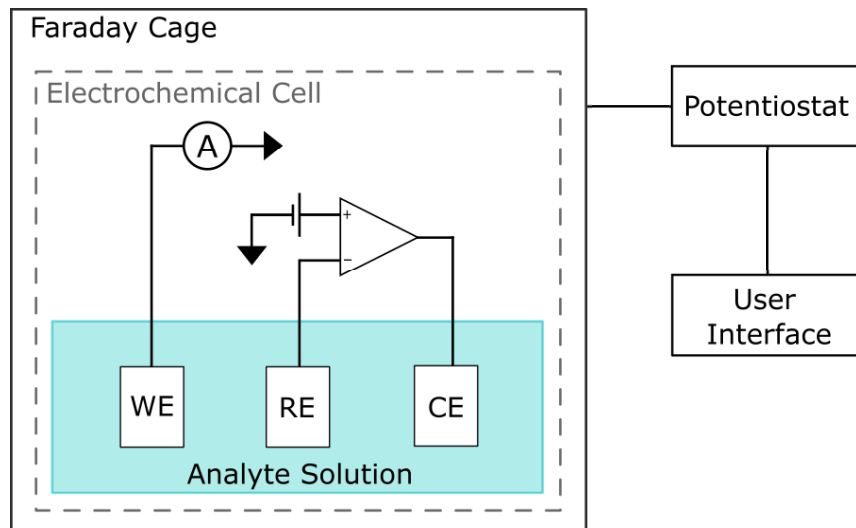


Figure 1.1: Schematic diagram of a standard three-electrode electrochemical setup within a Faraday cage, including the WE, RE, and CE, all connected to a potentiostat and a user interface (adapted from [39]).

Electrode reactions occur at the interface between the electrode and the solution. The separation of charge on the electrode can be modeled as a capacitor, while the charge transfer rate can be represented as a resistor. During electrochemical measurement, the double-layer capacitance cannot be neglected, as well as the presence of a charging current [46]. The Randles equivalent circuit to describe the electrode-electrolyte interface present in an electrochemical cell is depicted in Figure 1.2 [47, 48]. The solution resistance (R_{sol}), which is opposed to ion migration, can be represented as a resistor. This element is put in series since all the current passes through the solution. The double-layer capacitance can be modelled as a capacitor (C_{dl}) and is illustrated in Figure 1.3. This ca-

capacitance manifests at the electrode electrolyte interface, where the electrode acquires charge, drawing oppositely charged molecules from the electrolyte. The magnitude of C_{dl} is influenced by various factors, such as the applied potential, ionic concentration of the electrolyte, electrolyte temperature, and the roughness of the electrode surface.

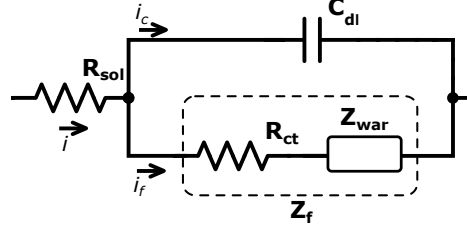


Figure 1.2: Circuit model of an electrochemical cell (adapted from [49]).

The faradaic process can be generally represented as an impedance (Z_f), which can be described as a series combination of the charge-transfer resistance (R_{ct}), and the Warburg impedance (Z_{war}) [46]. The charge transfer resistance signifies the movement of ions between the electrode and the solution, facilitated by redox reactions in both directions. Factors such as the concentrations of reactants, the applied potential at the interface between the WE and the solution, the characteristics of the redox reaction, the composition of the WE, and the temperature of the solution all affect R_{ct} . The Z_{war} denotes the ion diffusion occurring at the interface between the electrode and the solution. Its impedance value is related to the frequency of potential variations at this interface. The circuit elements that represent physical processes are arranged in parallel. This configuration allows for the total current to be the sum of the faradaic current (i_f), which flows through the charge-transfer resistance, and the capacitive current (i_c), which flows through the double-layer capacitance.

1.4.2 Potentiostats

A potentiostat is an electronic equipment required to control an electrochemical cell and perform electrochemical experiments [46]. Potentiostats are integral to electrochemical instrumentation and play a fundamental role in controlling and measuring electrochemical processes within an electrochemical cell [50, 51]. By understanding the principles and concepts behind potentiostats, insights are gained into how these devices enable the precise monitoring of targets, which is essential for the developed work in this thesis.

Potentiostats are responsible for controlling the voltage difference between a WE and a RE in an electrochemical cell. This system maintains the potential at the WE at a constant level with respect to the RE, thereby allowing precise con-

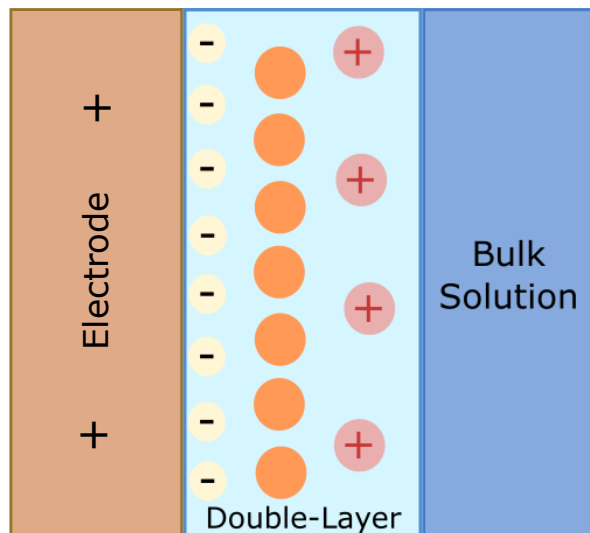


Figure 1.3: Diagram of the double-layer capacitance (adapted from [39]).

trol and measurement of the potential at the WE [52]. Such control is achieved by applying a current between the WE and the CE, with the CE typically being larger in size than the WE. The RE should maintain a constant potential towards WE, and have infinitely high impedance not to allow any current flow through it [46]. Figure 1.4 illustrates a schematic representation of the main components that integrate a potentiostat, colour-coded for clarity. These components include the electrochemical cell with its typical three-electrode configuration, the current-voltage converter resistor (R_m) as part of the current-voltage converter, the electrometer, and the control amplifier.

The **signal generator** acts as a computer-controlled voltage source and transforms a digital signal from the user interface into an analog signal. This generated signal is then applied to the electrochemical cell, with its complexity tailored to meet the specific requirements of the electrochemical application or measurement.

The **control amplifier** controls the voltage of the cell by comparing the measured cell voltage (E_r) with the desired voltage (E_i) and drives current into the cell through the CE, forcing the voltages to be the same, as illustrated in Figure 1.4. This control mechanism employs negative feedback to counteract any perturbations within the cell. Assuming a simplified potentiostat schematic, as illustrated in Figure 1.5, where Z_1 represents R_m in series with the interfacial impedance of the CE and the solution resistance between the CE and RE; and Z_2 represents the interfacial impedance of the WE in series with the solution resistance between the WE and RE [52], the role of the control amplifier can be expressed by the following equation [46]:

$$E_o = A \times (E^+ - E^-) = A \times (E_i - E_r) \quad (1.1)$$

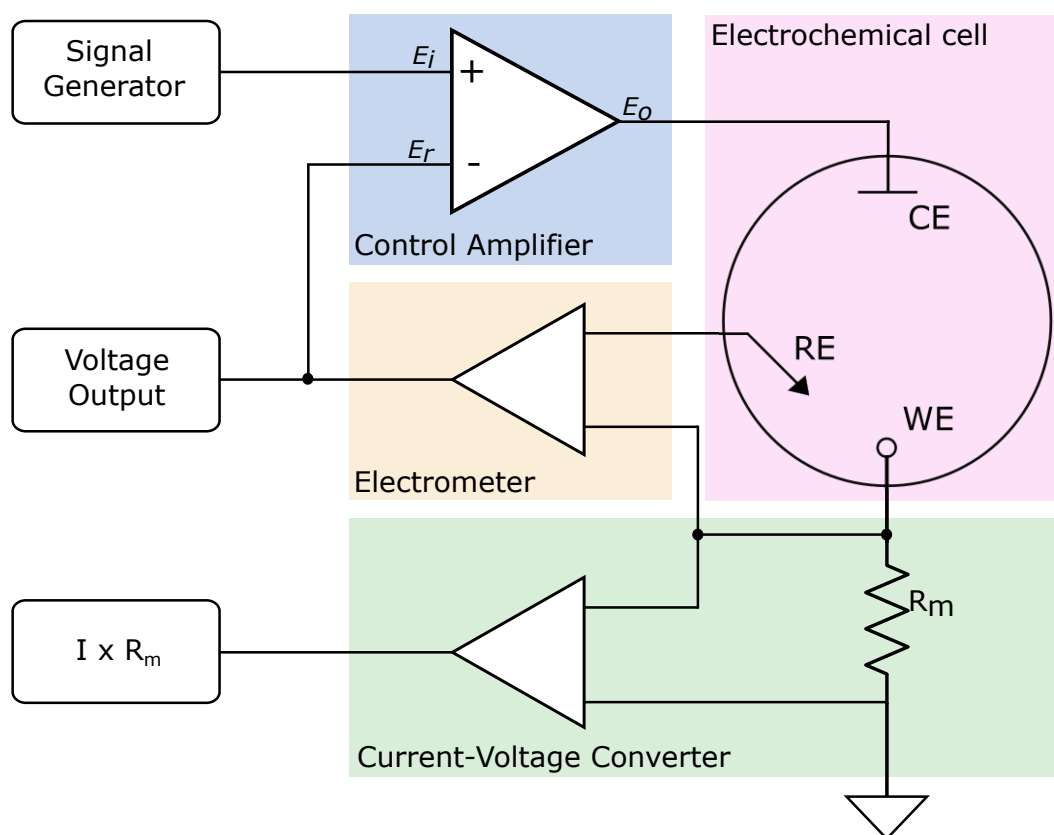


Figure 1.4: Schematic representation of a potentiostat illustrating the electrochemical cell with three electrodes, the current-voltage converter resistor (R_m), the control amplifier, and the electrometer (adapted from [49]).

Where E_o represents the output voltage of the control amplifier, and A denotes the amplification factor.

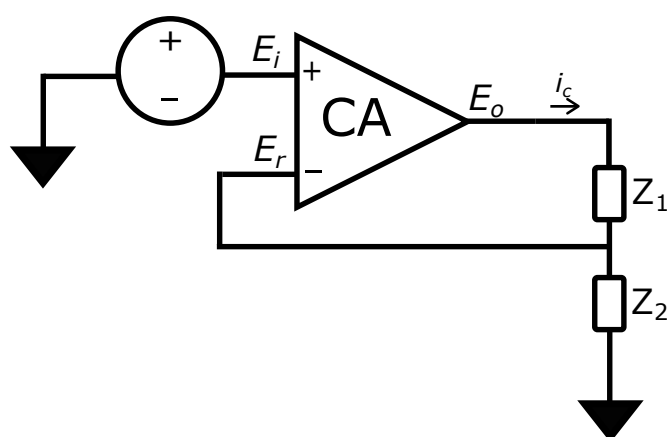


Figure 1.5: Simplified schematic of a potentiostat, including the control amplifier (CA) and two impedances.

Assuming a negligible current flowing through the RE, the following equations can be derived to determine the current cell (i_c):

$$i_c = \frac{E_o}{Z_1 + Z_2} \quad (1.2)$$

$$i_c = \frac{E_r}{Z_2} \quad (1.3)$$

and combining them yields:

$$E_r = \frac{Z_2}{Z_1 + Z_2} \times E_o \quad (1.4)$$

The feedback factor (β) indicates the fraction of the output voltage returned to the negative input, and can be calculated by Equation 1.5.

$$\beta = \frac{Z_2}{Z_1 + Z_2} \quad (1.5)$$

Merging Equations 1.4 and 1.5 yields:

$$\frac{E_r}{E_i} = \frac{\beta A}{1 + \beta A} \quad (1.6)$$

When βA becomes significantly larger than one, Equation 1.6 can be reduced to $E_i = E_r$, demonstrating that the control amplifier works to keep the voltage between the reference and working electrodes close to the input source voltage.

The **electrometer** measures the voltage difference between the RE and WE, acting as a voltage buffer connected to the RE. Ideally, this component should have a zero-input current and high input impedance to minimise its impact on current measurements. The bandwidth and input capacitance of the electrometer are critical considerations in its selection [52].

The **current-voltage (I/E) converter** measures the current flow in the cell by forcing it through the resistor R_m . Ideally, this amplifier exhibits infinite input impedance, meaning no current flows through it. The voltage drop (U) across the resistor is used to measure the cell current by applying Ohm's Law (Equation 1.7). This amplifier should also have a minimal bias current to minimise errors in the current measurements, particularly when dealing with small currents. Consequently, the measurement of small currents often necessitates the use of large resistor values [52].

$$U = I \times R_m \quad (1.7)$$

The exploration of potentiostats' theoretical background provided a solid foundation for understanding the principles underlying the development of electrochemical sensing platforms. By examining the operation of potentiostats and their key components, such as the control amplifier, electrometer, and current-

voltage converter, a deeper understanding of the intricate mechanisms involved in electrochemical measurements was achieved.

While the theoretical framework of potentiostats is essential for their use and applications, it is equally critical to evaluate their current State-of-The-Art (SoTA). Modern potentiostats have evolved to incorporate high precision, and advanced user interfaces, enabling techniques such as Cyclic Voltammetry (CV), CA, and Electrochemical Impedance Spectroscopy (EIS). Potentiostats are available in various forms, including benchtop systems, portable devices, and Integrated Circuits (ICs).

Commercially available potentiostats, such as the PalmSens4 [53], Gamry PAL [54], and Autolab PGSTAT302N [55], encounter several limitations, including high costs, size constraints, lengthy analysis times, limited battery life, and lack of configurability [56, 57]. A short overview of some commercially available off-the-shelf potentiostats is presented in Table 1.1. Benchtop systems, particularly those designed for laboratory research, are highly expensive, limiting their suitability for field-based applications [58]. Although portable potentiostats are available, they often sacrifice advanced functionality for reduced size [59]. For instance, the Autolab PGSTAT302N offers higher ranges and resolutions compared to its compact counterpart, the DropStat [60], but at the expense of portability. Moreover, commercially available portable potentiostats may not meet the specific needs of certain applications or sensors. For example, they may lack compatibility with technologies like near-field communication [61] or the detection of specific analytes such as hexavalent chromium [62]. Many devices are designed exclusively to function with commercial sensors, limiting their applicability for developing and testing custom sensors. These systems are generally intended for general-purpose use, leading to inefficiencies in specialised applications. Additionally, as noted in the literature, companies provide limited information about the systems' HW and software specifications, which complicates their use and restricts adaptability [63].

IC potentiostats, such as the ADuCM355 [64], have been employed to develop complete devices like the ACEstat [65]. However, these systems face challenges associated with specific communication protocols, which constrain the development of customised interfaces [66]. Another example is the AD5940 [67], which, in addition to the aforementioned constraints, requires a separate Microcontroller Unit (MCU) for data handling. Many of these systems do not require coding expertise, restricting users' ability to understand, control, and customise the device and electrochemical techniques.

The need for a bespoke potentiostat solution becomes evident when considering the capabilities and limitations of such commercially available potentiostats.

Table 1.1: Summary table of commercial off-the-shelf systems specifications.

System	Size (cm)	Weight (g)	Comm.	Voltage Range (V)	Current Range	Power Supply	Battery Time	Sensor	Conne- ctivity
PalmSens4 [53]	15.7×9.7× 3.5	500	USB / Bluetooth	±10	±100 pA – ±10 mA	USB / LiPo battery	16h idle, 4h active	Wired (1 sensor)	
PGSTAT 302N [55]	52×16×42	18,000	USB	±10	±10 nA – ±1 A	AC power supply	-	Wired (multiple sensors)	
DropStat [60]	6×2×8	71	USB	±2	±200 μ A	USB or Li- ion battery	Unknown	Direct connec- tion (1 sensor)	
EmStat3 [68]	6.7×5×2.8	85	USB	±5	±1 nA – ±10 mA	USB or lithium battery	8h idle, 6h continuous	Direct connec- tion (1 sensor)	
Sensit Wearable [69]	3.5×3.5× 1.2	10	USB-C / Bluetooth	±2	±3 mA	USB-C or battery	2h (idle or measuring)	Adaptable (1 sensor up to 2 working electrodes)	
Sensit BT [70]	7.5×5.5× 2.3	75	USB / Wireless	±2.2	±3 mA	USB / Battery	12h active	Direct connec- tion (2 sensors)	

Capabilities such as precision, dynamic range, bandwidth, and the set of electrochemical measurements offered are directly reflected in the price of these devices. However, almost all commercial solutions are tailored for single WE electrochemical measurements. Once a sensor is fully characterised, its response can depend on additional factors beyond those covered by commercial potentiostats. One factor to consider is the temperature of the environment, which affects electrochemical reactions [71]. Accurate readings necessitate the inclusion of external temperature measurements, which, in turn, invokes the need for a bespoke solution.

The need for a bespoke potentiostat solution becomes evident when considering the capabilities and limitations of such commercially available potentiostats. Capabilities such as precision, dynamic range, bandwidth, and the set of electrochemical measurements offered are directly reflected in the price of these devices. However, almost all commercial solutions are tailored for single WE electrochemical measurements. Once a sensor is fully characterised, its response can depend on additional factors beyond those covered by commercial potentiostats. One factor to consider is the temperature of the environment, which affects electrochemical reactions [71]. Accurate readings necessitate the inclusion of external temperature measurements, which, in turn, invokes the need for a bespoke solution.

A second reason for requiring a bespoke potentiostat is the need to integrate multiple sensors for specific applications. Some applications require a setup consisting of either a single sensor with multiple WEs or multiple sensors with a single WE [72]. Each WE provides sensitivity to a specific analyte, and most commercial potentiostats, which are typically designed for single WE measurements, are not suitable for such configurations. The only way to facilitate the use of multi-electrode setups is through a bespoke solution that accommodates these requirements.

A third crucial reason is related to the packaging and deployment requirements. In certain applications, such as the placement of a potentiostat inside a cow's stomach, the device must be designed with a specific form factor, such as a bolus-shaped enclosure, to meet deployment constraints. Furthermore, a bespoke potentiostat must support wireless communication to transmit data to an external device for analysis and processing, a feature often lacking or limited in commercial potentiostats.

Therefore, taking into account the currently available commercial potentiostats, this thesis investigated the potential to address the above requirements in a bespoke potentiostat, with customised FW tailored to the requirements of the selected electrodes and parameters. The resulting device, PULSE, offers full cus-

tomisation in both HW and FW, enabling collaborative development and in-depth exploration of sensor technologies. It is capable of measuring electrochemical analytes using up to two sensors, along with physical parameters such as acceleration and temperature. The hardware is also designed to support future expansion for additional sensing capabilities. While some commercial potentiostats allow integration with up to two sensors, they typically require screen-printed electrodes, which constrain the development of sensors optimised for bovine health monitoring. Moreover, as the aim of this work is to lay the foundation for a smart bolus form factor, commercial off-the-shelf potentiostats are unsuitable due to their lack of adaptability.

1.4.3 Electron Transfer

Typically, in electrochemistry, the electron transfer takes place at the interface between the electrode and solution through the application of a specific potential. This can happen in two ways: reduction (gain of electron) or oxidation (loss of electron) reactions, which can be described by the following equation:



Where O is the oxidised species, R is the reduced species, and n is the number of electrons (e^-) exchanged between the previous two. These reactions take place within a potential region where the electron transfer is either thermodynamically or kinetically favourable [73]. The potential represents the driving force required to facilitate electron movement. When the potential causes a reduction reaction, the electrode is referred to as the cathode, whereas it is called the anode when the potential induces an oxidation reaction. Applying a potential to an electrode makes the reaction thermodynamically favourable, a characteristic that can be determined through the change in Gibbs free energy (ΔG) described in the following equation [74]:

$$\Delta G = \Delta G^\circ + RT \ln \frac{R}{O} \quad (1.9)$$

Where ΔG° corresponds to the standard Gibbs free energy (J/mol), R is the gas constant (8.3145 J/molK), and T is the temperature (K). The proportion of reduced to oxidised species can be linked to the change in Gibbs free energy, providing a basis for deriving the potential, E (V) according to the following equation:

$$\Delta G = -nFE \quad (1.10)$$

Where n is the number of electrons involved in the reaction, and F is Faraday's constant (96.486 C/mol), which plays a crucial role, relating the quantity of electrical charge to the number of moles of electrons involved in a redox reaction. Alternatively, this equation can be expressed in terms of standard cell potential (E°), as follows:

$$\Delta G^\circ = -nFE^\circ \quad (1.11)$$

In cases where ΔG values are negative, reactions occur spontaneously. In contrast, when ΔG values are positive, reactions are non-spontaneous and require the application of voltage to proceed. The cell potential can be calculated by replacing Equations 1.10 and 1.11 into Equation 1.9:

$$-nFE = -nFE^\circ + RT \ln \frac{R}{O} \quad (1.12)$$

resulting in the simplification of the Nernst equation [39]:

$$E = E^\circ - \frac{RT}{nF} \ln \frac{R}{O} \quad (1.13)$$

1.4.4 Electrochemical Techniques

This section explores selected electrochemical techniques, focusing on potentiometric, amperometric, voltammetric, and impedimetric methods, with each offering unique insights into the study and analysis of electrochemical processes.

1.4.4.1 Voltammetric Techniques

Voltammetric sensors detect analytes through the application of a potential and the measurement of the resulting current [75]. The potential is applied between the CE and RE, measured at the WE relative to the RE, while the current flows between the WE and CE [40]. Data about the analyte can be deduced from the resulting graph, known as a voltammogram. A variety of techniques based on voltammetry are available, from simple methods such as Linear Sweep Voltammetry (LSV), to more complicated such as Square Wave Voltammetry (SWV) and Differential Pulse Voltammetry (DPV), where a series of pulses are applied over a potential staircase and the current is recorded. The two most used voltammetric techniques in this work are CV, and SWV.

Cyclic Voltammetry is based on the application of a stepped potential for the duration of the measurement and is monitored as a function of time [76], as shown in Figure 1.6 (a). If the experiment does not extend beyond the peak potential (E_{max}), the measurement is referred to as **Linear Sweep Voltammetry**.

try. This technique serves as a tool to derive both qualitative and quantitative insights into electrochemical reactions. Key parameters in the method encompass the maximum (E_{max}) and minimum (E_{min}) values of the voltage ramp, along with the scan rate (expressed in V/s), representing the speed at which the electric potential is swept [46]. In the examination of a CV curve, the primary focal points are the peaks associated with oxidation and reduction within the waveform and correlated with the concentration of the analyte in the solution, as illustrated in Figure 1.6 (b). The characteristics of these peaks serve as indicators of the kinetics of the electrochemical reaction occurring at the electrode-solution interface. This is one of the many advantages of CV scans, being able to detect both oxidation and reduction processes in a single experiment.

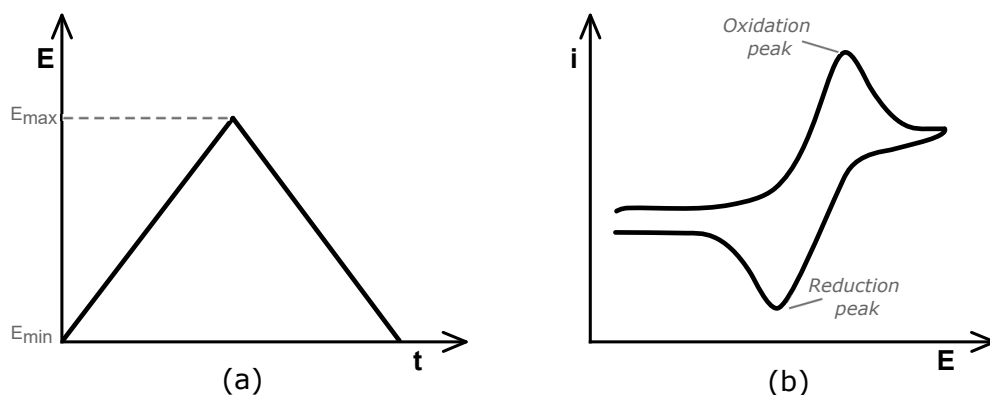


Figure 1.6: CV (a) potential (E) profile as a function of time (t), and (b) typical current (i) response over time (t) (adapted from [49]).

The Randles-Sevcik equation is a fundamental tool in electrochemistry for analysing the relationship between the peak current (i_p) and the solution's concentration during CV [77]. This equation is particularly useful when the diffusion coefficient (D) is known, as it allows the peak current to be predicted based on the analyte concentration. Similarly, if the concentration is known, the Randles-Sevcik equation can be employed to calculate the diffusion coefficient. The equation is expressed as follows:

$$i_p = 0.446nFAC \left(\frac{nFvD}{RT} \right)^{\frac{1}{2}} \quad (1.14)$$

Where n denotes the number of electrons transferred in the redox event, A is the electrode surface area in cm^2 , F is the Faraday constant in C/mol , D is the diffusion coefficient in cm^2/s , C represents the concentration in mol/cm^3 , v stands for the scan rate in V/s , R is the gas constant in J/Kmol , and T is the temperature in K . This equation does not consider capacitive currents and background noise; these factors should be addressed during data processing techniques, as applied in

this work. This equation benefits CV experiments by evaluating how variations in concentration, diffusion coefficient, or scan rate affect the peak current.

Due to its rapid and straightforward implementation, as well as its high sensitivity to changes in the electrode surface and surrounding environment [77], CV was extensively utilised in this thesis for investigating the electrode surface, determining the electrochemical active area of WEs, and analytes detection. Additionally, CV minimally affects the sensor, enabling repeated measurements and integration with other analytical techniques.

Square Wave Voltammetry involves the application of a square wave potential overlapped on a staircase waveform to the WE, allowing the current response to be measured. The square wave is characterised by specific parameters, including start and stop potential, a step size (ΔE_s), the amplitude of the square wave (ΔE_p), and frequency (f). These properties are represented in Figure 1.7 (a). During each cycle, the pulse width can be expressed as $T/2$, where $T = 1/f$. Two currents are recorded per cycle at the end of each pulse. The current measured in the first half of the cycle corresponds to the forward current (i_f), while the current measured in the second half corresponds to the reverse current (i_r). The difference between the reverse and forward currents provides the total current signal at the WE [78]. The SWV alternating positive and negative pulses enable the discrimination between faradaic currents and capacitive currents, facilitating the detection of trace analytes in complex matrices [39]. Analytical chemistry widely employs SWV for applications such as trace metal analysis [79], biosensing [80], and investigating electroactive species in various samples.

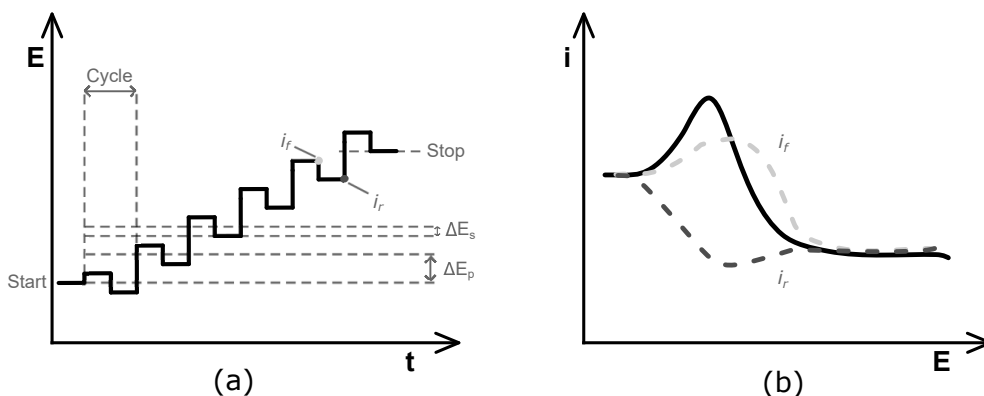


Figure 1.7: SWV (a) potential (E) profile as a function of time (t), and (b) typical current (i) response as a function of time (t) (adapted from [49]).

SWV is significantly more sensitive compared to other voltammetric techniques, such as CV, due to the measurement of both forward and reverse currents [81]. This makes it a processing method for detecting analytes associated with animal health monitoring. Additionally, this technique provides excellent LOD, enabling the quantification of electroactive species at low concentrations

[82]. Thus, this particular feature is relevant for the identification of trace-level biomarkers, which are often indicative of physiological or pathological conditions in animals. A significant benefit of SWV is its speed, as it measures the differential current response to potential pulses, allowing for quick data acquisition in a single sweep. This makes it ideal for applications requiring the monitoring of multiple parameters within short time frames. In this thesis, SWV was extensively employed to detect several key parameters relevant to animal health.

1.4.4.2 Amperometry

Chronoamperometry involves applying a constant potential to an electrode and measuring the resulting current over time. Utilising a stationary WE and an unstirred solution, the mass transport is exclusively driven by diffusion. Consequently, the current-time curve reflects alterations in the concentration gradient near the electrode surface. As time progresses, the slope of the concentration profile decreases due to reactant depletion associated with the gradual expansion of the diffusion layer. Subsequently, the current diminishes over time, following the principles outlined in the Cottrell equation [39]. Figure 1.8 illustrates the applied potential profile and the resulting current-time graph.

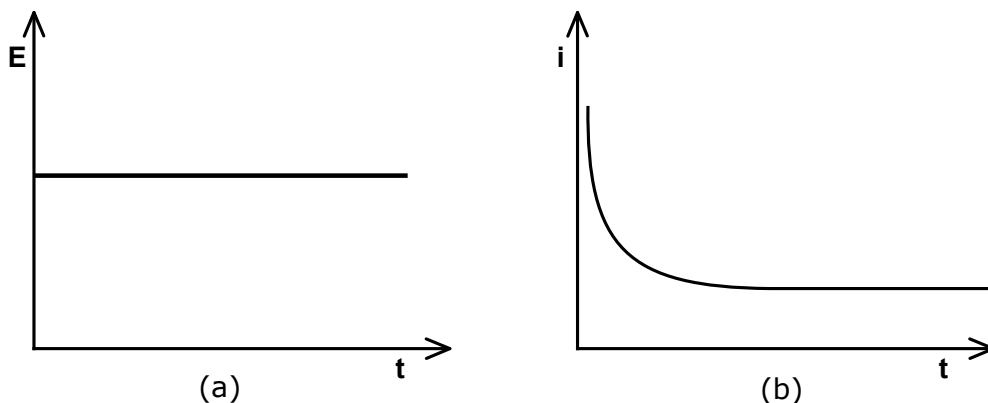


Figure 1.8: CA (a) applied potential (E) as a function of time (t), and (b) typical current (i) response as a function of time (t) (adapted from [49]).

This technique allows real-time monitoring of current changes, making it suitable for studying fast reactions at the electrode surface. Additionally, its simplicity, requiring the application of a constant potential and recording the resulting current, makes it an excellent starting point for FW development and an effective method for validating the accuracy of system readings.

1.4.4.3 Potentiometric Techniques

Open Circuit Potential is used to measure the potential difference between the WE and the RE, in conditions where no current flows in between [75]. The

typical observed graph is illustrated in Figure 1.9. The RE is needed to define the reference potential, whereas the WE potential depends on the analyte concentration [83].

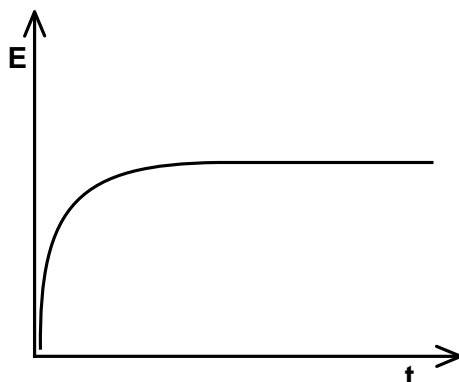


Figure 1.9: OCP typical voltage (E) response as a function of time (t) (adapted from [49]).

This technique is straightforward to implement due to its simplicity in setup. It offers a rapid and direct approach to measuring analyte concentrations, providing real-time results without extensive sample preparation. Additionally, this technique demonstrates high selectivity [84], resulting in minimal interference from other components in complex biological samples. Furthermore, the electrodes used in Open Circuit Potential (OCP) exhibit high stability, which makes them suitable for long-term measurements, as required in health monitoring systems [73].

1.4.4.4 Impedimetric Techniques

In impedimetric or conductometric sensors, the recognition of the element at the electrode surface starts a change in the ionic species concentration and measures conductance [85]. The **Electrochemical Impedance Spectroscopy** method can be initially applied to the determination of the double-layer capacitance, and used for the characterisation of electrode processes and complex interfaces [85]. Electrochemical impedance applies an Alternating Current (AC) signal to an electrochemical cell and monitors the resulting current magnitude and shift [86]. The resulting data is often represented in a Nyquist plot, as illustrated in Figure 1.10, where the real component of the impedance is plotted on the x-axis and the imaginary component on the y-axis. This technique also generates a simulated equivalent electric circuit, proving values for the components within the circuit.

EIS is particularly useful in biosensing applications, as it can detect Deoxyribonucleic Acid (DNA) [87], antigens [88], antibodies, and pathogens [89]. How-

ever, the high power demand and time-consuming nature of these measurements can be excessive, particularly when alternative electrochemical techniques can measure relevant analytes with lower power consumption. Consequently, the power consumption and lengthy measurement times do not align with the requirements for a fast real-time monitoring device. For the purpose of this thesis, EIS was used to study the electrochemical properties of cells, including double-layer capacitance and charge transfer resistance.

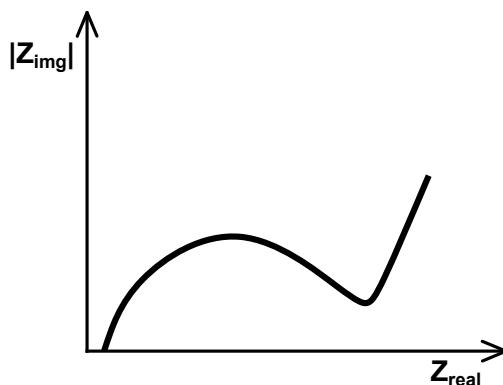


Figure 1.10: Typical Nyquist plot (adapted from [49]).

1.4.5 Conclusion

This chapter outlined the fundamental electrochemical principles and techniques essential for the research conducted in this thesis. An in-depth understanding of each technique, particularly voltammetric, amperometric, and potentiometric techniques, was conducted to gain a comprehensive understanding of the processes taking place at the electrodes. This knowledge informed the design and optimisation of both the Analog-Front-End (AFE) circuits, required for interfacing with electrochemical sensors, and the FW necessary to execute these techniques with precision.

A summary comparing the techniques— OCP, CA, CV, LSV, SWV, and EIS— was created based on characteristics including speed, complexity, and sensitivity, as described in Table 1.2.

OCP is the simplest and fastest electrochemical method, allowing real-time measurement of an electrode’s potential with moderate sensitivity. CA is also relatively straightforward and quick, offering valuable data on current as a function of time. However, its resolution is limited, and it is less sensitive for analysing complex reactions. CV provides higher sensitivity and is particularly effective for studying electrochemical behaviour over a range of potentials. Although it is more complex than OCP and CA, CV offers detailed insights into redox processes, making it highly suitable for intricate analyses. LSV shares similarities

Table 1.2: Comparison of electrochemical techniques based on speed, complexity, and sensitivity.

Technique	Speed	Complexity	Sensitivity	Ref.
OCP	+++	+	+	[75]
CA	++	++	++	[90]
CV	++	++	++	[90]
LSV	+++	++	++	[91]
SWV	+++	++	+++	[92]
EIS	+	+++	+++	[93]

+++ = High, ++ = Moderate, + = Low.

with CV, offering high sensitivity, especially for detecting oxidation and reduction peaks. LSV is slightly less complex than CV and is well-suited for precise potential measurements, though it provides less detailed kinetic information in comparison. SWV stands out as one of the fastest of the voltammetric techniques and is highly sensitive, particularly for detecting low concentrations and fast electron transfer kinetics. However, its implementation requires more complex FW development due to the use of square wave pulses. EIS is the most complex technique, requiring both extensive data analysis and modelling of impedance spectra. Despite its complexity, EIS offers unparalleled sensitivity and detailed information, making it particularly valuable for studying electrode processes, double-layer capacitance, and charge transfer resistance.

The successful integration of sensors, development of HW, and FW requires a deep understanding of electrochemical techniques and the various sensor and system factors that influence device performance. Electrochemical methods are particularly valuable due to their extensive use in healthcare, from early disease detection to continuous health monitoring. This expertise not only guided the precise design and development of HW and FW, but also enhanced measurement accuracy, robustness, and reliability, which are critical for advancing applications such as animal health monitoring.

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LITERATURE REVIEW

This chapter offers a comprehensive review of the key parameters and emerging technologies vital for effective bovine health monitoring. This serves as the foundation for the development of the smart bolus prototype technology discussed in this thesis. Divided into two main sections, the first, Biosensing, explores the physiological and biochemical parameters essential for assessing bovine health and well-being. The second, Sensing Devices, delves into the electronic components and modules used in health monitoring systems, offering insights into their operation and integration. By analysing the intersection of biosensors and electronics, this chapter identifies the knowledge gaps, ensuring a thorough understanding of the technologies that underpin the design and functionality of the proposed device. This literature review is pivotal in contextualising the research, guiding the technical development, and validating the relevance of the approaches adopted in this thesis.

The escalating global demand for animal-derived products has highlighted the criticality of monitoring livestock well-being to improve the dairy industry's efficiency [1, 2]. Besides this, cattle herd management requires numerous decisions to ensure the well-being of the animals, while optimising costs and minimising environmental impacts. Consequently, this requires improved data collection and analytic tools, which are coupled with animal health surveillance to enable decision support for herd management and ensure the quality of dairy products.

Health monitoring tools can enable timely interventions when anomalies are detected, thereby reducing costs and minimising animal losses. Once an ideal device is achieved, there will be opportunities to facilitate decision support tools on the farm that can reduce the economic impact of diseases [3]. In this light, the goal of this study is to identify the latest cutting-edge technologies and emerging trends in this domain, and critically evaluate them. Nevertheless, while primarily

focusing on devices and sensors used for continuous on-farm monitoring, these technologies also have the potential to be applied in research environments. For example, an effective health monitoring tool could become an asset to quantify directly and/or indirectly methane emissions, and take mitigation steps to reduce it as a source of Greenhouse Gas (GHG) emissions [4, 5].

Monitoring cattle health can be achieved through the employment of miniaturised electronic systems such as collars [6], ear tags [7], and wireless ingestible capsules, also known as smart boluses [8]. Researchers are putting effort into the development of sensors and associated systems needed to bring these technologies towards further advancement. When designing an ideal monitoring device for cattle health, certain specifications such as cost, data management, usability, safety and others are of concern. Some key standards that may be relevant include ISO 13485 (medical devices - quality management systems), ISO 9001 (quality management systems), IEC 60601 (medical electrical equipment), ISO/IEC 27001 (information security management systems), ISO 10993-1 (medical devices - biological evaluation), among others. The device should ensure animal safety and comfort during usage, coupled with easy deployment and removal [9–11]. Real-time health and well-being data acquisition is necessary, allowing for prompt detection of any anomalies [12, 13]. Additionally, it should facilitate multi-parameter measurement [13]. Secure data storage, easy retrieval, and durability to endure the rigors of farm environments and prolonged lifespans are key aspects for ensuring commercial viability [12]. Affordable maintenance and seamless integration with farm technologies, encompassing feeding and management systems, are indispensable for a holistic monitoring solution [12–14]. Nevertheless, data sharing with stakeholders like veterinarians, researchers, and agricultural professionals further amplifies the device’s utility [12, 14, 15]. Ultimately, the device should be easy-to-use, meaning it is user-friendly [13]. In addition, incorporating features like AI and advanced data analytics for data interpretation has recently become essential for farm management and disease detection [16].

2.1 Biosensing

Numerous commercially available biosensing systems have emerged to facilitate the monitoring of bovine health. Some examples include smaXtec Bolus [8], Cowlar [17], MooMonitor+ [18], among others. Beyond the current solutions, ongoing research is focused on biosensing for monitoring the health of cattle [19, 20]. Biosensing is a well-established technology, and its application to monitor the health of bovine animals through the assessment of a wide range of physical, physiological, chemical, and biochemical parameters is an emerging field

[21, 22]. Biosensors are transducers that can detect and measure these parameters by converting biological signals into electrical signals that can be analysed and interpreted [23]. By applying biosensors on farms, farmers and veterinarians can get real-time insights into the welfare of the herd.

Biosensors can be integrated into different platforms, such as wearable devices, implants, and remote monitoring systems. Depending on the parameters' requirements and the desired application, different types of biosensors and platforms may be used. Awasthi et al. [24] explored the most common wearable devices in correlation with the measured parameters within cattle. In recent years, significant advances have been made in developing devices to measure and improve animal welfare. These include wearable textile pieces [25], smart bolus [20], collars [26], and many others [27]. The broad range of measurable parameters highlights the growing need for advancements in biosensing systems, which are indispensable tools for monitoring bovine health.

2.1.1 Biometric Parameters

An overview of multiple studies is provided in Table 2.1, focusing on the monitoring of biometric parameters in cattle, encompassing temperature, activity, and heart rate. The table outlines the specific parameters measured, device types, key specifications, and their associated limitations and advantages.

2.1.1.1 Temperature

A cow's normal body temperature, which reflects its physiological condition, typically ranges between 38 °C and 39 °C; thus, fever became an early indicator of infection or other health issues [11, 28, 29]. Conversely, temperature fluctuations can also provide insights into reproductive status, as temperature changes are sometimes linked to ovulation or other reproductive cycles [30, 31]. However, interpreting elevated temperatures is challenging, because they can result from different causes, such as infections, environmental stress, or reproductive processes. For instance, elevated temperatures during pregnancy may indicate potential complications, including reduced fertility or embryonic loss. On the other hand, temperature fluctuations related to the reproductive cycle, such as those associated with ovulation, may be temporary and less concerning. Thus, if the temperature data is recorded over time, time series patterns associated with the cow's reproductive cycle can be compared to current data. Additionally, by combining temperature data with other factors, sensors can provide more context. Studies found another temperature-related parameter, the temperature-humidity index, which reflects the environmental conditions, and is associated

with advanced pregnancy (>90 days) when it exceeds 84 [32].

Wearable devices like collars [17, 18], and ear tags [7] measure temperature. However, these are external measurements and can be influenced by environmental factors like barn temperature, humidity, and the cow's behaviour, such as lying down or standing up. These external factors complicate the accuracy of body temperature readings [11]. In contrast, measuring temperature directly from within the cow provides a more accurate assessment of the cow's core body temperature, as it is less susceptible to external influences. Initial studies were focused on methods to access cattle's vaginal temperature using intravaginal sensor [33, 34]. Later, less invasive sensors were tested and used to alleviate the stress in the animals [35, 36]. Recently, Sakphrom and Chaimool [27] developed a rumen temperature bolus to assess the body temperature of the animals. However, this is limited to only temperature measurements and further studies need to be done to validate the technology used. Additionally, a study conducted by Vakulya et al. [20] has demonstrated how continuously monitoring temperature from the animal's rumen can be used to detect drinking patterns.

Furthermore, measuring temperature within the gut offers insights into the cow's digestive system function. The gut temperature reflects the activity of microorganisms involved in digestion, with temperature fluctuations indicating disruptions in these microorganisms and issues, such as acidosis or diarrhea [37]. Additionally, cattle have a unique thermoregulation mechanism that helps maintain a stable internal temperature. Hence, this measurement can also provide information about the cow's thermoregulation. Disruptions in this system can lead to heat stress, adversely affecting cow health and productivity [38]. The collection of this parameter aids farmers and veterinarians in taking prompt action to treat any issues, and improve the cow's well-being. Among commercially available devices for measuring gut temperature, the smaXtec Classic Bolus incorporated a temperature sensor with a range of 20°C to 60°C , and accuracy of $\pm 0.01^{\circ}\text{C}$ [39]. The high accuracy of these sensors ensures that even subtle changes in temperature related to specific health or reproductive conditions can be detected, supporting timely intervention by farmers and veterinarians. These devices are administered to each animal in the herd. Similarly, the eBolus by eCow employed a temperature sensor with an accuracy of $\pm 0.1^{\circ}\text{C}$ [40].

Ultimately, the integration of temperature data with other behavioural and physiological indicators allows for more informed decision-making. For example, a sudden increase in body temperature combined with reduced movement may indicate the early onset of illness, prompting immediate veterinary intervention. Similarly, detecting a temperature rise aligned with specific activity patterns can help identify oestrus, allowing timely and accurate insemination. Early detec-

tion of health issues or deviations in reproductive cycles enables farmers to take proactive actions that optimise herd health and productivity, improving overall farm management.

2.1.1.2 Motion

Cattle activity quantification is done through Global Positioning System (GPS) modules, accelerometers, and gyroscopes [41, 42]. These components can be incorporated into wearable devices such as tracking collars, as previously done by Benaissa et al. [43]. Collars are largely used due to affordability and efficiency to detect and track animal motion [44]. A recent study conducted by Yu et al. [26] demonstrated the development of a collar equipped with an accelerometer to detect behaviours using Machine Learning (ML) algorithms. Similarly, ear tags have the potential to incorporate motion sensors, as previously done by Pereira et al. [45]. Some of the commercially available systems included a variety of devices, such as CowManager [7], MooMonitor+ collar [46], and AfiAct Pedometer [6]. A review conducted by Riaboff et al. [47] revealed methods employed in diverse systems for livestock behaviour prediction. This study revealed the lack of model generalisation as a limitation in commercial deployment. According to the study, larger datasets with broader variability provide better generalisation. A study conducted by Vakulya et al. [20] demonstrated that acceleration data from a rumen bolus can be utilised to detect rumination and monitor motion activities through the application of algorithms.

By scrutinising activity levels, farmers can identify signs of illness or discomfort. Reduced activity might indicate an animal is unwell and may need to be checked by a professional veterinarian [41]. Additionally, by coupling activity data with AI, it is possible to identify calving events, as demonstrated by Miller et al. [48]. This study demonstrated that accelerometer data from tail-mounted sensors, when processed by ML models, can accurately predict calving. Besides this, AI can enhance artificial insemination success when combined with temperature measurements [49]. A study conducted by Wongvivatvaitaya et al., demonstrated that the combination of temperature and accelerometer data can be used to predict estrus [50]. Moreover, this information can be used to improve the efficiency of the breeding programme and increase the overall productivity of the herd. Cattle activity levels are also interlinked with feeding patterns. More active animals usually exhibit a greater appetite, thus, activity monitoring can be used as a tool to identify feeding needs and others. This approach can improve cow health and productivity, as well as grassland management [24, 51].

2.1.1.3 Heart Rate

Measuring heart rate in cattle yields crucial information about animal health. This physiological parameter can increase in response to stress, making it a valuable indicator of the animal's condition [52]. Variations in heart rate can signify a range of health issues, encompassing heart disease, respiratory problems, and pain. Therefore, its utility extends to veterinary procedures [53]. Numerous techniques exist to monitor bovine heart rate, including Electrocardiography (ECG), which involves placing electrodes on the animal's skin to record the electrical activity [54]. A study by Betancourth-Chaves et al. [25] developed a wearable textile vest for monitoring heart rates in cattle. The study used commercial devices to measure the animals' heart rates, establishing a method for heart rate measurements using vests. However, further investigation is required to evaluate long-term performance and to identify and eliminate factors that may affect the accuracy of the readings.

Technological progress has facilitated preliminary investigations into measuring heart rate within the cow's rumen; however, this research is still preliminary [55]. A rumen bolus equipped with an accelerometer demonstrated the potential to estimate heart rate exclusively from accelerometer data through algorithmic processing [20]. However, further advancements are necessary to reduce the current 10% error. A significant challenge in this approach is the elimination of noise. Heart rate signals in the rumen can be obscured by various sources of interference, including muscle contractions, ruminal movements, and other physiological processes.

Non-invasive heart rate monitoring devices, such as Polar Sport Tester, can be placed and fixed to the animal's body for continuous heart rate tracking [53]. These devices are also known as Photoplethysmography (PPG) and have been incorporated into ear tags as a promising technology for continuous animal heart rate monitoring [56]. Since PPG detects the blood volume changes, this method can be considered an indirect measurement of heart rate. Thus, PPG enables heart rate measurement from any location on the animal's body where blood vessels are close to the skin's surface [57].

2.1.2 Chemical & Biochemical Parameters

A vast variety of chemical and biochemical parameters can provide information about the animal's health. Several parameters related to the microbiome and its metabolic processes can be identified, such as blood metabolites, including glucose and lactate [58, 59]. Table 2.2 offers an overview of various studies.

2.1.2.1 Blood Glucose

Blood glucose levels are critical for understanding the metabolic health of dairy cows. Low glucose levels can indicate conditions like ketosis, and high levels can be related to insulin resistance, both of which affect the cow's overall productivity and health [60]. Glucose levels are related to both the quality and quantity of milk [61], as well as to the levels of lactose [62]. Glucose serves as the primary source of lactose production in the mammary gland [63]. Lactose is a key determinant of milk yield, influencing both milk quantity and composition. Low blood glucose may lead to reduced lactose synthesis, thus decreasing milk production.

Blood glucose can be measured using a glucose meter, which analyses a small blood sample typically obtained from the cow's ear or tail with a test strip. A recent study performed by Byrd et al. [64], demonstrated that human glucose monitors like FreeStyle Libre [65] and Dexcom [66], designed to measure ISF glucose levels, can be applied in cows to detect glucose variations, yet with less accuracy compared to blood-based systems. There are commercially available devices tailored for dairy cows, such as CentriVet GK Blood Glucose & Ketone Monitoring System, measuring blood glucose in 5 s within a range between 0.6–33.3 mmol/L [67].

Monitoring blood glucose levels is critical for the early detection of metabolic disorders, particularly during the transition period around calving, when cows are highly susceptible to energy imbalances. Regular glucose measurements help identify cows at risk of conditions such as ketosis, enabling timely intervention and facilitating the monitoring of recovery after treatment. Furthermore, as blood glucose is directly linked to lactose synthesis, tracking its levels provides valuable insights into milk yield and quality, supporting the optimisation of dairy production. As technology continues to advance, a preliminary study by Liu et al. [68] proved the potential to detect glucose from artificial ISF using a wearable device. However, this study is still in its early stages, and further analysis is needed to address challenges such as microneedle interference, repeatability, and stability. In addition, future research should evaluate the impact of microneedle size and the associated effects of skin thickness in bovine applications. Additionally, the significance of measuring glucose from bovine ISF needs to be thoroughly investigated.

Table 2.1: Comparative analysis of physical parameter measurement devices for bovine health monitoring.

Study	Parameter	Type of Device	Specifications	Limitations	Strengths
[17]	Temperature	Collar	No details on accuracy and precision.	Affected by surroundings and animal activity.	Non-invasive.
[18]	Temperature	Collar	No details on accuracy and precision.	Affected by surroundings and animal activity.	Non-invasive.
[7]	Temperature and activity	Ear tag	Operating temperature range from -4°C to 104°C .	Affected by surroundings and animal activity.	Non-invasive. Required solar routers in the farm.
[39]	Temperature	Smart bolus	Temperature range from 20°C to $60^{\circ}\text{C} \pm 0.01^{\circ}\text{C}$.	Invasive.	Accurate internal body temperature measurement.
[40]	Temperature	Smart Bolus	Temperature $\pm 0.1^{\circ}\text{C}$.	Invasive.	Accurate internal body temperature measurement.
[33]	Temperature	Intravaginal temperature device	Temperature sensor: ADT7320. Motion sensor: ADXL362.	Invasive.	Real-time measurements. Detected an increase of 0.3°C during estrus.

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Table 2.1 – continued from previous page

Study	Parameter	Type of Device	Specifications	Limitations	Strengths
[34]	Temperature	Intravaginal probe	Temperature sensor: KS103J2. Accelerometer: ADXL362. Probe diameter 20 mm and lengths of 120 mm and 160 mm.	An optimal probe size remains undetermined. The sizes used may induce internal bleeding. Challenges associated with measuring resistance.	Measured vaginal resistance associated with vaginal hydration levels. Also acquired data on acceleration.
[69]	Temperature	Implantable sensor	Temperature sensor integrated in a RFID LifeChip Microchip with a precision of 0.1 °C.	Invasive procedure - the device is implanted at the base of an ear and the insertion needs to be performed by a qualified veterinarian. Limited number of subjects (n=3).	Demonstrated accurate measurements of core body temperature.

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Table 2.1 – continued from previous page

Study	Parameter	Type of Device	Specifications	Limitations	Strengths
[35]	Temperature	Thermal infrared imaging of eye and vulva	Temperature sensor: FLIR A310. Operational range from -20°C to 120°C , and an accuracy of $\pm 2^{\circ}\text{C}$.	Limited estrus detection rate. Applicable mainly to small farms due to acquisition method constraints. The study lacked comprehensive measurements across the entire estrous cycle.	Non-contact approach to mitigate animal stress.
[70]	Temperature	Implantable sensors	Temperature sensor range 34°C to 43°C with an accuracy of 0.005°C .	Invasive method, the sensors are surgically implanted in 10 different spots on the animal.	Tail-based was identified as the best location for subcutaneous monitoring of body temperature in cows.
[36]	Temperature	Infrared photodiode thermometer	Operating ranges of -10°C to 50°C . Sensor is placed over the tail base and abdominal region.	Not suitable for wireless applications and large-scale farms.	Surface temperature measurements, non-invasive.

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Table 2.1 – continued from previous page

Study	Parameter	Type of Device	Specifications	Limitations	Strengths
[51]	Activity	RumiWatchSystem	Pressure sensor in head halter, and a pedometer on the leg. Raw data saved in an SD card for 4 months.	Operated best with continuous data instead of periodical.	Accurate detection of rumination time.
[71]	Activity	Noseband	RumiWatch	Low accuracy in calculating chews per minute and per bolus, and number of boli.	Accurate measurements of ruminating time.
[72]	Activity	Wearable motion sensor	6-axis Inertial Measurement Unit (IMU) placed on the leg. Apply ML to determine abnormal patterns.	Space for improvements in accuracy and sensitivity (74%). In abnormal patterns, 74.2% are detected, leaving 25.8% undetected.	Each animal's particular characteristics were taken into account.
[73]	Activity	Collar	Accelerometers from Afimilk Ltd.	Required a wider evaluation to determine accuracy.	Non-invasive. Detected heat stress.

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Table 2.1 – continued from previous page

Study	Parameter	Type of Device	Specifications	Limitations	Strengths
[74]	Activity	Inclinometer	Sensor from Moocall Ltd. placed at the top of the tail.	Limited sensitivity, instances of device detachment and displacement, device-induced pressure marks, and occurrences of necrosis.	Wearable and non-invasive.
[46]	Activity	Collar	No details provided.	Required its own base station.	Wearable.
[6]	Activity	Pedometer	Accelerometers attached to leg.	Offered intermittent data collection at 15-minute intervals and interfaces with the farmer's software only once per hour.	Wearable and non-invasive.

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Table 2.1 – continued from previous page

Study	Parameter	Type of Device	Specifications	Limitations	Strengths
[75]	Activity	Collar	Device MOOnitor with GPS, 3-axis accelerometer, and temperature sensor. Machine learning applied to classify animal behaviour.	Required enhancements in device weight and dimensions.	Transmitted data directly to a server and exhibits high precision in activity detection and identification.
[76]	Heart rate	Magnetocardiography	Sensor: QuSpin QTFM	Practicality - animal is enclosed in a metal structure.	Capable of capturing heart rate data, including beat timing and amplitude without contact.
[77]	Heart rate	Human heart pulse device	No details were given on the sensors used.	Absence of a device body placement specification, further testing and validation are needed.	Radio frequency identification module technology.

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Table 2.1 – continued from previous page

Study	Parameter	Type of Device	Specifications	Limitations	Strengths
[78]	Heart rate	Nose ring	Sensor: MAX30101 (TinyCircuit, Akron, OH)	Susceptible to environmental temperature impact. Constrained availability of visual breathing count methods for reference.	Quantifies breathing rate and blood oxygen levels. It preserves the cow's natural behaviour unaffected.

Table 2.2: Comparative analysis of chemical parameter measurement devices for bovine health monitoring.

Study	Parameter	Type of Device	Specifications	Limitations	Strengths
[79]	Glucose	Glucometer FreeStyle Libre Sensor and LibrePro Sensor	Sensor is placed at the base of the ventral tail. Lower LOD 40 mg/dL.	Requires calibration for cattle and lower measurement limits.	Capable of predicting calving within 24 hours.
[64]	Glucose	Interstitial glucose monitors	Sensors: FreeStyle Libre and Dexcom G6. Placed behind the cows' polls and beneath pin bones.	Limited accuracy (60.7%), inferior to blood glucometer. Limit of measurement not appropriate for bovines.	Identify sensor limitations.
[65]	Glucose	FreeStyle Optium Neo	No details on accuracy and precision.	Tailored only for humans.	Portable ketone measurement device.
[80]	Glucose	FASTest Bovine IgG	Sensitivity 95.5% and specificity 96.2%.	Restricted to veterinarians; expensive tool.	Applicable to calves and cattle.
[67]	Glucose	CentriVet™ GK Blood Glucose & Ketone Monitoring System	No details on accuracy and precision.	Not wearable or continuous; operator required.	Affordable and rapid results in seconds.

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Table 2.2 – continued from previous page

Study	Parameter	Type of Device	Specifications	Limitations	Strengths
[81]	Oxygen	VetScan Alinity v	iStat Capable of measuring multiple analytes from a blood sample.	Invasive and requires an operator.	Portable and delivers results in 2-3 minutes.
[82]	Oxygen	Pulse oximeter	Sensor M-LNCS TF-I A, Masimo Corporation placed in interdigital space.	Requires additional studies for continuous measurement across various activities.	Suitable for measuring oxygen levels and pulse rate.
[83]	Oxygen	Pulse oximeter	Sensor: MAXREFDES117 (Maxim Integrated) measured hemoglobin oxygen saturation and pulse rate placed close to the mammary region.	-	Low-cost device; detected post-milking oxygen level variation.
[84]	Methane	Respiration chamber	Animals are placed in chambers	Data collection required animal confinement in the chamber.	Approximation to methane emissions.

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Table 2.2 – continued from previous page

Study	Parameter	Type of Device	Specifications	Limitations	Strengths
[85]	Methane	Green feed	Estimated methane based on gas concentration and flux at a feeding stall.	Limited to measuring ruminal fermentation [86]. Interferences can occur due to methane in the background.	Strong agreement with respiration chambers.
[87]	Methane, pH	Laser methane detector and smaXtec bolus	Methane sensor: LMD HESAI HS4000. Cows were separated into groups according to ruminal pH, and methane emissions were measured.	Further validation studies are required.	Identified a potential link between pH and methane.

2.1.2.2 Blood Oxygen

Blood oxygen levels are equally pertinent, and reduced levels are associated with various respiratory or cardiovascular issues [88]. Reduced oxygen saturation, known as hypoxemia, can signal underlying health issues, such as respiratory distress, pneumonia, or circulatory problems, all of which can significantly impact the animal's productivity and overall well-being. Monitoring blood oxygen is essential, especially in high-risk periods such as after calving or during periods of heat stress, where respiratory demands may increase. Early detection of hypoxemia can help prevent the progression of serious conditions and guide timely interventions, improving animal health and reducing the likelihood of costly treatments or prolonged recovery times. In healthy cattle, the typical value for arterial plasma partial pressure of oxygen is above 80 mmHg [89]. Levels below 80 mmHg are considered hypoxemic, suggesting that the animal may be experiencing respiratory or circulatory distress. When oxygen levels drop below 60 mmHg, immediate veterinary attention is required, as such low oxygen levels can lead to organ failure and severe metabolic disturbances.

There are two primary methods for monitoring blood oxygen levels in cattle: invasive blood oximeters and non-invasive pulse oximeters. Blood oximeters require direct access to the blood and are commercially available for veterinary use, such as the VetScan iStat Alinity v [81]. In contrast, pulse oximeters utilise light beams through the skin to measure blood oxygen levels through the skin. These devices have been tested in cows and are considered effective for the continuous monitoring of bovine blood oxygen levels. Additionally, pulse oximeters can also measure pulse rate, providing further valuable physiological data [82].

2.1.2.3 Metabolites and microbiome

The gut microbiota complex community of microorganisms, encompassing bacteria, viruses, and fungi, is a key element in fermentation, synthesis of essential vitamins, immune system regulation, pathogen control, and regulation of metabolism [59]. Disruptions to the gut microbiota can lead to digestive and metabolic issues, and increased disease vulnerability. Therefore, real-time monitoring of the microbiome would be facilitated by a device capable of directly detecting disturbances in microbial communities. However, such a POC instrument is not yet available [90, 91].

To better understand the gut microbiome's influence on bovine health, researchers developed offsite measuring techniques to be undertaken in a laboratory environment [92]. From these techniques, DNA sequencing provides a comprehensive understanding of microbiome composition and diversity [93], while metage-

nomics identifies the genetic material within a sample [94]. Another technique was microarray-based analysis, such as phylogenetic microarray, and PCR-based methods, such as Quantitative PCR (qPCR) enabled researchers to identify specific microbial species based on DNA and Ribonucleic Acid (RNA) sequences, gene amplification, and other properties [94]. However, these methods require sophisticated equipment and are not suitable for continuous, real-time monitoring.

Although a POC system for direct microbiome monitoring is not yet available, wearable devices such as boluses could potentially be adapted to indirectly monitor parameters that indicate changes in the microbiome. Techniques like DNA sequencing and metagenomic analysis, while informative, are too complex to integrate into bolus or wearable sensor systems, as they require sophisticated laboratory equipment. However, simpler biochemical markers offer a promising alternative for real-time monitoring.

2.1.2.4 Methane

Methane forms within the digestive system through microbial fermentation of food in the rumen. It acts as an indicator of microbial activity in the rumen and the overall digestive health of ruminant animals [95]. Numerous methodologies assess methane emissions from cows, including direct measurement through air collection and analysis using chambers and indirect approaches by assessing feed intake and output [96]. Furthermore, remote sensing techniques, such as infrared cameras, and mathematical models also play a role in estimating methane emissions. An in-depth overview of various techniques for quantifying methane emissions in cattle is provided by Bekele et al. [97].

Methane production has a large contribution to global warming and climate change [5]; in particular, ruminal methane accounts for 6% of the global GHG emissions [2]. In 2022, cattle were responsible for 62% of the global livestock GHG emissions, with 54% attributed to CH₄ from enteric fermentation [98]. Therefore, accurately measuring and quantifying methane emissions from livestock, along with implementing mitigation strategies, such as dietary modifications [5], could contribute to reducing the environmental impact of livestock production. While current bolus technology may not yet include methane-specific sensors, advancements in methane sensing technologies could enable direct methane measurement within the rumen in the future. Alternatively, the detection of other analytes correlated with methane production, which may be easier to measure, could provide an indirect yet effective means of monitoring methane emissions.

2.1.2.5 pH

The bovine blood pH serves as an indicator of acidity or alkalinity and is typically around 7.37 [99]. Abnormal pH can indicate a problem related to acid-base balance and can be caused by a variety of health problems, such as respiratory or metabolic disorders [99]. However, measuring pH in the blood has several disadvantages, including being an invasive procedure, often causing stress to the animal and requiring skilled personnel for collection. This makes frequent monitoring challenging and less practical for routine health assessment.

The rumen's pH is crucial for assessing the health of the digestive system. The rumen's pH should typically fall between 6.0 and 6.5 [99]; however, in cases of high-grain diets, pH can range between 5.5 and 6.0 [100]. Nevertheless, pH values between 5.0 and 5.5 are considered indicators of Subacute Ruminant Acidosis (SARA), while pH values between 5.6 and 5.8 lack a clear definition and can be classified as marginal [101, 102]. On the other hand, elevated pH can suggest that the animal has difficulties breaking down feed, also leading to health problems [103]. Regularly monitoring the pH of the rumen allows for early disease identification, allowing proactive measures to maintain the overall health of the herd.

Several methods exist for measuring the rumen's pH; a procedure referred to as rumenocentesis was once commonly used. This method involved the use of a needle and syringe to collect a sample of rumen fluid for pH analysis [103]. More recently, a rumen pH sensor bolus appeared to avoid issues with lesions and sample contamination. These devices have a pH sensor that can be inserted into the cow's reticulum. Typically, the bolus is connected to a data logger that records pH values, and wirelessly transmits them to a computer or mobile device. This approach permits continuous monitoring of reticulorumen pH, providing detailed insights into pH changes over time [104]. SmaXtec offered a pH plus bolus, which is administered to between 6-10% of the herd, monitoring the group's feeding and health. The sensor can detect pH values from 3 to 9, with an accuracy of ± 0.2 during the first 90 days and ± 0.4 for up to 150 days [39]. Similarly, the eBolus incorporated a pH sensor with an accuracy of ± 0.1 and a lifetime of 90 to 150 days [40]. However, since these sensors have a limited lifespan of 90 to 150 days, they do not provide continuous monitoring throughout the cow's entire life cycle, leaving gaps in long-term health monitoring.

2.1.2.6 Lactate

Lactate, in particular D-lactate levels, can contribute to the detection of SARA, since it reduces the rumen pH [103]. High levels of ruminal lactate

are related to situations where the animal is under stress, such as heat stress, and infections [105]. Lactate is considered a byproduct of anaerobic metabolism that can indicate conditions of ketosis, where lactate levels rise due to the overproduction of glucose [106]. Devices based on Enzyme-Linked Immunosorbent Assay (ELISA) and spectrophotometry can be employed to measure lactate by analysing a small blood sample from the cow. However, these toolkits are expensive, limiting their accessibility for routine or widespread use in smaller farms or by individual farmers [107, 108]. The need for blood samples also presents the challenge of invasive testing, making frequent monitoring more complicated. This makes the tools less practical for continuous or on-site monitoring of lactate levels in real-world farming scenarios. To address the invasiveness of blood sampling, a study proposed the use of microneedles to measure lactate levels in artificial ISF [68]. However, further testing is required to evaluate the reliability of these measurements and the relevance of lactate monitoring in animal ISF. Additionally, the proposed method may not provide a viable solution for continuous or on-site monitoring, as its feasibility depends on the performance and durability of the microneedles.

2.1.2.7 Nitrite and Nitrate

Cattle are frequently susceptible to nitrate poisoning due to diet, negatively impacting reproductive performance and milk production [109]. Previous studies have reported that ingesting 0.22 g to 0.33 g of nitrate per kg of body weight can be fatal within three hours, corresponding to a nitrate concentration of 220 ppm to 330 ppm [110]. Hall [111] reported that poisoning was detected at 300 ppm of nitrate and 20 ppm to 25 ppm of nitrite in plasma or serum, with one-third less in ocular fluid.

Ruminants are especially vulnerable due to nitrate's conversion to ammonia, with nitrite as an intermediate product during digestion [111]. Nitrate ions are reduced to nitrite ions in the rumen, followed by rapid absorption and the formation of methemoglobin, causing health risks to the animal [110]. During gestation, elevated nitrate levels reduce oxygen supply to the fetus, potentially causing abortion [112]. Beyond its impact on animal health, nitrate also influences methane (CH_4) emissions as an electron acceptor in the rumen [113, 114]. Studies explored using nitrate as a dietary supplement to mitigate methane emissions [110]. This strategy is favoured since the conversion of nitrate to ammonia ($\Delta G^0 = -600 \text{ kJ/mol}$) is a more favourable pathway compared to the reduction of carbon dioxide to methane ($\Delta G^0 = -136 \text{ kJ/mol}$) [115]. Therefore, monitoring ruminal nitrate and nitrite levels is vital for cattle health, and mitigating GHG emissions.

Typically, ruminal fluid sampling for nitrate and nitrite detection is obtained through oral stomach tubing or fistulated cows, with samples subsequently stored for later analysis [113]. However, this method faces challenges due to practical execution, animal discomfort, saliva contamination, and potential sample quality compromise during storage. To overcome these issues and allow real-time detection, alternative techniques were proposed using electrochemical sensors [116]. A review by Lal et al. [117] highlighted the demand for robust sensors in multiparametric scenarios, emphasising repeatability, sensitivity, and selectivity. The literature presented solutions that mainly focused on water applications, requiring sampling.

2.1.2.8 Sodium

Sodium is responsible for maintaining the health and well-being of cows, alongside other analytes, as it is involved in various physiological processes. Sodium levels are associated with conditions such as diarrhea, where sodium loss occurs, and play a role in maintaining milk quality by ingesting sodium bicarbonate as a rumen buffer. Treatments for certain diseases, like salmonella, include the use of ceftiofur sodium, while sodium bicarbonate is employed to address dehydration related to diarrhea and to treat acidosis. Additionally, sodium thiosulfate can be administered to treat copper toxicity at doses of 300–1000 mg/head/day for three weeks [118]. A study showed that the rumen of a healthy cow contains approximately 116 mM of sodium, making it a valuable diagnostic parameter [119]. Given its consistent presence in the rumen and its frequent use in food supplements, sodium serves as an ideal analyte for monitoring cow health, providing insight into both dietary balance and potential metabolic issues. While devices like the VetStat Electrolyte Blood Gas Analyzer [120], 9180 Electrolyte Analyzer [121], and i-STAT 1 [122] are commercially available and capable of measuring analytes, including sodium, in blood samples, they come with several practical limitations in animal health monitoring. First, these devices are designed for analysis of extracted blood sampling, which is invasive and can cause stress to the animal, making frequent monitoring challenging. Additionally, there are significant costs involved, both in terms of the equipment and the need for trained personnel to operate them, which limits their accessibility to smaller farms. Moreover, they require regular calibration and maintenance to ensure accurate readings, adding to the overall expense and complexity.

2.1.2.9 Potassium

Potassium is an important electrolyte for monitoring the health and well-being of cows, especially in relation to dehydration and electrolyte imbalances caused by diarrhea and other health issues [118]. Animals suffering from diarrhea may initially exhibit elevated potassium levels in their blood due to dehydration and fluid shifts. However, they are often severely deficient in potassium overall because of continuous and significant losses through faeces. Thus, a continuous monitoring device is crucial to visualise the overall trend in potassium levels. To treat this, extra potassium (10–20 mM) is often added to rehydration fluids. Potassium is also used to treat conditions such as pregnancy toxemia and fatty liver syndrome, by administering an electrolyte solution containing potassium along with other essential electrolytes. A healthy cow typically maintains around 20 mM of potassium in its rumen, making this ion a key diagnostic parameter to regularly measure in the rumen [119]. This way, farmers can better assess an animal’s electrolyte balance, helping to ensure timely intervention and optimal well-being. This analyte can be measured from the bloodstream using the same tools mentioned for sodium.

2.2 Sensing Devices

Section 2.1 provided a comprehensive understanding of biosensing analytes and the key parameters for bovine health monitoring. That knowledge is complemented by this section on the technological front, where the review meticulously delves into the devices utilised for the monitoring process. Advances in technology have revolutionised bovine health monitoring [123]. Advanced electronic instruments are replacing manual methods, allowing for real-time monitoring and increased efficiency on the farm. These instruments comprise a number of technology building blocks, including sensors, a MCU, data storage components, and a wireless communication module. This technological integration bridges the gap between theoretical understanding and practical application, offering a holistic solution for enhancing bovine well-being and productivity.

A comprehensive analysis of currently available and under-development devices is detailed in Table 2.3 for bovine health monitoring. This table provided a breakdown of crucial attributes, including encapsulation material, overall dimensions, sensing parameters, communication frequency, battery lifetime, and weight to compare the different existing approaches, including a smart pill for application in human health for gastric mobility tests.

Monitoring devices architecture is similarly structured across different tech-

nologies, encompassing a data-capturing measurement unit, a base station as necessary, and, in selected instances, supplementary elements like wearable gateway devices or charging stations to enhance system support and user experience.

Battery selection significantly impacts the performance of these technologies. For example, the smaXtec bolus battery endures for about five years—coinciding with a cow’s fertilisation cycle—facilitating optimal fertilisation timing and calf birth prediction 15 to 20 hours in advance [124]. On the other hand, the eBolus, measuring the same variables, remained operational for only two years. Additionally, while smaXtec retained up to 6 days of data before overwriting, eCow stores data for 28 days. Accessing bolus data necessitates the animal’s proximity, typically 5 m to 10 m from the base station, extendable to 120 m using a secondary device. The boluses are not reusable or replaceable, and once the battery runs low or a problem occurs in the sensing system, the device remains in the animal. Extracting boluses from a cow’s gut remains challenging, possible only with fistulated cows due to rumen and reticulum accessibility. Despite being designed for a cow’s life span (15 to 20 years), none of these technologies guarantee such longevity [125]. As a result, a cow might require more than one bolus during its lifetime. The battery is not the major limiting factor, but rather the sensing systems. For instance, the pH sensor readings from the rumen become unreliable after 90 to 150 days of continuous use [126]. Therefore, advancements in electrochemistry are also necessary to address this challenge.

Wearable devices such as ear tags [7], leg braces [6, 127], and collars [6, 17, 18] are monitoring devices that supply real-time data to the final user. The ear tags are designed to be lightweight and compact, allowing them to be attached to a cow’s ear in various sizes and shapes. Chung et al. [128] demonstrated the use of an injectable temperature sensor placed behind the ear to predict the animal’s body temperature using regression models. Additionally, Hu et al. [129] showed how accelerometers integrated into ear tags can monitor the animals’ activity levels and patterns by calculating statistics such as the mean and Standard Deviation (SD) of the acceleration magnitude, as well as applying filters to process the data. Overall, ear tags can track location, movement, and health through embedded sensors, transmitting this data to a base station. However, studies have shown negative impacts on the animals, including infections, injuries, and scarring [130]. Although ear tags may, in theory, be a valuable tool for real-time monitoring, with rechargeable batteries and long-term use over the animal’s lifetime, further investigation is needed to minimise the associated health risks.

Table 2.3: Overview of the technology devices developed to monitor the health and well-being of the cattle and comparison with a human GI pill and sphere devices to monitor bioreactors.

Device	Type	Dimensions (mm)	Measured Variables	Communication Frequency (Hz)	Battery life-time	Weight (g)	Ref.
SmartPill	Smart pill for humans	26.8×11.7	pH, temperature, pressure	434 M	> 5d	4.5	[131]
smaXtec Classic	Smart bolus	105×35	Temperature	433 M	$\approx 5y$	210	[39]
smaXtec pH Plus	Smart bolus	132×35	pH, temperature	433 M	$\approx 5y$	220	[39]
Smart Rumen Bolus	Smart bolus	110×20	pH, temperature, acceleration	868 M	> 6y	≈ 200	[132]
eBolus	Smart bolus	135×25	pH, temperature	432 M	2y	150	[40]
MooMonitor+	Collar	-	Acceleration	13.56M	-	-	[46]
CowLar	Collar	$110 \times 62 \times 33$	Temperature, motion	-	6m (single charge)	240	[17]
CowManager	Ear tag	$50 \times 65 \times 19$	Temperature, acceleration	2.4 G	capacity 1200 mAh	30	[7]
SmartBow	Ear tag	$52 \times 36 \times 17$	Acceleration	-	-	34	[133]

Leg wearable devices, attached to the animal limb, detect changes in walking patterns, which may indicate health issues such as lameness. Haladjian et al. [72] demonstrated the application of gait analysis by utilizing acceleration and orientation data collected from the leg, which is processed by ML algorithms to detect walking patterns and identify any abnormalities. The collected data is transmitted to a base station and analysed to provide early alerts to farmers. A study conducted by Marques et al. [134] highlighted concerns associated with pedometers, including the overestimation of step counts. Additionally, any lifting of the leg is recorded as a step, regardless of whether there is actual body movement or if the animal remains stationary. Meanwhile, collar systems yield insights into animal behaviour and surroundings. Innovations like Dairymaster's Moomonitor+ [18], and CowManager's system [7] introduced methods for locating and tracking animals on the farm. Moomonitor+, a collar by Dairymaster commercialised in 2014, employed triaxial accelerometers to measure activity, feed intake, rumination, and lying time [46]. This device made use of Near Field Communication (NFC) to access an individual cow's information and a base station to transfer and store data in the cloud. A study by Versluijs et al. [135] demonstrated the use of GPS collar data to detect behaviours using ML models. Recent approaches have introduced a collar for measuring temperature, detecting activity, and counting steps [26]. However, further research is needed to minimise the influence of sunlight and to adapt the device's design to fit the animal's neck more effectively. Generally, collars contain sensors that measure the animal's temperature and track their movements, providing data on the animal's feed intake and activity levels. The data from the collars is transmitted to a base station for analysis and can be accessed by farmers through a smart device.

Smart bolus emerged as a tool for assessing and tracking changes within the ruminal complex. An understanding of bovine anatomy, particularly the stomach, is imperative to comprehend the deployment of these devices. The cow's stomach is comprised of four compartments: the rumen, reticulum, omasum, and abomasum, each with a distinct function. The four compartments allow ruminant animals to digest grass or vegetation without fully chewing it first. The rumen, facilitating fermentation and food storage, constitutes the largest stomach section. As a result of fermentation, Volatile Fatty Acids (VFAs) are generated in this segment of the stomach. The rumen and reticulum are not entirely separated, and this interlinked structure is also known as the ruminoreticulum [136]. The reticulum serves as a repository for foreign objects and dense feed particles [136]. The complexity of this compartmentalised stomach provides an opportunity to continuously monitor an animal's gut, providing insights into diet, nutrition, and overall health [126].

Smart boluses offer real-time internal monitoring of bovine health, providing valuable insights into an animal's well-being. A study conducted by Studer et al. [137] demonstrated the potential of these devices to detect SARA by collecting continuous pH data over 80 days. Despite their potential, commercially available smart boluses remain limited in their sensing capabilities, typically measuring only temperature and pH. However, in academic contexts, researchers have introduced alternative methods and techniques, such as using accelerometer data combined with data processing algorithms to detect heart rate [20]. A detailed examination of these devices, including their limitations and areas for improvement, will be presented in the following sections of this chapter. Expanding their functionality could enable these devices to track dietary trends, rumination patterns, and overall animal health, further enhancing their utility on the farm.

Innovative approaches have also been developed, such as vests [25], which offer a comfortable wearable solution for detecting heart rate. Other studies proposed a nose ring with a pressure sensor and accelerometer to measure rumination times and cycles [138]. Additionally, advancements in technology have facilitated the development of methods to monitor animals without relying on wearable devices. Pretto et al. [139] proposed a surveillance camera to detect basic animal activities. By employing the You Only Look Once version 3 (YOLOv3) model, animals are identified through their Ear ID tags, and information is collected regarding drinking habits. However, this approach provided only a superficial analysis of the animals' health compared to other systems used on farms.

2.2.1 Farm Integration

Several companies have developed advanced monitoring devices designed to integrate into modern farming operations, aiming to enhance efficiency and sustainability. Data-driven decision-making, enabled by real-time data acquisition and processing through advanced analytics and AI algorithms, facilitates the early detection of health issues, thereby enhancing animal welfare and optimizing farm management practices. However, the adoption of these technologies faces challenges, including cost, ease of use, and the need for farmer training to maximise their utility.

In 2007, eCow Limited (Glasgow, Scotland) introduced the eBolus, an ingestible device that measures temperature and pH in a cow's reticulum. Each animal can host a maximum of two capsules, with data transmitted to a base station accessible through smart devices. The eBolus has gained popularity in academic investigations for monitoring ruminal pH [40, 140, 141], but despite its adoption in research settings, its application remains limited to tracking only two

parameters. Additionally, the costs associated with deploying and maintaining the system on a large scale may pose significant challenges for many farmers. Furthermore, the dependence on a stable transmission network presents challenges in remote farming environments, where connectivity is often unreliable due to factors such as large farm distances, limited telecommunications infrastructure, and the range limitations of selected frequency bands.

Likewise, smaXtec provided a bolus technology for monitoring rumen pH and temperature [142, 143]. This system also includes external sensors for motion activity, core temperature, and humidity monitoring. The collected data is stored in an online database via a base station, with the option to extend the range using repeaters [39]. A messenger device is incorporated to inform the end-user on the animals' health status [142]. However, this technology is limited to animals weighing at least 300 kg. This weight limitation excludes younger animals, potentially delaying early interventions during critical growth phases. Despite this limitation, this technology has been used in research settings for ruminal pH monitoring [144].

Moonsyst Industrial Technologies (Cork, Ireland) introduced the Smart Rumen Bolus, which leverages AI to predict animal welfare events based on pH, temperature, and activity data. The collected information is accessible through cloud-based software (mooncloudTM) on a smart device, as illustrated in Figure 2.1 [132]. The information is linked to each cow via the bolus ID number.

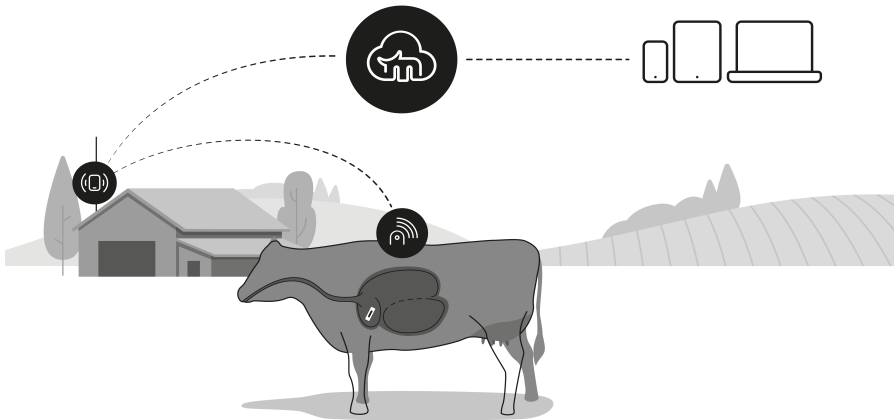


Figure 2.1: Data flow on the farm (created by Moonsyst).

Smart bolus technologies are a big investment, but can also return big profits due to efficient farm management [145]. Advanced monitoring systems hold great promise for enhancing farm management and improving animal welfare. However, their effectiveness hinges on addressing several limitations, such as accessibility, scalability, and sensing capabilities. Additionally, progressing these technologies is essential for promoting their widespread adoption in farming practices.

2.2.2 Power supply and lifetime

A critical evaluation of the power supply in smart devices highlights several challenges and opportunities for optimisation. One significant challenge is the choice of battery, which directly affects the device's functionality and sustainability. In scenarios requiring a short operating time, coin cell batteries, such as those with a capacity of 36 mAh, can be a viable option [146]. However, such disposable batteries raise concerns regarding environmental impact and long-term cost efficiency. Rechargeable batteries present a more sustainable alternative, particularly in wearable devices where frequent replacements can be avoided. For example, Deniz et al. [147] successfully integrated two 3.7 V Li-ion polymer rechargeable batteries of 2500 mAh (EB615268VU, Samsung) recharged through a 1 W solar panel, in a collar system. Similarly, the eGazor collar used a 3.6 V, 13.4 Ah Li-ion battery recharged via six photovoltaic modules [148]. However, the reliance on solar panels raises questions about their practicality in less sunny climates or during periods of low sunlight, potentially limiting their utility.

In encapsulated devices, such as capsules or boluses, the choice of battery and power consumption optimisation is even more critical due to restricted access. The 3.7 V Lithium Polymer (LiPo) battery (GM301014H, PowerStream) with 14 mAh capacity lasted for 30 hours [149]. The device consumed 25 mA in active mode, $2.5 \mu\text{A}$ when in idle state, and had a quiescent current of $0.58 \mu\text{A}$. While this demonstrated efficient power management, the limited operational duration necessitated frequent recharging or replacement, which could be impractical in agricultural settings.

For devices like smart boluses, where there is no direct access to the electronics, another challenge is the activation. Magnetic activation is a common solution, which involves the interaction of magnets with an internal power trigger to switch the state of the capsule between on and off [142, 150]. However, this approach might suffer from reliability issues in harsh environments. Hall switches offer an alternative option, acting as a power management tool [151]. Nevertheless, there is a dependence on consistent magnetic fields, which could also pose challenges in farm conditions. There is a constant trade-off between the size requirements and the limited small-size batteries that produce enough current for a long period. Hence, the choice of power management circuit and battery is crucial in determining the lifetime of a device. The smaXtec bolus incorporated an STM32L431KC MCU (STMicroelectronics), and a LIS2DW12 (STMicroelectronics) three-axis accelerometer powered by a lithium metal battery, consuming $1 \mu\text{A}$ in its active low-power mode [124]. At the time of writing, the smaXtec Classic Bolus has a lifespan of up to five years, with readings taken

every 10 minutes [142]. In comparison, the smaXtec pH Plus Bolus has the same battery duration with pH measurements lasting for 150 days (≈ 5 months) [126]. Moonsyst used a spiral battery with 6000 mAh capacity, providing a lifetime of 6 years, taking measurements every 10 minutes [132]. Identically, the battery in eBolus by eCow ran for 4 years, although no details were available on the battery type. However, the lifespan of its sensors, particularly pH sensors (90–150 days) and temperature sensors (2 years), is shorter than that of the battery itself [40]. This disparity indicates a critical limitation in sensor technology, highlighting the need for advancements to align sensor durability with the lifetime of the electronics.

Recharging encapsulated devices, as smart boluses, introduces another layer of complexity. In a study conducted by Zimmermann et al. [151], a coin cell powered a capsule for 400 hours, with a sampling rate of 240 per second via a hall switch. An approach to power up implanted capsules has been proposed by Fischer et al. [152], based on contactless charging via induction coils. One coil is printed in a capsule and is combined with a transmitting coil capable of synchronously charging up to five sphere capsules. Each capsule contained a Li-ion battery (MS412F, Seiko Instruments Inc., Japan), powering an EFM8SB1 MCU (Silicon Laboratories, Inc., U.S.A), with a nominal capacity of 1.0 mAh [153]. The induction coil and two capacitors created a parallel resonant circuit, generating a 13.56 MHz vibration used for capsule location identification [152]. This strategy also has practical challenges associated with it, such as ensuring efficient power transfer through biological tissues and fluids, which remain unresolved. Besides this, this technology can only work for small distances, up to 10 cm, which can be insufficient for the depth of the animal’s body [154].

Khan and Desmulliez [155] investigated the body attenuation effects by using a miniaturised Receiver (RX) wireless power transfer system powered by multiple Transmitter (TX) to power implantable devices. The spiral TX coils weigh 2.8 g, with a thickness of 35 μm , and an outer diameter of 200 mm. In contrast, the RX coil had a diameter of 8.9 mm, including the miniaturised RX, and 4 mm diameter ferrite to improve power transfer efficiency. A frequency of 1 MHz is selected due to its low cost compared to the industrial, scientific, and medical band frequencies and to ensure tissue safety. This study reported that 50 mW of power was supplied to a tissue phantom, with 28 cm of diameter, in the worst-case scenario of angular and translational alignment, with a power transfer efficiency of 0.7%. To validate this study, a simulation on a phantom was used, predicting a maximum error of 12% between the calculated and measured trajectories of the capsule. Tests performed on a porcine Gastrointestinal (GI) tract demonstrated that a power threshold of 100 mW is necessary for endoscopic capsules to prevent

damage to the surrounding tissue [156]. Despite these results being promising, the low efficiency and potential energy losses raise doubts about the viability of this technology for monitoring bovine health.

An alternative solution is to harness energy from the environment to power the device, making it self-powered and self-sufficient with the potential for an infinite lifespan, limited only by the lifetimes of the embedded components and sensors. This approach, known as energy harvesting, is a rapidly growing field of research and development. A review done by Jiang et al. [157] described implantable energy harvesting technologies for self-powered implantable devices. This included the work conducted by Nadeau et al. [158] on a self-powering ingestible capsule, utilising gastric fluid as an electrolyte for a galvanic cell. The system delivered an average power density of $0.23 \mu\text{W}/\text{mm}^2$ for 6.1 days, powering a temperature cell. The drawback of this type of battery was the lifespan of the electrodes (less than 24h), restricting the broader application of such systems. Additionally, Stuart et al. [159] conducted a comprehensive review of battery-free technologies used in human medical devices, which could potentially be applied to bovine health monitoring systems. Examples include ultrasonic transducers, thermoelectric power converters, and biofuel-based energy sources. More recently, a review of emerging technologies for energy harvesters and wireless charging of implantable devices in the context of human health identified potential future directions, such as harvesting energy from mechanical movement [160]. While significant progress has been made in power supply technologies for wearable and implantable devices, critical gaps persist. Although this thesis does not address issues such as energy efficiency or the practicality of recharging and energy harvesting systems, these areas remain vital for the continued advancement of the technology.

2.2.3 Communication Method

The transmission of real-time data is a highly attractive feature for health monitoring devices, enabling fast intervention when necessary. However, achieving this relies heavily on the design of a robust Radio Frequency (RF) system. Industrial, scientific, and medical applications benefit from a reserved frequency band ranging from 6.78 kHz to 245 GHz within the radio spectrum [161]. In Europe, the most commonly used is around 433 MHz, extending from 433.05 MHz to 434.790 MHz with a spacing of 25 kHz [161]. Similarly, the United States Federal Communication Commission defined the Medical Device Radiocommunications Service (MedRadio) band from 401 Hz to 457 MHz including sub-regions [162]. Frequencies like 433 MHz [163–165], and 434 MHz [166] are commonly incorporated in various systems, including wireless sensor networks for monitoring ani-

mal health, and intra-body communication. Other frequencies, such as 868 MHz have demonstrated utility in wireless data transmission [132]. Additionally, the 2.45 GHz band is globally recognised for its widespread availability and cost effectiveness, making it a standard choice for wireless Internet of Things (IoT) and Wireless Local Area Network (WLAN) applications [7, 161]. Despite the versatility of these frequency bands, each has limitations that must be critically evaluated before selection. Therefore, environmental and farm-specific conditions, such as structural barriers and electromagnetic interference, need to be taken into account in the choice of frequency.

Designing antennas for medical implant devices is a significant challenge. A lot of effort goes into making antennas as small as possible since the size of the antenna depends directly on the communication frequency - the higher the frequency, the smaller the wavelength, and therefore, the smaller the antenna can be. Antennas designed for intra-body communication must meet specific requirements, including high efficiency, ensuring minimal energy loss during signal transmission, patient safety and comfort, voiding any adverse effects from prolonged use, and technical specifications like high permittivity, and impedance matching [167]. Due to variations in the conductivity and permittivity of different body substances, designing robust antennas to ensure efficient data transfer poses a challenge. For example, the permittivity of skin tissue is 46.7, while that of muscle tissue is 58.8, making consistent signal transmission challenging [168]. To overcome these challenges, several antenna designs have been proposed for capsule applications, including the conformal chandelier meandered dipole antenna [169], and the conformal helix antenna [170]. Additionally, a study by Lauterbach et al. [165] demonstrated that placing an antenna at the centre of a sphere improved the gain by using the sphere's diameter as the antenna's size, achieved through a flexible Printed Circuit Board (PCB). Despite these advancements, further research is needed to test these designs under diverse environmental conditions and to assess their reliability and adaptability in smart bolus systems. Challenges remain in ensuring proper antenna alignment with an external receiver, particularly in dynamic environments where the orientation of the device may shift within the animal's digestive system.

Recent developments have investigated the use of the body's electrical conductivity for intra-body communication as a power-efficient alternative to traditional RF systems [171]. Using the body's electrical conductivity, techniques like capacitive [172] and galvanic [173] coupling have been investigated. A study carried out by Noormohammadi et al. [173] showed that galvanic coupling had the potential for deep implants due to its low path loss, simplicity in electronic design, and energy efficiency. In this technique, a weak electrical current was injected and

flew through tissue, transmitting the signal to the implant through modulation. The study used an on-body receiver antenna consisting of two 20 mm squared electrodes placed 120 mm apart and a transmitter circuit enclosed in a 7 mm by 40 mm capsule, powered by a 5 mAh battery and an in-body transmitter antenna. In vivo testing on porcine subjects was conducted at a frequency of 10 kHz to 10 MHz, a maximum of 2.5% bit error rate, and 45 μ W total power consumption at 10 cm distance, with a data rate of 64 kbps. While this approach shows potential, it faces limitations in scaling for deeper implants or longer transmission ranges [174]. Additionally, the developed system must meet the safety standards concerning the prolonged effects of weak electrical currents on biological tissue.

Efficient wireless data transmission from smart devices to base stations is critical for integrating monitoring systems on farms. Solutions like SIM cards in gateway devices, implemented by Moonsyst [132], and Long-Range (LoRa) Low Power Wide Area Network (LPWAN) protocols, used by eCow and Germani et al. [175], demonstrated significant advancements in this challenge. In the case of eBolus, LoRa protocol was used and offered a transmission range of 200–500 meters, with updates sent every two hours and data saved for up to 28 days if outside the range [40]. Similarly, CowManager utilised Radio-frequency Identification (RFID)-equipped sensors paired with solar-powered routers for continuous monitoring. These devices with QR-coded ear tags are connected to routers for data collection, and placed indoors or outside the parlour [7]. Recently, a study conducted by Sakphrom and Chaimool [27] highlighted the advantages of using LoRa over 433 MHz to transmit data from the rumen to the outside, achieving a range up to 5 m between TX and RX antennas. However, further improvements are required in optimising the shape and size of the transmitting device, which is currently designed as a box measuring $(2 \times 5 \times 7.5) \text{ cm}^3$.

Effective communication systems for livestock health monitoring require careful selection of frequency bands. While advancements in antenna design and intra-body communication techniques have improved efficiency and adaptability, significant challenges remain, particularly in safety and reliability. Wireless data transmission protocols, such as LoRa and RFID, showed promise, but require further optimisation for seamless integration into farm environments. Key gaps include testing antenna designs under varied conditions, evaluating the long-term safety of intra-body communication methods, and refining wireless transmission protocols for large-scale deployments. Even though addressing these challenges is outside the project's scope, overcoming them will lead to more reliable and efficient health monitoring systems for livestock.

2.2.4 Size and Material

When considering the size and material of a device designed for monitoring bovine health, it is crucial to distinguish whether the device is wearable or ingestible. For wearable devices, it is imperative to prioritise lightweight and compact designs to avoid causing discomfort for the animal. The device should also exhibit robust resistance to impacts and be waterproof to endure various environmental conditions. Consequently, this device should adhere to a minimum Ingress Protection (IP) code, as IP67, for health monitoring purposes of wearable devices [176]. This is essential to guarantee the animals' well-being and the monitoring equipment's longevity.

In the case of smart boluses, size-reduction techniques from human health devices, such as thin Land Grid Array (LGA) package, offer promising strategies [124]. This approach involved using metal pads arranged in a grid or array underneath the component's body for external electrical connections, achieving compact designs, such as boluses measuring $(105 \times 35) \text{ mm}^2$ (length $\times$ diameter) [124]. However, incorporating sensors influenced the bolus size, e.g., pH sensor addition increased the diameter by 27 mm [163]. While size reduction is a critical goal, there is a trade-off between reduced size and performance, such as signal transmission, battery life, and sensor integration. For example, eCow proposes the use of an additional metal cap to protect the pH sensor from rumen content. The bolus has dimensions of 25 mm in diameter, 135 mm in length, and a weight of 150 g, making it suitable for cows weighing over 80 kg with body condition score ranging from 1 to 3.5 [40]. The texture and shape of smart boluses are also essential for ease of ingestion and to minimise the risk of injuries to the animal. The sterilisation of smart boluses after being sealed is another crucial factor for their safe use. Common methods include ethylene oxide application and/or steam sterilisation at 120°C [177]. The boluses are orally introduced into the animal's reticulum using a standard bolus gun designed for safe and efficient placement [132].

The biocompatibility of the materials used in smart boluses is critical to avoid inflammation, irritation or harm [178]. Adherence to the US Food and Drug Administration (US) (FDA) ISO 10993-1 standard ensures rigorous biological toxicological evaluation of medical devices [179]. The choice of packaging material is a fundamental aspect of devices that remain within the rumen. In the case of the smaXtec bolus, the materials employed have undergone rigorous testing by the German Agricultural Society, confirming their resilience to rumen fluid [39]. The utilisation of acid-proof resin can be adopted to safeguard the electronic components from contamination and to assure the well-being of the animal [49].

Other materials used include polyurethane [150], Polyoxymethylene (POM) [49], MED610 [149], and Polypropylene (PP), such as Bormed HD810MO (Borealis A/S, Denmark) [165]. Advancements in compact designs have led to reductions in size through innovative techniques. Devices with 60 mm [149], and 25 mm with 8 g packaging [177], and in some cases, as small as 7.9 mm diameter, have been achieved, with some bioreactor monitoring devices. This compactness involved using techniques such as dividing the electronic circuitry into multiple PCBs.

Currently, smart boluses are retained within the animal's rumen. However, there is potential to investigate strategies that utilise advanced materials to design a smart bolus that remains in the rumen until it reaches a reliable operational period, considering sensor limitations, and subsequently safely exits the animal's body. For example, Liu et al. [180] proposed a method that could potentially be applied to a bolus. The strategy consisted of an ingestible pill encased in a material that expanded 100 times its original size within 10 minutes of entering the stomach, allowing it to remain there for up to a month. This material is comprised of two hydrogel components: superabsorbent particles called polyacrylic acid and a substance composed of minute crystalline polyvinyl alcohol chains. The latter acts as a protective layer, preventing the initial material from disintegrating during the expansion process. Upon exposure to a drinking solution containing calcium chloride ions, the hydrogel contracts within 10 minutes, rendering it suitable for further applications. This strategy can be beneficial in the context of dairy health monitoring within the rumen. By using a monitoring device with a lower density than traditional smart boluses, it would pass into the rumen rather than being retained in the reticulum. Once in the rumen, the material expands and remains there for an extended period until exposure to the drinking solution triggers shrinkage, ultimately facilitating its exit from the animal's digestive system. Applying this concept to bovine health monitoring could significantly reduce the environmental and health risks associated with retaining inactive devices in the rumen. However, the scalability of this technology, cost implications, and the feasibility of widespread adoption in farm settings need critical evaluation.

Another potential application of expandable materials could address the challenge of low battery life in smart bolus. When the battery nears depletion, a signal could be sent to notify the user that the bolus will become inactive within a few hours or days. This way, a shrinking process could be triggered, and the device can exit the animal for retrieval and recycling. This innovative approach aligns with sustainability goals but introduces new complexities, such as ensuring reliable retrieval of the device in farm environments and avoiding unintended activation of the shrinking process. Further studies on the practicality of such

systems are necessary.

2.3 Discussion & Conclusion

Designing a comprehensive system for bovine health monitoring presents significant challenges and requires a multidisciplinary approach. This literature review has helped to identify key analytes for assessing animal health, such as pH, temperature, nitrate, nitrite, sodium, and potassium, highlighting their underexplored potential in current systems. A summary of the currently known technical specifications for these parameters is shown in the Table 2.4. The limited exploration of these analytes within the ruminal complex represents a critical gap that, if addressed, could provide valuable insights into bovine health. This gap showcases the need for further interdisciplinary research combining engineering and electrochemistry to broaden the scope of measurable parameters in livestock monitoring.

Table 2.4: Summary of parameter specifications for bovine health monitoring.

Parameter	Healthy Range	Target Range	Accuracy / Precision	Relevance	Ref.
pH	6.0–6.5	3.0–9.0	± 0.2 (90 days); ± 0.4 (150 days)	pH 5.5 indicates SARA; 5.8 linked to metabolic issues	[39, 99, 100]
Temp. (°C)	38.0–39.0	20–60	± 0.1	$>39.4^{\circ}\text{C}$ suggests disease; early fever detection critical for health insights	[39, 40, 181]
Sodium (mM)	± 116	40–200	CV ^a : 0.38%	Electrolyte balance; monitoring treatment.	[119, 121]
Potassium (mM)	± 20	1–50	CV ^a : 0.57%	Electrolyte balance; medication monitoring.	[119, 121]
Nitrate	$<0.22\text{g/kg BW}^b$ in-take	20–330 ppm	1.21% RSD ^c	$>220\text{ ppm}$ indicates toxicity.	[110, 182]
Nitrite	$<20\text{ ppm}$	1–30 ppm	1.26% RSD ^c	High levels indicate toxicity; affects methane production; 10× more toxic than nitrate.	[111]

^a Coefficient of Variation (CV).

^b Body Weight (BW).

^c Relative Standard Deviation (RSD).

The review also identified smart boluses as a promising technology for monitoring bovine health. These devices provide the potential for continuous, real-time data collection, crucial for early disease detection and timely interventions.

However, the primary limitation of current smart boluses lies in their restricted sensing capabilities, typically limited to temperature and pH. While these parameters are undeniably important, they alone do not provide a full comprehensive view of the animal's internal state. This narrow focus constrains the practical utility of smart boluses and limits their contribution to precision farming. Additionally, due to their inability to be retrieved after deployment, smart boluses must be designed for long-term reliability and should leverage all interesting sensing capabilities to maximise their utility. Expanding the range of parameters to include additional analytes like those identified earlier, enhances these devices' contribution to animal health and welfare, enabling farmers to detect and manage health issues more effectively. Consequently, the availability of additional data requires not only technological innovation but also the integration of AI for data processing and advanced data analytics [183–185]. This approach should prioritise user-centred design to ensure the technology is accessible, practical, and readily adopted in real-world settings. For instance, understanding how farmers interpret and utilise this expanded data is essential for delivering actionable insights through intuitive decision-support tools. Thus, more studies into human factors are essential to assess the specific requirements, preferences, and challenges faced by farmers. These studies should explore how farmers interact with data-driven tools, the training needed for effective use, and how such tools can be tailored to align with diverse farming practices and technological literacy levels.

Furthermore, the potential for smart boluses extends beyond individual animal health. These devices could support broader goals, such as reducing GHG emissions. By monitoring digestive processes and analysing the impacts of dietary changes or medication, smart boluses could support strategies aimed at mitigating methane emissions, a significant contributor to GHG in livestock farming. This gap in smart boluses points to the need for the development of multi-parametric sensing technologies. Although this application is promising, it is largely theoretical at this stage. More focused research is required to validate the practical effectiveness of smart boluses in environmental management. This requires the development of multi-parametric devices capable of supporting such investigations, enabling a more comprehensive understanding of their functionality.

In conclusion, smart bolus technology holds immense potential, not only for advancing bovine health monitoring but also for contributing to livestock management and environmental sustainability. This technology showed great promise; however, there are still underdeveloped in key areas, particularly in expanding measurable parameters and optimising sensor integration. By leveraging knowledge from electronic engineering, software development, and electrochemistry, the development of a robust, multi-parametric smart bolus could revolutionise

livestock health management and contribute to global sustainability efforts.

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DEVELOPMENT OF A PORTABLE MULTI-PARAMETER ELECTROCHEMICAL SENSING PLATFORM

3.1 Introduction

The dairy industry has seen significant technological advancements in recent years, enhancing efficiency and improving overall livestock management [1]. However, challenges persist, and there are unresolved research questions, particularly regarding real-time health monitoring and disease prevention in cattle. Effective monitoring solutions require reliable, multi-parametric sensing capabilities to detect physiological changes promptly. Addressing these needs, this chapter presents the development of an advanced electronic system suitable for integration into a smart bolus prototype.

This system was designed to provide accurate, real-time electrochemical measurements, optimising system performance. It incorporated multiple sensing capabilities, including the design of AFE circuits for integrating electrochemical sensors. Additionally, it encompassed the development and implementation of FW for the MCU, along with a dedicated APP for device control and data visualisation, ensuring efficient operation and seamless user interaction.

To overcome the limitations of existing portable electrochemical sensing platforms, this study introduces the Portable Unit for Lab-on-Site Electrochemistry (PULSE). The study focuses on both HW and software advancements, ensuring reliable signal acquisition and efficient data processing. The approach involves optimising AFE circuits to improve performance, including a low input bias current amplifier in the potentiometric AFE to minimise leakage currents and improve signal reliability. Additionally, an Automatic Gain Control (AGC) mechanism is

incorporated in the voltammetric AFE to dynamically adjust the feedback loop to improve signal stability. Furthermore, an instrumentation amplifier is integrated to allow precise gain adjustments.

Beyond HW optimisation, the FW was developed to control the AFE, facilitating the execution of various electrochemical techniques, such as CV, CA, and OCP. Additionally, a custom-designed, user-friendly APP was implemented for real-time monitoring, device control, and data visualisation, ensuring seamless communication between the system and the end user.

Thus, this chapter describes the development, characterisation, and validation of the PULSE system, integrating HW and software to support high-performance electrochemical sensing for real-time bovine health applications. By successfully achieving the research objectives outlined above, this prototype has the potential to:

- Evaluate the sensing platform with a focus on a customisable AFE circuitry with adjustable feedback loop and fast detection capabilities during electrochemical measurements. Implement multi-parametric capabilities to monitor physical, chemical and biochemical parameters. This approach optimizes device performance, ensuring accurate, rapid, and reliable measurements across a range of sensing applications;
- Broaden the scope of electrochemical techniques by incorporating voltammetric and potentiometric AFE, enabling the system to provide accurate, real-time measurements for diverse sensing needs;
- Implementation of FW designed for electrochemical measurements, ensuring fast detection, efficient signal processing, and seamless data acquisition, which significantly enhance the system's overall performance and responsiveness;
- Offer an intuitive and user-friendly APP that facilitates device control, real-time monitoring, and data visualisation, enhancing the accessibility and usability of the PULSE system for end-users in real-world applications.

This chapter is structured into sections, where Section 3.2 describes the experimental procedures and techniques employed in the development, testing, and validation of the PULSE. The focus of Section 3.3 is to showcase the results obtained from the experiments, and to thoroughly evaluate, analyse, and compare them against the SoTA. Finally, Section 3.4 summarizes the main findings.

3.2 Methods

This section comprehensively describes the experimental procedures and techniques employed to design, develop, and validate the presented sensing platform. The methods can be divided into four key areas: HW development, FW development, APP development, and electrochemical validation. The subsequent subsections will delve into the specific details and procedures associated with each aspect.

3.2.1 Hardware Development

The HW development plays a pivotal role in the design and functionality of the electrochemical sensing platform developed in this research. At its core, the HW architecture encompasses the physical components and their interconnections, enabling the platform to perform electrochemical measurements with precision and reliability. This initial HW development serves as a proof of concept, allowing for validation of the design before advancing to greater miniaturisation and refinement in later stages of this thesis. Key elements of the HW development include sensor interfaces, through AFE circuits, data acquisition modules, power management circuitry, and communication interfaces, all connected to the chosen MCU - STM32F372R8T6 (STMicroelectronics, Geneva, Switzerland). The STM32F372R8T6 was selected for its high-performance capabilities, including advanced peripherals, 12-bit Analog-to-Digital Converter (ADC), low-power consumption, and sufficient processing power to manage the electrochemical measurements and communication interfaces required for the sensing platform. All HW development, including schematic designs, and PCB layout were made using Altium Software (California, U.S.). More details on the schematics are presented in Appendix B.1.

The electronic system was designed as a modular block system, as shown in Figure 3.1, allowing for flexibility and redesign to meet specific requirements. The system offered the possibility of a standard 3-electrode connection or 2-electrode configuration sensors, along with temperature and acceleration measurement capabilities. Acting as an intermediary within the system, the AFE, to which the sensors are connected, processes the sensor signals, conditioning them for conversion and ensuring compatibility with the subsequent stages of the system [2].

This platform can communicate with the user device, such as a computer, through a universal asynchronous receiver-transmitter to a universal serial bus USART-to-USB converter (FT232RQ from FTDI Chip, Glasgow, United Kingdom) or wirelessly. Wireless transmission operates at 433 MHz, a frequency within the industrial, scientific and medical band. Its favourable propagation

properties in conductive liquids make it an excellent choice for use in ingestible devices for animals and humans [3, 4]. The communication circuit also included the Si4455 transceiver from Silicon Labs Inc. (Texas, U.S.), which was directly interfaced with the MCU using the Inter-Integrated Circuit (I2C) communication protocol and connected to an antenna. The components were meticulously arranged on the PCB, with compact sizes selected and organised into functional blocks to optimise the use of space, ensuring suitability for a portable device. This layout not only minimised the overall footprint, but also accommodated rerouting and adjustments when necessary, maintaining a certain degree of design flexibility.

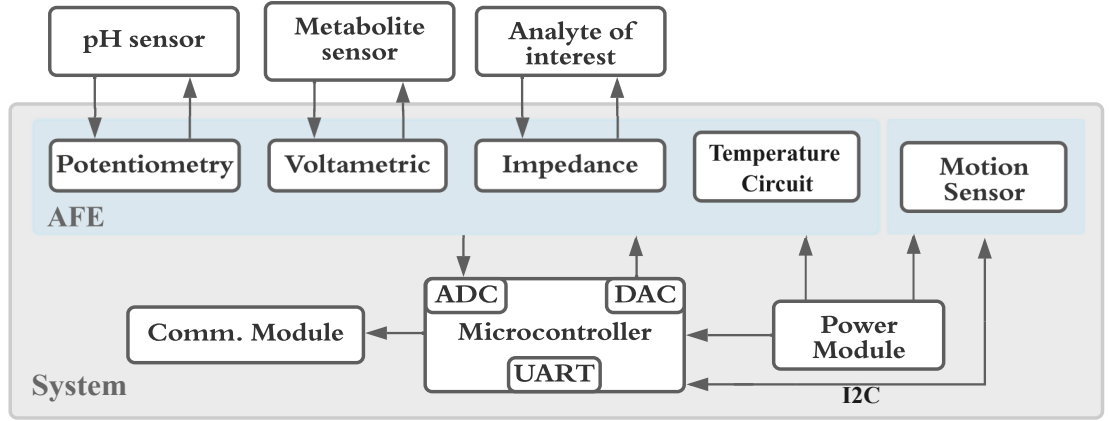


Figure 3.1: Overall system block diagram, including AFE (Analog Front-End), ADC (Analog to Digital Converter), DAC (Digital to Analog Converter), UART (Universal asynchronous receiver-transmitter), and I2C (Inter-Integrated Circuit).

To ensure reliable measurements, the electrochemical sensing platform underwent calibration of the MCU internal ADC of 12-bit resolution, using a benchtop voltage generator (Rigol DP1308A, Rigol Technologies, Beijing, China) for voltage sweeping. The voltage was recorded using a high-precision multimeter (Fluke 289 True-RMS Data Logging Multimeter, Fluke Corporation, Washington, U.S.) and the PULSE's serial port, followed by linear regression analysis. All measurement instruments used in the thesis work were calibrated. For precise voltage control, an external DAC (DAC8568ICPW by Texas Instruments, Texas, U.S.) was used, which required calibration to establish the relationship between the digital input code and the real output voltage. All the calibration equations were embedded into the APP. The ADC, and DAC calibration processes are explained in more detail in Appendix B.2, B.3, and B.4.

$$V_{BAT} = 2 \times VBATT_MON \quad (3.4)$$

To incorporate the concept of a smart bolus device with sealed electronics, an experimental feature was introduced by using a magnetic switch (S_1 , MK24-B-3 from Standex-Meder Electronics, New Hampshire, U.S.) to activate the device. This allowed the device to be turned on by using a magnet to open/close the power supply switch. As a prototype, a convenient and practical alternative was provided by using a jumper in header K_1 to close the circuit. Once the Metal–Oxide–Semiconductor Field-Effect Transistor (MOSFET) U2 (SSM3J306T(TE85L,F) from Toshiba Semiconductor and Storage, Tokyo, Japan) gate was connected to ground, the drain current was at its largest value, and the transistor was in the active region of conduction.

The boost regulator U4 (MCP1640B-IMC, Microchip Technology Incorporated, Arizona, U.S.) was added to boost the voltage coming from the coin cell battery since most of the selected components in this device require a voltage supply higher than 3.3 V. To adjust the output voltage to the desired voltage, Equation 3.5 was applied to calculate the resistor divider values, where R_{TOP} corresponds to R_7 , R_{BOT} to R_8 , and the voltage at the VB pin (V_{FB}) is 1.21 V.

$$R_{TOP} = R_{BOT} \times \left(\frac{V_{OUT}}{V_{FB}} - 1 \right) \quad (3.5)$$

As mentioned above, the PULSE can also be powered from a USB source. When 5 V from the USB source is applied, the MOSFET U1 disabled the upper circuit by pulling the enable signal from U4 down. This disabled U4 and allowed the power to flow from the USB source. To accommodate the components' voltage requirements, a voltage regulator (U3, NCP161ASN350T1G, Onsemi, Arizona, U.S.) was added to lower the input voltage from the USB, resulting in a stable output of 3.5 V. Then, an off-the-shelf cathode common Schottky barrier diode was used (D_1 , BAT54CHYFH, Rohm Semiconductor, Kyoto, Japan). This way, it was possible to select the preferred power supplier without worrying about reverse current, as the diodes act as isolators.

Furthermore, to monitor the device power consumption, a current sense power measurement circuit was introduced by U5 (MAX44284HAUT+T, Analog Devices Inc., Massachusetts, U.S.). This component has a fixed gain of 100; amplifying the voltage drop across the sense resistor (R_9 of 0.1 k Ω), which is placed between the power supply and the downstream circuitry. The amplified voltage signal is then divided by a voltage divider composed of R_{10} and R_{11} of 10 k Ω each to scale it down by half, before being monitored by the ADC on the MCU as

V_{curr} . This setup allows the system to accurately measure the current flowing through R_9 and, consequently, the power consumption of the device. Assuming $V_{OUT'}$ is the output voltage of U5, Equations 3.6, and 3.7 can be formulated.

$$R_{10} + R_{11} = \frac{V_{OUT'}}{I} \quad (3.6)$$

$$R_{10} = \frac{V_{OUT'} - V_{curr}}{I} \quad (3.7)$$

Combining the above equations and replacing the resistors values, $V_{OUT'}$ can be expressed as:

$$V_{OUT'} = 2 \times V_{curr} \quad (3.8)$$

Considering the ADC conversion, a coefficient (V_{REF}/ADC_{max}) of 8.056×10^{-4} was included, leading to:

$$V_{OUT'} = 2 \times V_{curr} \times \frac{V_{REF}}{ADC_{max}} \Leftrightarrow V_{OUT'} = 1.611 \times 10^{-3} \times V_{curr} \quad (3.9)$$

The voltage flowing through R_9 can be labeled as V_{sense} , and calculated by the following equation:

$$V_{sense} = \frac{V_{OUT'}}{Gain} \quad (3.10)$$

By merging Equations 3.9 and 3.10, and replacing the gain by 100, Equation 3.11 was generated.

$$V_{sense} = 1.611 \times 10^{-5} \times V_{curr} \quad (3.11)$$

The current across R_9 can be described using Ohm's Law with the following equation:

$$I = \frac{V_{sense}}{R_9} \Leftrightarrow I = 1.611 \times 10^{-4} \times V_{curr} \quad (3.12)$$

Additionally, when applying Ohm's Law to calculate power (P), and replacing the voltage by 3.5 V, the following equation can be utilised to calculate the system's power consumption.

$$P = 0.5639 \times 10^{-4} \times V_{curr} \quad (3.13)$$

Externally, a Power Profiler Kit II (PPK2) (Nordic Semiconductor, Trondheim, Norway) was used to measure the current driven by the PULSE, working as the Device Under Test (DUT). The PPK2 operated in source meter mode, mea-

measuring the current while providing power to the DUT (3.5 V). The current signal was time-averaged for 60 seconds. This method was applied across multiple measuring capabilities on the DUT to identify and address potential power-related anomalies. The power was calculated as the product of the supply voltage and the measured current, as shown in Equation 3.14.

$$P = V \times I \quad (3.14)$$

To maintain effective power management of the system, multiple power modules were implemented to supply current to the individual sub-sections, as presented in Figure 3.3. Component U1 (LM4132BMF-3.3/NOPB, Texas Instruments) is a precision voltage source, driving individual portions of the circuit. This component featured an enabling feature connected to the ground; thus, when the system was powered up, the individual modules were powered down. By placing jumpers on the headers, the respective portion of the circuit can be powered up with 3.3 V.

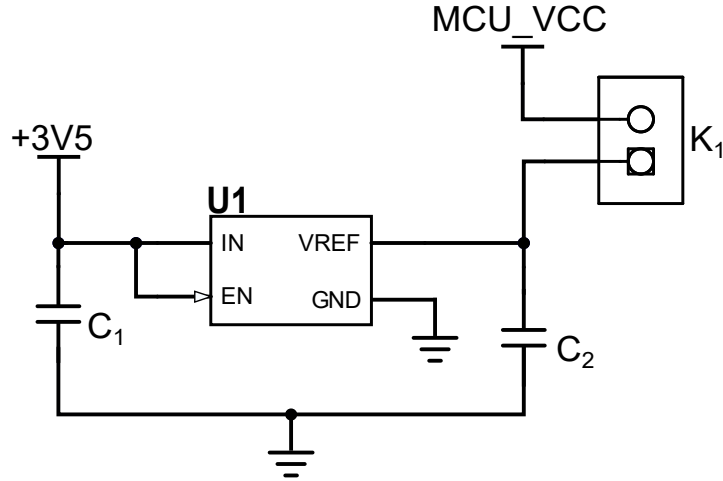


Figure 3.3: Single power module circuit.

3.2.1.2 Temperature

The temperature was measured using a thermistor, labelled as R_1 in Figure 3.4. The thermistor selected for this purpose was the NCP03WB473FRL from Murata Electronics (Kyoto, Japan). It is a Negative Temperature Coefficient (NTC), meaning its resistance decreases as the temperature increases. To convert this change in resistance into a measurable voltage, a second resistor (R_2) was placed in series with the thermistor, forming a voltage divider circuit.

The output voltage (V_{temp}) was calculated using the following equation:

$$V_{temp} = V_{cc} \times \frac{R_1}{R_1 + R_2} \quad (3.15)$$

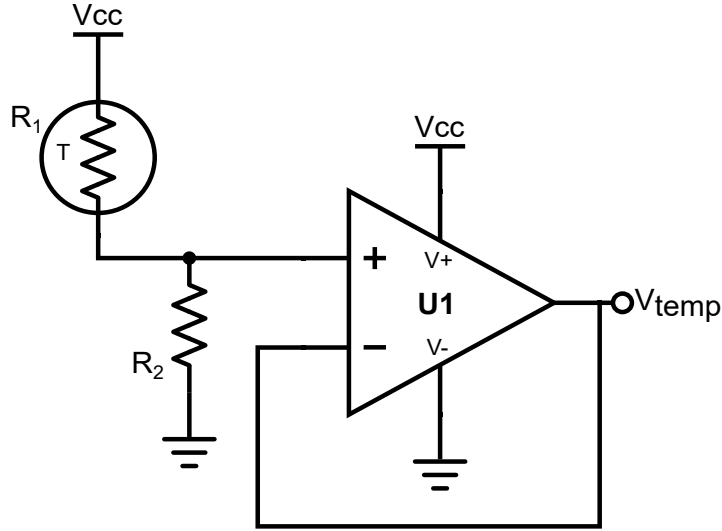


Figure 3.4: Temperature sensor circuit schematic.

This expression can be rearranged to solve the thermistor resistance (R_1):

$$R_1 = R_2 \times \left(\frac{V_{cc}}{V_{temp}} - 1 \right) \quad (3.16)$$

An alternative way to determine the value of R_1 involved applying Ohm's Law to the voltage divider, resulting in the following relationship:

$$R_1 + R_2 = \frac{V_{cc}}{I} \Leftrightarrow I = \frac{V_{cc}}{R_1 + R_2} \quad (3.17)$$

Similarly, the current (I) can also be expressed as:

$$R_1 = \frac{V_{cc} - V_{temp}}{I} \Leftrightarrow I = \frac{V_{cc} - V_{temp}}{R_1} \quad (3.18)$$

Equating both expressions for I leads to Equation 3.16. With a known value of R_2 as $47\text{ k}\Omega$ and V_{cc} as 3.3 V , the final equation to calculate the thermistor resistance can be written as:

$$R_1 = 47k \times \left(\frac{3.3}{V_{temp}} - 1 \right) \quad (3.19)$$

To accurately measure the temperature associated with this change in resistance, the thermistor's temperature coefficient, also known as beta value (β) must be considered. This value defines the change in resistance between two specific temperature points. For the selected component, the β -value in the range of 25°C and 50°C was 4050. This range was chosen to align with the typical internal body temperature of the animal, which is approximately $38\text{--}39^\circ\text{C}$ [5]. The relationship between temperature and resistance for an NTC thermistor is described by the following equation:

$$\frac{1}{T} = \frac{1}{T_0} + \frac{1}{\beta} \times \ln\left(\frac{R}{R_0}\right) \quad (3.20)$$

Where T_0 is the reference temperature (usually 25 °C or 298.15 K), R_0 is the thermistor's resistance at this reference temperature, T is the measured temperature in Kelvin, R is the thermistor's resistance at the measured temperature, and β is the thermistor's material-specific constant.

The output voltage is buffered using an amplifier (U1 - LTC6081HMS8 from Analog Devices Inc.) to ensure stable readings. The thermistor's resistance (R) can be calculated using the exponential form of the thermistor equation:

$$R = R_0 \times e^{\beta\left(\frac{1}{T} - \frac{1}{T_0}\right)} \quad (3.21)$$

For a measurement at T equals ∞ , $1/T$ can be neglected. Replacing the known values, including $R_0 = 47 \text{ k}\Omega$, $\beta = 4050$, and $T_0 = 298.15 \text{ K}$, the following is obtained:

$$R_i = 47k \times e^{(-4050/298.15)} \approx 0.05925 \Omega \quad (3.22)$$

Where R_i represents the infinite resistance. The temperature in Kelvin can be determined from the resistance using the following equation:

$$T(K) = \frac{4050}{\ln\left(\frac{R_1}{0.05929}\right)} \quad (3.23)$$

This formula provided the temperature in Kelvin based on the measured resistance (R_1). Equations 3.19 and 3.23 were implemented in the APP to obtain the measured temperature. The temperature in Celsius is then obtained by subtracting 273.15 from the Kelvin value.

3.2.1.3 Motion

The ADXL345 from Analog Devices Inc. was selected as the accelerometer for motion sensing [6]. The accelerometer was integrated into the device as a proof of concept to demonstrate motion-sensing capability; however, no further testing or data processing was conducted. This ultralow-power device integrates a triaxial accelerometer, making it suitable for Inertial Measurement Unit (IMU) applications. The schematic design incorporating the ADXL345 is presented in Figure 3.5. Communication between the accelerometer and the MCU is facilitated via I2C digital interface.

In the implemented configuration, resistors R_1 and R_2 were connected to the voltage supply (V_{cc}) and act as pull-up resistors for the I2C bus. These resis-

tors ensure that the I2C bus lines are pulled high when inactive, allowing proper communication between the accelerometer and the MCU. Choosing the correct pull-up resistor values is crucial, as improper values can result in signal degradation or loss, negatively impacting communication reliability. To ensure reliable performance of the I2C bus, the values of the pull-up resistors were calculated using the following equations, which define the minimum ($R_p(\min)$) and maximum ($R_p(\max)$) allowable resistance for optimal operation [7]:

$$R_p(\min) = \frac{V_{cc} - V_{OL}(\max)}{I_{OL}} \quad (3.24)$$

$$R_p(\max) = \frac{t_r}{0.8473 \cdot C_b} \quad (3.25)$$

Here, C_b represents the estimated bus capacitance, t_r is the maximum rise time for the I2C signal, $V_{OL}(\max)$ is the maximum low-level output voltage, and I_{OL} is the low-level output current.

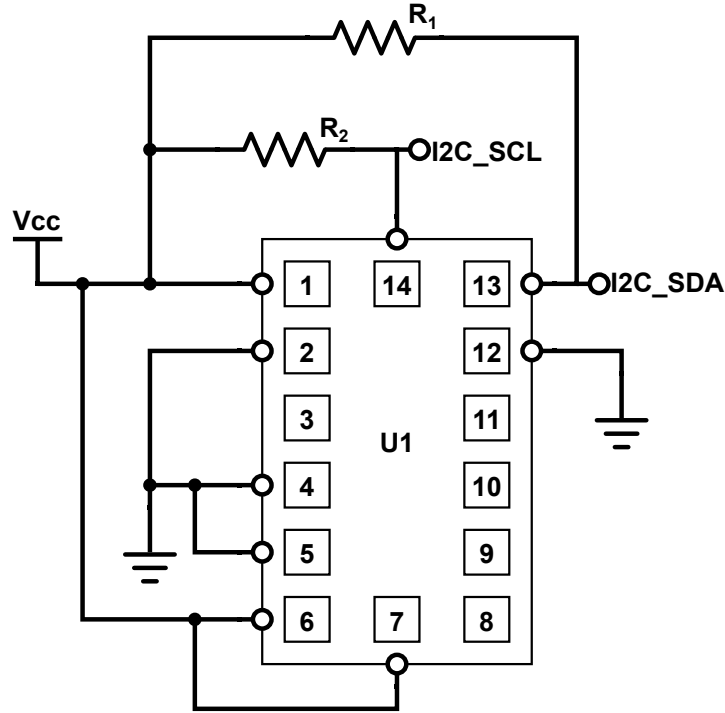


Figure 3.5: Accelerometer schematic circuit.

The accelerometer operates within a selected range of $\pm 4g$, where g represents the acceleration due to gravity, approximately 9.80665 m/s^2 . The device outputs data with a sensitivity scale factor of 7.8 mg/LSB [6]. To convert this scale factor from mg to standard units of m/s^2 , the following conversion is applied:

$$\text{scale factor}(\text{m/s}^2) = \frac{7.8}{1000} \times 9.80665 = 0.07649187 \text{ m/s}^2 \quad (3.26)$$

This means that each Least Significant Bit (LSB) from the accelerometer corresponds to approximately 0.07649 m/s^2 of acceleration. At the APP level, this scale factor is multiplied by the raw accelerometer data from each axis (x, y, and z) to convert the readings into physical units of acceleration (m/s^2) in all three directions (a_x , a_y and a_z). To determine the total acceleration experienced by the sensor, the acceleration values in the x, y, and z directions are combined using the following equation, which calculates the magnitude of the resultant vector:

$$a_T(\text{m/s}^2) = \sqrt{a_x^2 + a_y^2 + a_z^2} \quad (3.27)$$

In addition to measuring total acceleration (a_T), the accelerometer data can be used to calculate the orientation of the device in space. For this application, the object was considered to be in a static position, assuming the only force applied was gravity ($a_T=1g$). Since this component provides data in all three axes (x, y, and z), this allows for the calculation of angular positions, also known as Euler angles, specifically roll (ϕ), and pitch (θ) [8]. The roll angle describes the rotation around the x-axis, while the pitch angle describes the rotation around the y-axis [9]. The third Euler angle, yaw (Ψ), cannot be determined using an accelerometer alone, as it requires measurements of rotational velocity, which would typically be provided by a gyroscope. These angles provide information about the tilt of the device relative to the horizontal plane.

To compute the roll and pitch tilt angles, when the total acceleration is approximately 1 g, the angles can be determined as follows:

$$\text{roll}(\phi) = \arctan\left(\frac{a_y}{a_z}\right) \quad (3.28)$$

$$\text{pitch}(\theta) = \arctan\left(\frac{a_x}{a_z}\right) \quad (3.29)$$

The roll and pitch were included in the APP for demonstration purposes. These formulas have limitations addressing the entire 360° for the multiple quadrants detection. For more accurate analysis, a robust algorithm could be implemented, complemented by an IC equipped with an accelerometer and gyroscope.

3.2.1.4 Voltammetric AFE

A voltammetric circuit was designed to measure the current response to a varying applied voltage in an electrochemical process. The design of a voltammetric circuit was crucial to ensure accurate measurements of current [10]. The implemented circuit is depicted in Figure 3.6, incorporating three essential com-

ponents: the control amplifier (U5 - ADA4807 from Analog Devices Inc.) [11], the electrometer (U6 - LTC6081 from Analog Devices Inc.) [12], and the current-voltage converter or Trans-Impedance Amplifier (TIA) (U1 - LTC6081 from Analog Devices Inc.) [12, 13].

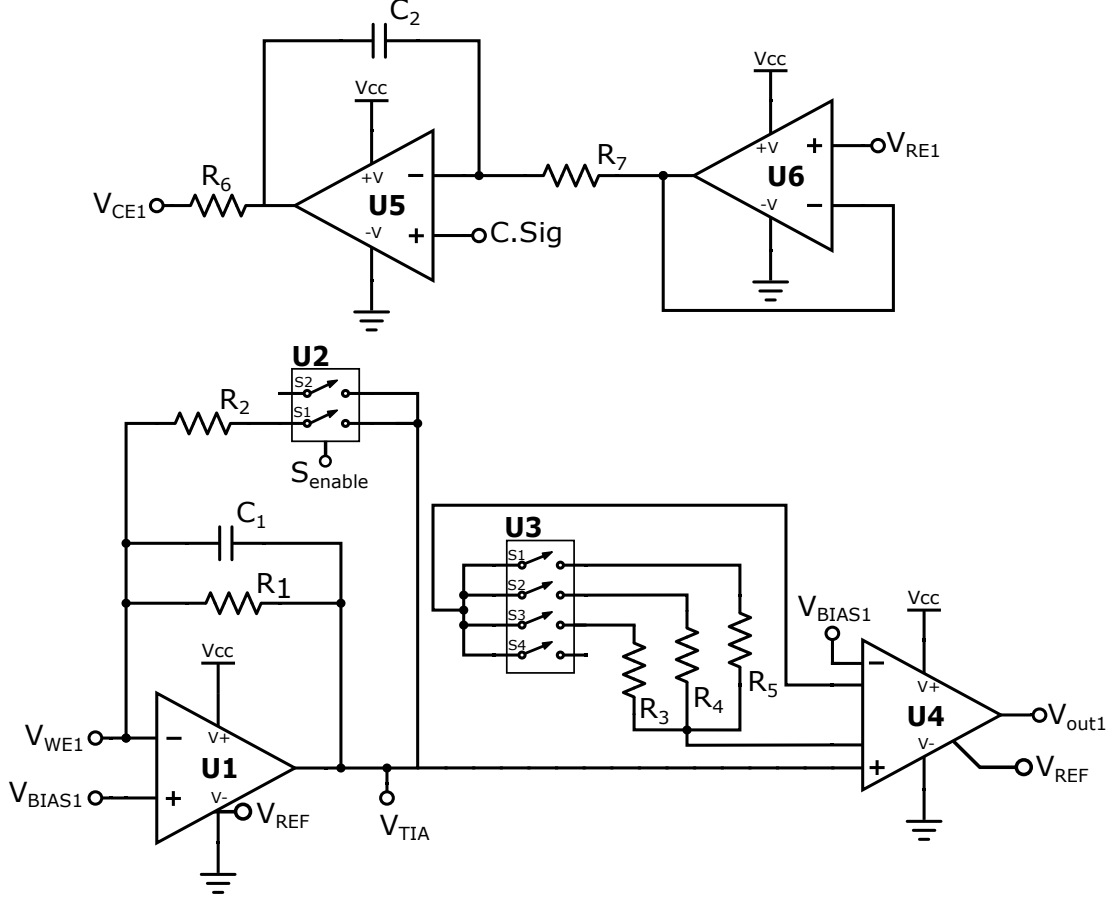


Figure 3.6: Simplified schematic circuit design of voltammetric AFE.

The control amplifier (U5) was used to drive large capacitive loads in the electrochemical cell, while the electrometer (U6) acted as a buffer. The primary role of C_2 (470 pF) is to reduce the bandwidth of U5. This bandwidth reduction is crucial to mitigate the risk of oscillation and instability, which can arise when two operational amplifiers are configured in such a feedback arrangement—here, with U6 operating in the feedback loop of U5 [14]. The combination of C_2 and R_7 (10 k Ω) introduces a pole that modifies the unity-gain frequency, improving the phase margin and stabilising the system. To further ensure stability, especially when driving large capacitive loads such as C_{dl} , a resistor R_6 (10 Ω) is included at the output of U5. Acting as an isolation resistor, R_6 prevents ringing and distortion by decoupling the Operational Amplifier (OpAmp) output from the capacitive load, placing it outside the feedback loop. This configuration reduces the likelihood of oscillations when driving high capacitance. Additionally, the properties of U5 allowed the user to switch on and off the component, based

on the enabling signal. Activation of the component occurred when this signal exceeded 1.7 V, while deactivation was triggered by a voltage below 1.3 V. When active, the amplifier drove $C.Sig$ into the CE. In contrast, in the inactive state, the amplifier exhibited high impedance.

The TIA (U1) was responsible for generating an output voltage proportional to the input current from the WE. This was accomplished by incorporating a feedback resistor (R_f), as illustrated in Figure 3.7. Additionally, a feedback capacitance (C_f) was included to maintain system stability by shifting the noise gain zero towards lower frequencies. Equation 3.30 can be utilised to determine the minimum value of the compensation capacitor required to eliminate oscillation and minimise ringing. Besides this, it is common practice to overcompensate to ensure stability [15].

$$C_f = \frac{1}{4\pi R_f f_{GBWP}} \times \left(1 + \sqrt{1 + 8\pi R_f C_i f_{GBWP}} \right) \quad (3.30)$$

Where C_i represents the total input capacitance, and f_{GBWP} is the unity gain bandwidth of the U1 amplifier. Figure B.1.12 provides a graphical representation of the open-loop gain, and the reciprocal of the feedback loop versus frequency, offering a visual insight into the variables mentioned above. The TIA had a feedback loop composed of two branches. The first branch was fixed, consisting of R_1 in parallel with C_1 , while the second branch, which can be activated as needed by the analog switch U2 (ADG779 from Analog Devices Inc.), consisted of R_2 . The R_2 -U2 circuit served as an AGC mechanism for the TIA regulated by the MCU signal (S_{enable}), which enabled the selection of the appropriate branch in U2. Once the measured signal exceeded a certain threshold, R_2 was activated, allowing measurements across a wider range. This ensured that the TIA remained compliant and maintained a linear response without drifting. The activation status of the switch was specified in Table B.1.1. When S1 was enabled, the R_f was set to 9.9 k Ω , achieved by the parallel of R_1 and R_2 [16]. The resistor R_1 of 1 M Ω , served the purpose of facilitating electrode cleaning. When S1 was activated, the current value increased, resulting in a cleaning effect on the electrode surface, a phenomenon that has been previously explored in the literature [17, 18]. Figure 3.7 provides a simplified version of an electrochemical cell's equivalent circuit connected to the TIA. In this circuit, the solution resistance was omitted, while R_{ct} represents the charge transfer resistance, and C_{dl} denotes the double-layer capacitance. By applying Ohm's Law, the output voltage (V_{out}) is given by:

$$V_{out} = -R_f \times I \quad (3.31)$$

At maximum current, the equation can be expressed as:

$$V_{cc} = R_f \times I_{max} \quad (3.32)$$

Therefore, for a constant voltage supply (V_{cc}), a decrease in the value of R_f by approximately 100, resulted in an increase by a factor of 100 in the maximum current.

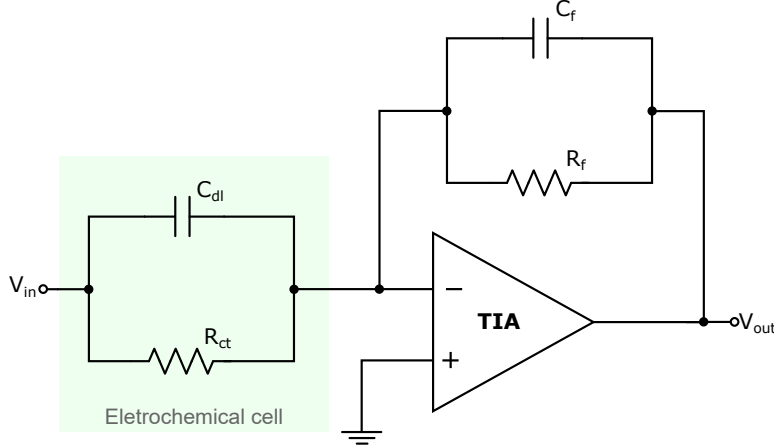


Figure 3.7: Simplified circuit diagram showing an electrochemical cell equivalent circuit connected to the TIA.

Furthermore, the feedback components of the TIA worked as a frequency compensation voltage divider in conjunction with the electrochemical cell. The resistors divided the voltage in the Direct Current (DC) domain, while the capacitors acted in the AC domain. By examining the diagram in Figure 3.7, two Equations 3.33 and 3.34 were derived to describe the circuit's behaviour in the DC and AC domains, respectively. The negative input voltage of the amplifier was denoted as V^- .

$$V^- = \frac{R_f}{R_{ct} + R_f} \times V_{in} \quad (3.33)$$

$$V^- = \frac{C_f}{C_{dl} + C_f} \times V_{in} \quad (3.34)$$

Ideally, these two domains are equal, meaning that the voltage at the negative input remains consistent across both domains. This equality leads to the frequency-compensated voltage divider expression for an optimal compensation [19, 20]:

$$R_f \times C_f = R_{ct} \times C_{dl} \quad (3.35)$$

If the product $R_f \times C_f$ exceeded $R_{ct} \times C_{dl}$, it resulted in a case of over-compensation, suggesting that C_f might be excessively large. In contrast, if

$R_f \times C_f$ fell below $R_{ct} \times C_{cdl}$, it represented under-compensation, implying that C_f may not be sufficiently large.

The selection of C_f in the TIA was application-dependent and required careful consideration of the trade-off between bandwidth and output stability. To determine C_f , two approaches can be used. Equation 3.30 yields a smaller C_f value, which maximises the bandwidth of the TIA. However, this smaller capacitance can contribute to overshooting and ringing at the TIA output, potentially affecting signal integrity. Equation 3.35 provides a larger C_f value, which helps suppress overshoot and improve the stability of the TIA output. The trade-off, however, is a reduction in the TIA bandwidth. The choice between these two methods depends on the specific application requirements.

To enhance the system's versatility, the study integrated an instrumentation amplifier (U4 - INA333 from Texas Instruments) with a variable gain, connected to a Complementary Metal–Oxide–Semiconductor (CMOS) analog multiplexer (U3 - ADG704 from Analog Devices Inc.) featuring low channel resistance. This multiplexer was responsible for the selection of the gain resistor (R_G). Consequently, the gain of the signal was determined by the value of R_G as per Equation 3.36, meaning the resistor value was variable due to the component U3.

$$G = 1 + \frac{100 \text{ k}\Omega}{R_G} \quad (3.36)$$

The truth table presented in Table 3.1 outlines the activation status of each switch, the associated gain resistor value, and the corresponding gain of the instrumentation amplifier U4. For instance, when switch S1 was on, R_G was set to R_5 (100 k Ω), resulting in a gain of 2 for the INA333. When switch S2 was on, R_G became R_4 (11 k Ω), amplifying the input signal by a factor of 10. Similarly, when switch S3 was on, R_G was R_3 (1 k Ω), providing a gain of 100. Lastly, when the switch was connected to S4, no resistor was connected, and the amplifier operated as a unity gain buffer.

Table 3.1: Truth table of ADG704 according to its logic inputs (A1 and A0), and its relationship with INA333 gain (NC - not connected).

A1	A0	EN	Switch	R_G (Ω)	Gain
X	X	0	NONE	NC	1
0	0	1	1	100k	2
0	1	1	2	11k	10
1	0	1	3	1k	100
1	1	1	4	NC	1

The output voltage (V_{out1}) of the circuit in Figure 3.6 can be calculated using the Equation 3.37, as an adaptation from the components' data sheet [21].

$$V_{out1} = V_{REF} + G \times (V_{TIA} - V_{BIAS1}) \quad (3.37)$$

This equation can be rearranged to express the output voltage signal of the TIA as follows:

$$V_{tia} = \frac{V_{out1} - V_{ref}}{G} + V_{Bias1} \quad (3.38)$$

Using the fundamental principles of virtual ground in operational amplifiers (OpAmps) operating in linear mode, V_{Bias1} was found to be equivalent to the negative input signal of the TIA (V_{WE1}) [22]. Consequently, the current can be expressed as:

$$I = \frac{V_{TIA} - V_{BIAS1}}{R_f} \quad (3.39)$$

Where R_f is the value of the feedback resistor of the TIA. Additionally, this equation was used for a comprehensive study of the operational range of this circuitry.

The noise of the voltammetric and potentiometric AFE was evaluated with an R_f of 9.9 k Ω and a C_f of 10 nF. The AFE circuitry was connected to a dummy cell (Metrohm, UK), which mimicked the properties of an electrochemical cell. The input voltage was varied to obtain the full signal range from 0 V to 3.3 V, and the noise was assessed by analysing the peak-to-peak differences in the time-domain recorded data, which was then used to calculate the Signal-to-noise Ratio (SNR). SNR calculations were made to assess signal quality using Equations 3.40 and 3.41.

$$SNR = \frac{P_{signal}}{P_{noise}} \quad (3.40)$$

$$SNR_{dB} = 10 \times \log_{10}(SNR) \quad (3.41)$$

3.2.1.5 Potentiometric AFE

The developed circuit shown in Figure 3.8, was an adaptation of the 2-electrode methodology previously reported in [23]. This circuit was designed to measure the voltage drop between the reference and working electrodes. To achieve this, the voltage signal was first buffered by the U7 (LMP7721 from Texas Instruments) connected to the WE, chosen due to its low input bias current of 20 fA at 25 °C, which provided high input impedance and ensured no losses or alter-

ations in the signal [24]. The instrumentation amplifier U8 (INA333 from Texas Instruments) can be considered high-impedance with a low input bias current of typically 70 pA. However, this input bias current can negatively affect the pH sensor's stability and durability; an input bias current lower than 1 pA is desirable for pH sensing [25].

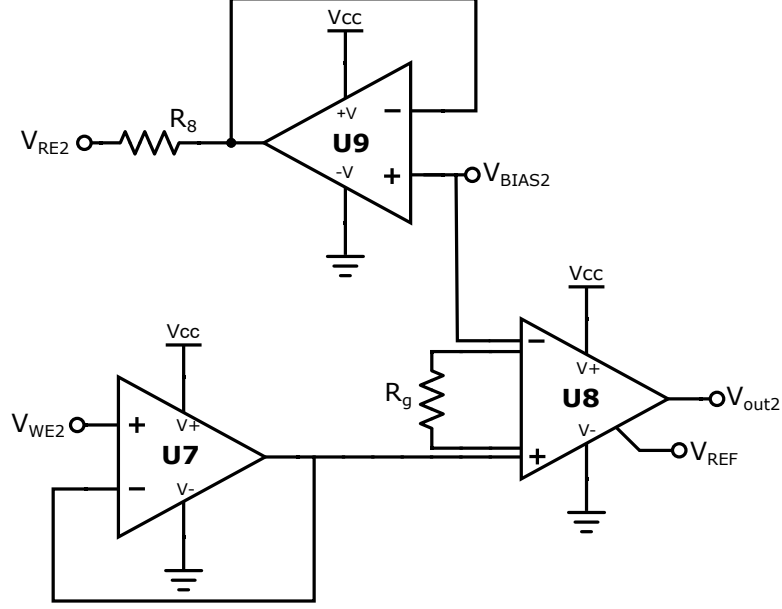


Figure 3.8: Simplified schematic circuit design of potentiometric AFE.

The RE is maintained at a constant voltage using the U9 (ADA4807 from Analog Devices Inc.) amplifier, which can be enabled and disabled, minimising interference with other sensors [11]. By connecting the non-inverting input of U9 to V_{BIAS2} , the circuit can be used for independent measurements and in conjunction with other sensors. This signal acts as a virtual ground, and the output voltage (V_{out2}) can be calculated as:

$$V_{out2} = V_{ref} + G \times (V_{WE2} - V_{Bias2}) \quad (3.42)$$

The voltage difference between the WE and RE was measured by calculating $V_{WE2} - V_{RE2}$. These equations facilitated a comprehensive study of the operational range of this AFE. Additionally, the circuit was tested for pH measurements to assess its ability to capture pH changes, including detection speed. The pH measurement capabilities are relevant for applications in environmental monitoring [26], biomedical research [27], and industrial processes [28].

3.2.2 Firmware Development

This section focuses on the implementation of FW, which is responsible for controlling the HW, acquiring and processing data from sensors, and provid-

ing user-interface functionalities. The chosen MCU was STM32F372R8T6 from STMicroelectronics, which has sufficient processing power for the required tasks [29]. The FW development was carried out using the STM32 Cube IDE platform in C language, which provided the necessary resources. The FW was uploaded to the device through the Joint Test Action Group (JTAG) interface using the ST-LINK V2 programmer/debugger. This method enabled direct communication with the MCU, facilitating efficient programming and real-time debugging during the development process. For more detail on the implemented FW code, consult Appendix E.

The flowchart in Figure 3.9 outlined the overall FW development implemented in the MCU [30].

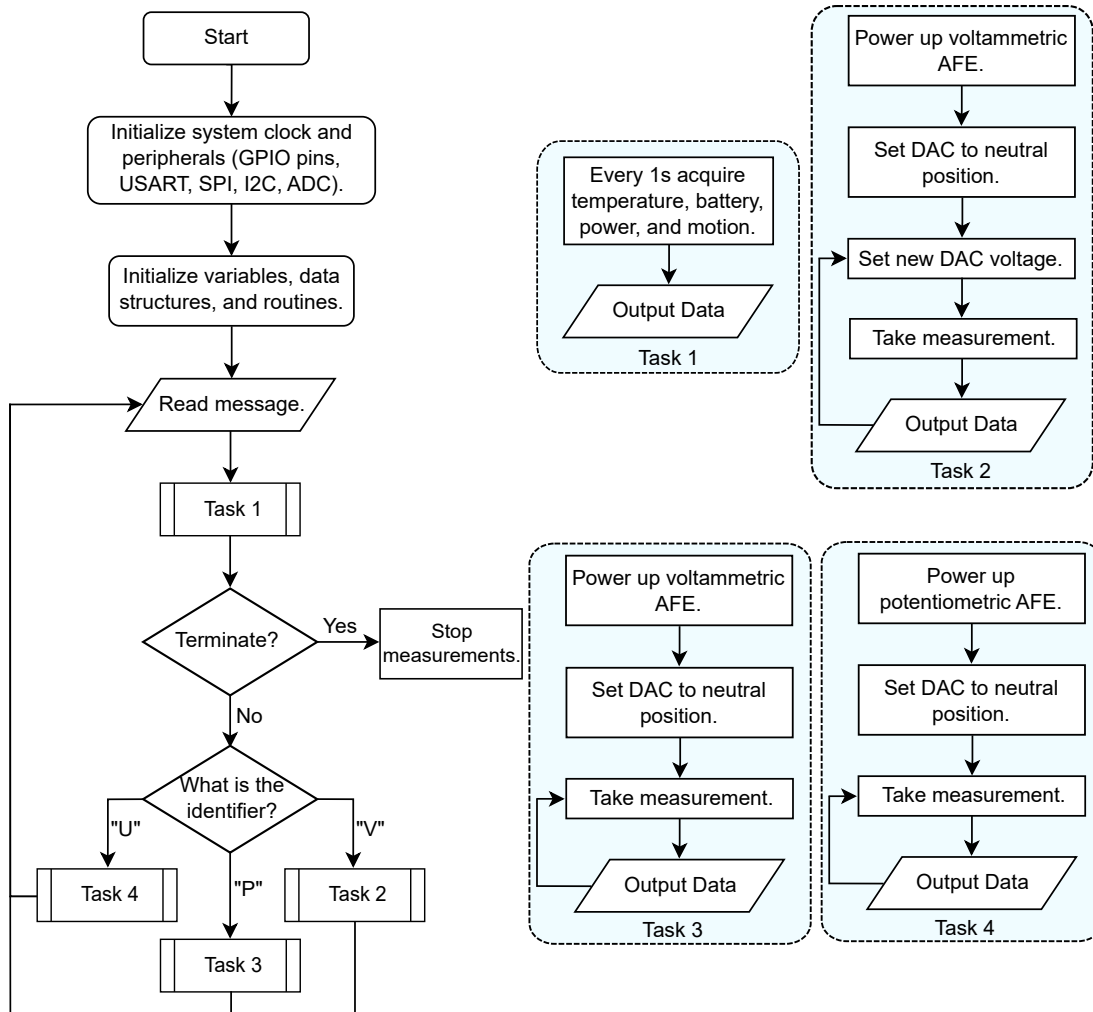


Figure 3.9: Overview flowchart of the FW procedure, with Task 1 for continuous measurements after the start signal and Tasks 2–4 summarizing the FSMs for CV, CA, and OCP measurements, respectively.

Once the start signal was received, periodic data acquisition started, measuring temperature, battery level, motion, and power data at one-second intervals. The collected data was then outputted for analysis. Simultaneously, the system

processed input data from the APP and checked for termination conditions. If no stop signal was received, data acquisition began for specific measurements according to the received signal. More details on the APP development are provided in Section 3.2.3. A Finite-State-Machine (FSM) approach for CA, CV, LSV, SWV and OCP were implemented, executing every 100 ms. Communication with the APP was established via a serial port operating at 115,200 Bd, with a robust serial protocol for reliable communication. The protocol structure included a start index, a channel identifier, data in a hexadecimal format, and a stop index. Acquired data was then outputted and analysed at the APP level. This process continued until termination conditions were met, either upon receiving a stop signal or completing the specified number of cycles.

To guarantee the integrity of the measurement, a checksum value was calculated by performing an Exclusive OR (XOR) operation, and the result was appended to the message before the stop index. The receiver verifies the message integrity by performing the same XOR operation and comparing the calculated checksum with the received checksum. If the values matched, it indicated that the message had been received without errors or data corruption [31].

As mentioned above, the implemented software enabled key electrochemical techniques: CV, CA, LSV, SWV, and OCP. Depending on the technique used, V_{REF} , V_{BIAS1} , V_{BIAS2} , and $C.Sig$ were set to 0 V or 1.65 V using the external DAC capabilities. These voltage settings were chosen to be at $V_{cc}/2$, where V_{cc} is 3.3 V, allowing for both polarity measurements on the electrochemical cell, effectively biasing the voltage at the midpoint of the signal range. The integration of these electrochemical techniques into the FW allowed for versatile and comprehensive electrochemical analysis.

3.2.2.1 Chronoamperometry

As described previously by Bard et al. [32], CA focuses on quantifying the steady state current resulting from an electrochemical reaction, providing quantitative information about the analyte concentration or reaction rate [33]. Figure 3.10 depicted a detailed flowchart of this procedure. Once the FSM was started, the AFE circuit was enabled, and V_{ref} , V_{Bias1} , and $C.Sig$ were set to 1.65 V. Following a 5-second delay for cell stabilisation, $C.Sig$ adjusted to the user-input voltage. The MCU started data acquisition, continuously sending acquired data back to the user every 100 ms until the stop signal was received. Upon termination, the AFE circuit was powered down, and signals reset, halting data acquisition. This approach ensured precise amperometric measurements with voltage control flexibility.

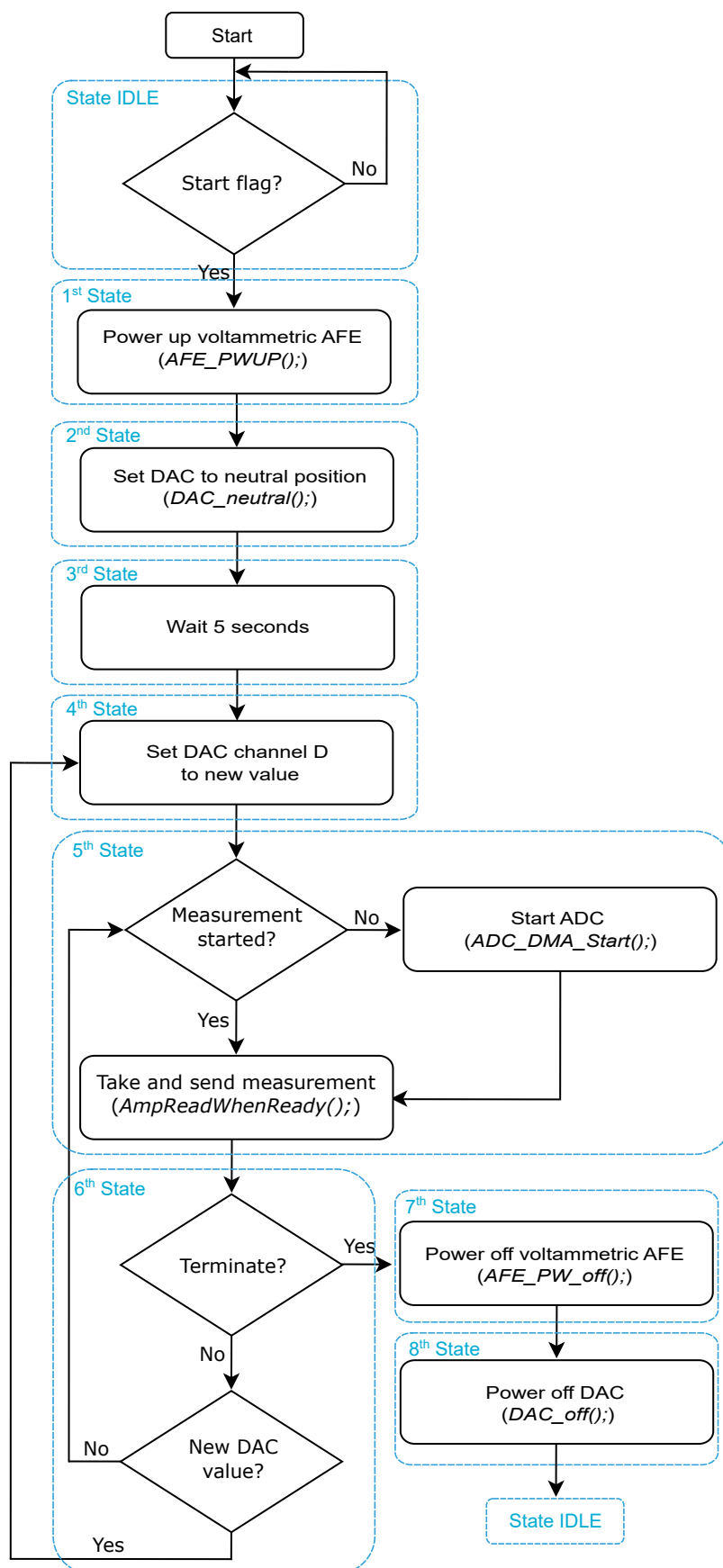


Figure 3.10: Flowchart of the CA procedure.

3.2.2.2 Cyclic Voltammetry

CV involved sweeping the applied potential repetitively to observe redox reactions [32, 34]. This process is graphically illustrated in Figure 3.11.

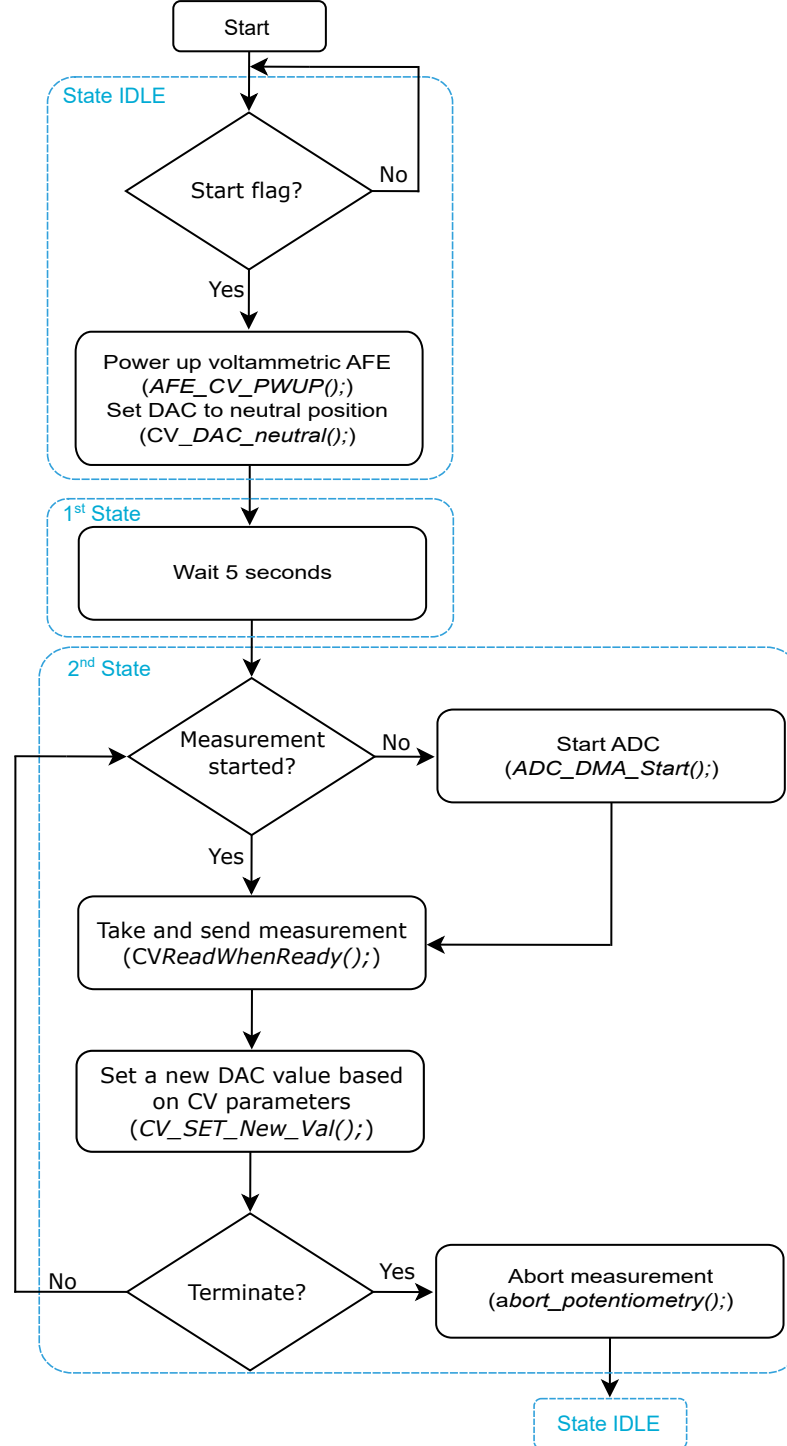


Figure 3.11: Flowchart of the CV procedure.

Through the APP, the user can set initial, maximum, and minimum voltages, slope, and the number of cycles. Once the start signal was received, the voltammetric AFE was enabled, setting V_{ref} , V_{Bias1} , and $C.Sig$ to 1.65 V. After a

5-second delay for system stabilisation, measurements began, sweeping from initial to maximum and back to minimum voltage at the specified rate for one cycle. The process continued until a stop signal was received or the predetermined cycle number was reached. Upon completion, the AFE was disabled, and signals were reset.

3.2.2.3 Linear Sweep Voltammetry

LSV involved varying potential from start to stop to an electrochemical cell while measuring the resulting current response [32]. The implemented process is graphically illustrated in Figure 3.12.

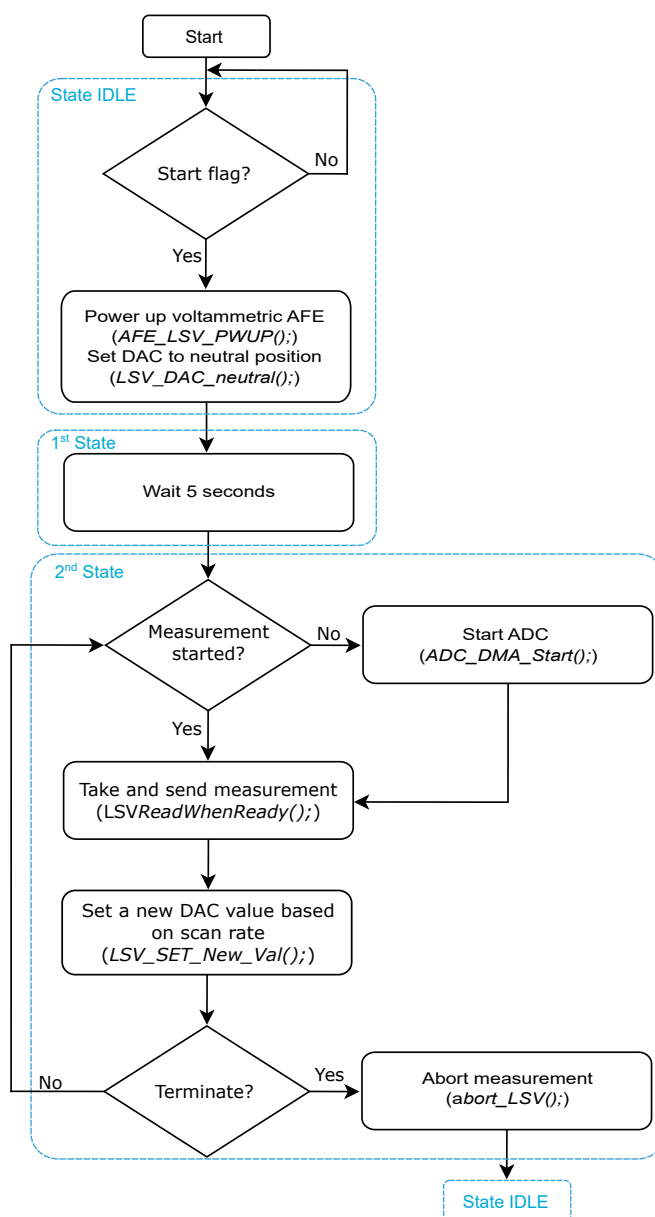


Figure 3.12: Flowchart of the LSV procedure.

During this sweep, either the oxidation or reduction current was recorded,

depending on the direction of the voltage sweep. Through the APP, the user can set initial and stop voltages, and scan rate, which controls the speed of the voltage sweep. Once the start signal was received, the voltammetric AFE was enabled, setting V_{ref} , V_{Bias1} , and $C.Sig$ to 1.65 V. After a 5-second delay for system stabilisation, measurements begin, sweeping from initial to stop voltage at the specified rate. Once stopped, meaning when the applied voltage is equal or superior to the stop voltage, the AFE was disabled, and signals reset.

3.2.2.4 Square Wave Voltammetry

SWV involved applying a square-wave modulated potential to the electrochemical cell while recording the current response [35]. Through the APP, the user can configure the initial, stop, and step voltages. In the FW, the frequency was set to 50 Hz, corresponding to a period of 20 ms.

Once the start signal was received, the voltammetric AFE was enabled, setting V_{ref} , V_{Bias1} , and $C.Sig$ to 1.65 V. After a 5-second delay for system stabilisation, the firmware defined two values: initial voltage $\pm$ modulation. The modulation value was set in the FW to 25 mV. First, the initial voltage plus the modulation value was applied to the CE, and after a 5-second delay, the current was measured and stored as the forward current. Next, the initial voltage minus the modulation value was applied, and the resulting current was measured as the reverse current after another 5-second delay. Both the forward and reverse current values were then sent to the APP for further processing. If no termination conditions were met (such as user cancellation or error), the voltage continued sweeping stepwise with the $\pm$ modulation applied at each step until the stop voltage was reached or exceeded. Once the stop condition was met, meaning the applied voltage equalled or surpassed the stop voltage, the AFE was disabled, and all signals were reset to default. This process is graphically illustrated in Figure 3.13.

3.2.2.5 Open Circuit Potentiometry

OCP involved measuring the potential between the WE and RE, enabling the investigation of various analytes [32, 36]. Similarly to the previous techniques, using the APP, the user had control over initiating and terminating the measurement. When the MCU received the start signal, the potentiometric AFE was enabled, setting the signals V_{ref} and V_{Bias2} to 1.65 V, and initiating the data acquisition. Upon reception of the stop signal, the procedure aborted the measurement, which consisted of disabling the AFE, setting signals to 0 V, and going back into an idle state. Figure 3.14 shows the described chain of events.

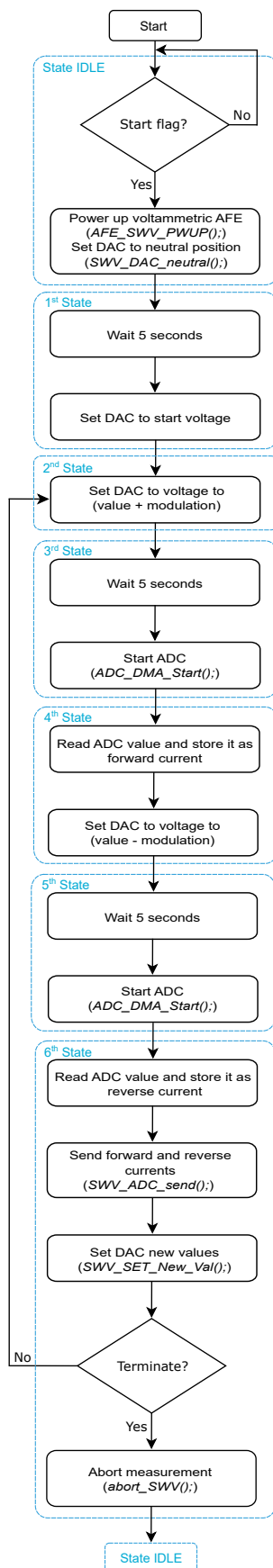


Figure 3.13: Flowchart of the SWV procedure.

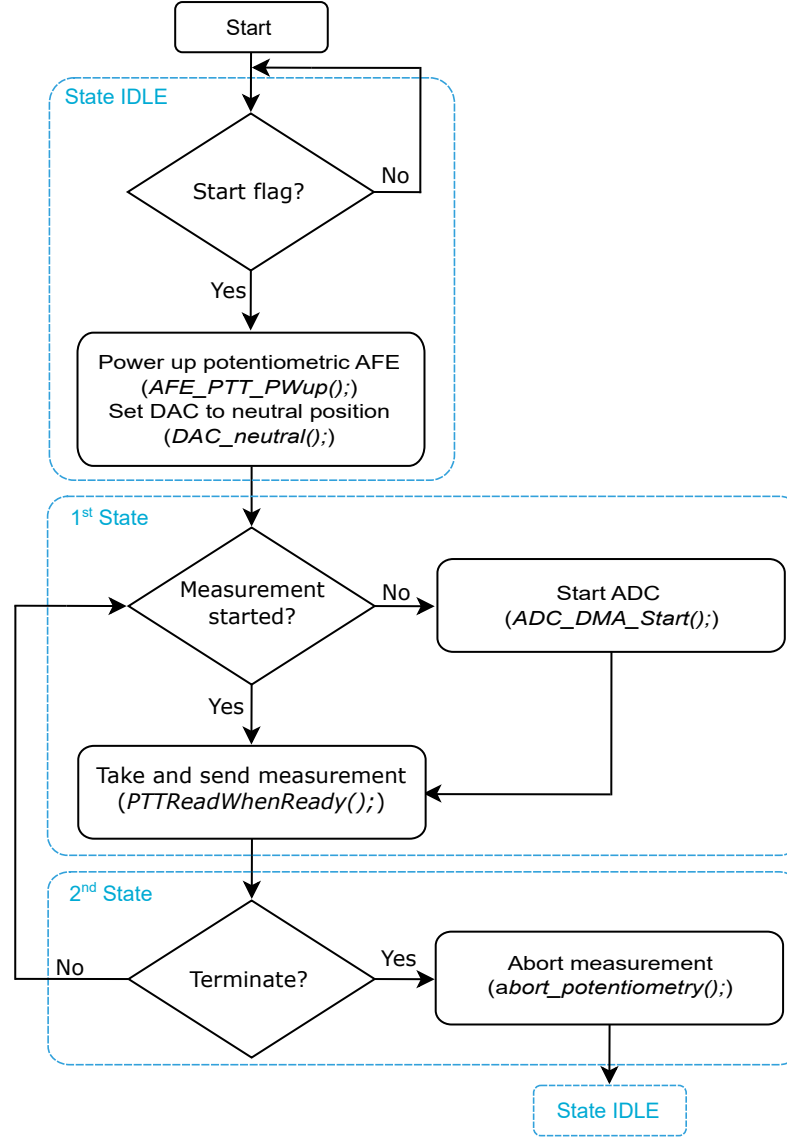


Figure 3.14: Flowchart of the OCP procedure.

3.2.2.6 Serial Communication Protocol

In the implemented serial communication protocol, Universal Synchronous Asynchronous Receiver Transmitter (USART) communication was utilised to facilitate data exchange between the user's device and the MCU. The MCU is configured to operate at a baud rate of 115200 Bd, using 8 data bits, 1 stop bit, and no parity. This configuration ensured reliable and efficient communication between the devices. Table 3.2 shows the serial port settings.

The message format used in this situation consisted of the following:

- Start message index "\$": This character marked the beginning of the message and served as a delimiter to indicate the start of data transmission.
- Index identifier (1-byte size): A single-character identifier responsible for

Table 3.2: Serial port settings.

BaudRate	115200 Bd
Word Length	8 bit
Parity	None
Stop Bit	1

specifying the target module or task within the MCU. This identifier ensured that the following parameters were correctly applied to the intended AFE or other modules in the MCU.

- Data in hexadecimal (variable size, minimum 4 bytes): This section contained a variable number of parameters, each representing a 4-byte hexadecimal value. It carried the specific data or settings related to the identified task.
- XOR-checksum (2-byte size): A two-character XOR-checksum was included in the message. This allowed the MCU to verify the accuracy of the received message by performing an XOR operation on the received data.
- Stop index "\n": This character marked the end of the message, indicating the completion of data transmission. It acted as a clear delimiter, helping the MCU recognize the message's conclusion.

This structured format enabled precise communication between the user interface and the MCU for various tasks and configurations. The implemented commands can be seen in more detail in Appendix B.5 along with the error-handling protocols implemented.

3.2.3 APP Development

An APP was developed using Qt software (Qt, Helsinki, Finland) to enable device control, plotting, and saving data. This APP is supported by Linux, OS, Windows, Android, iOS, and other operating systems. The implementation encompassed the development of a comprehensive communication protocol, incorporating an extensive command list (described in Appendix B.5), and integrating a GUI within the APP to facilitate the presentation of measurement results. For additional insights into the developed code, please refer to Appendix E.

The APP is available on the user's computer called "PULSE", as shown in Figure 3.15 (a). Data collected by the APP is automatically saved as an Excel file in the "data" folder, organised based on the type of measurement and the time it was collected, as illustrated in Figure 3.15(b).

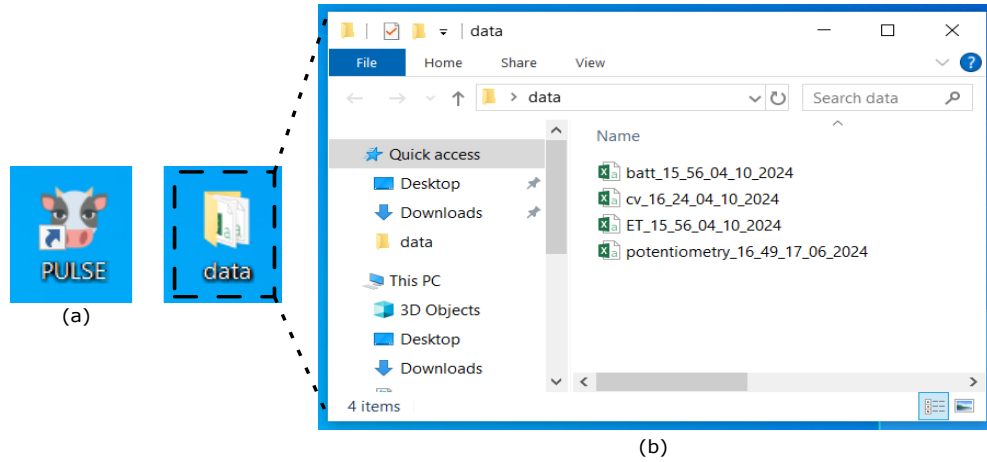


Figure 3.15: (a) APP logo, and (b) data folder with Excel files according to data type and timestamp.

Upon launching the APP, two windows appear: the main window "PULSE", depicted in Figure 3.16 (a), and a secondary window "Orientation", shown in Figure 3.16 (b). The main window serves as the primary interface for controlling the device and monitoring data, while the secondary window shows the device orientation based on accelerometer data.

Once the serial port is selected in the main window and the "Start" button is pressed, the APP opens the selected serial port and begins communication. As data is received, the APP performs an error check using the XOR-checksum technique. If the data is valid, it is parsed based on its identifier. If the data is incomplete or invalid, it is temporarily stored in a buffer until the full message is received. The parsed data is then processed according to the type of measurement, such as temperature, CV, or OCP. Once processed, the data is plotted in real-time and saved to an Excel file. The file is named based on the measurement type, as well as the exact timestamp (including hour, minute, day, month, and year) when the data was recorded. This cycle of receiving, processing, plotting, and saving data continues until the "Stop" button is pressed, at which point communication is halted and data collection ends. This process is illustrated in Figure 3.17.

The APP also has the capability to send instructions to the device, enabling it to start and stop specific measurements. For example, during OCP, the user can define the duration for which the measurement will be conducted. Once this time period elapses, the APP automatically sends a stop signal to the MCU to terminate the measurement. In addition, the user can also configure parameters associated with each specific measurement within the APP. More details on how to operate the APP can be found in Appendix B.6, and the implemented code can be consulted in Appendix E.

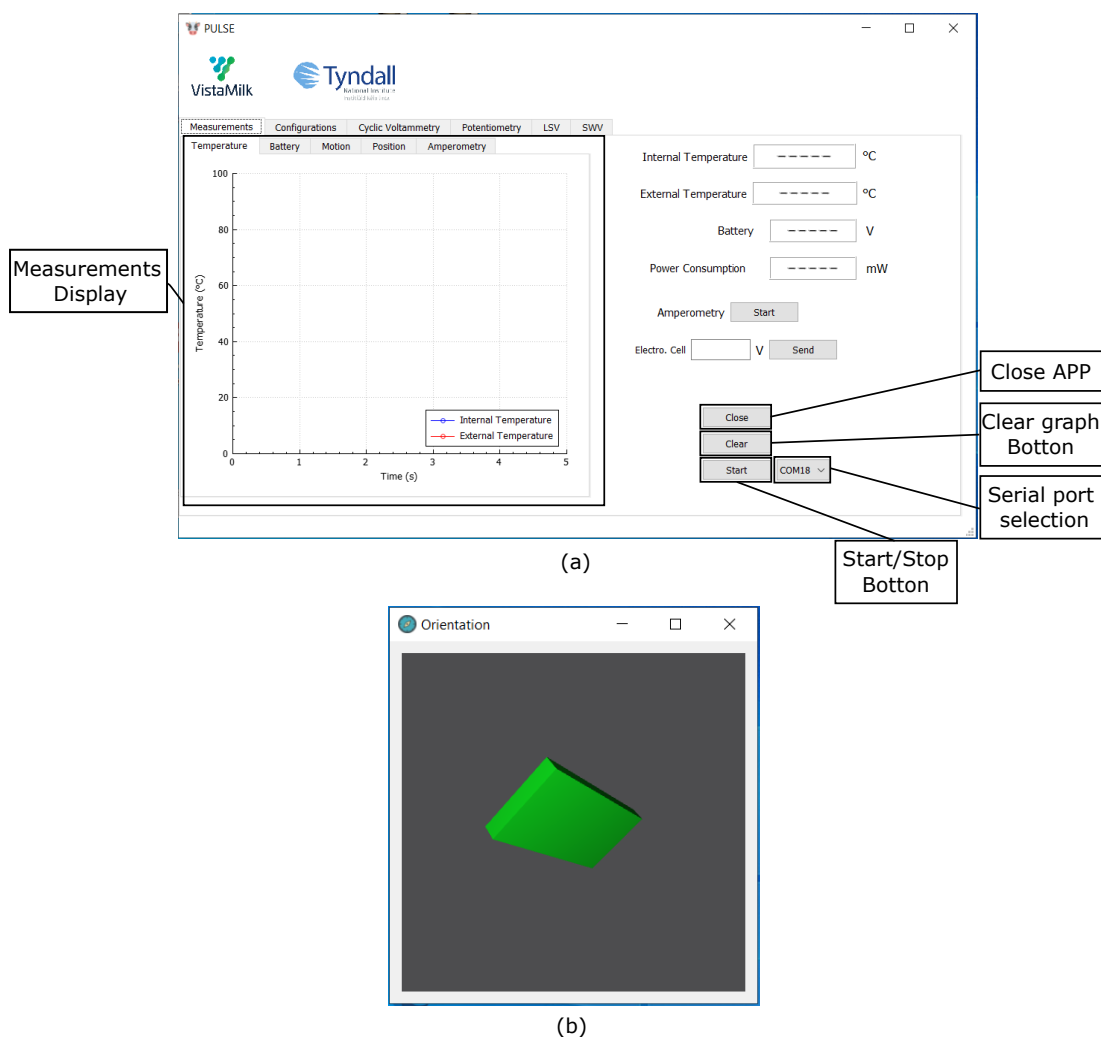


Figure 3.16: APP (a) main window "PULSE", and (b) secondary window "Orientation".

In general, when the user initiates a command - such as starting a measurement or adjusting parameters - the APP first checks whether the serial port is open. If the serial port is not open, the APP generates an error message advising the user to open the serial port before proceeding. Once the serial port was confirmed to be open, the transmitted data was processed and converted into a hexadecimal format. Before the data was sent to the MCU, an XOR-checksum was calculated to ensure data integrity, and the checksum value was appended to the data package. Finally, the complete data package, including the checksum, was transmitted to the device's MCU. This entire process is illustrated in Figure 3.18.

The procedure described in Figure 3.18 as "Process", varies depending on the type of measurement being performed. For instance, in the case of SWV, the start, stop, and step voltages are sent as a single data package. Initially, the APP retrieves the values entered by the user and converts them from text to double

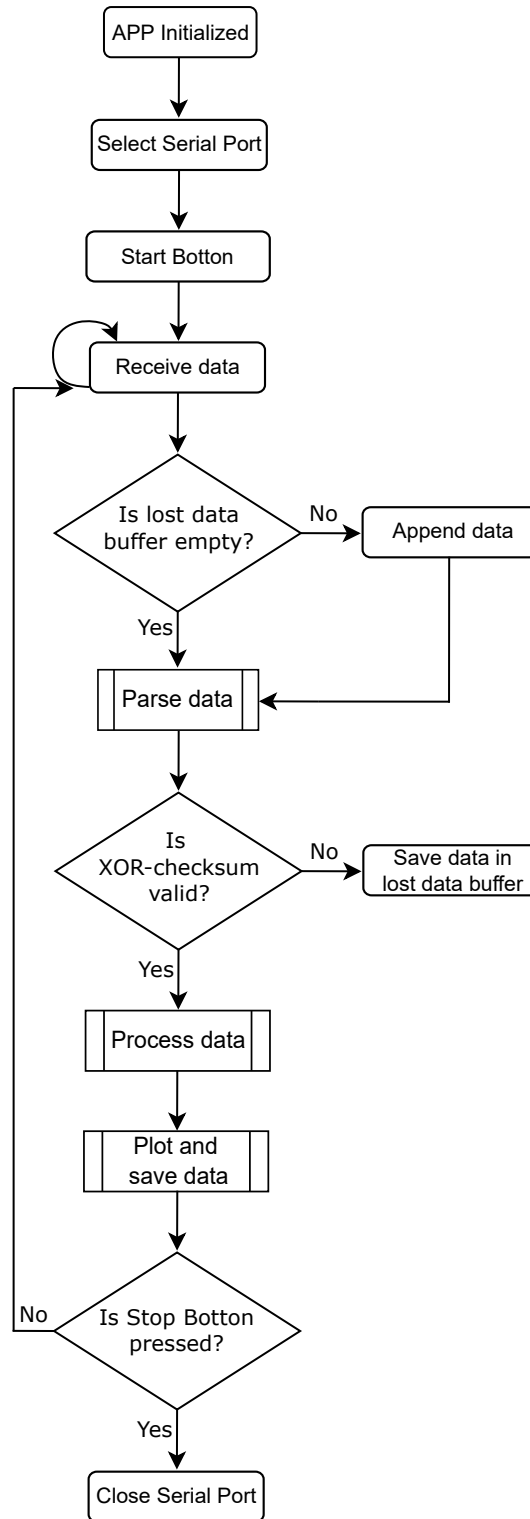


Figure 3.17: Simplified overview APP flowchart.

precision floating-point for accurate processing. Subsequently, it is necessary to adjust the raw voltage values to account for the characteristics of the DAC, particularly its offset and slope. This adjustment ensures that the values sent to the DAC correctly reflect the desired analog output. The following equation is applied to perform this adjustment:

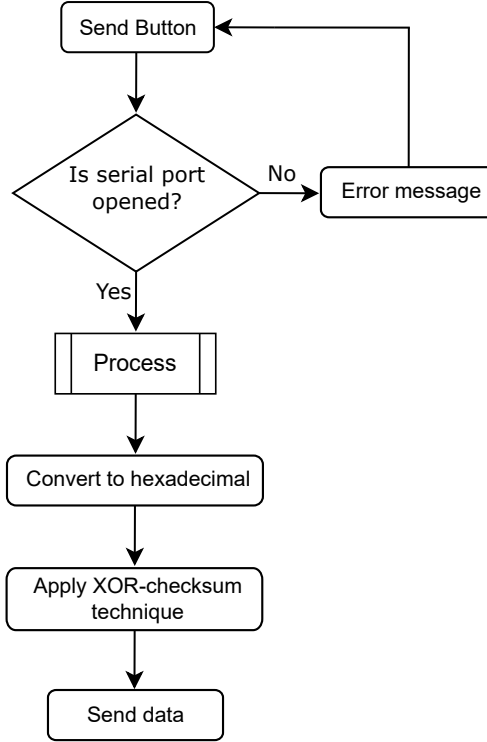


Figure 3.18: Simplified flowchart illustrating the process of sending data from the APP to the device's MCU.

$$DAC_{value} = \frac{value - DAC_{offset}}{DAC_{slope}} \quad (3.43)$$

In this equation, DAC_{value} corresponds to the corrected digital value, $value$ is the raw input value, DAC_{offset} is the offset of the DAC, specified as 0.005498; and DAC_{slope} is the slope parameter that accounts for the linear response of the DAC, specified as 0.000076. This ensured that the value sent to the MCU accurately reflects the desired analog output.

3.2.4 Electrochemical Validation

All the electrochemical measurements were performed in the PULSE system. To validate the PULSE, the device was benchmarked against a calibrated commercial benchtop potentiostat - Metrohm Multi Autolab M101 (Cheshire, UK) - as shown in Figure 3.19 (a), performing CA, CV, and OCP. The electrochemical measurements were carried out using carbon Screen-Printed Electrodes (SPEs) obtained from DropSens (DRP – 110GPH) (Figure 3.19 (c)). These SPEs consisted of a carbon WE (4 mm diameter), a carbon CE, and a silver/silver chloride (Ag/AgCl) RE. CA, CV, and OCP were performed in triplicate.

Potassium hexacyanoferrate III ($K_3[Fe(CN)_6]$), potassium hexacyanoferrate II ($K_4[Fe(CN)_6]$), PBS, iridium (IV) chloride ($IrCl_4 \cdot H_2O$), hydrogen peroxide

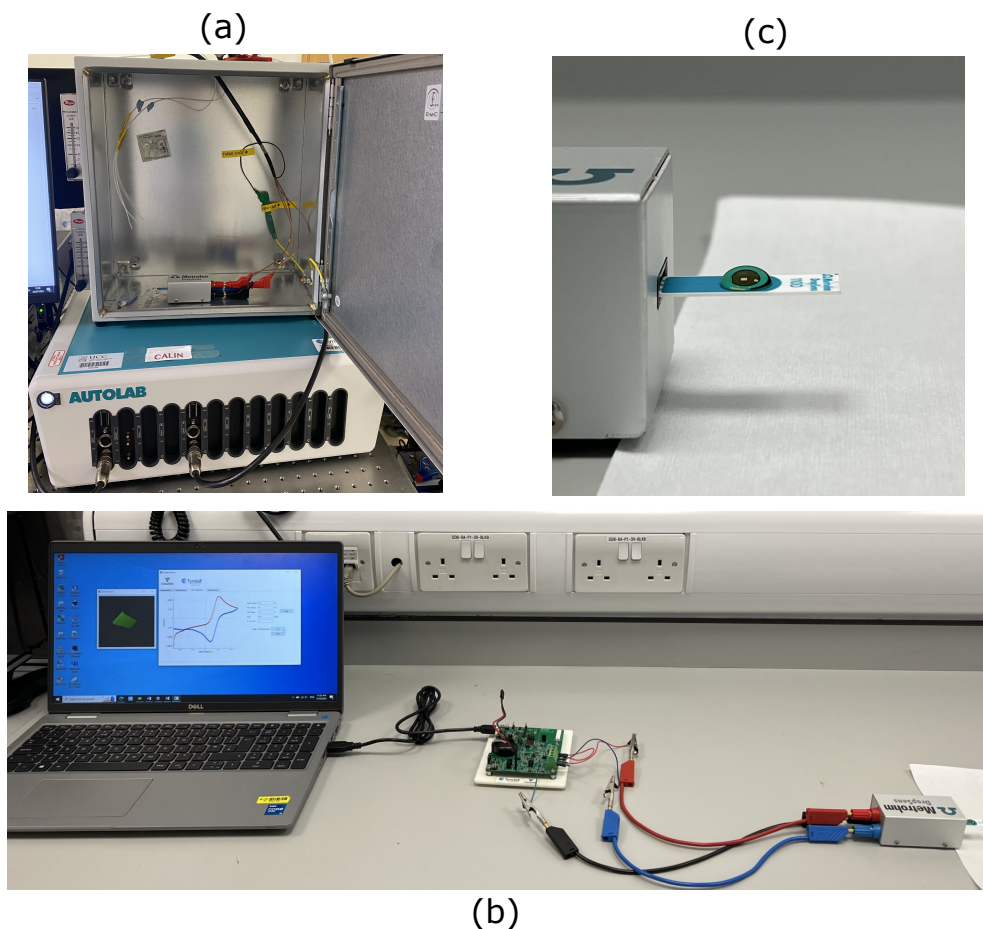


Figure 3.19: Laboratory setup for validation experiments using (a) a commercial benchtop potentiostat (Metrohm Multi Autolab M101), (b) the PULSE system with the developed APP, and (c) SPEs from DropSens (DRP-110GPH).

(H_2O_2), oxalic acid ($\text{C}_2\text{H}_2\text{O}_4$), sodium carbonate ($\text{Na}_2\text{-CO}_3$), sodium hydroxide (NaOH), and hydrochloric acid (HCl) were obtained from Sigma Aldrich (Gillingham, UK). PBS solutions (10mM) were obtained by dissolving 1 PBS tablet of pH 7.4 in 200 ml of Deionized (DI) water. All solutions were prepared using DI Milli-Q water (resistivity $18.2 \text{ M}\Omega\text{cm}$).

Ferri-ferrocyanide solutions were prepared in PBS pH 7.4 with concentrations ranging from 1 mM to 5 mM, and analysed with CV. Prior to conducting CA, the voltammograms obtained from CV at various solution concentrations were examined to determine the voltage corresponding to peak oxidation, which was subsequently applied during CA experiments. For CV measurements performed with the PULSE, the experimental setup is illustrated in Figure 3.19 (b). The voltammetric circuitry was configured with a gain of 1, R_f of $9.9 \text{ k}\Omega$, and C_f of 10 nF . pH measurements were conducted using OCP in PBS, where NaOH or HCl was added to adjust the pH accordingly. A calibrated pH probe from Mettler Toledo (Greifensee, Switzerland) was used to ensure the accuracy of the measurements. The potentiometric circuitry of the PULSE system was also configured

with a gain of 1. All the tests performed with the PULSE were conducted without the use of a Faraday cage, to better replicate real-world scenarios. The collected data was processed, and regression models were generated for datasets obtained from both the benchtop potentiostat and the PULSE system [37].

3.2.4.1 IrOx Functionalization

Prior to the analysis of pH with OCP, the WE of the commercial SPEs was functionalized with an Iridium Oxide (IrOx) sensing layer. This layer was added due to the advantages of IrOx in pH detection, including prolonged stability, rapid response time, and minimised chemical interference, among others [38]. IrOx was made using an adaptation of the Yamanaka method [39], with modifications such as extended stirring periods and the use of Sodium carbonate (Na_2CO_3) to adjust the solution pH. For further details on IrOx preparation, refer to Appendix B.10.

CV is a widely used technique for IrOx deposition [40]. A prior evaluation was conducted before the start of this project to determine the deposition parameters. CV scans were performed sweeping the potential from -0.7 V to 0.6 V at a scan rate of 10 mV/s for a total of 80 scans.

3.3 Results & Discussion

This section provides a comprehensive analysis of the HW performance, characterisation, and validation of the developed PULSE platform, and comparison with the existing SoTA.

3.3.1 Hardware

The PCB layout presented in Figure 3.20 (a) maximised the top layer utilisation for ease of testing and repairs, while ensuring a robust ground return. The routing used a $0.16\text{ }\mu\text{m}$ trace width for cost-effectiveness, re-routing away from passive components. Additional ground vias were introduced, with unrestricted multi-layer routing. Although noise was not a critical issue, these vias were added as a preventative measure, following best practices to minimise potential coupling, particularly around sensitive analog components. Polygon pours were used for signal integrity and structural prevention, as well as a split between analog and digital ground and power planes. Additionally, capacitors were carefully selected and positioned for power supply decoupling and filtering [41]. More details on the PCB can be found in Appendix B.7.

The fabricated PCB illustrated in Figure 3.20 (b) measured approximately $(78 \times 100 \times 2)\text{ mm}^3$ and 53 g, highlighting its compact and lightweight design,

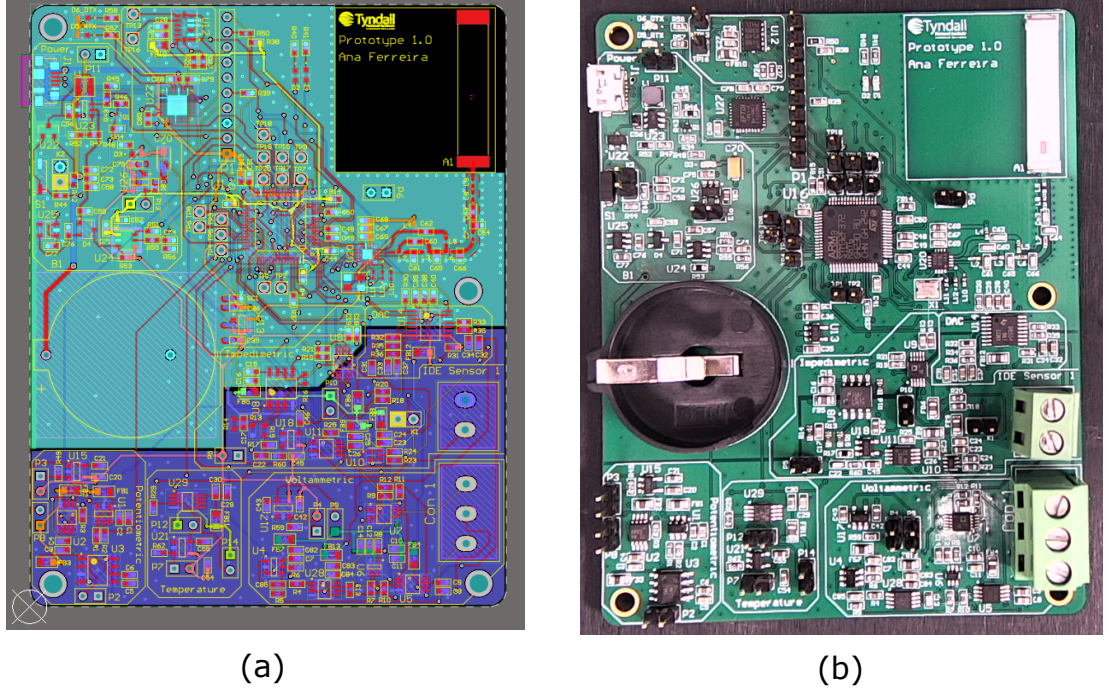


Figure 3.20: Printed circuit board: (a) Altium's 2D PCB routing interface, and (b) fabricated PCB.

crucial to ensure the device's portability and suitability for in-situ applications. From the comparison in Figure 3.21, although indirect, since the benchtop potentiostat's size includes its housing while the PULSE PCB represents only the circuit board, it is evident that PULSE is smaller than the benchtop potentiostat. In particular, the potentiometric circuit occupied an area of approximately 426.56 mm^2 , while the voltammetric circuit took approximately 771.309 mm^2 , including connectors. The PCB featured a coin cell battery holder of diameter 24 mm, micro USB-B connector, antenna, and connectors for both 3-electrode and 2-electrode configurations. The PCB served as a solid foundation for the subsequent stages of electronic system development and characterisation.

From the calibration of the ADCs, Equations 3.44 and 3.45 were taken for voltammetric and potentiometric AFEs, respectively. These equations represent the linear relationship between real-world voltage values (V_{real}) and serial port voltage values (V_{serial}), both with $R^2 = 0.999$.

$$V_{real} = 1.003919 \times V_{serial} + 0.037731 \quad (3.44)$$

$$V_{real} = 1.004436 \times V_{serial} - 0.0527213 \quad (3.45)$$

Similarly, the calibration equation of the DAC, representing the linear ($R^2 = 0.999$) relationship between the digital input code (D_{in}) and actual output

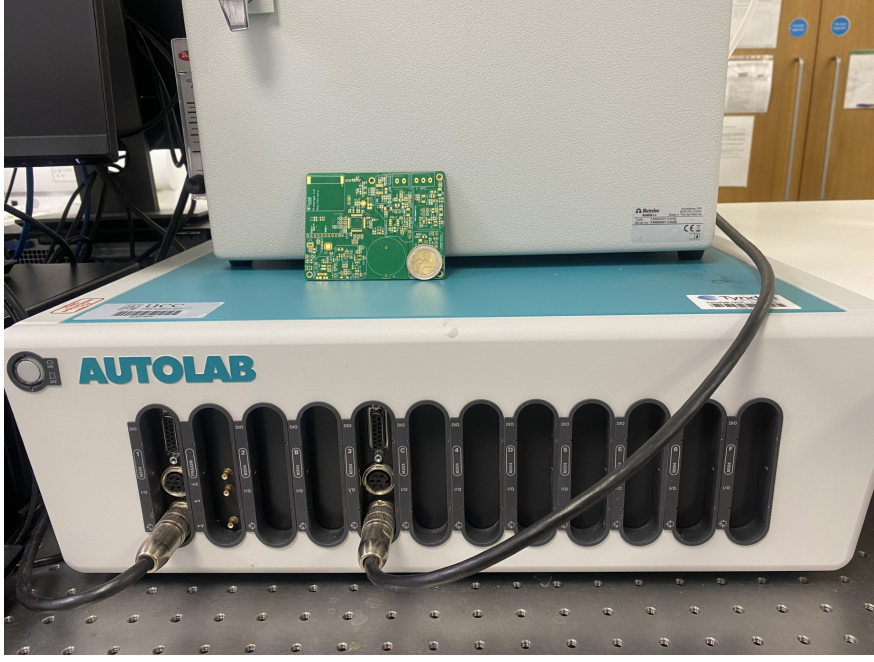


Figure 3.21: Size comparison of the PULSE PCB, benchtop potentiostat (including its housing), Faraday cage, and a 2€ coin.

voltage (V_{out}) is expressed as follows:

$$V_{out} = 0.000076 \times D_{in} + 0.005498 \quad (3.46)$$

3.3.2 Characterisation

3.3.2.1 Operational Range

The power supply of 3.3 V, with a minimum voltage of 0 V and a maximum of 3.3 V, combined with the TIA's rail-to-rail output margin of 100 mV, results in a TIA output voltage range from 0.1 V to 3.2 V. To calculate the TIA's output voltage (V_{TIA}), Equation 3.37 was rearranged as:

$$V_{TIA} = \frac{V_{out1} - V_{ref}}{G} + V_{Bias1} \quad (3.47)$$

Since V_{TIA} dependent of R_f , i.e. $V_{TIA} = -R_f \times I$, meaning it determines the gain of the TIA for a given current. To ensure accurate measurement ranges, the linear operating range of the INA333 must be considered. The analysis began from the INA333's output and worked back toward the TIA input to account for the supply voltage constraints and the op-amps' linear operating regions. Table 3.3 summarizes the V_{TIA} range required to keep the INA333 in linear mode of operation.

The current range analysis for the voltammetric AFEs is presented in Ta-

Table 3.3: Summary table of output voltage of the TIA (V_{TIA}), according to different gains (G).

Signal	$G = 1$	$G = 2$	$G = 10$	$G = 100$
V_{TIA} (V)	[0.1; 3.2]	[0.85; 2.45]	[1.49; 1.81]	[1.63; 1.67]

ble 3.4. This table outlines the current ranges for which the TIA operates in linear mode for different values of R_f . The operational ranges were characterised in terms of current (I) for various configurations of R_f and INA333 gain (1, 2, 10, 100). As mentioned before, the TIA's output voltage ranges from 0.1 V to 3.2 V, with the maximum output voltage of the circuit spanning 0.05 V to 3.25 V.

Table 3.4: Operational range of the voltammetric AFE by gain (G) and feedback resistor (R_f) values.

R_f (Ω)	$G=1$	$G=2$	$G=10$	$G=100$
1 M	$\pm 1.55 \mu A$	$\pm 800 nA$	$\pm 160 nA$	$\pm 16 nA$
100 k	$\pm 15.5 \mu A$	$\pm 8 \mu A$	$\pm 1.6 \mu A$	$\pm 160 nA$
9.9 k	$\pm 156.57 \mu A$	$\pm 80.81 \mu A$	$\pm 16.16 \mu A$	$\pm 1.62 \mu A$
1 k	$\pm 155 mA$	$\pm 0.80 mA$	$\pm 160 \mu A$	$\pm 16 \mu A$

From the analysis of the table, it was noticeable that the same measurement range can be achieved with different combinations of R_f and INA333 gain. Selecting a lower R_f is often preferable, as it generates less thermal noise, improving overall signal quality. In this scenario, the INA333 gain should be set higher to compensate for the smaller TIA output. While this flexibility in circuitry design allows for a range of configurations, each comes with distinct performance trade-offs: one configuration may be optimised for bandwidth, while another prioritises noise reduction.

The current range extended from microamperes to nanoamperes, achieving smaller currents compared to other devices, which is further explored in Section 3.3.6 [42]. The user can conveniently adjust different settings of gain and R_f values at the APP level (as shown in Appendix B.6), allowing customisation for specific electrochemical analysis requirements in terms of sensitivity and dynamic range. This flexibility optimised the system for multiple electrochemical applications. Additionally, the ability to modify these parameters via the GUI enhanced the system's versatility and adaptability to diverse experimental conditions.

The potentiometric circuitry was characterised by input voltage at the WE (V_{WE2}) and sensed voltage (V_{out2}) between WE and RE. To calculate the values of V_{WE2} , the analysis started from the output of the INA333 and worked backwards

towards the voltage at the WE, considering the linear operating region of the OpAmp, using the following equation:

$$V_{WE} = \frac{V_{out2} - V_{REF}}{G} + V_{BIAS2} \quad (3.48)$$

Table 3.5: Operational ranges of the potentiometric AFE, including WE voltage (V_{WE2}) and output voltage (V_{out2}) by gain (G).

Signal	G=1	G=2	G=10	G=100
V_{WE2} (V)	[0; 3.3]	[0.825; 2.475]	[1.485; 1.815]	[1.634; 1.667]
V_{out2} (V)	± 1.65	± 0.83	± 0.165	± 16.5 m

For the potentiometric circuit, which operates within a voltage range of ± 1.65 V, the 12-bit ADC provides a resolution of $805 \mu\text{V}/\text{step}$ at a gain of 1, being the step the input current per digital code increment. This resolution ensures precise voltage measurements, which are critical for detecting subtle changes in the potentiometric signal. On the other hand, the resolution of the voltammetric measurements depends on the selected gain and the value of the R_f , which together define the measurement range. For example, within an operational current range of $\pm 156.57 \mu\text{A}$, the resolution of the ADC is calculated to be $76.45 \text{ nA}/\text{step}$, while at a range of $\pm 16 \text{ nA}$, the resolution is as low as $7.81 \text{ pA}/\text{step}$. This ultra-high resolution can be crucial for detecting extremely low current signals in sensors operating at picoampere levels. These specifications highlighted the adaptability of the system for both potentiometric and voltammetric applications, ensuring robust performance across a variety of sensing scenarios. The resolution values provided reflect the balance between range and precision, tailored to meet the demands of multi-parametric sensing applications.

3.3.2.2 Noise Levels

The noise levels of the voltammetric and potentiometric AFEs circuits were assessed, which demonstrated SNR levels ranging from 25.02 dB to 33.08 dB, and 28.06 dB to 33.13 dB, respectively. These measurements were taken across the entire voltage range, indicating the performance and reliability of the circuits under various operational conditions.

3.3.2.3 Power Consumption

The power consumption associated with performing single measurements is represented in Table 3.6, as recorded using PPK2. The idle state, meaning a state where the DUT is operational but not actively engaged in performing any

specific task or process, exhibited a consumption of 81.4 mW. The highest values were observed when performing electrochemical techniques. Power consumption increased to 87.2 mW during CV, 90.4 mW during CA, and 89.9 mW OCP measurements. The difference in the current signal can be observed in Figure 3.22, where the PULSE was initially in an idle state before starting a CA measurement. These values provided insight into the energy demand of these specific electrochemical techniques compared to the idle state. These techniques involve actively controlling and monitoring the electrochemical system, thereby being more power-demanding. The current spikes observed in the figure were due to voltage regulation caused by rapid load changes in the DUT, resulting in peaks that cancel each other out, making the average current the key metric to consider.

Table 3.6: Idle-state power consumption and individual measurements, including acceleration, temperature (internal/external), battery voltage, power monitoring, and electrochemical techniques (CV, CA, OCP).

Measurement	Idle	Acceleration	Temp. External	Temp. Internal	Battery	Power Consp.	CV	CA	OCP
Power Cons. (mW)	81.445	83.335	82.250	82.495	83.020	83.965	87.185	90.440	89.915

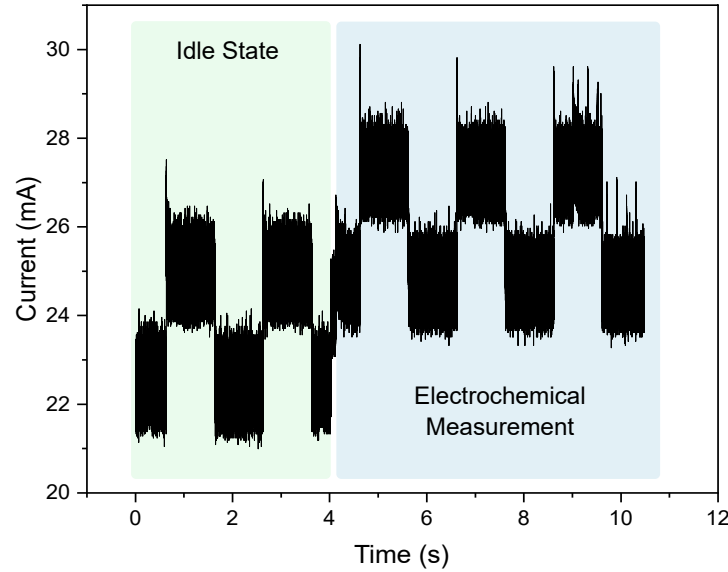


Figure 3.22: Current over time, showing the transition from the idle state to perform an electrochemical measurement.

Moreover, a study was conducted to evaluate the power consumption associated with multiple simultaneous measurements, as detailed in Table 3.7. Simultaneous execution of all individual non-electrochemical measurements resulted in a total power consumption of 83.86 mW, reflecting an approximate 3% increase from the idle state. The results indicate that the most power-intensive measurement involved performing CV, while using the external temperature sensor,

and accelerometer. This combined activity consumed 97.3 mW, representing a 19.5% increase in power consumption compared to the idle state. For example, a 3.0 V coin cell battery with a capacity of 220 mAh would allow the device to perform temperature measurements, acceleration sensing, and CV scans in continuous mode for approximately 6.8 hours. However, in practice, CV scans are typically not performed continuously, which extends the overall operation time. Ultimately, battery choice also plays a crucial role in determining the device's runtime.

Table 3.7: Power consumption of multiple simultaneous measurements, where *Acc* denotes accelerometer, *TE* refers to external temperature, *TI* corresponds to internal temperature, and *All* signifies the combined power demand of all physical measurements.

Measurement	Acc+TE	Acc+TE+TI	All	OCP+TE	OCP+TE+ACC	CV+TE	CV+TE+Acc
Power Consp. (mW)	82.950	82.880	83.860	93.100	96.145	88.025	97.300

3.3.3 Cyclic Voltammetry

The results obtained from performing CV using the benchtop potentiostat and the PULSE system to detect 5 mM of ferri-ferro are displayed in Figure 3.23. Although simultaneous measurements were possible, they were conducted sequentially for practicality, as the tests were performed manually. All the key measurements including voltage and current values recorded during three cycles, are presented in Table 3.8 for both systems. Additionally, average (avg) and SD calculations provided insights into the performance of the respective devices.

Table 3.8: Comparison of oxidation/reduction peak potentials and currents over three cycles for the benchtop instrument and PULSE, including average (Avg) and standard deviation (SD).

System	Benchtop Potentiostat				PULSE System			
Peak	Oxidation		Reduction		Oxidation		Reduction	
Cycle	Pot (mV)	I (μ A)	Pot (mV)	I (μ A)	Pot (mV)	I (μ A)	Pot (mV)	I (μ A)
1	171.8	150.7	51.7	-152.4	171.9	141.6	58.2	-146.6
2	174.3	155.8	54.2	-151.4	171.9	152.5	53.3	-145.3
3	174.3	157.9	54.2	-151.7	166.9	155.5	53.3	-145.9
Avg	173.4	154.8	53.3	-151.8	170.2	149.9	54.9	-145.9
SD	1.18	3.05	1.16	0.44	2.33	5.97	2.33	0.53

From the analysis of the data in Table 3.8, it was observed that the peak positions and intensities exhibited slight changes across the cycles, a common

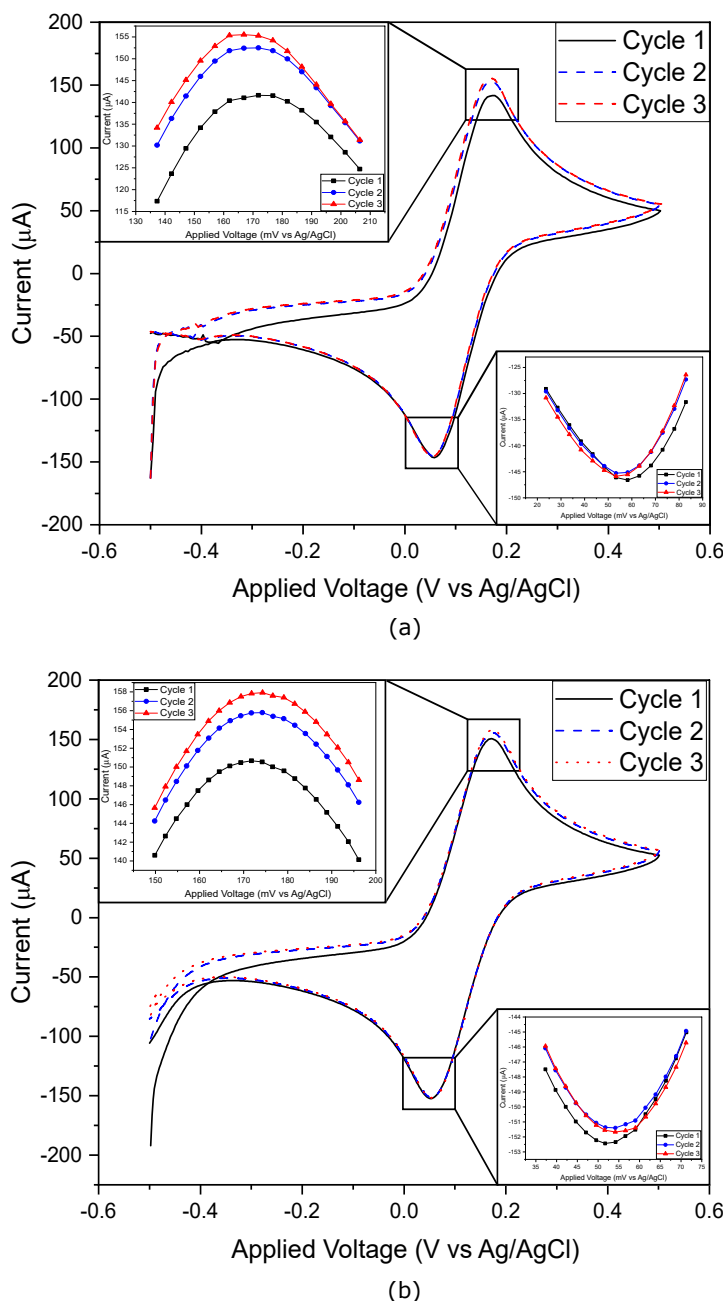


Figure 3.23: Cyclic voltammograms using (a) the PULSE system and (b) the benchtop potentiostat, where the potential was swept from -0.5 V to 0.5 V at 50 mV/s with a 5 mM concentration of $[\text{Fe}(\text{CN})_6]^{3-/4-}$.

phenomenon in reversible redox reactions, which stabilise after the third cycle [43, 44]. This behaviour is often attributed to evolving mass transport conditions and surface effects, which influence the analyte's diffusion and interaction with the electrode during the first few scans [45]. Firstly, regarding the oxidation peaks, both systems exhibited similar peak potentials, and the correlation coefficient between the two datasets was 0.99. The PULSE system showed an average oxidation potential of 170.2 mV , and the benchtop potentiostat recorded 173.4 mV . This corresponded to a difference of 3.2 mV , representing a 1.85%

decrease in the PULSE system. The averaged oxidation peak currents were comparable: the PULSE system recorded $149.9 \mu\text{A}$, and the benchtop potentiostat recorded $154.8 \mu\text{A}$, meaning a difference of $4.9 \mu\text{A}$, which corresponded to a 3.2% decrease in the PULSE. For reduction peaks, the benchtop potentiostat and the PULSE system averaged 53.3 mV and 54.9 mV , respectively, with a difference of 1.6 mV . Reduction peak currents were alike: the PULSE recorded $-145.9 \mu\text{A}$, and the benchtop potentiostat $-151.8 \mu\text{A}$, resulting in a difference of $5.9 \mu\text{A}$, which corresponds to 4% increase in the PULSE. SD provided insights into data dispersion, and the results suggested that the PULSE offered stable and reliable measurements. Nevertheless, these are preliminary tests conducted using a single sensor and a single concentration; therefore, further experiments are required to validate the findings.

The observed differences between the PULSE system and the benchtop potentiostat could be attributed to sensor variability, including slight differences in electrode surface properties, and unintentional variations in sensor fabrication. It is important to note that this experiment was conducted using a single sensor, serving as a preliminary comparison between the two systems. A more comprehensive evaluation was conducted next, involving multiple sensors and different concentrations to fully characterise the accuracy of the PULSE system. Despite these small discrepancies, the high correlation and minimal deviations in peak values suggested that the PULSE system performed comparably to the benchtop potentiostat.

To further validate the system's performance, this work tested different concentrations of $[\text{Fe}(\text{CN})_6]^{3-} / \text{Fe}(\text{CN})_6^{4-}$ in triplicate ($n=3$), using CV measurements. Conducting experiments in triplicate ($n=3$) is a common practice in electrochemical studies, as reported in the literature [46–49]. The concentrations ranged from 1 mM to 5 mM at a scan rate of 50 mV/s from -0.5 V to 0.5 V . The results obtained with the PULSE are shown in Figure 3.24 (a), while the results using the benchtop instrumentation are illustrated in Figure 3.24 (b). For further comparison of these systems, the peak oxidation currents were analysed and presented in Table 3.9, and Figure 3.25, where the oxidation peak currents were plotted against the solution concentrations. More details about the data are shown in Appendix B.8.

The PULSE calibration line exhibited a slope of $25.66 \mu\text{A}/\text{mM}$. The model exhibited an excellent fit to the data with an R^2 of 0.9996, indicating that 99.96% of the variance in the current can be explained by the linear regression model. Similarly, the linear regression analysis performed on the dataset obtained from the benchtop potentiostat resulted in a slope of $26.69 \mu\text{A}/\text{mM}$ and an R^2 of 0.999. A statistical analysis was performed, yielding a correlation coefficient of 0.999

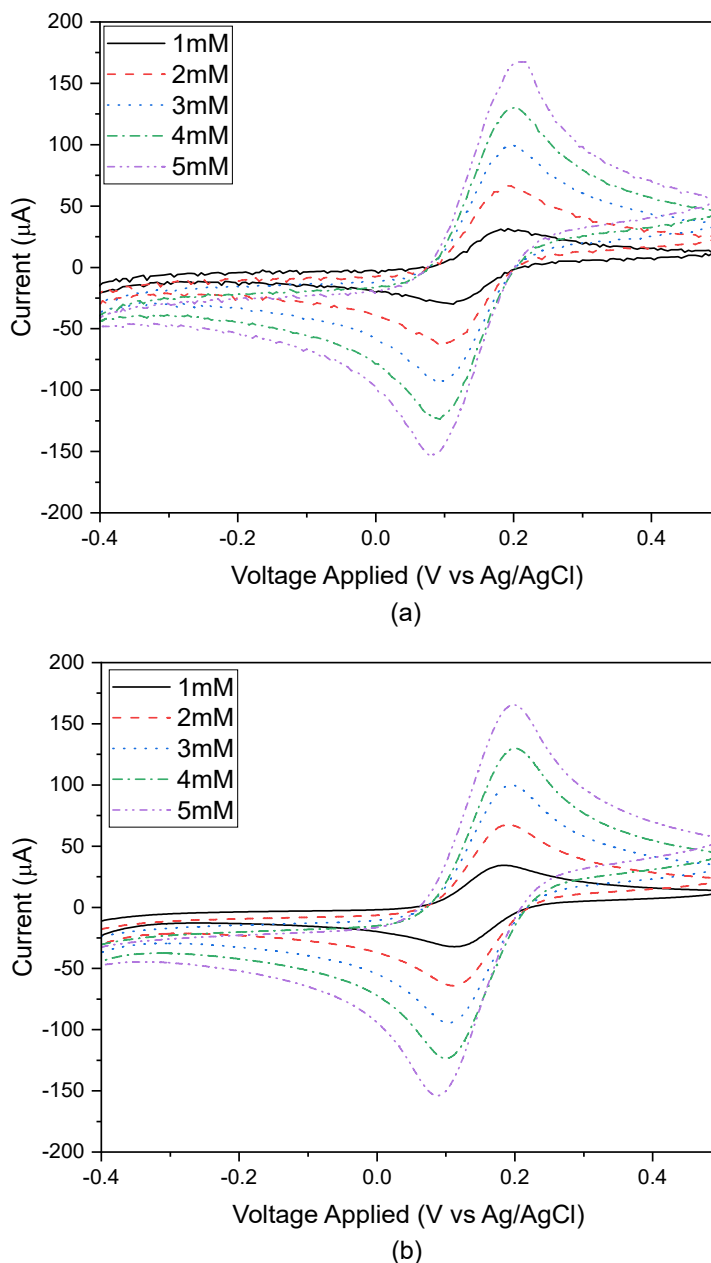


Figure 3.24: Cyclic voltammograms (a) using the PULSE, and (b) the commercially available benchtop potentiostat, where the potential was swept from -0.5 V to 0.5 V at 50 mV/s with varied concentrations of $[\text{Fe}(\text{CN})_6]^{3-} / [\text{Fe}(\text{CN})_6]^{4-}$ in 10 mM PBS.

between the datasets from both systems. Therefore, it is justified to conclude that the data obtained from the benchtop potentiostat and the PULSE system are in agreement.

3.3.4 Chronoamperometry

CA was also used during the validation of the PULSE system. In this technique, the potential was held constant over a 60 s period in the region where

Table 3.9: Oxidation peak currents presenting the average and standard deviation (SD) of three sensors using the PULSE and the benchtop instrument.

System	PULSE		Benchtop Instrument	
Conc. (mM)	Average (μA)	SD	Average (μA)	SD
5	131.152	15.975	131.124	20.634
4	105.465	10.889	105.352	15.083
3	77.562	6.220	79.051	14.378
2	53.092	4.341	53.033	8.500
1	27.387	3.536	26.115	5.140

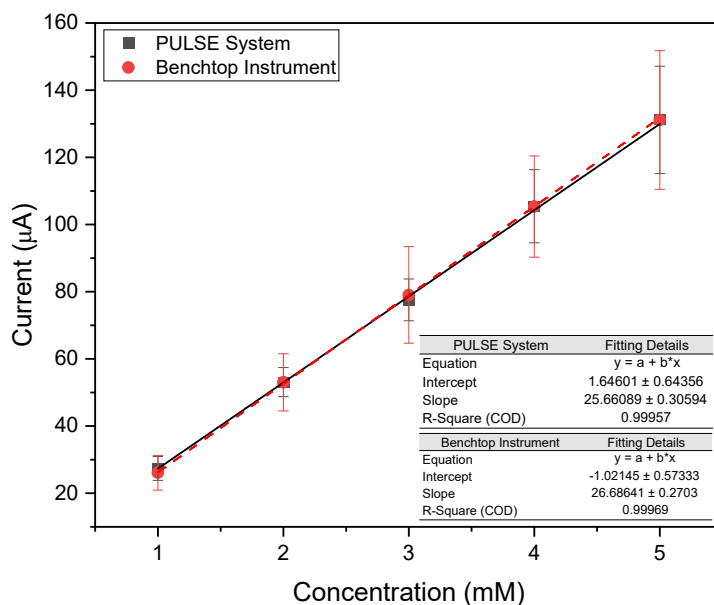


Figure 3.25: Calibration curves of oxidation peak current against $[\text{Fe}(\text{CN})_6]^{3-} / [\text{Fe}(\text{CN})_6]^{4-}$ concentration using the PULSE and the commercial instrument (n=3, mean $\pm$ SD).

the oxidation potential occurs. The resulting current was a function of the solution concentration. The results obtained using the PULSE are shown in Figure 3.26 (a), while the results from the benchtop potentiostat are illustrated in Figure 3.26 (b). The experiments revealed that the PULSE system reached the same rate of change faster, approximately within ~ 20 s, compared to the benchtop potentiostat, which required around ~ 45 s. This difference can be attributed to the fundamental design and operational principles of each system. The benchtop potentiostat is a more complex instrument that employs high-precision electronics and low-noise circuitry, which require prolonged stabilisation times to ensure accurate and reproducible measurements. Additionally, it often incorporates filtering and signal-processing mechanisms that introduce a delay before reaching a steady-state response. In contrast, the PULSE system is designed for

rapid measurements, prioritising speed and efficiency, and reducing the need for extended periods of stabilisation.

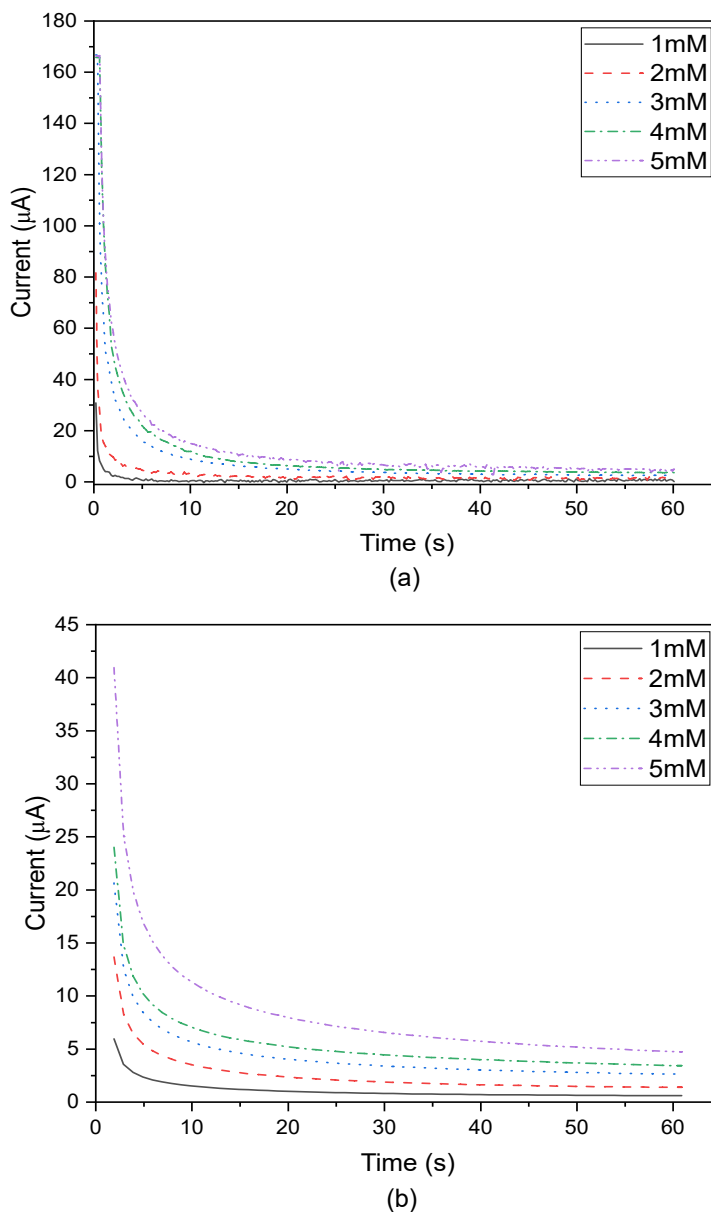


Figure 3.26: CA results using (a) the PULSE, and (b) the commercial benchtop instrument, where a constant potential was applied to solutions of varying $[\text{Fe}(\text{CN})_6]^{3-/4-}$ concentrations for 60 s.

The linear regression analysis performed on each dataset is illustrated in Figure 3.27, and more detail can be found in Appendix B.9. Regarding the resulting calibration curve of the PULSE, the slope was $1.09 \mu\text{A}/\text{mM}$. The model exhibited an excellent fit to the data with R^2 of 0.988, which indicates that 98.8% of the variance in the current can be explained by the linear regression model. In comparison, the benchtop potentiostat showed a slope of $1.08 \mu\text{A}/\text{mM}$, and its model exhibited a R^2 of 0.996. Additionally, the datasets from both systems exhibited a coefficient > 0.99 , indicating that the PULSE performance closely aligned with

that of the benchtop instrument.

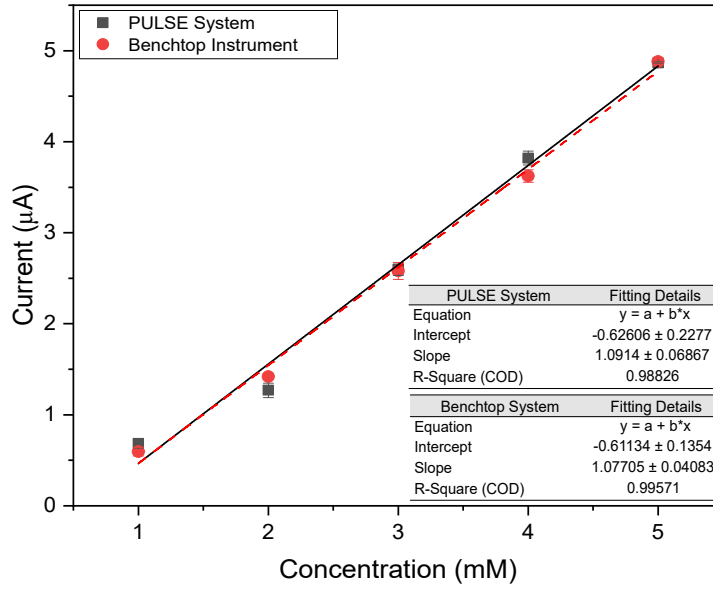


Figure 3.27: Calibration curves for the resulting current against $[\text{Fe}(\text{CN})_6]^{3-/4-}$ concentrations using the PULSE and a benchtop instrument ($n=3$, mean $\pm$ SD).

3.3.5 pH Measurements

OCP was used to validate the PULSE against the benchtop potentiostat. pH was chosen as the target analyte due to its importance in animal health monitoring, among other applications [27, 50]. For OCP detection of pH, the WE generated a potential as a function of the surrounding solution. This potential was measured against that of the RE, which provided a constant potential. Prior to analysis, the WE of the SPE (DRP-110GPH from DropSens) was functionalized with an IrOx sensing layer. PBS solutions with pH values ranging from 3 to 11 were prepared. The OCP for each pH solution was assessed over a 60 s period in triplicate. The results obtained are shown in Figure 3.28.

From the data taken, it was observed that the PULSE system rapidly achieved the same rate of change (mV/s) in approximately 2 s, whereas the benchtop potentiostat took around 50 s. This speed can be attributed to the sensor stabilisation facilitated by the low input bias current, which does not affect the sensor. In contrast, the benchtop potentiostat, being a more complex instrument, required prolonged stabilisation times. Additionally, due to the benchtop instrument's capabilities in recording current at the WE while performing OCP measurements, currents in the range of the pA were observed, as illustrated in Figure 3.29. This highlighted the importance of incorporating component U7 (LMP7721) from Figure 3.8 into the AFE circuitry, due to its input bias current of 20 fA.

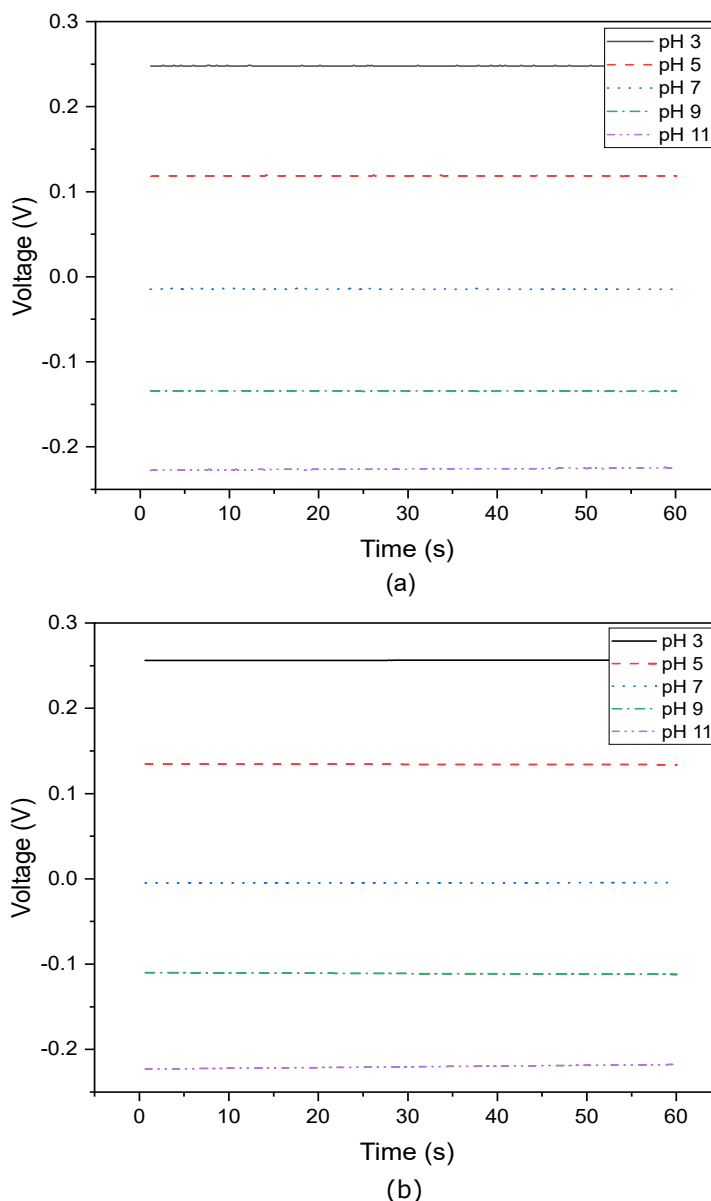


Figure 3.28: OCP results for various pH solutions over a 60 s period for (a) the PULSE, and (b) benchtop instrument.

The calibration curve in Figure 3.30 represented the potential of each solution plotted against the pH value and includes linear regression analyses obtained from both the PULSE and the benchtop potentiostat. More details on the results obtained can be found in Appendix B.10. For the PULSE system, the regression analysis revealed a slope of -60.43 mV/pH with an R^2 of 0.996, which is close to the ideal -59 mV/pH according to Nernst equation [51]. This can be translated into an accuracy of 97.6%. Similarly, for the benchtop potentiostat, the regression analysis showed a slope of -61.08 mV/pH with an R^2 value of 0.998. Supporting the visible overlap in the datasets, a correlation analysis revealed a coefficient of 0.999 between the datasets from both systems, further emphasising the accuracy of the PULSE.

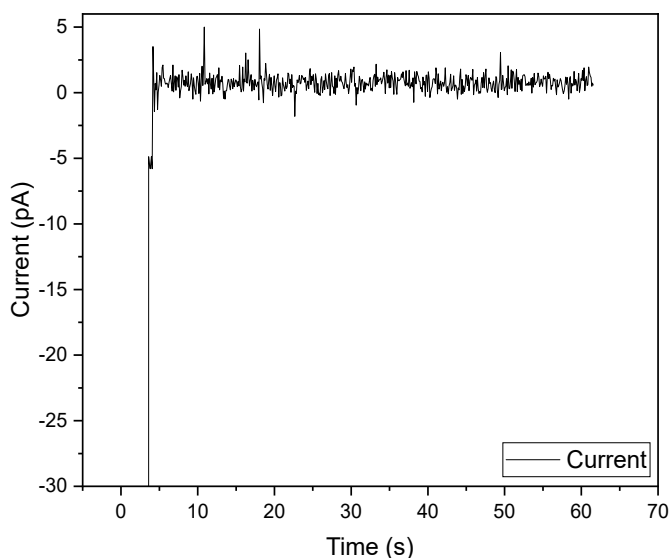


Figure 3.29: Current at the WE during OCP measurements for pH detection over 60 s period, obtained using the benchtop potentiostat.

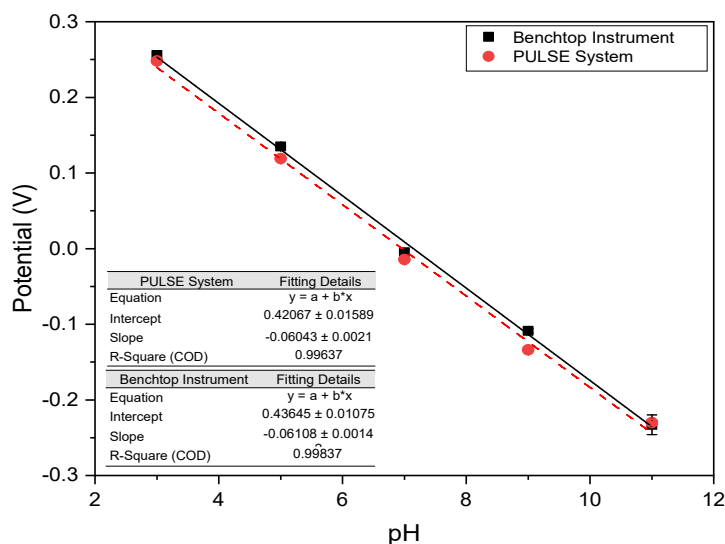


Figure 3.30: Voltage response to pH changes between pH 3 and 11 in PBS ($n=3$, mean $\pm$ SD).

3.3.6 Related Work

In this section, the proposed device, PULSE, was compared against other SoTA systems relevant to this study. This comparison focused on their specifications, as detailed in Table 3.10.

The measurement range highlights the current sensing ability of the PULSE to read output currents in the nA range. For example, the KAUSTat [54] system demonstrated a measurement range of $\pm 500 \mu\text{A}$, while the MiniStat [52] measured $\pm 100 \mu\text{A}$. However, the PULSE system, with a measurement range extending down to $\pm 10 \text{ nA}$, enables the detection of low currents, which is crucial for applications involving highly sensitive electrochemical sensing. Additionally,

Table 3.10: Comparison of portable potentiostats, including size, measuring techniques, communication (Comm.), operational voltage range, and measuring range.

System	Size (mm)	Techniques	Comm.	Voltage Range (V)	Meas. Range
MiniSat [52]	27×20	CA, CV, SWV	Bluetooth	±1.2	±100μA
DStat [53]	92×84	CV, SWV, DPV	USB	±1.5	±600fA
KAUSTat [54]	50×35	CV	Bluetooth	-	±500μA
Wei et al. [55]	64×39	CV, DPV, CA	Bluetooth	-	±10nA
PULSE (This work)	78×100	Temp., Acc., CV, OCP, CA, LSV, SWV	USB, 433MHz	±1.65	±800nA^a ±1.65V^b

^aWhen voltammetric circuit is set with $G = 2$, and R_f of $1\text{ M}\Omega$.

^bWhen potentiometric circuit is set with $G = 1$.

the proposed device featured an adaptable circuit design that allows customisation according to the specific sensor/analyte under investigation, which is not explicitly mentioned in most of the previously developed devices.

Additionally, many SoTA devices primarily focus on electrochemical techniques, including CV, CA, SWV, and DPV. While systems such as MiniStat [52], DStat [53], and others support these techniques, PULSE integrated a potentiometric circuit for OCP measurements, thereby broadening its measuring capabilities beyond standard current analysis.

Regarding the working voltage range, the PULSE system offered a broader operational window of $\pm 1.65\text{ V}$, whereas previously reported devices such as MiniStat ($\pm 1.2\text{ V}$) and DStat ($\pm 1.5\text{ V}$) operate within a narrower range. This extended voltage range enhances the applicability of PULSE. However, it is essential to acknowledge that the effective voltage range is influenced by the specific electrochemical cell and system compliance voltage. Compliance voltage, which dictates the maximum voltage that can be applied to the CE, ensures the system operates within safe limits but may slightly constrain the actual measurement range depending on the experimental setup.

Another key advantage of PULSE is its multi-functional capability, integrating both electrochemical and physical parameter sensing. Unlike prior systems that focus solely on electrochemical techniques, PULSE includes real-time monitoring of temperature and acceleration, making it more versatile for field-deployable and multi-sensing applications. In particular, temperature and mo-

tion sensing capabilities were integrated to enhance the device’s functionality; however, these features were not subjected to further testing in this study. Although PULSE was not explicitly designed for miniaturisation, its footprint (78×100) mm² is comparable to DStat (92×84) mm² and remains only slightly larger than some other devices, despite integrating significantly more functionalities [53]. Moreover, while most portable potentiostats feature a single sensor connector supporting the 3-electrode configuration, PULSE extends its capabilities by enabling simultaneous connections for two sensors, supporting both 3-electrode and 2-electrode configurations. This design contrasts with the dual-channel device developed by Wei et al. [55], which primarily focused on voltammetric and amperometric measurements. By offering enhanced multi-sensor connectivity, PULSE provides increased flexibility for applications requiring parallel sensing or multi-analyte detection.

3.4 Conclusion

This chapter detailed the development, characterisation, and validation of the PULSE system, a portable, multi-parametric electrochemical sensing platform engineered specifically for real-time applications in the complex environments of rumen health monitoring. Unlike conventional laboratory potentiostats, which are bulky, single-purpose, and lack field applicability, the PULSE introduces several key innovations that position it beyond the capabilities of current SoTA portable devices.

One of the primary contributions of this work is the development of a versatile sensing architecture that supports voltammetric, amperometric, and potentiometric techniques, whereas most portable potentiostats are limited to only voltammetric or amperometric sensing. The integration of potentiometric functionality into a compact form factor, along with a customisable AFE capable of supporting both 3-electrode and 2-electrode configurations, is a notable advancement. The system can interface with up to three electrochemical sensors, when including the impedimetric AFE.

Moreover, the PULSE incorporates physical sensing modalities, including temperature and acceleration monitoring, which enable broader health monitoring capabilities beyond chemical detection. This fusion of electrochemical and physical sensing in a single portable device distinguishes it from existing portable potentiostats. This feature supports more comprehensive assessments, particularly in veterinary and agricultural settings, in comparison with currently available bovine health monitoring devices. Unlike existing systems, which are often constrained by wired connections or rely on short-range Bluetooth, the PULSE

employs 433 MHz wireless communication, specifically selected for future integration into a smart bolus designed for operation in aqueous environments like the bovine rumen.

In terms of performance, the PULSE was benchmarked against a gold-standard benchtop potentiostat, showing a high correlation coefficient (>0.9) in electrochemical measurements, while offering significantly faster response times (48s faster than the gold standard benchtop potentiostat), a critical improvement for time-sensitive diagnostics. The improved speed and accuracy are attributed to the optimised AFE and the development of custom FW algorithms for efficient signal processing and data acquisition. These enhancements allow the system to operate reliably across a wide range of voltages and currents, further validating its robustness.

The system also demonstrates clear practical advantages, it is compact (78×100) mm²), and user configurable. It includes both USB and wireless interfaces and is controlled via a custom APP that supports real-time monitoring, data visualisation, and device control, ensuring usability even for non-technical operators in field environments.

Crucially, the in-house development of the PULSE, rather than relying on commercial solutions, enabled complete HW and FW customisation. This allowed the platform to be tailored to the specific needs of bovine health monitoring, offering compatibility not only with commercial sensors but also supporting the co-development of sensing technologies. This level of adaptability is not offered by existing commercial potentiostats, as sensor operation is typically constrained by the predefined capabilities and limitations of the device.

In conclusion, this work demonstrated that the PULSE successfully fulfils its intended research objectives by delivering a high-performance, customisable, and field-deployable sensing system. It enabled real-time, multi-parametric analysis across a combination of electrochemical and physical domains. Through its innovative integration of functionalities, rapid detection capabilities, and system adaptability, the PULSE represents a significant advancement in portable electrochemical instrumentation, offering strong potential for broader use in complex fluid analysis and interdisciplinary research.

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MULTI-PARAMETRIC ELECTROCHEMICAL SENSING PLATFORM: APPLICATIONS IN ANIMAL WELFARE

4.1 Introduction

Following the development and validation of the PULSE device in Chapter 3, this chapter explores its application for livestock health monitoring. Designing and integrating a smart bolus for livestock health monitoring poses several challenges, including ensuring device durability within the rumen, achieving accurate measurements, and maintaining cost-effectiveness for farmers. While not all of these challenges are within the scope of this work, this chapter focuses on the initial integration of multiple physical, chemical and biochemical sensors into a single device, which has not been reported before, evaluating the feasibility of combining key functionalities in one embedded system [1]. Since smart boluses are used throughout the animal's life, incorporating multi-parametric capabilities enhances their utility, ensuring users derive maximum value from their investment.

This study specifically investigates the integration of sensors for pH, temperature, nitrate, and nitrite, aiming to address the limitations of existing monitoring tools. Nitrate and nitrite were prioritised due to their significant impact on both cattle health and environmental concerns, such as methane emissions as a GHG, as discussed in Chapter 2.

Electrochemical sensors for detecting nitrite are often fabricated using materials such as carbon (C), copper (Cu), platinum (Pt), gold (Au), and diamond. However, bare electrodes often have performance limitations, which are commonly addressed by modifying their surfaces to enhance sensitivity. A comprehensive

review by Ibrahim et al. [2] revised multiple materials employed in modified electrodes, including gold nanoparticles, carbon nanotubes, and chitosan, among others. The most commonly employed electrochemical techniques for the detection of nitrite are voltammetric methods, such as SWV, DPV, and CV, as well as amperometry [3]. Similarly, a study on electrochemical sensors for nitrate detection emphasised the critical role of modified electrode surfaces for enhancing surface area, electrical conductivity, and catalytic activity [4]. Nitrate detection was primarily conducted using voltammetric techniques, though some studies reported the use of potentiometry [5]. The choice of technique is often linked to the properties of the modified electrode material. It is important to note that most of these studies mainly focused on food safety and soil applications. No research has been identified that addresses the detection of nitrate and nitrite associated with monitoring livestock welfare.

This chapter validated the accuracy of nitrate and nitrite measurements in fluids of increasing complexity, starting with PBS, followed by artificial ISF. Rumen fluid was available only for pH analysis, aimed at assessing the system behaviour in a complex fluid. The key objectives of this chapter are:

- Evaluate a multi-sensor platform capable of monitoring pH, temperature, nitrate, and nitrite levels for continuous livestock health assessment and early disease detection.
- Validate the accuracy of the PULSE device for pH, nitrate, and nitrite measurements across different fluid media, from simpler solutions (PBS) to pH analysis in complex ruminal fluid.

This chapter is organised as follows: Section 4.2 describes the materials, experimental procedures, and techniques used for testing and validating the PULSE system. Section 4.3 presents the results from detecting pH, temperature, nitrate, and nitrite. Finally, Section 4.4 summarizes the main findings of this work.

4.2 Materials & Method

This section outlines the materials, equipment, and methodologies used to test and validate the PULSE system for real-time cattle health monitoring. Key procedures include pH, nitrate, and nitrite analysis, and a double-layer capacitance study to optimise system performance. Additionally, real-world testing of pH in the ruminal fluid was conducted to validate the device's functionality under practical conditions.

4.2.1 Materials and Equipment

4.2.1.1 Reagents

Magnesium chloride (MgCl_2), sodium chloride (NaCl), potassium chloride (KCl), magnesium sulphate (MgSO_4), calcium chloride (CaCl_2), HEPES, mono-sodium phosphate ($\text{NaH}_2\text{-PO}_4$), sucrose, sodium hydroxide (NaOH), nitric acid (HNO_3), potassium dicyanoargentate ($\text{KAg}(\text{CN})_2$), potassium dicyanoaurate ($\text{K-Au}(\text{CN})_2$), lithium nitrate (Li-NO_3), sodium nitrite (NaNO_2), iridium tetrachloride, and oxalic acid were obtained from Sigma Aldrich (Arklow, Ireland). Iridium (IV) chloride was purchased from Alfa-Aesar (Gillingham, UK). All solutions were prepared with double distilled DI water with a resistivity of $18.2 \text{ M}\Omega\text{cm}$. Sterilised bovine rumen fluid was purchased from Bar Diamond, Inc. (Idaho, U.S.).

Stock solutions of 200 ppm LiNO_3 in 1 M NaOH , and 200 ppm NaNO_2 in 10 mM PBS were made up for nitrate and nitrite analysis. The stock solutions were subsequently diluted to test various concentrations.

Artificial ISF was prepared using an adaptation of the procedure provided in the literature [6]. A 200 ml artificial ISF solution was prepared by measuring out 0.7 mM of magnesium sulphate, 2.5 mM of calcium chloride, 1 mM HEPES, 3.5 mM potassium chloride, 123 mM sodium chloride, 1.5 mM mono-sodium phosphate, and 7.4 mM sucrose, in 200 ml DI water. The pH of the artificial ISF was 7.5.

4.2.1.2 Equipment

The PULSE was used for all electrochemical measurements, except for the pH sensing repeatability test and the double-layer study, both of which were conducted using a benchtop potentiostat. These exceptions were made to enable other measurements to be performed concurrently with the repeatability test. The double-layer study was carried out on the benchtop instrument to optimise the performance of the PULSE. The PULSE communicated with the computer via the custom APP, previously described in Section 3.2.3. For benchmarking and validating the results obtained by the PULSE, Metrohm Multi Autolab (MAC90389, Metrohm, UK) was used with NOVA 2.1 software. When using the benchtop instrument, the measurements were carried out at room temperature in a Faraday cage (Metrohm, UK). In-house electrodes were fabricated using the Voltera V1 printer (Voltera, Canada). A pH probe from Mettler Toledo (Greifensee, Switzerland) was used as a gold standard calibrated reference to ensure the accuracy of the measurements. A temperature sensor (NCP03 from Murata Electronics, Kyoto, Japan) was used, and a calibrated digital thermometer with a probe from Testo (Titisee-Neustadt, Germany) ensured measurement

accuracy.

4.2.2 Electrochemical Setup and Materials

Preliminary results indicated that nitrate could not be detected using the commercial carbon SPE (see Figure C.4.1), as no reduction was observed associated with nitrate detection. While nitrate and nitrite are typically detectable with gold sensors [3], commercial gold sensors (C220AT from DropSens) were found to be ineffective for nitrate detection. This inefficiency was attributed to potential reactions between the carbon CE and silver RE, which led to unexpected peaks and failed to provide clear reduction peak differentiation between concentrations (for reference, see Figure C.4.2). Therefore, in-house gold-printed electrodes were fabricated to measure nitrate and nitrite concentrations [7]. The sensors were printed in polyimide kapton films (50 μm thickness), and silver paste purchased from Radionics RS (Dublin, Ireland). The gold polymer paste (C204120-6P2) was purchased from Sun Chemicals (New Jersey, U.S.). These electrodes were printed in a flexible material to facilitate seamless integration into a smart bolus in the future.

The 3D-printed holder, shown in Figure 4.1 (a), was designed previously to this work to facilitate electrochemical measurements using the flexible gold-printed electrodes. To avoid leakages O-rings (7 mm diameter) were used as a seal between the well within the electrochemical cell holder and the printed electrode. These measurements were conducted using an external commercial Ag/AgCl RE and a platinum wire as the CE (from IJ Cambria Scientific Ltd, CH Instruments, U.S.). Additionally, Figure 4.1 (b) shows the setup used for measurements with the PULSE.

Commercial carbon SPEs were purchased from DropSens (DRP-110GPH), consisting of a 4 mm diameter carbon WE, carbon CE, and silver/silver chloride (Ag/AgCl) RE. These sensors are commonly used for pH detection, as previously utilized in Chapter 3 for pH calibration in PBS. A 3D-printed holder was fabricated to accommodate the thermistor for temperature sensing and a pH sensor, as illustrated in Figure 4.1 (c). This design allows the sensors to be fully immersed in liquid, as depicted in Figure 4.1 (d). All 3D-printed structures were fabricated using a Formlabs Form 3 printer with standard white or transparent resin purchased from Formlabs (Massachusetts, U.S.).

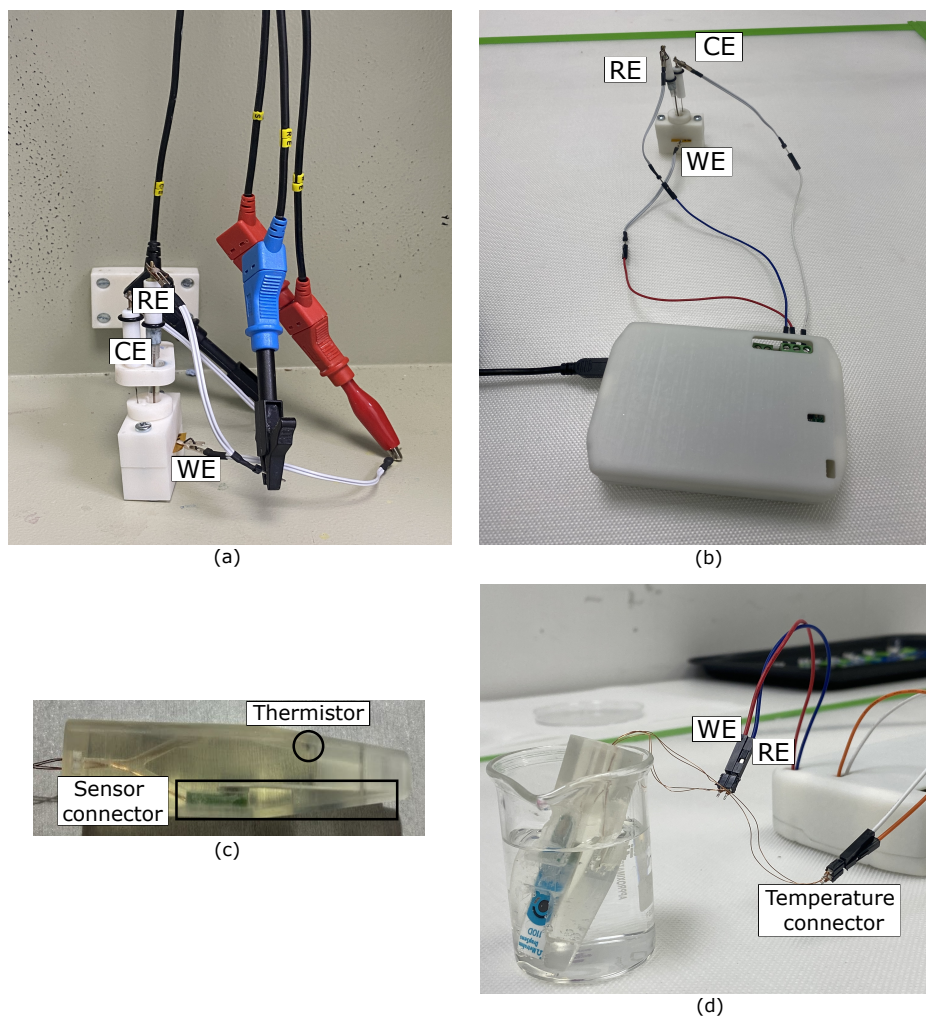


Figure 4.1: (a) Electrochemical setup inside the Faraday cage, featuring the 3D-printed holder designed to facilitate electrochemical measurements; (b) setup for measurements using the PULSE; (c) 3D-printed holder for immersive in liquid measurements; and (d) setup for measurements using the PULSE for pH measurements.

4.2.3 Methods

4.2.3.1 IrOx Functionalization

Prior to the analysis of pH, the commercial SPEs was functionalized with an IrOx sensing layer. The IrOx functionalization process used was the same as that described in Section 3.2.4.1.

4.2.3.2 Flexible Gold Electrodes Modification

The deposition of Nanoporous Gold (NPG) followed the method reported by Nagle et al. [8].

4.2.3.3 Electrode Characterisation

Assessing the electrochemically active area of the electrodes is crucial for determining the sensitivity achieved during measurements. The Randles-Sevcik equation was employed to calculate the electrochemical active area of the WE in both the flexible gold-printed electrodes and the commercial SPEs, as detailed in Appendix C.1. Additionally, the Randles-Sevcik equation was further used to evaluate the accuracy of the PULSE in detecting peak oxidation currents compared to theoretical values. For more details, see Appendix C.2.

4.2.3.4 Double-layer Study

To study the electrochemical double-layer in the solution of interest, EIS was used to assess double-layer capacitance (C_{dl}) and charge-transfer resistance (R_{ct}). Impedance measurements were conducted using the benchtop potentiostat, which applied a signal across a range of frequencies. The obtained data was analyzed by fitting it to an equivalent circuit model representing the electrochemical cell, enabling a detailed interpretation of the specific characteristics of the electrochemical cell. From this analysis, C_{dl} and R_{ct} were extracted and used to construct an electrochemical dummy cell, simulating the interface conditions observed in nitrite environments. Subsequently, the dummy cell was interfaced with the PULSE device to evaluate and calibrate the system's response, resulting in the generation of a calibration equation tailored to these specific electrochemical sensors and environments. This calibration is crucial to ensure the PULSE accurately detects nitrite concentrations.

4.2.3.5 pH Measurements

OCP measurements were performed at room temperature ($\approx 20^\circ\text{C}$) on artificial ISF solutions of pH varying from 3 to 11 in duplicate. These solutions were made using artificial ISF pH 7.5, and the pH was adjusted using hydrochloric acid and sodium hydroxide measured with a pH calibrated commercial benchtop probe, as applicable. The pH and temperature sensors were mounted on a 3D-printed holder to enable their immersion in a beaker containing the solution of interest.

To advance the characterisation of the developed system, OCP measurements were conducted for 200 seconds, with the pH gradually increased from 3 to 11, and subsequently decreased back to 3. In addition, given the final application, the long-term stability of the IrOx deposition was verified by performing pH measurements with a benchtop potentiostat every 7 days over a period of 42 days ($n=3$).

Interference tests were conducted to assess the system's response to the presence of other ions. For this, 80 mM MgCl₂, 140 mM NaCl, and a 30 mM KCl were separately dissolved in 10 ml DI water. These solutions were mixed separately with artificial ISF pH 3 solutions, using a 50/50 ratio. OCP measurements were carried out in triplicate on the four solutions.

4.2.3.6 Nitrate and Nitrite Measurements

Analysis of nitrate was performed using CV with a potential window of 0 V to -1.3 V, while nitrite swept voltage from 0 V to 0.9 V at 50 mV/s. Nitrate and nitrite analysis were carried out with NPG-modified electrodes, with an external Ag/AgCl reference and an external platinum wire as CE. For the validation of the measurements, the experiments were initially carried out in PBS solutions with the PULSE and benchtop devices. Then, exclusively carried out using the PULSE in artificial ISF to demonstrate its reliability for measurements in complex media. The PULSE was set up with a C_f of 1 nF, R_f of 1 k Ω , and gain of 1.

To evaluate the multi-parametric performance of the device, nitrite, pH, and temperature were measured. Commercial SPEs modified with IrOx, and NPG-modified electrodes were employed in the same beaker to enable the detection of pH and nitrites, respectively. To evaluate the influence of nitrite concentration on pH measurements, varying nitrite concentrations were tested while maintaining a fixed pH of 7. Additionally, to explore the reciprocal impact of pH on nitrite detection, a 100 ppm nitrite solution was examined across different pH levels. Initially, these measurements were conducted in PBS, and subsequently in artificial ISF, where a temperature sensor was added using the 3D printed holder. The LOD was determined based on the calibration curves using the following equation [9]:

$$LOD = 3.3 \times \frac{SD}{m} \quad (4.1)$$

Where SD represents the standard deviation of the blank samples, and m is the slope of the calibration curve.

Additionally, sensitivity was calculated to assess the electrodes' response to changes in analyte concentration. The slope of the calibration curve, which reflects the relationship between current response and analyte concentration, was used to determine sensitivity. Sensitivity is also influenced by the electrochemical active area of the WE electrode, as a larger active surface can enhance the current response for a given analyte concentration. Therefore, sensitivity is defined as the ratio between the slope of the calibration curve and the electrochemical active area of the WE [10].

4.2.3.7 Real-World Application

To demonstrate the capability of the PULSE device in real-world scenarios, pH measurements were conducted in sterilised rumen fluid. IrOx-functionalized SPEs were employed in triplicate to accurately assess the pH of the ruminal complex fluid across a range of 3 to 11.

4.3 Results & Discussion

This section provides a comprehensive analysis of the results obtained from measuring pH, temperature, nitrate, and nitrite, progressing from less complex fluid media to real-world sample applications.

4.3.1 Electrode Fabrication/ Modification

The commercial SPEs were effectively modified with an IrOx deposition layer to facilitate pH detection. Additionally, in-house fabricated flexible gold electrodes were modified with NPG to enable the detection of nitrate and nitrite. These modified electrodes were integrated with the PULSE, which interfaces with smart devices via the developed APP. This configuration enables real-time monitoring, as detailed in Figure 4.2.

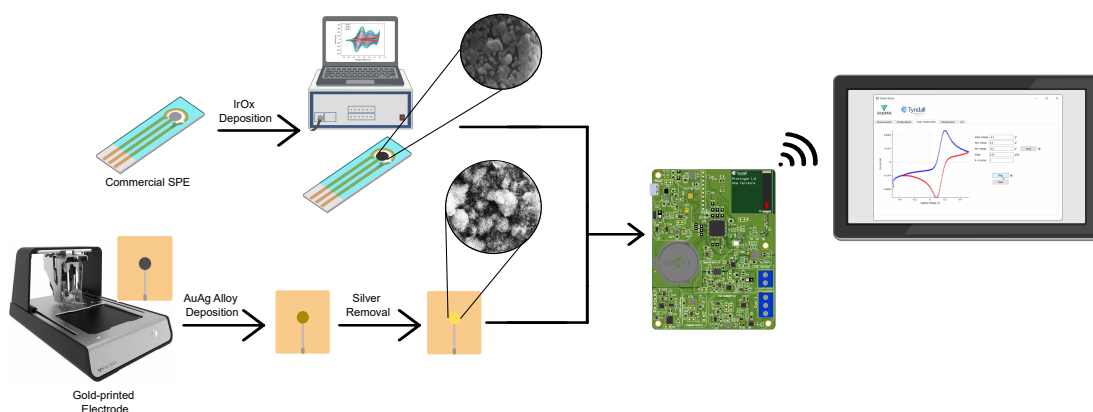


Figure 4.2: Schematic overview of the SPEs functionalized with IrOx, in-house fabrication of flexible gold electrodes, and the deposition of the NPG layer, both applied to the PULSE device, with results displayed through the APP.

4.3.2 pH Measurements in ISF

OCP measurements were conducted on artificial ISF with various pH values, ranging from 3 to 11. This study aimed to investigate the pH behaviour of complex solutions and benchmark the PULSE against the benchtop instrument.

Temperature readings were simultaneously recorded with a thermistor integrated into the PULSE system, adding valuable insight into the thermal behaviour of the solutions. For additional details, refer to Appendix C.3.

The calibration curves obtained from both the PULSE system and benchtop instrument, along with the corresponding temperature measurements, are shown in Figure 4.3. The regression analysis conducted on the PULSE data revealed a slope of -64.1 mV/pH with an R^2 value of 0.996, which is close to the ideal -59 mV/pH , as predicted by the Nernst equation [11]. Consequently, the accuracy of the measurement performed by the PULSE was 91.4%. Similarly, the benchtop instrument yielded a Nernst response with a slope of -70.6 mV/pH and an R^2 value of 0.996. The results showed a strong correlation (> 0.99) between the datasets from the benchtop instrument and the PULSE system, highlighting the PULSE's potential as an alternative for pH sensing in complex media.

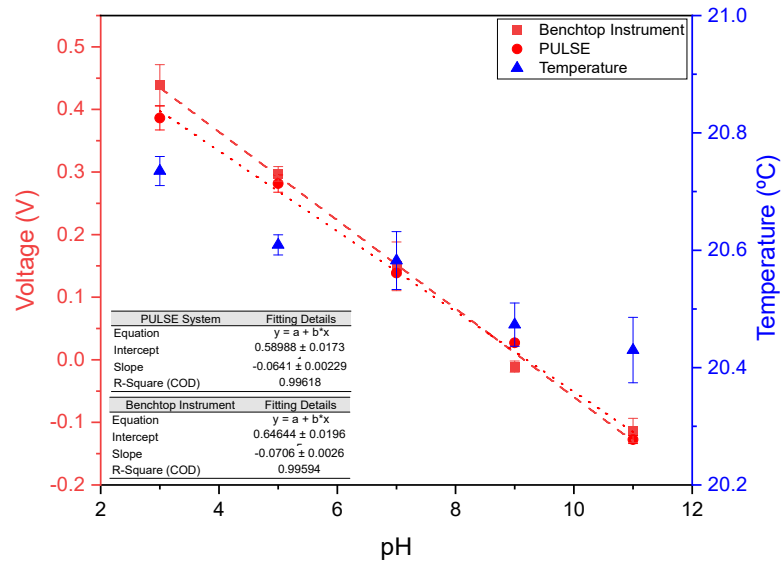


Figure 4.3: Voltage response to pH changes between pH 3 and 11 in artificial ISF, for both the benchtop instrument and the PULSE system ($n=2$, mean $\pm$ SD), along with the average temperature over a 200 second interval.

Furthermore, the temperature measurements aligned with the expected responses described in the literature, demonstrating a minor decrease in temperature as pH increased [12]. This trend indicated that, while the PULSE measured temperature as expected, external environmental factors may also contribute to slight variations in readings.

4.3.2.1 Repeatability

A repeatability study was conducted in the benchtop potentiostat to assess sensor stability in artificial ISF pH solutions over a period of 41 days. Measurements were recorded on days 1, 7, 14, and 21, at 7-day intervals, with an additional

measurement on day 41, as illustrated in Figure 4.4. The values obtained at pH 7 exhibited high stability throughout the study period. In contrast, pH 5 readings initially exhibited higher variability, particularly from day 1 to day 7; however, the subsequent days converged towards day 7. This early variation can be attributed to the IrOx stabilisation time. Consequently, it is recommended that future IrOx depositions should be performed 7 days after preparing the solution. Strong correlations (>0.9) were consistently observed between the datasets from different days, supporting the sensors' use for long-term applications.

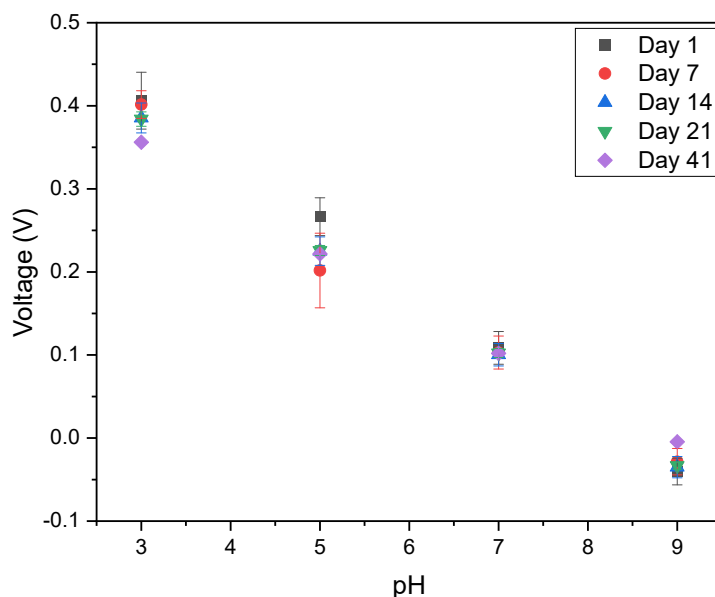


Figure 4.4: Voltage response to pH changes over 41 days ($n=3$, with $n=2$ on day 41, mean $\pm$ SD).

4.3.2.2 Interferences

An interference study was performed, and the results obtained are presented in Figure 4.5. The voltage differences measured between the artificial ISF solution at pH 3 and various interference solutions are summarized in Table 4.1. This highlighted the performance of the benchtop instrument and the PULSE device when exposed to MgCl_2 , NaCl , and KCl solutions.

Table 4.1: Voltage difference (V) between the artificial ISF pH 3.0 solution and interference solutions (MgCl_2 , NaCl , and KCl) for the benchtop instrument and the PULSE.

System	MgCl_2	NaCl	KCl
Benchtop Inst.	0.0119 V	0.0058 V	0.0156 V
PULSE	0.0274 V	0.0265 V	0.0316 V

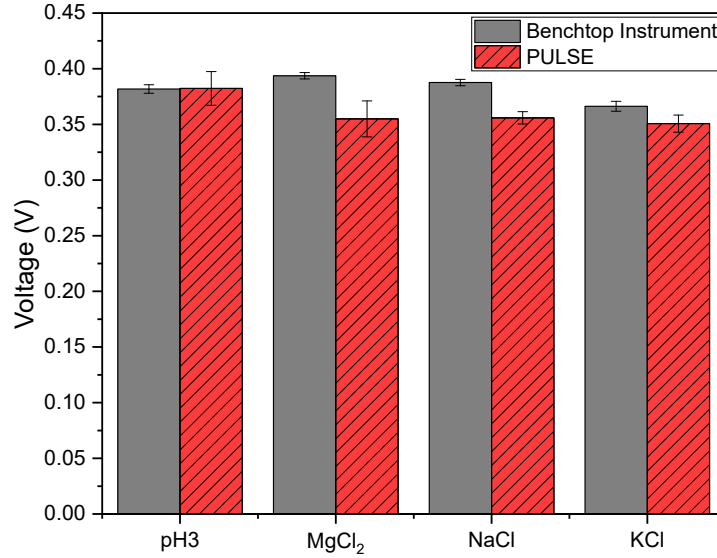


Figure 4.5: Voltage responses to ISF pH 3 solution in the presence of MgCl₂, NaCl, and KCl, using the benchtop instrument and the PULSE system in triplicate (n=3, mean $\pm$ SD).

As shown in Table 4.1, the highest voltage difference using the benchtop instrument was observed with KCl (0.0156 V, meaning a 4.7% interference), indicating an interaction with the main solution [13]. Similarly, the PULSE system exhibited its largest voltage difference with KCL (0.0316 V, representing an 8.3% interference). The behaviour of the systems in the presence of KCl suggested that potassium ions, known to increase solution basicity, contributed to a decrease in measured potential, consistent with findings reported in previous studies [14]. Similarly, magnesium ions from MgCl₂ are also known to have alkalinising properties [15], as evidenced in the PULSE measurements. These interference results have been previously observed in sensors with an IrOx deposition layer, as reported by Huang et al. [16]. In this context, the PULSE system appeared more responsive to changes in the solution. In contrast, the benchtop instrument showed a different response, which could be attributed to its more complex circuitry and advanced signal processing. Despite these differences, both systems accurately measured the pH of the artificial ISF solution at pH 3 in the presence of interfering ions, with percentage errors below 10% - within the acceptable range reported in the literature [17, 18].

4.3.2.3 pH staircase

To evaluate the performance of the system in a dynamic environment, artificial ISF solutions with different pH levels were prepared. OCP measurements were conducted for 200 seconds in each solution, with the pH values incrementally increasing from 3 to 11, and then decreasing back to 3. Figure 4.6 illustrates

the distinct voltage changes observed each time the solution's pH was altered, resulting in a stair-step shape. In some instances, the readings required a brief period to stabilise after the pH change, due to the sensor's behaviour. Calibration curves were constructed for both the increasing and decreasing pH scenarios, yielding slopes of -66.56 ± 2.47 mV/pH and 68.72 ± 4.5 mV/pH, respectively. In both cases, the calibration curves were consistent with Nernstian behaviour [11].

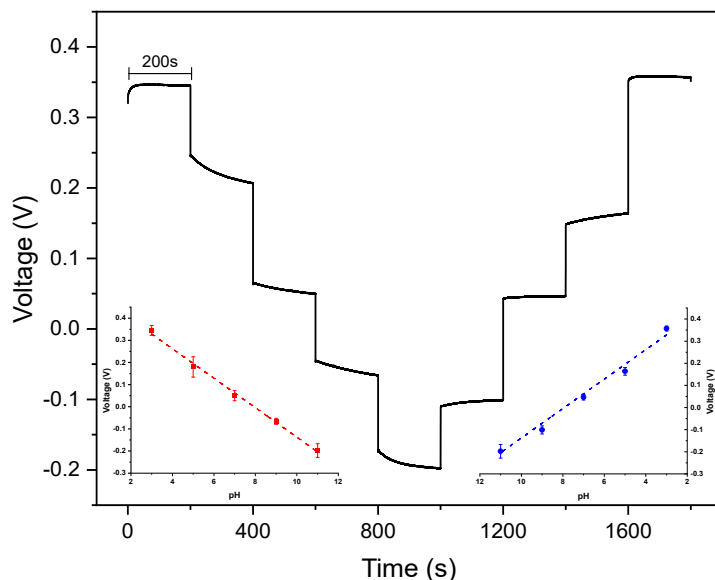


Figure 4.6: Voltage response pH changes in artificial ISF, increasing and decreasing the pH levels, and corresponding calibration curves obtained using the PULSE system ($n=3$, mean $\pm$ SD).

The differences in the slopes observed during this experiment can be attributed to several factors. One factor, commonly reported in literature, is hysteresis in the sensor response, where the sensor's response to increasing pH levels differs slightly from its response during a reverse pH change [19]. Another contributing factor can be drift over time due to prolonged exposure to the solution. As the pH staircase experiment progresses, the electrochemical properties of the sensor can subtly change because of extended exposure to the solutions at different pH levels [20]. These factors combined likely contributed to the slight difference of 2.16 mV/pH observed in the electrochemical response going up and down in pH. Additionally, a difference of 2.46 mV/pH was noted between the slope obtained from the pH staircase from pH 3 to 11 (-66.56 ± 2.47 mV/pH) and the initial ISF calibration slope (-64.1 ± 2.29 mV/pH). The overlapping standard deviations of these slopes indicate strong agreement between the measurements, suggesting consistency in the system's performance. The PULSE system consistently demonstrated being capable of accurately measuring pH, maintaining responses within the Nernstian range. This indicated that the system can track pH changes under varying conditions. Overall, the PULSE showed reliable elec-

trochemical behaviour across a broad pH range, confirming its suitability for applications requiring precise pH monitoring in dynamic environments.

4.3.3 Nitrate Detection

To evaluate the detection of nitrate, a series of solutions with nitrate concentrations ranging from a control sample (10 mM PBS containing 0 ppm nitrate) to 200 ppm of nitrate in 10 mM PBS were assessed. NPG modification significantly increased the electrochemical active area of the gold-printed electrodes, enhancing detection performance. Figure 4.7 revealed that the bare gold WE did not exhibit a clear reduction peak, even in the presence of 200 ppm nitrate. This indicated minimal or no catalytic activity towards nitrate reduction with the unmodified electrode. In contrast, the NPG-modified electrodes demonstrated significantly enhanced reduction peaks. This improvement was attributed to the increased electrochemical active area provided by the nanoporous structure, which was measured as $9.48 \times 10^{-2} \text{ cm}^2$ for the control electrode and $13.4 \times 10^{-2} \text{ cm}^2$ after NPG deposition.

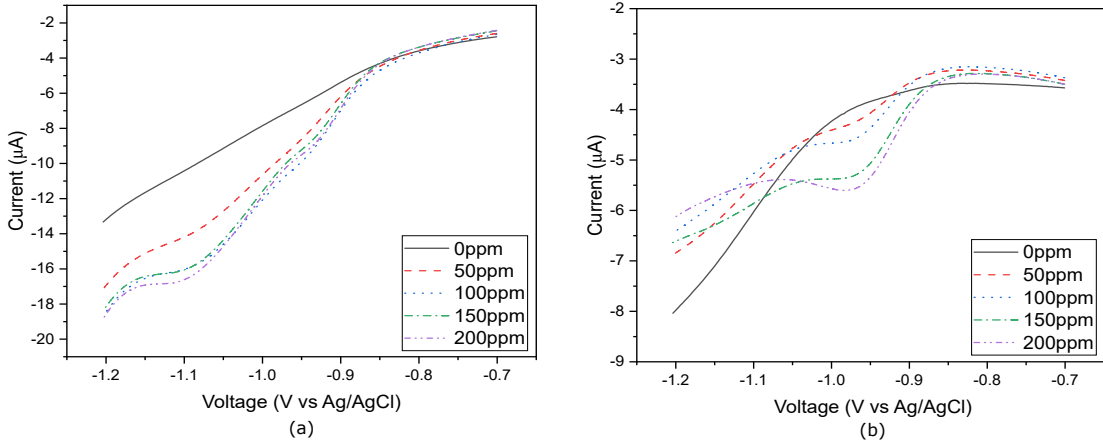
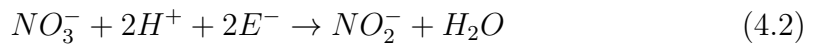


Figure 4.7: CV scans detecting nitrate from 0 ppm to 200 ppm in PBS, conducted using (a) control gold flexible electrode, and (b) after NPG deposition.

For both the benchtop instrument and the PULSE, NPG-modified printed electrodes were used ($n=2$). CV scans were taken ranging the voltage from 0 V to -1.3 V at 50 mV/s, where the typical reduction reaction of nitrate (NO_3^-) to nitrite (NO_2^-) occurs as follows:



The reduction potential of interest was analysed within the range of -0.8 V and -1.1 V, focusing on the lowest measured current to identify the peak reduction current, which represents the maximum electrochemical activity of nitrate reduc-

tion. The selected voltage window captured the characteristic reduction peak, as shown in Figure 4.8 for both systems. For more details consult Appendix C.4.

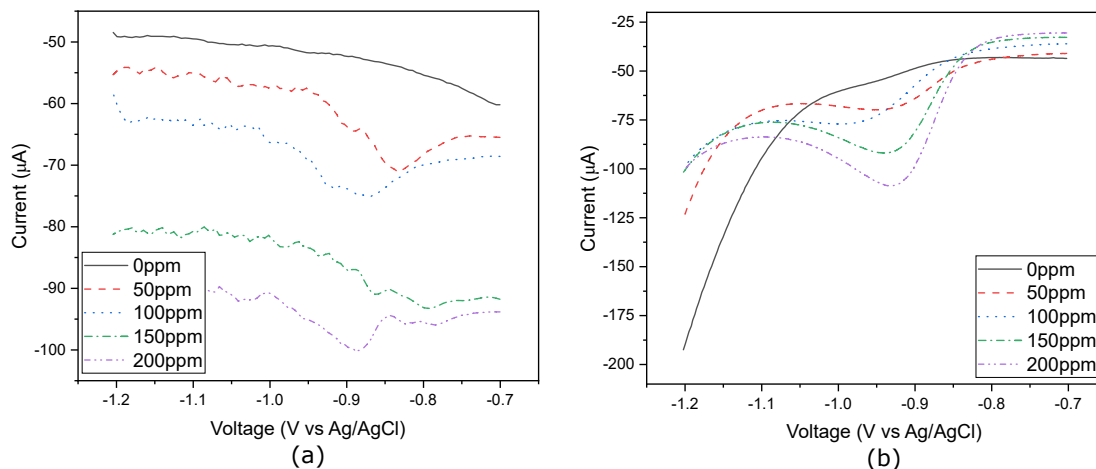


Figure 4.8: CV scans detecting nitrate from 0 ppm to 200 ppm in PBS, conducted using (a) the PULSE system, and (b) the benchtop instrument.

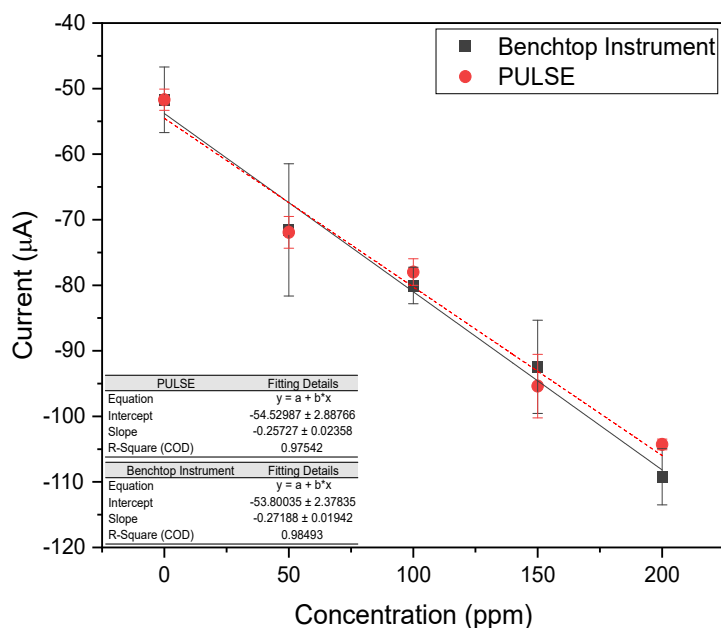


Figure 4.9: Calibration plots of peak reduction currents across multiple concentrations of nitrate in PBS, using the benchtop instrument, and the PULSE ($n=2$, mean $\pm$ SD).

A calibration plot was made by comparing the measured current against the nitrate concentration, as shown in Figure 4.9. The benchtop instrument and the PULSE system exhibited high linearity in the calibration curves, with R^2 values of 0.985 and 0.975, respectively. To improve precision, the background current difference between the datasets of the two systems, calculated as $31.6 \mu\text{A}$, was subtracted from the PULSE measurements. This adjustment ensured that the

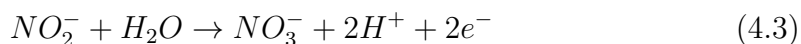
baseline currents were aligned, allowing the comparison of the systems' performance. Following this, a correlation analysis was made between the datasets, revealing a high correlation coefficient (> 0.99), supporting the agreement between the systems.

The PULSE demonstrated a LOD of 20.75 ppm (0.335 mM) and a sensitivity of $1.92 \mu\text{A}/\text{ppm}/\text{cm}^2$ ($0.119 \text{ mA}/\text{mM}/\text{cm}^2$). These results highlighted the PULSE system's capability for accurate nitrate quantification, reinforcing its potential for use in animal health applications. The benchtop instrument yielded a LOD of 60.73 ppm (0.979 mM) and a sensitivity of $2.03 \mu\text{A}/\text{ppm}/\text{cm}^2$ ($0.122 \text{ mA}/\text{mM}/\text{cm}^2$). The PULSE achieved a 65.83% lower LOD compared to the benchtop instrument, meaning the PULSE can detect smaller concentrations of nitrate. The slightly higher (2.46% in mM) sensitivity of the benchtop instrument suggested it generated a stronger signal per unit concentration. Nevertheless, the PULSE offered better detection limits with comparable sensitivity, reinforcing its suitability for real-world applications.

4.3.4 Nitrite Detection

As described in Section 4.2.3.4, a double-layer study was performed to obtain the double-layer capacitance (C_{dl}) and electron-transfer resistance (R_{ct}). EIS conducted in 200 ppm nitrite in ISF yielded values of $C_{\text{dl}} = 27.7 \mu\text{F}$ and $R_{\text{ct}} = 1.07 \text{ k}\Omega$. A dummy cell was constructed based on these parameters and interfaced with the voltammetric AFE to generate a calibration curve. The results indicated an amplification factor of 1.999 for nitrite, which was applied to the data to ensure accurate measurements. To improve precision, the background current difference observed among control samples was calculated as $25.57 \mu\text{A}$, and subtracted from the PULSE system data. Further details can be found in Appendix C.5.

An initial analysis of nitrite was conducted in PBS using the benchtop instrument and the PULSE to validate accurate nitrite measurement with the PULSE system. CV measurements were conducted sweeping voltage from 0 V to 0.9 V at 50 mV/s. The nitrite oxidation (NO_2^-) to nitrate (NO_3^-) typically occurs around 0.77 V [21], and follows the reaction:



Thus, the potential range analysed was set between 0.5 V and 0.9 V, with the maximum current measured within this range. Nitrite concentrations were varied, starting with a control solution of 10 mM PBS containing 0 ppm nitrite and increasing up to 200 ppm nitrite. The characteristic response curves obtained from both the PULSE platform and the benchtop instrument are shown in Figure 4.10,

demonstrating the consistency between the two systems.

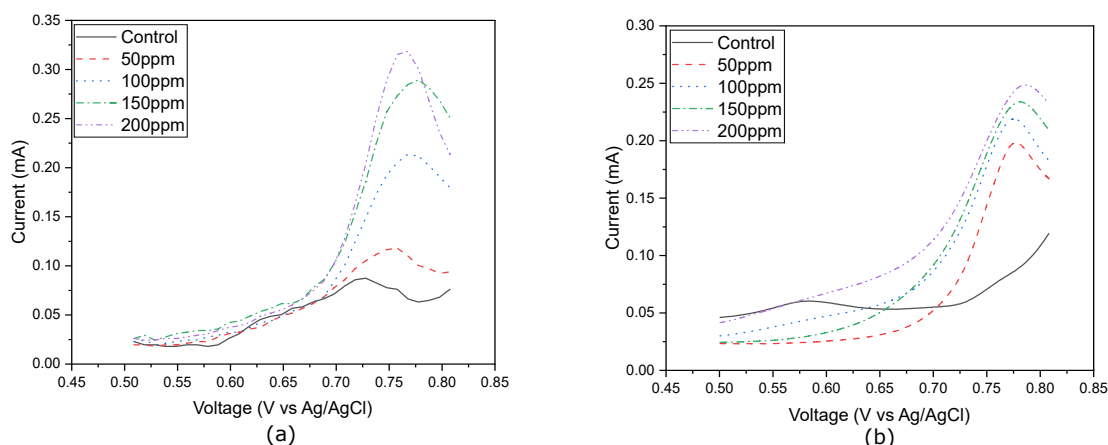


Figure 4.10: CV scans detecting nitrite from 0 ppm to 200 ppm in PBS, conducted using (a) the PULSE system, and (b) the benchtop instrument.

A calibration plot was generated, comparing the measured current against the nitrite concentration, as shown in Figure 4.11. The benchtop instrument and the PULSE exhibited high linearity, with R^2 values of 0.85 and 0.98, respectively. The datasets from both systems were closely aligned, and an additional correlation analysis indicated a strong correlation (>0.9) between the systems. These results confirmed that the PULSE system can accurately measure nitrite concentrations.

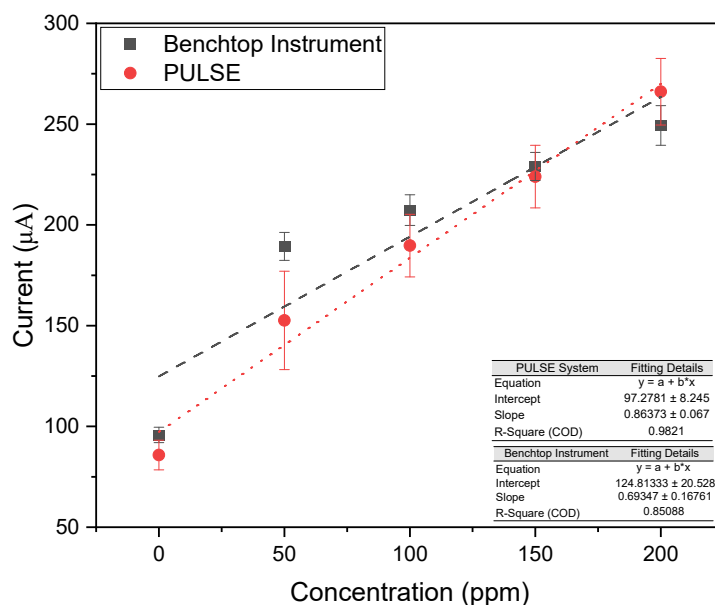


Figure 4.11: Calibration plots of peak oxidation currents for varying nitrite concentrations in PBS measured with the benchtop instrument and the PULSE system ($n=3$, mean $\pm$ SD).

The PULSE yielded a LOD of 14.62 ppm (0.318 mM) and a sensitivity of $6.45 \mu\text{A/ppm/cm}^2$ (0.297 mA/mM/cm^2). In comparison, the benchtop instrument demonstrated a LOD of 35.08 ppm (0.762 mM) and a sensitivity of $5.18 \mu\text{A}$

/ppm/cm² (0.238 mA/mM/cm²). Similarly to nitrate detection, the PULSE achieved lower LOD, representing a 58.3% improvement in the LOD. This means that the PULSE can detect significantly lower concentrations of the target analyte. The PULSE exhibited a 24.5% higher sensitivity than the benchtop instrument, meaning a better signal response per unit concentration. The significantly lower LOD and higher sensitivity reinforce the PULSE's practical utility for real-world deployment.

An analysis of varying nitrite concentrations in a fixed pH 7 solution was conducted in the PULSE to assess whether nitrite levels impact pH readings. Figure 4.12 (a) shows that at pH 7, different nitrite concentrations could be measured without affecting the pH readings. The potential remained constant across increasing nitrite concentrations, indicating that nitrite does not interfere with pH detection. To further explore this relationship, measurements were taken with a fixed 100 ppm nitrite concentration solutions with varying pH levels, as shown in Figure 4.12 (b). In this scenario, the system successfully measured pH in the presence of nitrite, producing a calibration curve with a slope of -63.32 mV/pH, corresponding to an accuracy of 92.7%. According to the nitrite calibration curve at pH 7.5, a 100 ppm nitrite produced a current of approximately 165.02 μ A. When the pH was adjusted to 7, the current increased to approximately 175.79 μ A, resulting in a difference of 10.7 μ A attributed to pH variation. The nitrite oxidation peak remained detectable, though slightly reduced, even in more acidic solutions, suggesting that the solution's pH significantly influences nitrite oxidation behaviour.

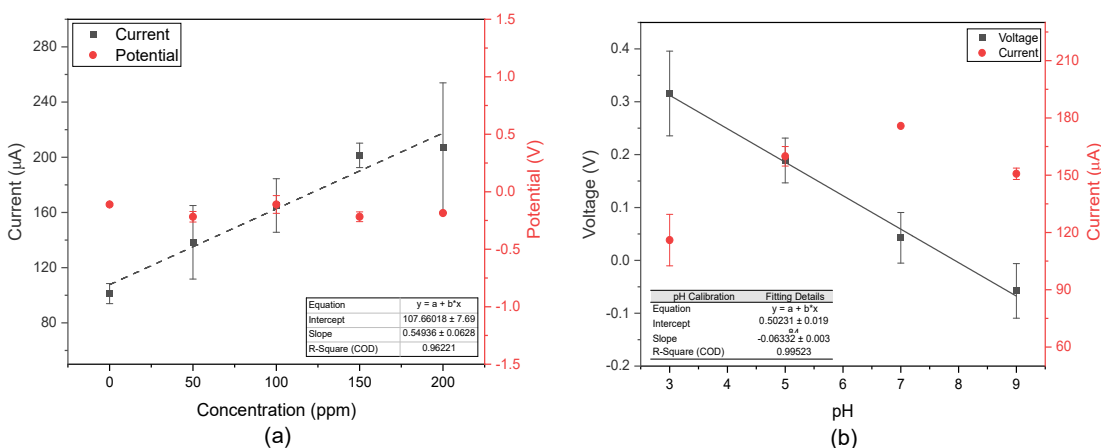


Figure 4.12: (a) Calibration plots of peak oxidation currents across multiple concentrations of nitrite in PBS at pH 7.0 ($n=2$, mean $\pm$ SD) and (b) pH calibration plot in 100 ppm nitrite solution in PBS ($n=2$, mean $\pm$ SD), with detection of 100 ppm nitrite across different pH levels.

Subsequent analyses were performed in artificial ISF using the PULSE to assess the system's response to various nitrite concentrations in a more complex

fluid. Figure 4.13 (a) shows the characteristic response curves obtained from the PULSE at increasing concentrations of nitrite in ISF, each conducted in triplicate. A calibration plot is illustrated in Figure 4.13 (b) showing the relationship between the measured current and nitrite concentration. The system exhibited high linearity, with R^2 of 0.997, indicating a strong, consistent response across the tested concentration range. The LOD was calculated to be 39.27 ppm (0.854 mM), and the sensitivity was $6.32 \mu\text{A}/\text{ppm}/\text{cm}^2$ ($0.291 \text{ nA}/\text{mM}/\text{cm}^2$), indicating the precision of the current response per unit concentration of nitrite.

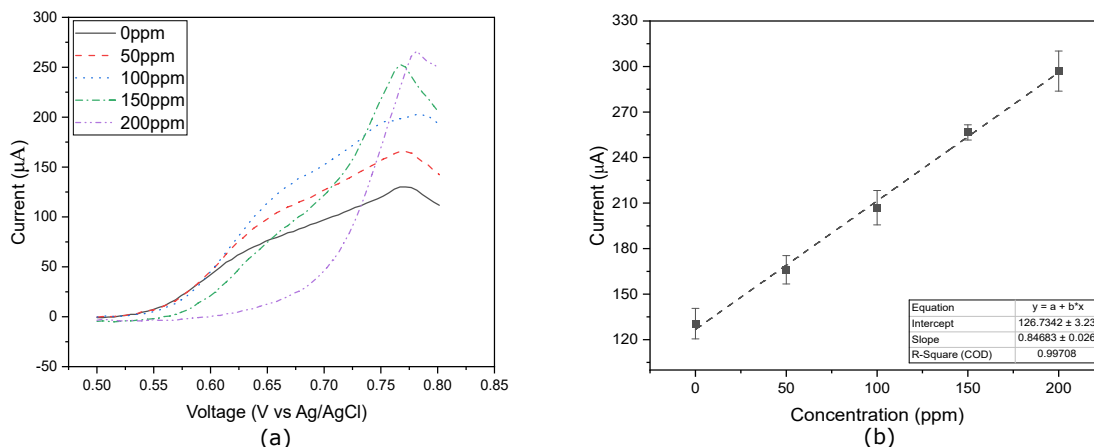


Figure 4.13: (a) CV scans detecting nitrite (0–200 ppm) in artificial ISF; (b) calibration plot of peak oxidation currents versus nitrite concentration ($n=3$, mean $\pm$ SD).

Furthermore, an examination was conducted to evaluate the influence of nitrite on pH measurements in this media and vice versa. Figure 4.14 (a) revealed that varying levels of nitrite could be detected accurately at a fixed pH of 7. According to the pH calibration curve in the ISF staircase (Figure 4.6), the measured voltage at pH 7 was approximately 0.0499 V. In the control solution, meaning only artificial ISF and 0 ppm of nitrite, a voltage of 0.0494 V was measured, aligning with the previous results. In the presence of nitrite, the voltage increased slightly to ≈ 0.088 V, and remained stable across different nitrite concentrations, indicating that nitrite presence does not significantly interfere with pH detection. Additionally, temperature measurements remained stable and were minimally affected by external factors.

In contrast, Figure 4.14 (b) illustrates the behaviour of a 100 ppm nitrite solution in ISF across varying pH levels. According to the nitrite calibration curve in artificial ISF at pH 7.5 (Figure 4.13 (b)), the measured current for 100 ppm was $206.97 \mu\text{A}$. At pH 7, this current decreased to $188.72 \mu\text{A}$, representing a difference of $18.25 \mu\text{A}$. This discrepancy can be attributed to changes in pH, as it has been previously reported in the literature that nitrite concentrations vary with pH [22]. This variation arises from the equilibrium between nitrite ions (NO_2^-)

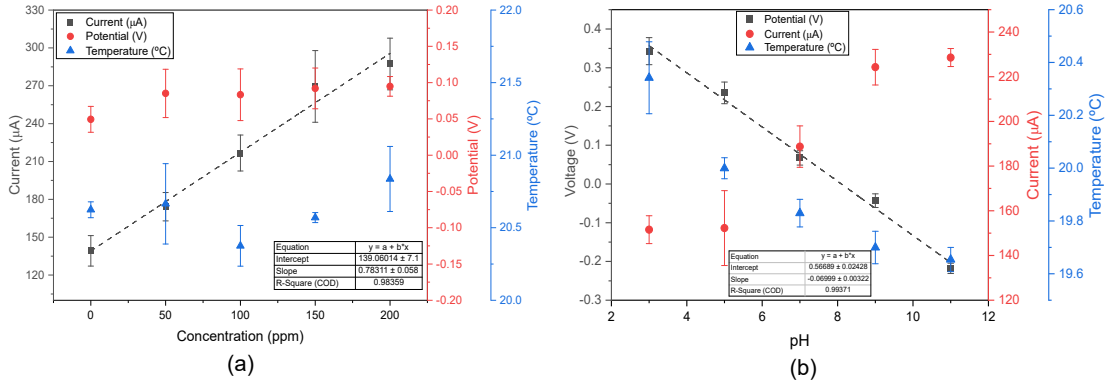


Figure 4.14: (a) Calibration plots of peak oxidation currents across multiple concentrations of nitrite at pH 7.0 ($n=3$, mean $\pm$ SD) and (b) pH calibration plot in 100 ppm nitrite solution ($n=3$, mean $\pm$ SD), with detection of 100 ppm nitrite across different pH levels and temperature measurements.

and nitrous acid (HNO_2), where lower pH levels favor the formation of HNO_2 , while higher pH levels shift the balance towards (NO_2^-) [23]. Even in more acidic solutions, the nitrite peak oxidation remained detectable, despite being reduced. Nitrite peak oxidation exhibited pH-dependent behaviour, decreasing in acidic conditions and increasing in basic environments, as reported in literature [24, 25]. These findings highlighted the importance of considering pH effects on nitrite detection, suggesting that future studies should establish a calibration matrix with distinct nitrite calibration curves for different pH levels. Moreover, pH detection remained unaffected, and a calibration curve with a slope of -69.9 mV/pH was established, following a Nernst behaviour [11]. Additionally, the temperature results were supported by literature, where a temperature decrease is observed with an increasing pH [12].

4.3.5 Real-World Proof of Concept

The primary objective of this chapter is to demonstrate the suitability of the PULSE system for bovine health monitoring. To this end, this study evaluated the system's capabilities for deployment in animal health monitoring by performing pH measurements in ruminal complex fluid, a critical parameter for monitoring the health of cattle, as explained in Chapter 2.

The typical OCP measurements over a 60-second period are illustrated in Figure 4.15 (a), showing an evident decrease in voltage with increasing pH. This inverse relationship aligns with expected electrochemical behaviour as demonstrated in previous sections. The resulting calibration curve derived from these measurements, shown in Figure 4.15 (b), yielded an R^2 value of approximately 0.96. The slope of the curve, measured at -59.63 mV/pH , was consistent with Nernstian behaviour [11]. The system demonstrated an accuracy of 98.9%, fur-

ther supporting its reliability for precise pH detection. These findings emphasised the accuracy of the PULSE system and provided strong support for its practical application in real-world animal health monitoring. For more details, refer to the Appendix C.6. Beyond its application in livestock health monitoring, the PULSE system presents opportunities for broader use, including water quality monitoring and microneedle-based technologies, as detailed in Appendices C.7 and C.8, respectively.

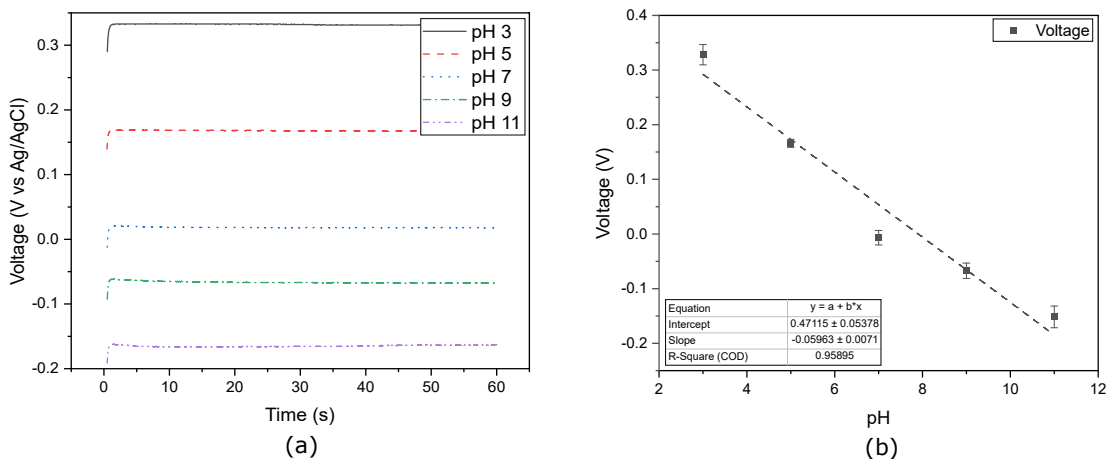


Figure 4.15: (a) OCP measurement over 60 seconds in ruminal fluid with pH ranging from 3 to 11, and (b) pH calibration curve ($n=3$, mean $\pm$ SD).

4.4 Conclusion

This study successfully demonstrated the integration of pH, temperature, nitrate, and nitrite sensors into the PULSE system, creating a robust multi-parametric platform for real-time livestock health monitoring. This multi-sensor approach marks a significant advancement toward enhancing continuous health monitoring in cattle and supporting sustainable herd management practices.

The system's capability to measure pH, nitrite, and nitrate was validated in various fluid media, from PBS to artificial ISF. In artificial ISF, pH detection demonstrated excellent linearity ($R^2 > 0.99$) with a sensitivity of -64.1 mV/pH and 91.4% accuracy. This system demonstrated accurate nitrite and nitrate detection with sensitivities of $6.32 \mu\text{A/ppm/cm}^2$, and $1.92 \mu\text{A/ppm/cm}^2$, respectively. Additionally, the strong correlation between the datasets from both systems highlighted the high agreement of the PULSE with the gold-standard device, reinforcing its reliability. Furthermore, compared to the benchtop instrument, the PULSE achieved a 65.83% lower LOD for nitrate. Similarly, for nitrite measurement, the PULSE exhibited a 58.3% lower LOD and a 24.5% increase in sensitivity, demonstrating its improved capability for detecting lower analyte con-

centrations. These results emphasised its capability to accurately measure nitrate and nitrite, which are critical indicators of animal health and have an indirect relationship with GHG emissions. These findings established this technology as a valuable asset for supporting strategies aimed at reducing environmental impact and improving economic sustainability in the dairy industry.

The PULSE was tested in complex ruminal fluid, demonstrating its practicality for real-world applications in bovine health monitoring. pH detection in rumen fluid achieved an accuracy of 98.9%, highlighting the system's potential to support early disease detection, advance sustainable practices in the dairy sector, and improve overall cattle health, with potential impacts on economic efficiency and human health. While the pH sensor has demonstrated high repeatability for over 40 days, further studies are needed to evaluate how rumen fluid affects the sensor's long-term durability.

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PULSE 2.0: A NOVEL POINT-OF-CARE MULTI-PARAMETRIC PLATFORM FOR LIVESTOCK HEALTH MONITORING

5.1 Introduction

Building upon the development and validation of the PULSE system in Chapter 3 and its demonstrated capability to measure pH, temperature, nitrate, and nitrite established in Chapter 4, this chapter focuses on advancing the platform through miniaturisation and enhanced functionality. The second-generation prototype, PULSE 2.0, was designed to address the need for portability, ease of use, and adaptability in field applications for on-site bovine health monitoring. Designed as a POC device, this version was intentionally adapted away from the specific constraints of smart bolus deployment to better suit handheld or externally worn use cases. Although previous work demonstrated the feasibility of 433 MHz communication for integration in smart bolus systems, PULSE 2.0 instead incorporates Bluetooth connectivity, prioritising ease of deployment in non-ingestible applications. Nevertheless, an overview of bolus communication has been explored in Section 2.2.3, and Table 2.3. Additionally, the system includes a rechargeable Li-ion battery and a compact enclosure, enhancing its portability and usability in practical dairy farm and veterinary environments.

The PULSE 2.0 prototype not only retained its ability to measure temperature and motion, and to perform electrochemical techniques, but also expanded its sensing capabilities to potassium and sodium through SWV. Sodium and potassium are two key analytes that are essential for assessing ruminal health and electrolyte balance [1]. These additions were guided by insights presented in Chapter 2, which emphasised the importance of these ions in bovine health. By

broadening the range of measurable analytes, PULSE 2.0 addresses critical gaps in livestock health monitoring and supports precision agriculture practices. This chapter further evaluates the prototype’s performance and accuracy in detecting these parameters in bovine ruminal fluid. This step ensured that the PULSE 2.0 is not only suitable for controlled environments, such as laboratory settings, but also robust for practical applications in real-world settings.

Electrochemical sensors have been utilised for monitoring sodium levels in human health applications, including the analysis of sweat [2], saliva [3], and other biofluids. These sensors are often modified to enhance analyte detection with Ion Selective Membranes (ISMs), nanoparticles such as gold nano dendrites, among others [2]. While these technologies show significant potential for sodium detection in animal health contexts, progress in this area remains limited [4]. Similar to sodium, potassium has been studied in human health monitoring through the analysis of sweat using electrochemical sensors [5, 6]. These ion-selective sensors employ techniques such as potentiometry or EIS, depending on the sampling fluid [7]. Recent advancements in agriculture have explored soil analysis using SWV and CV to detect potassium in soil samples [8]. However, the application of sodium and potassium ion-selective sensors in rumen fluid for bovine health monitoring remains unaddressed.

Portable potentiostats have become essential tools for electrochemical analysis across many applications [9]. Recent developments have introduced various devices with different levels of functionality, sensing capabilities, and data management approaches. For example, UnpadStat demonstrated single-sensor operation validated for CV [10]. The device relied on USB communication and stored data on an SD card, limiting real-time data accessibility and flexibility. Similarly, the device developed by Vidotto et al. [11] supported a single sensor interface and multiple voltammetric techniques. However, it lacked support for potentiometric measurements and did not integrate additional sensing modalities, such as temperature sensing. These limitations restricted its usability in multi-sensing environments or more complex monitoring applications, where monitoring multiple analytes is necessary. Communication was established via Bluetooth to a smartphone, and subsequently transmitted data to an online server for storage. While this approach enabled data saving and remote access, it still relied on the availability of a stable internet connection via Wi-Fi or mobile data. Unlike some other IoT systems that may store data locally and sync once a connection is available, this device requires continuous network access for real-time data transmission. In areas with poor network coverage or cases of transmission failure, data loss could occur.

To increase the sensing capabilities, Boni et al. [12] introduced a potentio-

stat capable of interfacing with up to four voltammetric sensors. This potentiostat communication leaned exclusively on Wi-Fi, which introduced limitations in remote areas or situations where network connectivity is unreliable. The use of external wiring and off-the-shelf connectors limited its portability, making it less practical for POC applications. The device operated within a current range from $\pm 620 \text{ nA}$ to $\pm 62 \mu\text{A}$ and did not support potentiometric measurements or additional sensing modalities, limiting its application scope, especially in multi-parametric settings. In comparison, devices with the ability to support potentiometric measurements and physical parameters would allow for wider sensing capabilities, such as pH, temperature, and acceleration, among others, thus enhancing their versatility in complex monitoring applications.

Restricting its application scope for the monitoring of multiple analytes that require potentiometric techniques as pH, as well as other multi-parametric applications which require physical parameters to be monitored. Another alternative, NanoStat, was a compact device designed to interface with a single electrochemical sensor via wired connections [13]. Despite being suitable for certain applications, NanoStat was restricted to voltammetric techniques and did not support potentiometric measurements or offer additional sensing capabilities. This device was tested against a standard instrument using a single concentration of a solution, without benchmarking across diverse conditions or concentrations. Additionally, its validation relied on observational comparisons without statistical analysis, making it challenging to conclusively determine its performance relative to a standard potentiostat.

Moreover, to address the sensing limitations of previously described technologies, Irving et al. [14] presented MYSTAT, a potentiostat supporting both voltammetric and potentiometric techniques for single-sensor interfaces with up to four electrodes. While MYSTAT offered a measurement range from $\pm 200 \text{ mA}$ down to $\pm 2 \mu\text{A}$, and $\pm 12 \text{ V}$, it lacked wireless communication, relying solely on USB data collection. Despite demonstrating its capability to measure temperature using a resistance temperature detector connected to the sensor interface, this feature could not be utilised simultaneously with electrochemical measurements. The electrochemical validation of this device was limited to CV, using a single analyte concentration tested against a commercial potentiostat. This validation process highlights a common limitation in the literature, where a narrow validation approach may lack the robustness required for reliable performance in diverse sensing conditions.

The reported SoTA potentiostat technologies reveal several limitations, including constrained sensing capabilities, portability challenges, reliance on wired communication, and insufficient validation approaches. While a more in-depth

comparison is provided later in this work, these observations highlight key gaps that motivate this chapter’s approach. To address these challenges, this study focuses on enhancing the functionality and expanding the sensing capabilities of the PULSE 2.0.

The main objectives of this chapter are:

- Design and develop the second-generation PULSE 2.0, enhancing portability by reducing the size of the PCB, incorporating Bluetooth connectivity for wireless communication, and integrating a rechargeable circuit to allow independent operation for extended periods. The system was housed within a compact enclosure, along with the development of an Android APP for field applications and user accessibility.
- Expand sensing capabilities by incorporating pH, temperature, sodium, and potassium sensors to enable multi-parameter analysis of bovine ruminal health and electrolyte balance. FW development of SWV technique for sodium and potassium detection.
- Validate platform performance through extensive testing, ensuring the system’s accuracy and functionality in detecting pH, sodium, and potassium within complex ruminal fluid.

In alignment with the goals of precision livestock monitoring, this study aims to bridge the gap between technological development and real-world application, showcasing the evolution of the PULSE system into a practical and versatile tool for livestock health monitoring. The integration of multiple sensing capabilities, combined with its compact design and wireless communication, addresses key challenges in livestock health management, including the need for real-time, on-site data collection in remote settings.

This chapter is organised as follows: Section 5.2 provides an overview of the HW and FW development associated with PULSE 2.0, and the Android APP development. Additionally, this section presents the materials, experimental procedures, and techniques used for testing and validating the system. Section 5.3 presents the results from the system characterisation and the detection of pH, temperature, potassium, and sodium, along with a comprehensive discussion. Finally, Section 5.4 summarizes the main findings of this work, emphasising the novelty of the PULSE 2.0 system.

5.2 Materials & Methods

5.2.1 Hardware Development

The PULSE 2.0 represents the second generation of the previously reported sensing prototype, with significant advancements aimed at enhancing functionality and portability. The electrochemical AFE circuits remained unchanged to those described in Chapter 3, due to their excellent performance reported in previous chapters. The sensing capabilities of this device include a motion sensor, a thermistor interface circuit, and a voltammetric and a potentiometric AFEs.

This section focuses exclusively on the new features introduced in the PULSE 2.0 design. A rechargeable Li-ion battery has been incorporated, enabling greater flexibility and independence from external power sources. Additionally, a Bluetooth communication module has been included to facilitate wireless communication, enhancing usability and compatibility with modern devices. All the schematic circuits and PCB design were done in Altium Designer software, and can be consulted in Appendix D.1. The PCB layout has also been optimised to achieve a more compact design, reducing 70% of the overall device size and making it practical for real-world applications. These enhancements aim to create a versatile, all-in-one device and mark a significant step forward in advancing the PULSE 2.0 as a reliable, portable, and user-friendly multi-parametric sensing platform.

5.2.1.1 Power Supply

The power supply schematic shown in Figure 5.1 outlined a versatile circuit designed to power the device either through a rechargeable Li-ion battery or via USB, providing flexibility for portable applications.

The USB connected through the J_1 , provided a 5 V power supply via the *VBUS* line. In addition to supplying power, the *D+* and *D-* data lines are routed to an FT232R USB UART interface IC to facilitate communication for data transfer and the reception of device configuration settings. However, in this design, the primary function of the USB connection is to serve as a power source.

A rechargeable Li-ion battery is connected to the *VBAT* via the P_1 connector. To isolate the USB power from the battery circuit, a Schottky diode (D_1 , BAT54CHYH from SMC Diode Solutions) is employed. This ensured that the USB and battery power sources do not interfere with each other. A key component in the circuit was the Li-ion battery charging IC, U2 (MCP73831T-2ACI/OT from Microchip Technology, Arizona, U.S.). This component manages the charging process when USB power (*VBUS*) is available, working as the input for the

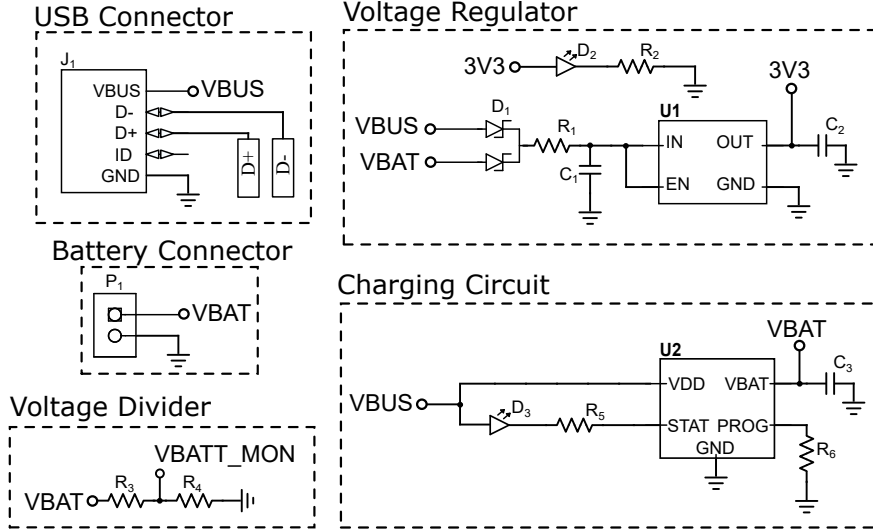


Figure 5.1: Power supply circuitry for the PULSE 2.0 device.

charger. The charging current is configured via the *PROG* pin by selecting an appropriate resistor value for R_6 . According to the component's datasheet, the charging current $I(mA)$ is determined by the following equation:

$$I(mA) = \frac{1000V}{R} \quad (5.1)$$

For this design, a charging current of 300 mA was selected, resulting in a calculated R_6 value of 3.3 k Ω . The *STAT* pin of the component is used to drive the LED (D_3 , LTST-C190KRKT, from Lite-On Inc., Taipei, Taiwan), which provided a visual indication of the charging status.

To regulate the output voltage, NCP161ASN330T1G (labelled as U1) was integrated, providing a stable 3.3 V output, regardless of whether the power source is the USB or the battery. The regulator's input is connected to the *VBUS* line via D_1 when USB power was available or to the *VBAT* line when operating on battery power. A resistor (R_1) served as an open path to incorporate a switch that allowed the device to be turned on and off. A power-on indicator is implemented using an LED (D_2 , LTST-C190KRKT, from Lite-On Inc.) connected to the 3.3 V output. This provided a clear visual indication that the device was operational.

The circuit also incorporated a voltage monitoring system to track the battery's charge level. This was achieved using a voltage divider composed of two 1 M Ω resistors (R_3 and R_4), which connect to the *VBAT_MON*. This configuration reduced the voltage at *VBAT_MON* to half the battery's voltage. This feature was critical for monitoring battery levels in portable devices and ensuring reliable operation over extended periods.

This design balanced performance, safety, and compactness while incorporating essential features such as power source switching, battery level monitoring,

and clear status indications, making it highly suitable for many applications.

5.2.1.2 Communication Module

The PULSE 2.0 device incorporated both wired and wireless communication options to enhance user flexibility and convenience. Communication between the device and the user interface can be established either through a USB cable or via Bluetooth. This dual communication option ensured that the PULSE 2.0 is well-suited for both laboratory and field applications. Bluetooth communication was implemented to enable wireless data transmission during the development and validation phase. This feature allows real-time monitoring and user interaction while ensuring portability and ease of testing in various environments. Bluetooth communication functioned as a solution for assessing system performance, optimising sensing capabilities, and refining data handling strategies, prior to the transition to a smart bolus with an appropriate wireless frequency for intra-body use. Additionally, it offers compatibility with standard mobile devices and laptops, making it suitable for other applications that require on-site electrochemical measurements.

For wireless communication, the HC-05 Bluetooth (from ITEAD Studio, Shenzhen, China) module was integrated into the system, enabling serial communication between devices. To ensure proper identification and ease of connection, the module was configured with the name "PULSE". Additionally, a password was defined to provide a layer of security and prevent unauthorized access. The HC-05 was set to operate with a baud rate of 115200 Bd, 1 stop bit, and no parity, adhering to the previous communication protocol settings for simplicity and compatibility.

5.2.1.3 PCB Design and Encapsulation

The PCB for the PULSE 2.0 was meticulously designed, featuring a six-layer structure with a total thickness of 1.91 mm. On the top layer, the primary components, including sensor connectors, were strategically placed for easy accessibility and optimal signal routing. The bottom layer housed the Bluetooth module and the rechargeable battery, ensuring effective utilisation of space. The design was made to include a connector reserved for potential future expansions. This connector allows the possibility of stacking an additional PCB to enhance sensing capabilities or integrate new functionalities without the need for a complete redesign. The final PCB design occupied a total area of $(49.4 \times 48.5) \text{ mm}^2$, achieving a balance between compactness, portability, and sufficient space for component placement. This represents a 70% reduction in size compared to the

first prototype, as it can be seen in Figure 5.2. More details on the PCB design can be consulted in Appendix D.2.

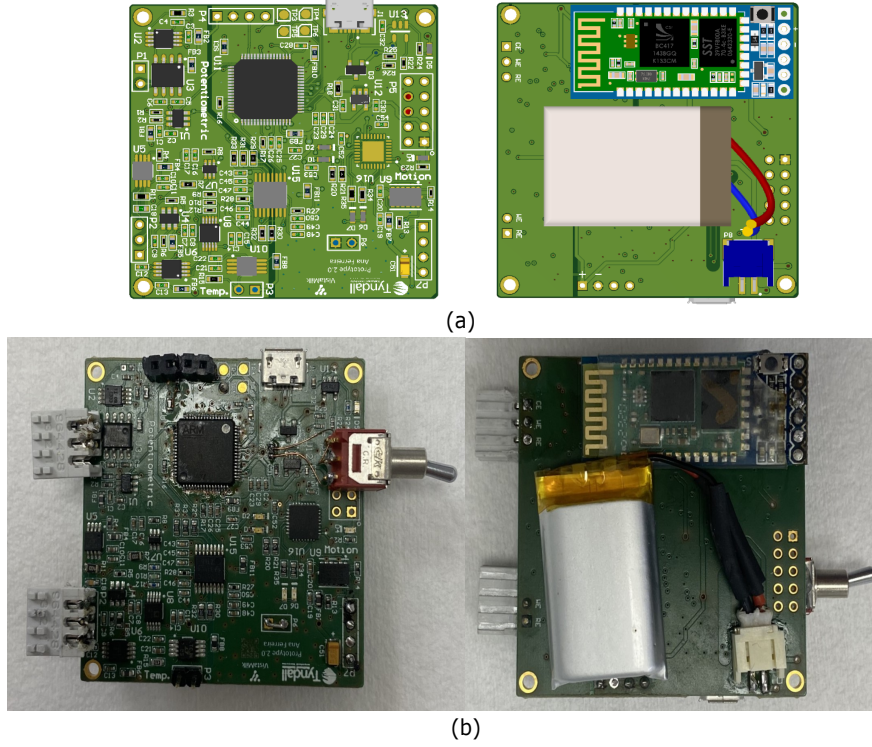


Figure 5.2: (a) 3D view of the PCB layout in Altium Designer, and (b) fabricated PCB.

An enclosure was designed to house the device using SolidWorks software to create a compact and functional design. The enclosure was subsequently fabricated using an in-house 3D printer, offering a cost-effective and efficient prototyping method. The Formlabs Form 3 with color base resin mixed with 80 ml cyan pigment and 20 ml white pigment purchased from Formlabs (Massachusetts, U.S.). The final enclosure, as shown in Figure 5.3, has dimensions of $(67.3 \times 52.9 \times 4.3) \text{ mm}^3$, ensuring a robust fit for the internal components while maintaining a compact form factor.

5.2.1.4 Operational Characterisation

To characterise the PULSE 2.0 device, its operational range, power consumption, and noise performance were evaluated. The operational ranges of the voltammetric and potentiometric AFE remain consistent with those reported in Chapter 3 and are detailed in Tables 3.4 and 3.5.

Similar to the methodology described in Chapter 3, a PPK2 (Nordic Semiconductor, Trondheim, Norway) was employed to measure the current consumed by the PULSE 2.0 during operation. The PPK2 was configured in source meter mode, supplying 3.5 V to the device while recording the current for a duration of

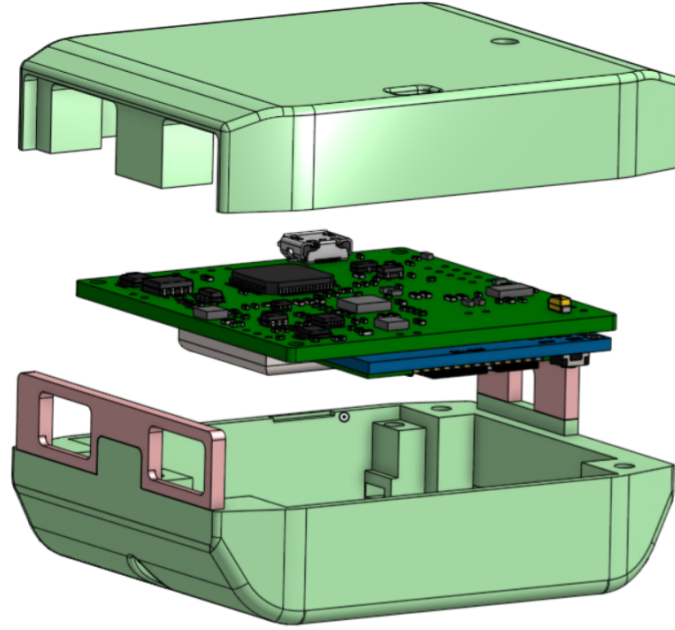


Figure 5.3: 3D view of the PULSE 2.0 enclosure.

60 seconds. This approach was applied across various measurement capabilities. The power was calculated as the product of the supply voltage and the average current.

The noise performance of the voltammetric and potentiometric AFE was assessed using an R_f of $9.9\text{ k}\Omega$ and a C_f of 10 nF . The AFE circuitry was connected to a dummy cell (Metrohm, UK), designed to mimic the properties of an electrochemical cell. The input voltage was varied across the full operational range of 0 V to 3.3 V . The noise was quantified by analysing the peak-to-peak differences in the time-domain recorded data, which was used to calculate the SNR, according to Equation 3.41.

5.2.2 Firmware Development

The PULSE 2.0 utilised the same MCU as the initial prototype, (STM32F372-R8T6 from STMicroelectronics). FW development was conducted using the STM32 Cube IDE platform with the C programming language. The PULSE 2.0 retained the same FW capabilities as the previous device, where the primary difference lies in the pinout configuration, reflecting changes made to the HW design. The FW was uploaded to the MCU via the Serial Wire Debug (SWD) interface, utilising a 4-wire connection for programming and debugging, using the ST-LINK V2. The FW development process has already been comprehensively detailed in Chapter 3, Section 3.2.2.

For this prototype, the FW was updated and optimised to PULSE 2.0, en-

hancing several key functionalities. These updates included improvements to the debugging process, adjustments to the electrochemical measurements were made, particularly to SWV, and simplification of wireless communication capabilities. Additionally, the control of external DAC voltage signals was refined and tailored, and internal battery and temperature measurements were removed. Temperature data is exclusively sourced from the thermistor circuit, and battery updates are handled through the power management circuit. For additional insights into the developed code, please refer to Appendix E.

5.2.3 APP Development

Based on the APP developed and described in Chapter 3 Section 3.2.3, an android APP (Figure 5.4) was adapted for prototyping using Kodular (Dover, U.S.). The APP is designed to identify and connect to the device named "PULSE" via Bluetooth, where the status is updated to "Connected". Once connected, it enables the user to initiate measurements and graphically visualise the results in real-time. Additionally, calibration equations for the analytes of interest can be incorporated within the APP, allowing the measured data to be automatically converted into meaningful outputs, such as concentration values or pH readings, which are displayed directly to the user. For additional insights into the developed code, please refer to Appendix D.



Figure 5.4: Android APP (a) logo, and (b) home screen.

5.2.4 Electrochemical Testing

5.2.4.1 Materials

Potassium ferrocyanide ($K_4[Fe(CN)_6]$), potassium ferricyanide ($K_3[Fe(CN)_6]$), phosphate buffered saline tablets (PBS 10 mM, pH 7.4), sodium chloride (NaCl), potassium chloride (KCl), Poly(3,4 - ethylenedioxythiophene) (PEDOT), sulfuric acid (H_2SO_4), polyvinyl chloride (PVC), potassium ionophore, potassium tetrakis(4-chlorophenyl) borate, 2-nitrophenyloctyl ether, tetrahydrofuran (THF), sodium ionophore, sodium tetrakis[3,5-bis(trifluoromethyl)phenyl]borate (Na - TFPB), sodium hydroxide (NaOH), hydrochloric acid (HCl) were obtained from Sigma Aldrich (Arklow, Ireland). Sterilised bovine rumen fluid was purchased from Bar Diamond, Inc. (Idaho, U.S.).

PBS solutions (10 mM) were obtained by dissolving 1 PBS tablet (pH 7.4) in 200 ml of DI water. Potassium hexacyanoferrate III and potassium hexacyanoferrate II solutions were prepared in PBS. All solutions were prepared with double distilled DI water with a resistivity of 18.2 M Ω cm.

5.2.4.2 Equipment

Electrochemical measurements were carried out using the PULSE 2.0. For the purposes of benchmarking this device against a standard well-established benchtop potentiostat, Methrom Multi Autolab MAC90389 (Metrohm, UK), with NOVA 2.1 Software was used. When using the benchtop instrument, the measurements were carried out at room temperature in a Faraday cage (Metrohm, UK). Graphene-SPEs were purchased from DropSens (DRP-110GPH), consisting of a 4 mm diameter carbon WE, carbon CE, and silver/silver chloride (Ag/AgCl) RE. A pH probe CHECKER1 pH meter from Hanna Instruments (Bedfordshire, UK) was used to verify the accuracy of the measurements. An electrochemical sensor holder, accommodating a temperature sensor (NCP03 from Murata Electronics, Nagaokakyo, Japan) was 3D-printed with standard white resin purchased from Formlabs. Flexible printed electrodes were fabricated in-house using a Voltera V1 printer (Voltera, Canada) [15], allowing for seamless integration into a future smart bolus prototype.

5.2.5 Electrochemical Methods

5.2.5.1 IrOx Functionalization

The IrOx functionalization process used was the same as that described in Section 3.2.4.1.

5.2.5.2 Flexible Electrodes Modification

The flexible in-house sensors were fabricated in Polyimide (Kapton) films ($50\text{ }\mu\text{m}$ thickness), with a 2 mm diameter WE and CE printed using gold (gold polymer paste: C204120-6P2, from Sun Chemicals, Heywood, UK), and a platinum RE (platinum polymer paste: C2050804P9, from Sun Chemicals). For the use of these sensors, O-rings (Radionics) with a 7 mm diameter created a seal between the well within the electrochemical cell holder and the printed electrode.

To prepare the flexible electrodes for the detection of potassium and sodium, a series of steps were undertaken to deposit ISMs onto the WE surface. The process began with sensor cleaning, performed using 0.5 M sulfuric acid, a well-known step for cleaning gold electrodes. The electrodes underwent 10 CV scans within a potential range of -0.5 V to 0.5 V at a scan rate of 50 mV/s, following an adaptation from the literature [16]. The cleaned sensors served as the control for subsequent modifications.

Subsequently, the conductive polymer Poly(3,4-ethylenedioxythiophene) (PEDOT) was deposited on the electrode surface through CA, which was selected prior to this work. This was achieved by applying a constant potential of 0.05 V for 90 seconds. The PEDOT deposition process began by preparing a 0.1 M potassium chloride (KCl) solution, into which 1 mL of PEDOT was thoroughly mixed to produce a final concentration of 10 mM PEDOT in the solution.

Following the deposition of PEDOT, the potassium and sodium ISMs were prepared using the methods reported by Ozer et al. [17]. Finally, the ISMs were applied to the electrodes by drop-casting, using approximately $3\text{ }\mu\text{l}$ for the potassium selective membrane and $2\text{ }\mu\text{l}$ for the sodium selective membrane, and left to dry for over eight hours. These steps resulted in functionalized flexible electrodes capable of selectively detecting potassium and sodium ions.

This complete process is depicted in Figure 5.5, outlining each step of the sensor modification along with the CV scans used to characterise the sensors at each stage of the procedure. To determine the electrochemical active area of the final prepared electrodes, the Randles-Sevcik equation was used. CV measurements were performed in triplicate using 5 mM solution of ferri-ferro. The measurements were conducted at various scan rates, ranging from 10 to 200 mV/s, to assess the relationship between scan rate and peak current, as shown in Equation C.1.1.

5.2.5.3 Double-layer Study

The double-layer study was the same as that described in Section 4.2.3.4.

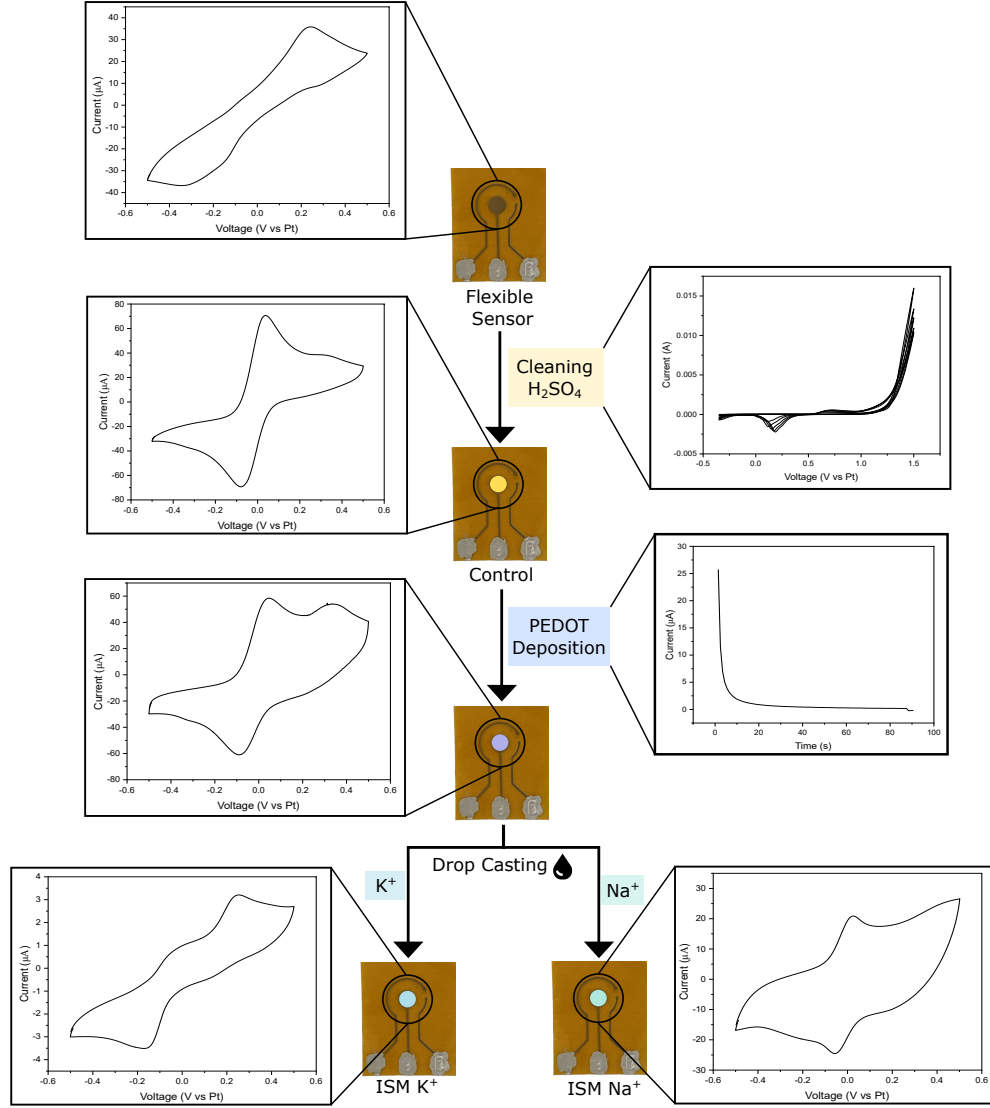


Figure 5.5: Schematic diagram of the ISMs deposition process for potassium (K^+) and sodium (Na^+), including the sensor cleaning with sulfuric acid (H_2SO_4), PEDOT deposition by CA, and CV scans in ferri-ferrocyanide solution at each stage.

5.2.5.4 Peak Oxidation Analysis

This test was designed to characterise and determine the relative error of the PULSE 2.0 detecting peak oxidation currents. CV scans were performed using the commercial SPEs ($n=3$) in a 10 mM PBS buffer at pH 7.4 with multiple concentrations of ferri-ferro ranging from 1 mM to 5 mM. The potential was swept from -0.5 V to 0.5 V at 50 mV/s. This procedure was carried out on both the benchtop instrument and the PULSE 2.0 system for comparison. PULSE 2.0 was setup with a gain of 1, R_f of 1 k Ω , and C_f of 10 nF. As described in Appendix C.2, the Randles-Sevcik equation was used to calculate the theoretical values of the oxidation peak current (i_p) as a function of analyte concentration [18]. The

calculations utilised Equation C.2.1 and the parameters listed in Table C.2.2.

5.2.5.5 pH Detection

pH measurements were carried out in PBS solution, adjusted as needed using NaOH or HCl, across a pH range of 3 to 11, in triplicate ($n=3$). Measurements were conducted using the PULSE 2.0 with a gain setting of 1, and the results were benchmarked against the benchtop potentiostat for comparison.

5.2.5.6 Potassium Detection

SWV was conducted in triplicate to detect potassium concentrations in DI water solutions, ranging from 0 mM (DI water only) to 25 mM. The voltage sweep was conducted from -0.4 V to 0.4 V, with a step size of 0.005 V and a frequency of 50 Hz. The PULSE 2.0 was configured with a C_f of 10 nF, R_f of 9.9 k Ω , and gain 10. This provided an operational current range of $\pm 16.16 \mu\text{A}$ (consult Table 3.4 for reference), which was well-suited for the signal amplitudes during potassium detection.

5.2.5.7 Sodium Detection

SWV was conducted in triplicate to detect sodium concentrations in DI water solutions, ranging from 0 mM (DI water only) to 200 mM. The voltage sweep was conducted from -0.6 V to 0.4 V, with a step size of 0.005 V and a frequency of 50 Hz. The PULSE 2.0 was configured in the same way as for potassium detection.

5.2.5.8 Ruminal Fluid Analysis

To prove the capabilities of the PULSE 2.0 for real-world animal welfare monitoring, bovine sterilised ruminal samples were analysed for pH, temperature, potassium, and sodium. pH measurements were performed using IrOx functionalized SPEs in triplicate. The pH of the samples was adjusted across a range of 3 to 11 using either NaOH or HCl, depending on the required pH shift. In-house flexible sensors were used to detect potassium and sodium concentrations in the ruminal sample in triplicate.

5.3 Results & Discussion

5.3.1 Noise Levels

The noise levels of the voltammetric and potentiometric AFEs circuits were evaluated, with an R_f of 9.9 k Ω and a C_f of 10 nF. The AFE circuitry was con-

nected to a dummy cell (Metrohm, UK), which mimicked the properties of an electrochemical cell. The input voltage was varied to obtain the full signal range from 0 V to 3.3 V, and the noise was assessed by analysing the peak-to-peak differences in the time-domain recorded data, which was then used to calculate the SNR. This yielded SNR values ranging from 25.27 dB to 36.08 dB for the voltammetric AFE, and 28.3 dB to 36.03 dB for the potentiometric AFE. These measurements aligned closely with the performance observed in the initial prototype.

5.3.2 Power Consumption

Table 5.1 represents the power consumption observed during single measurement operations, recorded using PPK2. In idle state, meaning when the device is powered on but not executing any specific tasks or measurements, a power consumption of 109.06 mW was recorded. This reflected an increase compared to the 81.4 mW observed in the initial prototype. This higher idle power consumption was likely attributed to additional HW components, such as the HC-05 Bluetooth module, which has an operating current of approximately 30 mA at 3.5 V. Additionally, the MCU supply current in run mode, executing code from Flash with both High-Speed Internal (HSI) and Phase-Locked Loop (PLL) disabled at 8 MHz, is approximately 98 μ A. Although low-power consumption is not a focus of this work, it remains a key consideration for future development of a smart bolus. The MCU’s power usage can be significantly reduced by leveraging low-power modes such as standby, where current consumption drops to 4.8 μ A, or by selecting an alternative MCU from the STM family designed for ultra-low-power applications. Among the different measurement tasks, electrochemical techniques exhibited the highest power consumption, with SWV requiring 195.4 mW, making it the most power-intensive operation due to the intensive signal generation and processing required for this technique.

Table 5.1: Idle state power consumption and individual measurements, encompassing the acceleration sensor, temperature sensor, battery monitoring, CV, SWV, LSV, CA, and OCP.

Measr.	Idle	Motion	Temperature	Battery	OCP	CV	LSV	SWV	CA
Power (mW)	109.06	158.97	159.57	159.64	168.74	179.76	179.66	195.405	171.43

The power consumption of simultaneous measurements was also evaluated, as shown in Table 5.2. When multiple non-electrochemical measurements were performed simultaneously, specifically motion sensing, temperature monitoring, and battery level monitoring, the power consumption was 160.2 mW. This indi-

cates a marginal increase compared to individual measurements of these components. On the other hand, when combining non-electrochemical measurements with electrochemical techniques, the power consumption increased. Among these combinations, SWV combined with non-electrochemical tasks recorded the highest value of 193.52 mW. This was consistent with the finding that SWV was the most power-demanding operation.

Table 5.2: Power consumption of multiple simultaneous measurements, where *NE* denotes for non-electrochemical.

Measr.	NE	OCP+NE	CV+NE	LSV+NE	SWV+NE	CA+NE
Power (mW)	160.2	170.77	177.8	178.15	193.52	171.05

The operational lifetime of the PULSE 2.0 was estimated based on its power consumption and the specifications of a Li-ion battery. In idle state, a 500 mAh battery with a nominal voltage of 3.7 V could sustain the device for approximately 17 hours. In contrast, during non-electrochemical measurements, including motion sensing, temperature monitoring, and battery level tracking, the same battery is estimated to last for 11.5 hours of continuous operation. If the device continuously measured only pH through OCP, consuming 168.74 mW, the same battery would last for 10.96 hours. The estimated battery life was calculated using the following relationship:

$$t = \frac{C}{I} \quad (5.2)$$

Where t is the operational time (h), C is the battery capacity (mAh), and I is the average current draw of the device (mA). This calculation underscored the direct dependence of the operational lifetime on the battery's capacity. For instance, employing a larger battery with a capacity of 1000 mAh would extend the device's run time to approximately 23.1 hours for continuous non-electrochemical measurements. These estimations highlighted the importance of selecting an appropriate battery capacity to meet specific operational requirements, particularly for applications requiring prolonged or intensive device usage. Depending on the application specifications, a power bank could be used to maintain the continuous operation of the device.

Additionally, the frequency of measurements also influences the battery's lifetime. Using the same 500 mAh, 3.7 V Li-ion battery with a duty-cycling operation, the lifetime will vary. Continuous operation of SWV would result in a battery life of 9.5 hours. In duty-cycling scenarios where the device enters idle mode between measurements, the battery life is approximately 16.9 hours, determined by the idle state, without exceeding the maximum operational time for

that state. However, if the device is powered off instead of entering idle mode, the battery life is extended. For example, with the same battery, performing SWV and non-electrochemical measurements every hour for 15s would result in an operational time of 95.6 days. If the measurements were conducted every 6 hours, the battery life would increase to 573 days, or approximately 1.57 years. Further research is required to determine the optimal number and frequency of measurements needed for a smart bolus system to effectively support advanced decision-making algorithms.

5.3.3 Sensor Integration

The modification of commercial SPEs with an IrOx deposition layer for pH detection and the integration of ISMs on in-house flexible electrodes for sodium and potassium detection were successfully achieved. A flexible material was selected to facilitate seamless future integration into the form factor of a smart bolus prototype. The electrochemical active area, which influences the sensor's sensitivity, was calculated to be $1.37 \times 10^{-2} \text{ cm}^2$ for potassium ISM, and $0.536 \times 10^{-2} \text{ cm}^2$ for sodium ISM. A detailed characterisation of the sensors can be found in Appendix D.3. These sensors were seamlessly integrated with the PULSE 2.0, which communicates wirelessly to a smartphone APP, enabling real-time data acquisition and visualisation. The integration of the sensors highlighted the versatility of the PULSE 2.0 system, providing a compact, portable, and efficient solution for electrochemical sensing in practical settings, as depicted in Figure 5.6.

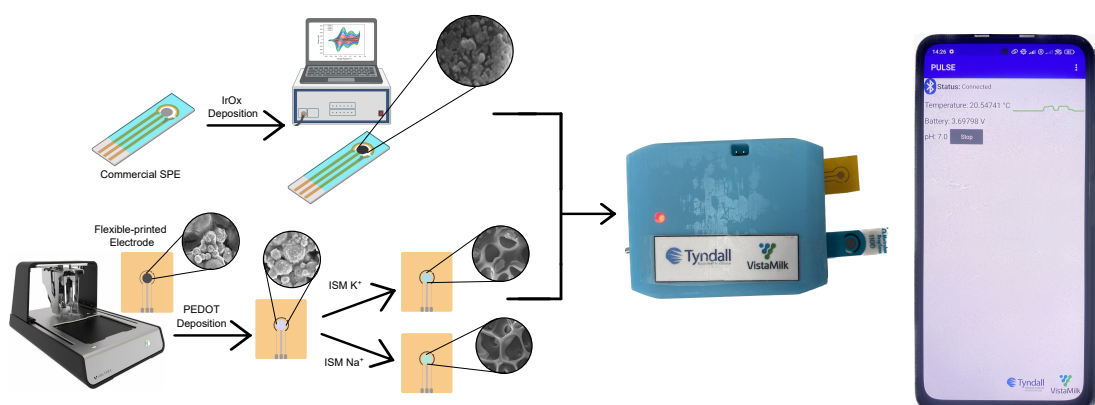


Figure 5.6: Schematic overview of the commercial SPE functionalized with IrOx, fabrication and modification of flexible sensors, and integration with PULSE 2.0, with results displayed through the APP.

5.3.4 Peak Oxidation Analysis

The electrochemical performance of the PULSE 2.0 was evaluated by analysing ferri-ferrocyanide concentrations ranging from 1 mM to 5 mM with commercial SPE sensors. Comparative CV scans using the PULSE 2.0 and the benchtop potentiostat revealed the typical characteristic shape of voltammograms, as shown in Figure 5.7. These results demonstrated that PULSE 2.0 can effectively replicate the standard electrochemical behaviour observed in traditional laboratory instruments. For more information about the results, refer to the Appendix D.4.

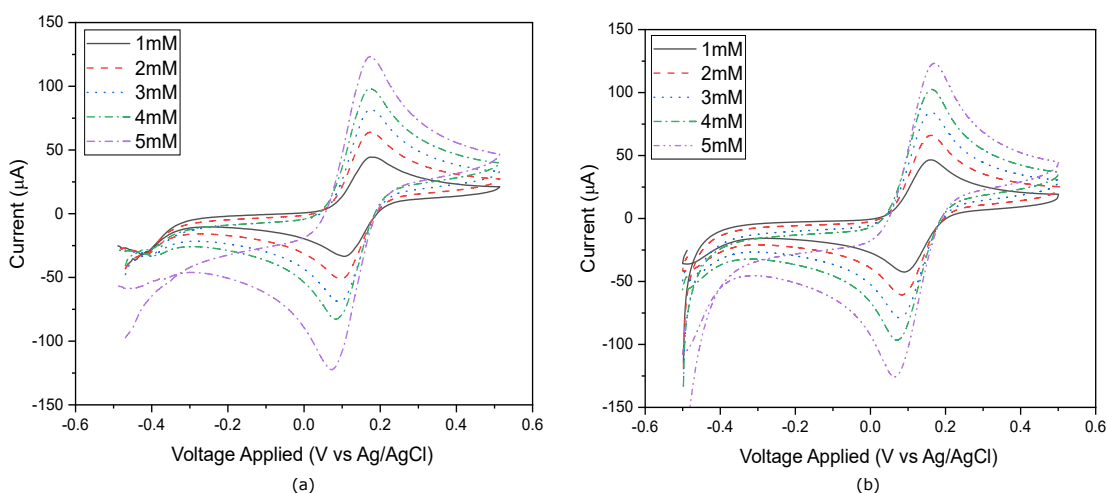


Figure 5.7: CV scans detecting ferri-ferrocyanide from 1-5 mM in 10 mM PSB, with potential swept from -0.5 V to 0.5 V at 50 mV/s, using (a) the PULSE 2.0 and (b) the benchtop instrument (n=3).

The oxidation peak currents were plotted against the ferri-ferrocyanide concentrations to construct calibration curves, as shown in Figure 5.8. Both systems exhibited strong linear trends with R^2 values of 0.96 and 0.98 for the PULSE 2.0 and the benchtop instrument, respectively. As observed in the figure, most of the peak oxidation points overlapped between the systems, suggesting a high correlation between the datasets. Additionally, a correlation analysis was conducted, revealing a correlation coefficient of 0.999, which supported the high agreement between the two systems. These findings justified to conclude that the data obtained from the benchtop potentiostat and the PULSE 2.0 are highly comparable.

Theoretical oxidation peak currents were calculated for each ferri-ferrocyanide concentration, and background noise was subtracted. Table 5.3 provides a detailed summary of these values. The relative errors were calculated using the standard formula for relative error, and the results are displayed in Figure 5.9.

The relative error analysis revealed that the PULSE 2.0 exhibited consistently higher errors than the benchtop potentiostat across the ferri-ferrocyanide concentrations. Commercial SPEs sensors are typically mass-produced, and while they

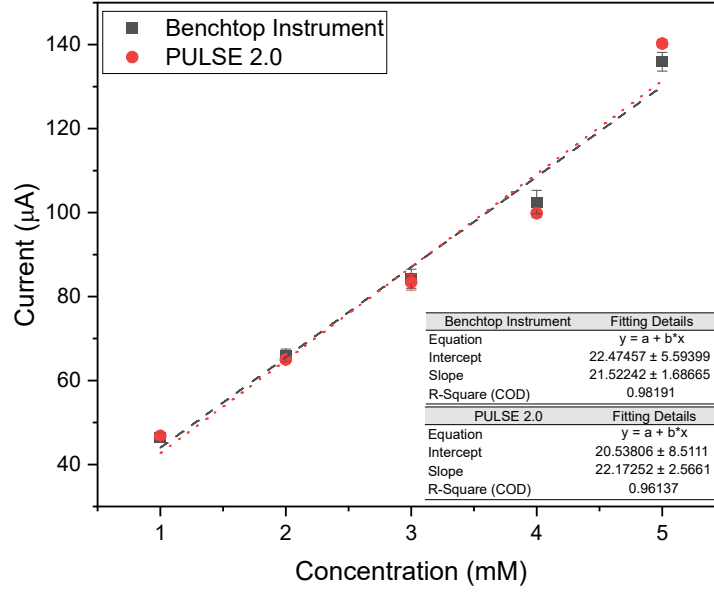


Figure 5.8: Calibration curves of oxidation peak current against ferri-ferrocyanide concentrations using the PULSE 2.0 system and the benchtop instrument ($n=3$, mean $\pm$ SD).

Table 5.3: Theoretical and background-removed oxidation peak currents in μA for multiple concentrations of $[Fe(CN)_6]^{3-}/[Fe(CN)_6]^{4-}$ in 10 mM PBS.

Concentration (mM)	Theoretical Value	Benchtop Instrument	PULSE 2.0
5	95.611	95.610	98.279
4	76.489	74.9294	73.691
3	57.367	56.680	57.0793
2	38.244	38.420	39.0921
1	19.122	18.989	19.649

offer convenience and reproducibility, they may have inherent batch-to-batch variability in terms of material composition, electrode surface characteristics, or baseline performance [19]. These factors could account for the observed differences in errors between this experiment and the one illustrated in Figure C.2.3. Despite the higher relative error observed in the PULSE 2.0 measurements, the errors remained less than 6%, meaning an overall accuracy superior to 94%.

5.3.5 pH Detection

OCP measurements were performed in PBS solutions over a pH range of 3 to 11 to assess the performance of the PULSE 2.0 system, which was compared to a standard benchtop instrument. The results are illustrated in Figure 5.10, demonstrating that both systems exhibited a strong linear response across the

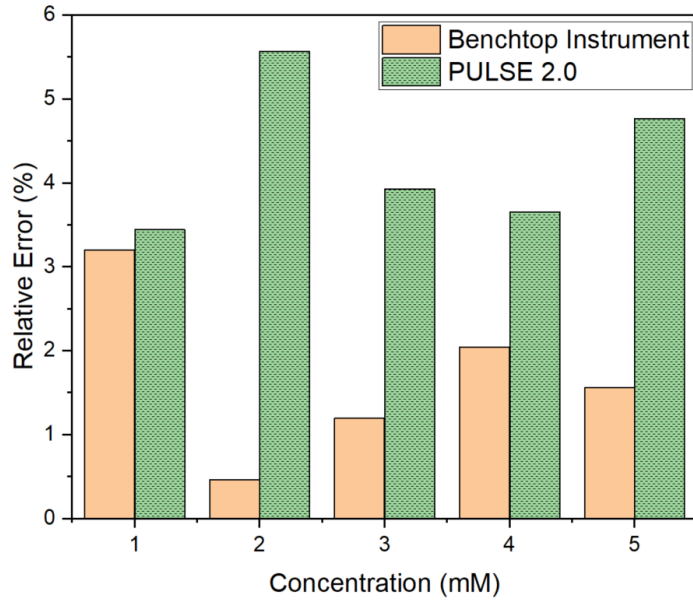


Figure 5.9: Relative error analysis of oxidation peak current measurements against ferri-ferro concentrations for the benchtop instrument and the PULSE 2.0 ($n=3$, mean $\pm$ SD).

tested pH range, with a R^2 value of 0.98 for both calibration curves. Detailed data supporting these findings are provided in the Appendix D.5. A correlation analysis between the two systems revealed a high correlation coefficient of 0.997, indicating excellent agreement among the responses.

The calibration curve obtained with the PULSE 2.0 system displayed a slope of -70.38 mV/pH , demonstrating a super-nernstian behaviour [20]. This behaviour indicated that the system was highly sensitive to pH changes, surpassing the theoretical -59 mV/pH predicted by the Nernst equation at room temperature. These results highlighted the capability of the PULSE 2.0 system to deliver accurate pH measurements, comparable to those obtained with a benchtop instrument. The excellent linearity, sensitivity, and correlation make it a viable alternative for applications requiring portable pH sensing.

5.3.6 Potassium Detection

As described in Section 5.2.5.3, a double-layer study was performed to obtain the double-layer capacitance (C_{dl}) and electron-transfer resistance (R_{ct}). EIS conducted in 25 mM potassium in DI water yielded values of $C_{dl} = 20.1 \text{ nF}$ and $R_{ct} = 3.40 \text{ k}\Omega$. A dummy cell was constructed using these parameters and interfaced with the voltammetric AFE to generate a calibration curve. The results indicated a reduction factor of 0.6652 for potassium, which was applied to the data to ensure accurate measurements. Further details on the calibration process can be found in Appendix D.6.

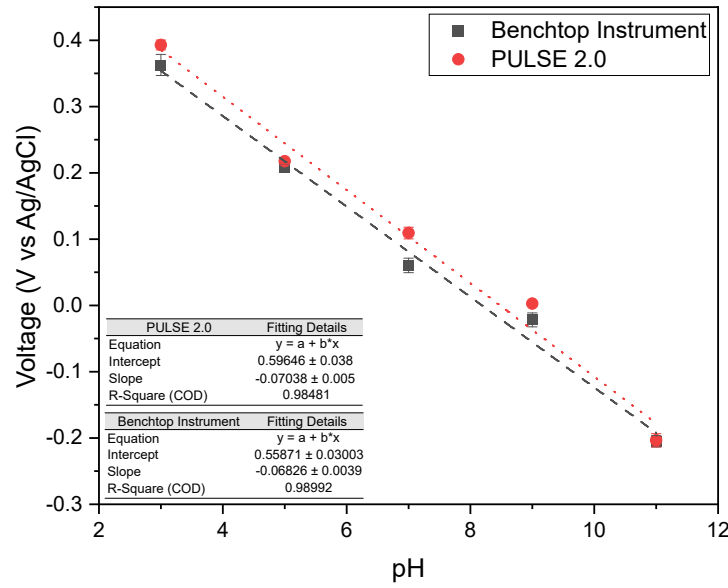


Figure 5.10: pH calibration curves of PBS solution (pH 3–11) using the benchtop instrument and PULSE 2.0 ($n=3$, mean $\pm$ SD).

Potassium oxidation typically occurs on the positive side of the potential range [21]; thus, the observed voltage range spanned from 0 V and 0.4 V. Representative voltammograms obtained during the experiments are presented in Figure 5.11, using SWV to measure oxidation peak currents across various potassium concentrations. These scans demonstrated the expected peak behaviour associated with potassium oxidation.

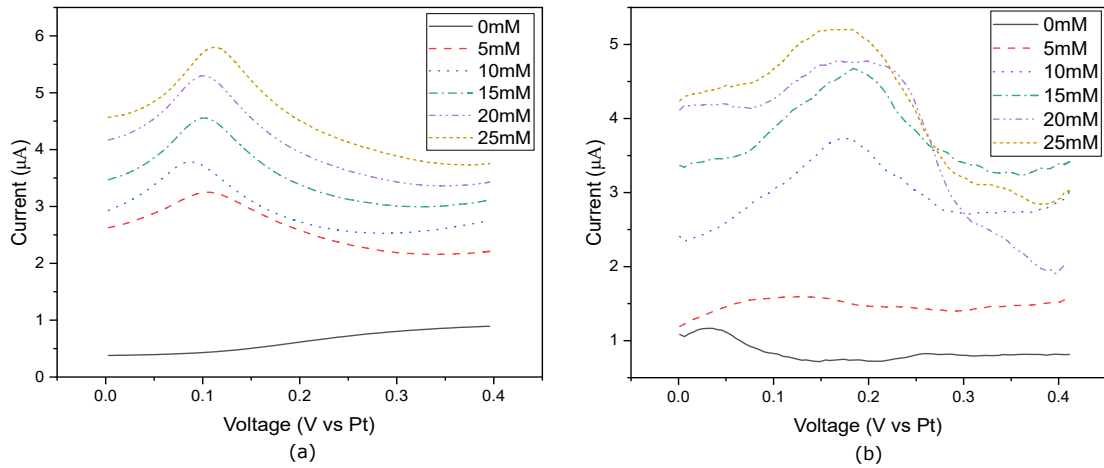


Figure 5.11: Voltammograms of potassium in DI water, with concentrations from 0 mM to 25 mM, obtained using (a) the benchtop potentiostat and (b) the PULSE 2.0 system.

A calibration curve for potassium detection was established to further validate the PULSE 2.0 system's performance, as shown in Figure 5.12. This curve was generated using SWV to measure oxidation peak currents across a range of potassium concentrations (0 mM to 25 mM). The experiments were conducted in

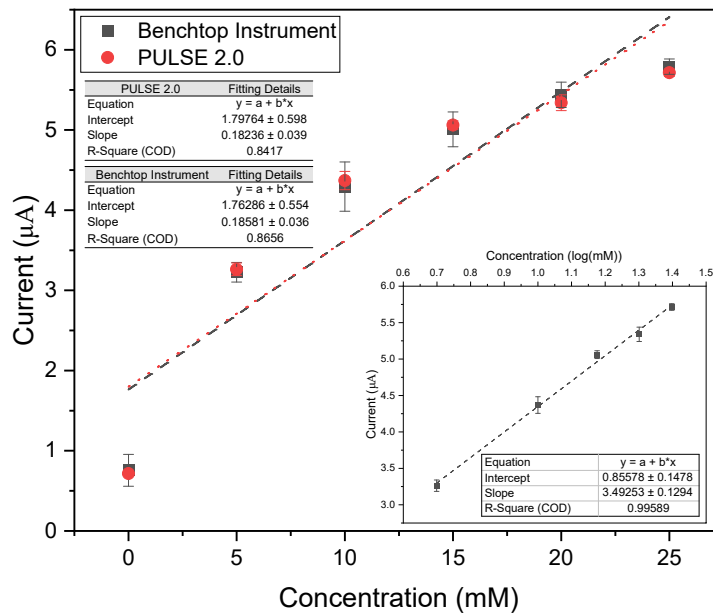


Figure 5.12: Potassium calibration curves in DI water (0 mM to 25 mM) using the benchtop potentiostat and the PULSE 2.0 system. Inset: relationship between logarithmic concentration of potassium and peak oxidation current measured with PULSE 2.0 ($n=3$, mean $\pm$ SE).

triplicate using both the PULSE 2.0 system and the benchtop potentiostat to assess the reliability and robustness of the PULSE 2.0 system.

The results demonstrated low Standard Error (SE), and strong consistency between the PULSE 2.0 system and the benchtop potentiostat, with a correlation coefficient of 0.999, indicating a strong agreement between the datasets. The calibration curve obtained from the PULSE 2.0 system exhibited a slope of $0.182 \mu\text{A}/\text{mM}$, with a sensitivity of $13.3 \mu\text{A}/\text{mM}/\text{cm}^2$, and a calculated LOD of 0.240 mM . In comparison, the calibration curve conducted with the benchtop instrument yielded a slope of $0.186 \mu\text{A}/\text{mM}$, with a sensitivity of $13.6 \mu\text{A}/\text{mM}/\text{cm}^2$, and a LOD of 1.79 mM . The PULSE 2.0 exhibited an 86.6% lower LOD compared to the benchtop instrument, demonstrating its ability to detect lower analyte concentrations. In terms of sensitivity, a 2.2% difference was observed, with the PULSE 2.0 showing lower sensitivity. However, this difference was negligible as the calibration curve slopes were highly comparable, further reinforcing the system's reliability.

The obtained results are not only statistically robust but also hold significant practical implications. The system's ability to detect potassium levels within this range makes it promising for real-world applications in bovine ruminal fluid. The typical potassium concentration in the rumen of a healthy cow is approximately 20 mM [1]. The PULSE 2.0 system demonstrated significant potential to effectively be used to monitor such physiological fluid, which was further investigated

later in this chapter.

5.3.7 Sodium Detection

For sodium detection, the voltage was analysed between -0.5 V to 0 V, as sodium oxidation typically occurs at negative potentials [22]. Representative voltammograms illustrating the relationship between sodium concentration and peak current are shown in Figure 5.13.

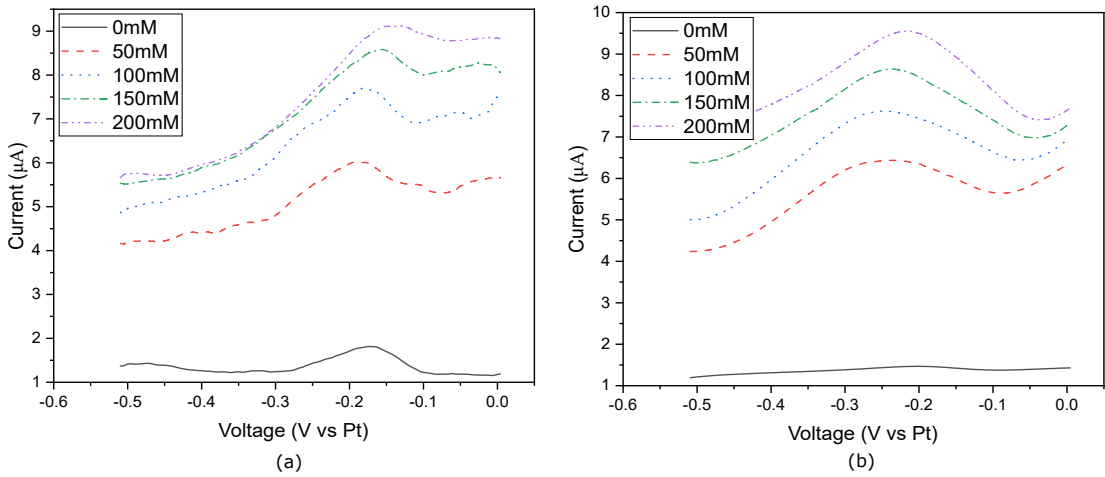


Figure 5.13: Voltammograms of sodium in DI water solutions with concentrations ranging from 0 mM to 200 mM, obtained using (a) the PULSE 2.0, and (b) the benchtop potentiostat.

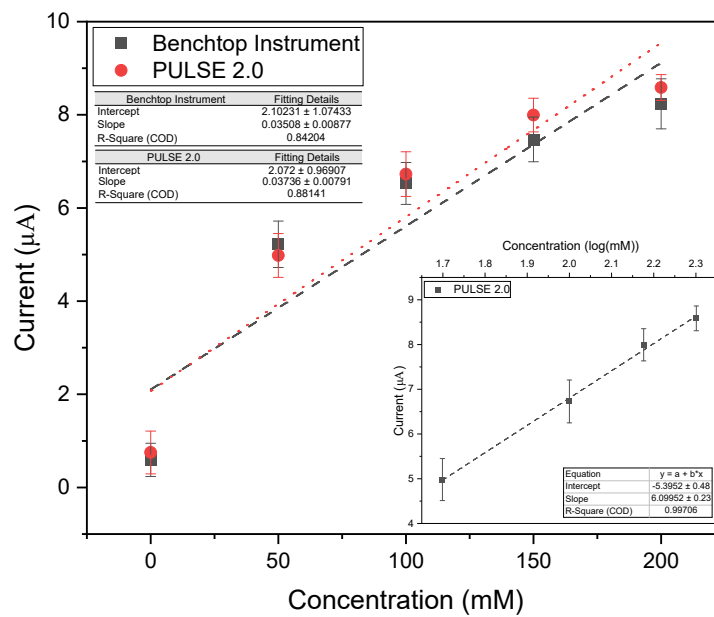


Figure 5.14: Sodium calibration curves in DI water (0 mM to 200 mM) using the benchtop potentiostat and PULSE 2.0. Inset: relationship between the logarithmic concentration of sodium and peak oxidation current measured with PULSE 2.0 ($n=3$, mean $\pm$ SE).

A calibration curve for sodium detection was generated, as shown in Figure 5.14, using SWV to measure oxidation peak currents across various sodium concentrations, ranging from 0 mM (DI water only) to 200 mM. The experiment was performed in triplicate using both the PULSE 2.0 system and the benchtop potentiostat to ensure the reliability of the PULSE 2.0 performance. Further details on the data can be found in Appendix D.7.

The results revealed a strong correlation between the two systems, with a correlation coefficient of 0.99, highlighting the reliability of the PULSE 2.0 for sodium measurements. The PULSE 2.0 system exhibited a slope of 37.4 nA/mM and a sensitivity of 6.96 $\mu\text{A}/\text{mM}/\text{cm}^2$, while the benchtop potentiostat showed a slightly lower slope of 35.1 nA/mM and a sensitivity of 6.54 $\mu\text{A}/\text{mM}/\text{cm}^2$, representing a 6.4% increase, respectively, for the PULSE 2.0. This indicated that the PULSE 2.0 provided a marginally stronger electrochemical response per unit concentration. In terms of detection limits, the PULSE 2.0 achieved a LOD of 2.69 mM, which was comparable to the benchtop instrument's LOD of 2.58 mM (4.26% difference). These results suggested that the PULSE 2.0 performed equivalently with the benchtop system, demonstrating its reliability and suitability for sodium detection.

Furthermore, these findings suggested that the PULSE 2.0 system was capable of reliably measuring sodium levels. Sodium concentrations in the rumen of a healthy cow typically measure approximately 116 mM [1]. The PULSE 2.0 system's sensitivity and detection range position it as a viable tool. This application was explored in more detail in subsequent sections of this chapter.

5.3.8 Rumen Analysis

Simultaneous pH and temperature measurements were performed using a custom-designed sensor holder that allowed the immersion of both sensors in a beaker containing ruminal fluid, as illustrated in Figure 5.15. The pH calibration, conducted in triplicate over the range of 3 to 11, is shown in Figure 5.16. The calibration yielded a slope of -71.1 mV/pH, indicative of a super-Nernstian behaviour [20]. The temperature measurements showed a decreasing trend with increasing pH, as expected from literature [23].

Using the pH calibration data from ruminal fluid, the calibration equation was rearranged to calculate pH from the measured voltage (M_v):

$$pH = \frac{M_v - 0.5596}{-71.1} \quad (5.3)$$

To assess the accuracy of the PULSE 2.0, a control ruminal fluid sample was measured and compared to the values of a commercial pH probe as illustrated

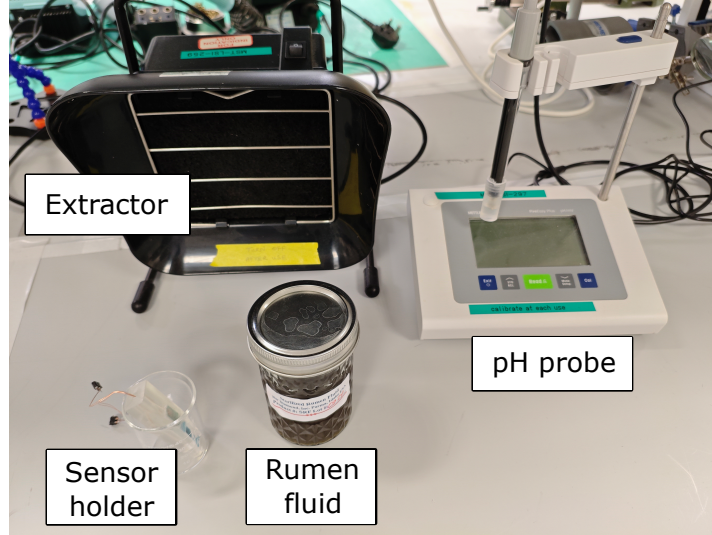


Figure 5.15: Experimental setup for rumen analysis, including the rumen fluid, pH and temperature holder on a beaker, a pH probe, and an air extractor.

in Figure 5.17. The average pH value obtained using the PULSE 2.0 was 9.256, compared to 9.267 obtained using a standard pH probe, translating to an accuracy of 99.88%. The measured pH was more alkaline than typically expected for a healthy bovine rumen, which may be attributed to the sterilisation process of the fluid [24].

Potassium and sodium concentrations in the ruminal fluid were quantified using both the PULSE 2.0 system and a benchtop instrument. The concentrations were calculated using logarithmic calibration equations derived from prior experiments:

$$[K^+](\log(mM)) = \frac{M_K - 8.5578 \times 10^{-7}}{3.493 \times 10^{-6}} \quad (5.4)$$

$$[Na^+](\log(mM)) = \frac{M_{Na} - 5.395 \times 10^{-6}}{6.099 \times 10^{-6}} \quad (5.5)$$

Here, $[K^+]$ and $[Na^+]$ represent the potassium and sodium concentrations in $\log(mM)$, respectively, based on the measured currents M_K and M_{Na} .

The results are summarized in Figure 5.17. For potassium, the PULSE 2.0 system measured a concentration of 2.646 mM, compared to 2.62 mM using the benchtop instrument, yielding an accuracy of 99.02%. For sodium, the PULSE 2.0 detected a concentration of 7.43 mM, closely aligned with the 7.36 mM obtained with the benchtop instrument, resulting in an accuracy of 99%. A t-test revealed no statistically significant difference between the PULSE 2.0 and benchtop instrument datasets (tested at $p < 0.05$), indicating comparable values across both measurement systems. These results highlighted the high accuracy of PULSE

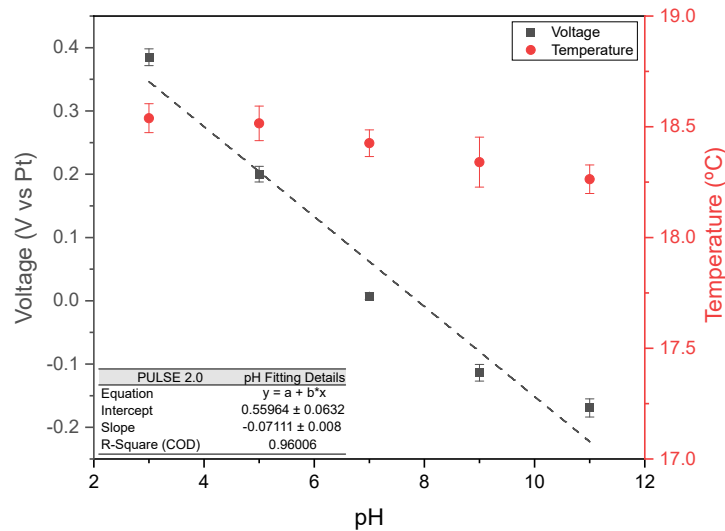


Figure 5.16: pH calibration curve of ruminal fluid from pH 3 to 11 using the PULSE 2.0 (n=3, mean $\pm$ SD).

2.0 system, confirming its suitability for real-world applications in animal welfare monitoring. All the results in this section can be found in Appendix D.8.

5.3.9 Discussion

This section provides a detailed comparison with the SoTA in potentiostats development. Table 5.4 presents a comprehensive summary of key features, including the device size or PCB footprint (depending on the available information), communication protocols, type of user interface, measurement range, supported measurements, and the number of sensors that can be interfaced or integrated simultaneously.

Table 5.4: Comparison of SoTA potentiostats against the PULSE 2.0, including size, communication (Comm.), user interface, measuring range, measurement capabilities, and sensors interfaced or integrated.

System	Size (mm)	Comm.	Interface	Meas. Range	Measurements	Sensors
UnpadStat [10]	150×95×45	USB	Touch-screen	$\pm 5V$; $\pm 100nA$ - $\pm 10mA$	CV	1
Vidotto et al. [11]	-	Bluetooth	APP/Website	$\pm 1.5V$; $\pm 1mA$	CA, CV, LSV DPV, SWV	1
Boni et al. [12]	-	Wi-Fi	Website	$\pm 1.65V$; $\pm 620nA$ - $\pm 62\mu A$	DPV, CA, CV	4
NanoStat [13]	4×40×20	USB, Wi-Fi	Website	-	CV, SWV, CA, PV	1
MYSTAT [14]	60×81	USB	APP	$\pm 12V$ $\pm 200mA$ - $\pm 2\mu A$	Potentiometry, CA, CV	1
PULSE 2.0	67.3×52.9×4.3	USB Bluetooth	APP	$\pm 1.65V$ $\pm 155mA$-$\pm 16nA$	OCP, CV, CA, SWV, LSV, Temperature Motion	4

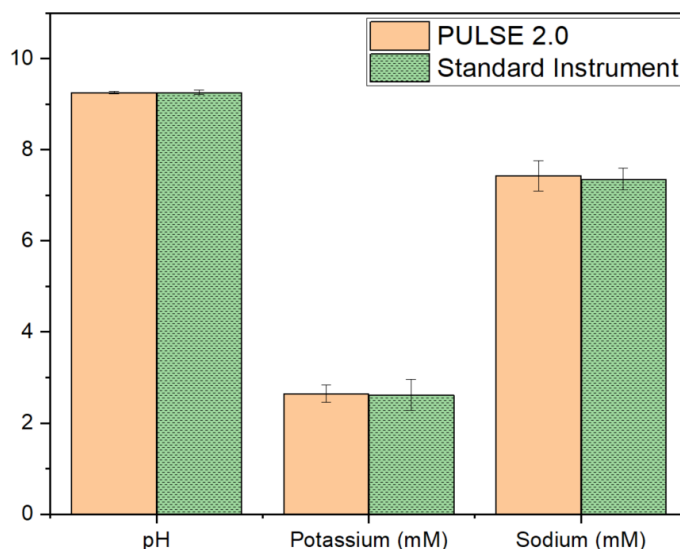


Figure 5.17: Comparison of pH, potassium, and sodium measurements from PULSE 2.0 and a standard instrument ($n=3$, mean $\pm$ SD).

Setiyono et al. [10] developed the UnpadStat, a device capable of interfacing with a single electrochemical sensor. This device operated continuously for 14 hours using three lithium-ion batteries (3.7 V, 2500 mAh each), indicating significantly higher power consumption compared to the PULSE 2.0, which achieved comparable operation times with a single, smaller-capacity battery (3.7 V, 500 mAh). The user interface of the UnpadStat was integrated into a touch screen on the device, which added to the power demand by consuming 410 mA during operation, and increased the device's footprint due to its size (133.50(L) $\times$ 84(W) $\times$ 6.8(H)). In terms of testing, the UnpadStat was validated against a standard device using a single concentration of analyte, which could be considered insufficient for comprehensive validation and strong conclusions. Moreover, the study lacked statistical tests to validate the performance of the device. In contrast to the PULSE 2.0, which provided wireless connectivity via Bluetooth to enhance versatility and user-friendliness, this device relied exclusively on USB communication and stored data on an SD card, thereby lacking wireless connectivity options.

Vidotto et al. [11] developed a potentiostat for a single sensor interface, which, while functional, presented critical limitations compared to the PULSE 2.0. The device operated within a current range of 30 μ A to 1 mA, which is narrower than the range supported by the PULSE 2.0, limiting its applicability for detecting lower current signals. The device supported multiple voltammetric techniques, several of which overlapped with the capabilities of the PULSE 2.0. Data was transferred to a smartphone via Bluetooth and subsequently to a website, whereas the PULSE 2.0 offered more robust data management options, providing local storage and improving reliability in disconnected environments. No information

was provided about the physical size of the device, making direct comparisons to portability and compactness difficult.

Boni et al. [12] developed a potentiostat capable of interfacing with four voltammetric sensors, operating within a current range of ± 620 nA to ± 62 μ A and a voltage range of ± 1.65 V. The device was powered by two 1.5 V AA batteries with a capacity of 2700 mAh, enabling continuous operation for approximately 15 hours. This run-time is comparable to the PULSE 2.0 performance and utilised larger size batteries that contributed to a bulkier form of approximately (10×10) cm² based on figure analysis. However, this footprint excluded sensor connectors, which rely on external wiring and off-the-shelf box connectors. These additional components further reduce the portability of the device, making it less suitable for POC applications compared to the compact and integrated design of the PULSE 2.0. As mentioned before, this device supported four electrochemical sensors but was limited to performing DPV, CA, and CV. Despite these techniques being common, the lack of support for potentiometric measurements and the absence of additional sensing capabilities restricted its application scope.

Lee and Burke [13] introduced NanoStat, a device whose simplicity may suit specific applications; however, it was limited to voltammetric techniques, many of which overlap with the capabilities of the PULSE 2.0. The NanoStat operated for approximately six hours, powered by a 280 mAh lithium polymer battery. No details about the measurement range are provided, leaving its applicability for many electrochemical detection tasks uncertain. This omission contrasted with the PULSE 2.0, which explicitly supported a broad range of current and voltage measurements, demonstrating its adaptability to a wide array of sensing scenarios. Furthermore, the subjective validation of NanoStat undermined confidence in its reliability, whereas PULSE 2.0 offered a more comprehensive validation approach, ensuring robust and reproducible results.

Irving et al. [14] presented MYSTAT, a potentiostat capable of interfacing with a single electrochemical sensor supporting up to four electrodes. While it accommodated both voltammetric and potentiometric techniques similar to the PULSE 2.0, it offered fewer supported techniques, limiting its application range. The PCB measured (60×81) mm², significantly larger than the PULSE 2.0's compact (49.4×48.5) mm² PCB. Despite its larger footprint, this device accommodated fewer sensing capabilities, whereas the PULSE 2.0 integrated multi-sensing capabilities, including temperature, motion, potentiometric, and voltammetric sensors within a more compact design. Regarding the measurement range, MYSTAT supported currents from ± 200 mA down to ± 2 μ A, which was narrower than the extensive range supported by the PULSE 2.0, extending into the nanoampere scale for high-precision applications. For voltage, MYSTAT

can measure a broader range of ± 12 V compared to the PULSE 2.0's ± 1.65 V, making it suitable for applications requiring higher voltage measurements. However, no electrochemical measurement conducted in this study required such high voltages. With MYSTAT, data collection was made through a USB cable, lacking wireless connectivity options. This limitation reduces the portability and ease of use, particularly in field applications where wireless communication is advantageous. Although MYSTAT supported only a single sensor, the PULSE 2.0 enabled simultaneous multiple sensors operation, integrating temperature, motion, potentiometric, and voltammetric measurements, thereby offering greater efficiency and versatility. The validation conducted in Irving et al. [14] reflected a narrow approach, whereas the PULSE 2.0 emphasised comprehensive validation, ensuring reliable performance across diverse sensing scenarios and analytes.

The PULSE 2.0 demonstrated a clear advancement over existing portable potentiostats and multi-sensor interfaces by addressing several key limitations of comparable devices. Unlike the competing systems, which primarily focus on voltammetric techniques with limited sensor integration, the PULSE 2.0 integrated multiple sensing capabilities, including potentiometric, voltammetric, motion, temperature, and battery monitoring, all within a compact and portable design. This integration allowed for simultaneous multi-parameter measurements, offering versatility for a wide range of applications. In terms of power efficiency, PULSE 2.0 achieved longer operational durations using a single 500 mAh Li-ion battery, outperforming most of its counterparts in continuous-use scenarios. Furthermore, the device supported both USB and Bluetooth communication, ensuring reliable communication even in remote or Wi-Fi deficient areas, unlike other systems constrained by wired or exclusive Wi-Fi connectivity. The robust electrochemical validation of PULSE 2.0, conducted with rigorous correlation analysis, underscored its reliability and precision, contrasting with the often limited validation of other devices. The compact size, extensive operational range, and multi-parametric capabilities highlighted the novelty of the PULSE 2.0, making it a pioneering tool for POC and portable sensing applications in livestock welfare.

5.4 Conclusion

The development and evaluation of the second-generation PULSE system, PULSE 2.0, marked a significant advancement in portable, multi-sensor platforms tailored for livestock health monitoring. This chapter successfully achieved its objectives, demonstrating the potential of PULSE 2.0 to address critical challenges in precision agriculture.

The PULSE 2.0 system exhibited robust sensing capabilities, expanding upon the first prototype (as detailed in Chapter 3) by integrating sensors for pH, temperature, sodium, and potassium detection. Performance evaluations revealed high accuracy in these measurements, with a pH calibration sensitivity of -70.38 mV/pH , following a super-Nernstian behaviour, and reliable sodium and potassium detection. Sodium detection achieved a LOD of 2.69 mM with a sensitivity of $6.96 \mu\text{A/mM/cm}^2$, while potassium detection exhibited a LOD of 0.240 mM and a sensitivity of $13.3 \mu\text{A/mM/cm}^2$. Benchmarking against a standard benchtop potentiostat yielded correlation coefficients exceeding 0.999, underscoring the reliability of the PULSE 2.0 system. In particular, when detecting potassium, the PULSE 2.0 demonstrated an 86.6% lower LOD in comparison with the benchtop instrument, demonstrating its ability to detect lower analyte concentrations.

The device demonstrated potential for application in bovine health monitoring by performing in complex ruminal fluid, a challenging biological medium. pH measurements in rumen fluid exhibited a sensitivity of -71.1 mV/pH , consistent with super Nernstian behaviour. Additional evaluations in ruminal fluid revealed high accuracy for all analytes, with pH measured at 9.26 (99.88% accuracy), potassium at 2.646 mM (99.02% accuracy), and sodium at 7.36 mM (99.00% accuracy). These results validate the PULSE 2.0 as the first reported demonstration of detecting these critical analytes in ruminal fluid, positioning it as a pioneering tool in bovine health monitoring.

The system's versatility is further emphasised by its operational range, multi-parametric capabilities, and dual communication capabilities, including USB and Bluetooth, which enhance usability by offering options for real-time communication to mobile devices or computers, as well as offline data storage for remote locations with limited connectivity. The inclusion of dual power supply options, including a rechargeable battery, ensured uninterrupted operation during extended field deployments, addressing the practical challenges of on-site livestock monitoring. The choice of battery and duty cycle is crucial for the battery's lifespan, potentially exceeding one year under specific conditions. Additionally, the compact size improved portability, making it convenient for handheld use. The PULSE 2.0 sets a new benchmark for portable POC devices by integrating physical sensors (temperature and motion) and chemical sensors (pH, sodium, and potassium) alongside voltammetric and amperometric techniques. These features not only differentiated it from previously reported systems but also highlighted its potential to revolutionise field-based livestock health diagnostics.

In summary, this work presented a novel portable POC device capable of integrating multi-parametric sensing within a compact, field-ready platform tailored

for real-time bovine health monitoring in complex biological media.

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CONCLUSION

This thesis presented the design, development, characterisation, validation, and testing of two innovative sensing platforms for multi-parameter health monitoring in livestock. Bridging the fields of electrochemistry, embedded systems, and bovine health, this doctoral research addressed the growing need for reliable, field-deployable devices capable of real-time, accurate measurements in complex farming environments.

The key contributions of this work include the design and fabrication of robust HW incorporating AFE circuitry for voltammetric and potentiometric measurements, the development of FW to enable precise and versatile electrochemical techniques, and the integration of a user-friendly APP for data visualisation, analysis, and device control. The optimisation of the first prototype led to a second-generation device with enhanced functionality and portability.

A major achievement of this research is the ability to obtain highly accurate measurements in ruminal fluid, with accuracies of 99%, including sodium (99.00%), potassium (99.02%), and pH (99.88%). Additionally, this work enabled rapid detection using OCP, achieving a response time of ≈ 2 seconds. Furthermore, a novel POC system was developed for real-time analysis of key analytes in ruminal fluid, contributing to advancements in monitoring bovine welfare.

This chapter summarizes the research conducted, highlighting the key achievements and original contributions of each chapter while situating the findings within the broader context of bovine health monitoring and precision farming. Additionally, it explores potential future directions to enhance the platform's capabilities, expand its applications, and address remaining challenges.

6.1 Summary

Chapter 1 established the context and foundation for this PhD research by presenting the problem statement, objectives, and theoretical underpinnings. The chapter outlined the main objective and goals of the thesis, emphasising the development of a comprehensive approach, including the development of HW, FW, and system integration to address current challenges. Additionally, it provided an overview of fundamental electrochemical principles and techniques, which served as a critical knowledge base for designing and optimising the AFE circuits essential for sensor interfacing and the FW required for precise execution of electrochemical techniques.

Chapter 2 provided a critical foundation for this thesis by offering an in-depth review of the key physiological parameters and sensing technologies relevant to bovine health monitoring. This comprehensive literature review identified essential analytes, such as pH, temperature, nitrate, nitrite, sodium, and potassium. These all hold potential for improving bovine health monitoring and early disease detection. The chapter also highlighted significant knowledge gaps in current smart bolus systems, particularly the limited scope of measurable parameters, which restricts their practical utility. The review emphasised the importance of integrating additional analytes, long-term reliability, and real-time data collection capabilities to enhance the functionality of smart boluses. Furthermore, the chapter discussed how these devices could play a pivotal role in precision farming and environmental sustainability by enabling methane emission monitoring or associated analytes and supporting dietary or veterinarian interventions to reduce GHG emissions. By synthesising insights from biosensing and electronics, the review underscored the need for multidisciplinary research to advance the development of next-generation smart boluses.

Chapter 3 described the development of the PULSE, a portable multi-parameter electrochemical sensing platform tailored for bovine health monitoring. Unlike existing solutions, this system was designed from the ground up to provide unprecedented adaptability to the application and sensors. The work introduced several key innovations, including a fully customisable HW architecture with an adaptive AGC AFE circuitry capable of operating across a wide dynamic range — from milliamps to nanoamps and $\pm 1.65\text{ V}$ - with high-resolution performance, including $805\text{ }\mu\text{V/step}$ for potentiometric sensing. In addition, the chapter detailed the integration of robust FW and a bespoke user interface through a dedicated APP, enabling real-time, multi-parametric measurements. Notably, the platform supports voltammetric, amperometric, and potentiometric techniques, as well as the measurement of acceleration and temperature, setting it apart from current

SoTA devices that typically offer limited electrochemical capabilities. This chapter establishes PULSE as a foundational step toward more scalable, responsive, and field-deployable health monitoring tools in precision livestock farming.

The PULSE was benchmarked against a gold-standard benchtop potentiostat while performing CV and CA, measuring ferri-ferrocyanide solutions from 0 mM to 5 mM, achieving correlation coefficients >0.99 , and demonstrating highly accurate measurements. Similarly, a statistical analysis in OCP measurements for pH levels ranging from 3 to 11 in PBS solutions achieved a correlation coefficient of 0.999. These results firmly established the PULSE system's reliability in electrochemical sensing applications. This chapter further highlighted the PULSE system's key operational advantages: rapid response times (improved in 48 s), portability, and wireless communication capabilities, paired with custom data processing and tailored APP. Unlike conventional laboratory devices, the PULSE system's design prioritised field usability, offering real-time analysis and reducing the need for sample transportation, storage, and specialised personnel. The in-house development approach ensured greater control over cost, design customisation, and compatibility with specific sensor requirements. These contributions position the PULSE as a scalable, efficient solution not only for livestock health management but also for broader applications in environmental monitoring and healthcare.

Chapter 4 established a major advancement in livestock health monitoring by demonstrating the successful integration of pH, temperature, nitrate, and nitrite sensors into the PULSE platform. This work is the first to report the real-time detection of both nitrate and nitrite in the context of bovine analysis using a portable sensing system, filling a critical gap in the literature where no prior studies have combined the measurement of these biochemical markers with physical and chemical parameters in a smart bolus-compatible format. The system exhibited excellent performance in diverse fluid environments, including PBS, artificial ISF, and complex ruminal fluid, with pH accuracy reaching 98.9% and strong linearity ($R^2 > 0.99$). Notably, sensitivities of $6.32 \mu\text{A}/\text{ppm}/\text{cm}^2$ and $1.92 \mu\text{A}/\text{ppm}/\text{cm}^2$ for nitrite and nitrate, respectively, were achieved, demonstrating the system's capability to accurately quantify these environmentally and physiologically significant analytes.

Comparative analysis against a commercial benchtop potentiostat revealed superior performance by the PULSE system, including 65.83% and 58.3% lower LOD for nitrate and nitrite, respectively, and a 24.5% increase in sensitivity for nitrite. These results not only validate the precision of the system but also highlight its potential as a field-deployable solution. The ability to monitor key biochemical parameters alongside pH and temperature sets PULSE apart from existing plat-

forms, none of which currently support a comprehensive multi-analyte detection strategy. This integration paves the way for early disease detection, improved animal welfare, and more sustainable livestock management through real-time data insights that can drive methane and GHG mitigation strategies. The findings of this chapter lay the groundwork for future research exploring multi-modal sensing in precision agriculture, with PULSE positioned as a pioneering platform in this emerging field.

Chapter 5 advanced the PULSE system through the development of its second-generation prototype, PULSE 2.0. Designed as a portable, multi-parametric platform for livestock health monitoring, PULSE 2.0 serves as a POC device intended to inform future iterations of an implantable smart bolus system. Nevertheless, it is important to note that this current version is not designed for internal use within animals. This enhanced version prioritised miniaturisation, functionality, and user accessibility by incorporating a compact form factor, Bluetooth communication, and a rechargeable Li-ion battery to enable extended autonomous operation in real-world farming environments. Expanding beyond the capabilities of the original system, PULSE 2.0 integrated SWV for the detection of critical electrolytes such as sodium and potassium, alongside continued pH and temperature measurements. This combination of analytes, essential for monitoring ruminal health, electrolyte balance, and overall animal welfare, has not been previously demonstrated in any portable platform targeting livestock applications in ruminal fluid. Performance evaluations conducted in complex ruminal fluid confirmed high accuracy across all parameters, with pH achieving 99.88%, sodium 99%, and potassium 99.02% accuracy. Sensitivities and LOD were fully adequate for field monitoring needs, further validating the platform’s robustness.

Unlike existing systems that typically monitor only limited biochemical markers or rely solely on laboratory conditions, PULSE 2.0 enables simultaneous real-time sensing of physical, chemical, and biochemical parameters in complex biological environments. Dual communication modes (USB and Bluetooth), combined with a custom-developed APP, provided a complete ecosystem for efficient, remote livestock monitoring. The portability and multi-parametric capability of PULSE 2.0 position it as a novel POC solution for bovine health diagnosis. The PULSE 2.0 marked a significant advancement in precision agriculture, providing a scalable and efficient solution to support sustainable bovine health monitoring and management practices.

6.1.1 Use Case Scenario

The first-generation PULSE prototype was developed primarily as a laboratory-based evaluation platform to validate the feasibility of multi-parameter electrochemical sensing for livestock health monitoring. Its use case focused on controlled environment testing, enabling the assessment of sensor performance in ideal and semi-realistic biofluids such as PBS, and artificial ISF. This approach allowed the validation of critical functionalities, such as measurement's accuracy, real-time communication, HW robustness, and FW stability. Thus, PULSE served as a crucial proof-of-concept platform, demonstrating that real-time, multi-analyte electrochemical monitoring is technically feasible, and highlighting the system's adaptability for eventual integration into a smart bolus form factor.

The second-generation PULSE 2.0 prototype was designed to transition the system from a laboratory device to a field-ready platform. Its use case centred on portable, on-site monitoring of complex biological samples such as rumen fluid, leveraging enhanced miniaturisation, battery-powered operation, and Bluetooth communication. By incorporating dual communication modes, rechargeable battery management, extended analyte coverage, and FW capabilities, the PULSE 2.0 moved forward to the integration of a smart bolus. The successful performance of PULSE 2.0 in rumen fluid conditions strongly supports its suitability for further encapsulation into a smart bolus, thus demonstrating both technical and application relevance for real-world livestock health monitoring.

The iterative development and validation of these two prototypes provided critical insights into the practical challenges and opportunities associated with real-time, multi-parameter sensing in agricultural environments. Through this work, it was possible to progressively bridge the gap between electrochemical sensors and electronics for bovine health monitoring, demonstrating the technical feasibility, adaptability, and future potential for integration into a smart bolus device. Building upon these, the key contributions of this thesis are discussed below, highlighting the novel advancements made and the successful achievement of the project objectives.

6.2 Contributions

This work significantly advances the current SoTA in the field of multi-parameter sensing for livestock health monitoring by delivering a novel, integrated, and versatile system that directly addresses limitations identified in existing technologies. The primary objective — to develop and validate a novel multi-parametric embedded system with customised HW, FW, and APP — has

been successfully achieved, with the system validated through extensive laboratory and biological media testing. This research offers important contributions by addressing specific technological gaps and proposing validated solutions aligned with the challenges of precision livestock farming.

The development of the first-generation PULSE system introduced a portable, multi-parameter electrochemical sensing platform capable of monitoring critical health parameters such as pH, temperature, nitrate, nitrite, sodium, and potassium. Unlike existing systems reported in the literature, which are often limited in parameter range, the PULSE integrated physical, chemical, and biochemical sensing capabilities into a single platform. It also offered a robust and reliable solution with versatile electrochemical techniques, including OCP, CA, CV, SWV, and LSV. This capability allows for accurate, real-time data acquisition and potential application in agricultural environments. Combined with a user-friendly APP, the platform provided real-time wireless communication and intuitive device control, enhancing accessibility and convenience for the user.

The first-generation PULSE system was validated through rigorous testing, demonstrating its accuracy and reliability. Measurements of pH, nitrate, and nitrite in laboratory conditions and ISF yielded excellent results, with correlation coefficients exceeding 0.99 when benchmarked against standard laboratory instruments. Testing in rumen fluid further validated the system's practical applicability, achieving high accuracies, including 98.9% for pH. This achievement directly addressed the project's performance validation objective and demonstrated the system's robustness beyond ideal laboratory conditions.

The development of the second-generation PULSE 2.0 prototype marked a significant advancement, featuring miniaturisation, enhanced portability, and improved usability tailored to the challenges of on-site, real-time monitoring. The inclusion of dual communication modes (USB and Bluetooth) and a rechargeable battery further optimised the system for on-site, real-time health monitoring in remote farm environments. This device also expanded its sensing capabilities, adding the ability to monitor sodium and potassium through SWV, parameters essential for electrolyte balance monitoring in cattle and previously underexplored in embedded ruminal systems. The system's accuracy in complex ruminal fluid samples, achieving over 99% accuracy for pH, sodium, and potassium measurements, positioned it as a highly reliable tool for field-based diagnostics.

The novelty of this work lies in its unique integration of multiple electrochemical techniques into a single platform tailored for future smart bolus deployment, and its demonstration of nitrate, nitrite, pH, potassium, and sodium sensing for rumen fluid analysis, a field with limited prior exploration. Thus, this thesis advances both the sensing methodology and the application context, opening new

research questions for early disease detection in bovine welfare and sustainable livestock management. Furthermore, by addressing the challenges of real-world deployment and biological media testing, this thesis demonstrated the feasibility of a multi-parameter sensing platform for precision livestock monitoring. The PULSE system bridged the gap between laboratory diagnostics and practical field applications, offering a potential solution for sustainable herd management. Its ability to monitor key analytes associated with animal health, nutrition, and GHG emissions positions this technology as a valuable tool to improve economic efficiency and environmental sustainability in the dairy industry. Moreover, the modular and adaptable nature of the PULSE 2.0 extended its potential application beyond agriculture to diverse POC applications in human health, aquaculture, and industrial monitoring. By integrating electronic engineering and electrochemistry, this thesis contributed a novel POC system for real-time analysis of key analytes in rumen fluid.

In conclusion, this research has made significant advancements in the development of real-time, multi-parameter sensing systems for livestock health monitoring, providing a robust, portable, and versatile tool for precision farming. These contributions pave the way for future innovations in livestock management, improving both economic efficiency and environmental sustainability within the dairy industry and beyond.

6.3 Future Work

The development and validation of the PULSE 2.0 system marked a significant advancement in portable, multi-parameter sensing platforms for precision livestock monitoring. However, several opportunities remain for future enhancements and expansions. These improvements could further increase its applicability in real-world agricultural settings and support its transition toward deployment as a smart bolus device.

Starting with the optimisation of power consumption to improve the practicality of PULSE 2.0 for extended field deployments, future work should focus on reducing its power consumption. By implementing low-power HW components, optimising FW for energy efficiency, and selecting an appropriate battery, the device's battery life can be significantly extended. This would enable longer operational periods, whether integrated into a smart bolus or used as a wearable device, making it well-suited for continuous livestock monitoring in remote locations without frequent recharging. Additionally, future iterations of the PULSE 2.0 can use multi-layered sensor integration. The existing connector offers opportunities to enhance the system's sensing capabilities by supporting modular

expansion. Future designs could accommodate additional sensors through stackable PCBs, enabling a multi-layered architecture for more comprehensive health monitoring. This would allow the device to be easily customised, for example, to detect additional biomarkers such as glucose or lactate, which are also relevant to livestock health. Expanding the library of compatible sensors and analytes would further enhance the device's capabilities, allowing it to address a wider range of agricultural and environmental monitoring challenges. This could include sensors for additional electrolytes, biomarkers, gases, and disease-related indicators. By broadening its sensing capabilities, PULSE 2.0 can become a more powerful and holistic tool for herd management and environmental sustainability.

Another important direction for improvement and future work is the wireless connectivity, such as integrating IoT capabilities. This would allow for real-time data transmission to cloud-based platforms, facilitating remote monitoring and data sharing among veterinarians, farm managers, and researchers. Future iterations could also explore alternative communication protocols and antenna designs to support deployment in areas with limited coverage and potentially reduce the device's footprint.

Moreover, it is essential to investigate user-specific data communication requirements, as the frequency and volume of data transmission directly impact the device's battery life and operational longevity inside the animal. While PULSE 2.0 is already compact and portable, future designs will focus on adapting the platform for integration into a smart bolus encapsulation.

Before the technology can be deployed inside the animal, several critical steps must be completed. Mechanical studies are required to evaluate the structural design of the smart bolus, including assessments of impact resistance, pressure endurance, and thermal stability under ruminal conditions. Robust sealing and encapsulation strategies must be developed and validated to prevent fluid ingress and chemical leakage over extended operational periods. Long-term durability testing and biofouling resistance studies of all sensing elements are also essential, alongside the development of cleaning strategies, if necessary, to maintain sensor performance over time. In addition, an in-depth power budget analysis tailored to the smart bolus deployment must be carried out to ensure that the device can operate autonomously for the intended lifetime inside the animal. Wireless data transmission performance must be validated within biological tissues, including ruminal fluid, with a focus on optimising antenna designs and communication protocols for reliable operation. Finally, preliminary regulatory, ethical, and animal welfare considerations must be addressed to enable future in vivo deployment of the technology.

Finally, the PULSE can be adapted for applications beyond bovine health

monitoring. Future work could explore its use in milk quality evaluation, aquaculture, environmental monitoring, like water quality assessment, and human health applications, such as minimally invasive wearable biosensors.

Appendices

RESEARCH EXCELLENCE OUTPUTS

A.1 Published

- **Ferreira, C.**, Barry, F., Todorović, M., Sugrue, P., Teixeira, S. R., & Galvin, P. (2025). PULSE: A Fast Portable Unit for Lab-on-Site Electrochemistry. *Sensors*, 25(3), 762. <https://doi.org/10.3390/s25030762>
- **Ferreira, C.**, Todorovic, M., Sugrue, P., Teixeira, S., & Galvin, P. (2025). Emerging Sensors and Instrumentation Systems for Bovine Health Monitoring. *animal*, 101527. <https://doi.org/10.1016/j.animal.2025.101527>

A.2 Publications Under Review

- **Ferreira, C.**, Lynch,E., O’Herlihy, A., Barry, F., Nagle, L., Teixeira, S., Galvin, P., “Multi-parametric electrochemical sensing platform: application in animal welfare”.
- **Ferreira, C.**, Todorović, M., Sugrue, P., Teixeira, S., Galvin, P., “A Novel Point-Of-Care Multi-parametric Platform for Livestock Health Monitoring”.
- Thatte, C., Barry, F., **Ferreira, C.**, O’Keeffe, E., Cioce, V., Galvin, P., Teixeira, S., ”Microneedle based electrochemical sensor with multiplex detection in physiological environment and incorporated in a portable device”

A.3 Publications in Preparation

- **C. Ferreira**, S. R. Teixeira, P. Galvin, “A novel compact multi-parametric platform for livestock health monitoring”.

- C. Thatte, F. Barry, **C. Ferreira**, E. O’Keeffe, V. Cioce, P. Galvin, S. R. Teixeira, “Multiparametric Microneedle Sensors for Potassium, pH, and Calcium Monitoring”.

A.4 Conferences

- Ferreira C.R., et al. (2025). "Multi-parametric electrochemical sensing platform for the detection of pH, temperature, nitrate and nitrite in live-stock welfare". [Oral Presentation], 2025 Early Career Researcher Electrochemistry Symposium (SERRS), Maynooth, Ireland.
- Ferreira C.R., et al. (2024). "Development of a Portable Electrochemical Multi-Parametric Platform". [Oral Presentation], EPoSS Annual Forum 2024: Sensing the Future of Smart Systems: Bridging Minds and Micro-electronics, Cork, Ireland.
- Ferreira C.R., et al. (2024). "Development of PULSE Multi-Parametric Platform". [Oral Presentation], 8th International Conference on Bio-Sensing Technology, Seville, Spain.
- Ferreira C.R., et al. (2023). "Development of a Portable Multi-Parameter Electrochemical Sensing Platform". [Oral Presentation], 2023 Early Career Research Regional Symposium on Electrochemistry, Galway, Ireland.
- Ferreira C.R., et al. (2023). "1st Generation Portable Electrochemical Platform". [Demonstration], VistaMilk Internal Conference.
- Ferreira C.R., et al. (2023). "Ingestible Multi-Parametric Biosensing Smart Bolus". [Oral Presentation, remote], Biosensors 2023, Korea.
- Ferreira C.R., et al. (2022). "Multi-Parameter Smart Bolus System for Health Monitoring in Cattle". [Poster Presentation], IEEE Region 8 Student and Young Professional Congress 2022, Tunis, Tunisia.
- Ferreira C.R., et al. (2022). "Development of a Multi-Parameter Smart Bolus Device". [Poster Presentation], 44th Annual International Conference of the IEEE Engineering in Medicine & Biology Society (EMBC 2022), Glasgow, Scotland.
- Ferreira C.R., et al. (2022). "1st Generation Smart Bolus Systems". [Demonstration], VistaMilk Industry Day, Ireland.

- Ferreira C.R., et al. (2022). "Development of a Smart Bolus System for Multi-Parameter Monitoring of the Bovine Microbiome". [Oral Presentation], VistaMilk Annual Conference 2022, Ireland.
- Ferreira C.R., et al. (2021). "Development of a Smart Bolus System for Multi-Parameter Monitoring of the Bovine Microbiome". [Oral Presentation], VistaMilk Annual Conference 2021, Online Event.

A.5 Awards

- Awarded Wrixon Research Excellence Award by Tyndall National Institute (2024).
- Finalist and shortlisted for Graduate of the Year 2024 by Women in STEM Ireland Awards.
- Winner of the Tyndall Annual Recognition Award (TARA) in the category of Education & Public Engagement/Outreach Person of the Year 2023, by Tyndall National Institute.
- Best Scientific Picture award by VistaMilk SFI Centre (2023).

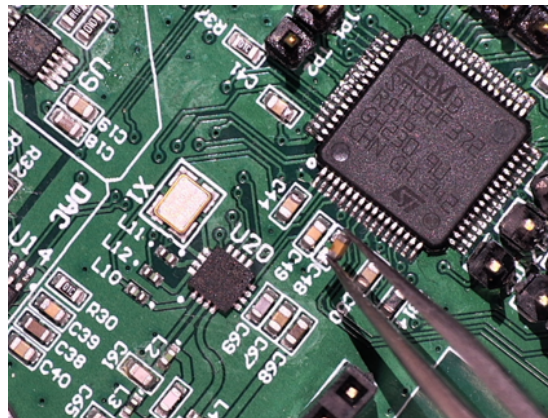


Figure A.5.1: 1st place picture.

- Awarded Education and Public Engagement (EPE) Activities Champion by VistaMilk SFI Centre (2023).
- Nominated for the TARA Award in the category of Education & Public Engagement/Outreach Person of the Year 2022, by Tyndall National Institute.
- Granted conference fees and student travel funding for the IEEE Region 8 Students and Young Professionals Congress, by IEEE (2022).

- Awarded Best PhD 3-Minute Presentation by VistaMilk SFI Centre (2022).

PULSE DEVELOPMENT

B.1 Schematic design

Table B.1.1: ADG779 analog switch truth table.

ADG779 IN	Switch S1	Switch S2
0	On	Off
1	Off	On

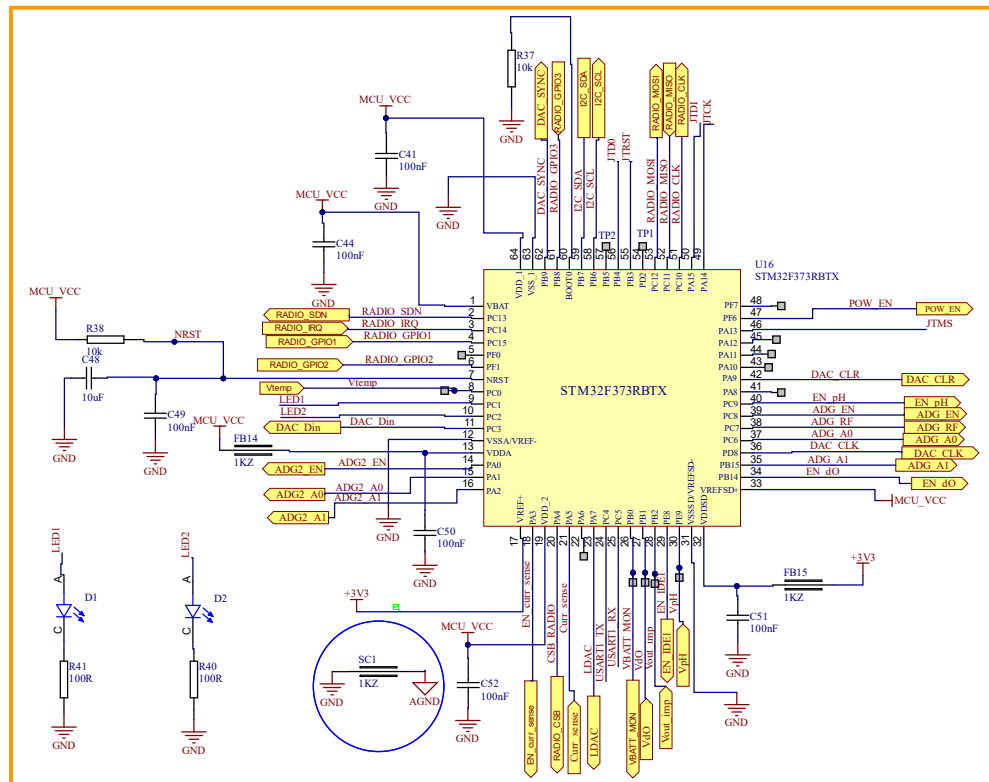


Figure B.1.1: MCU schematic design, using Altium Designer.





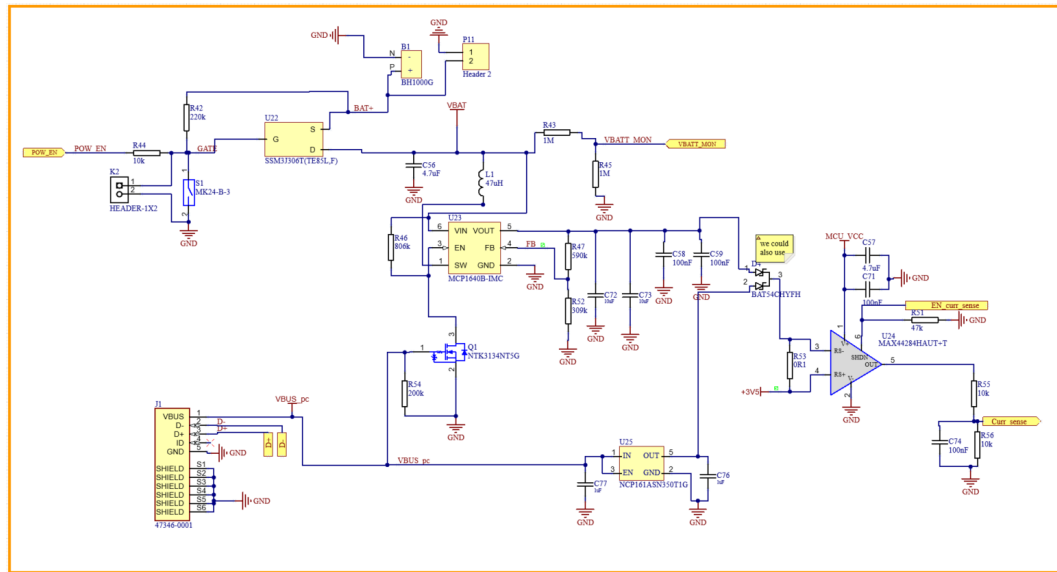


Figure B.1.5: Schematic design of power supply circuit, using Altium Designer.

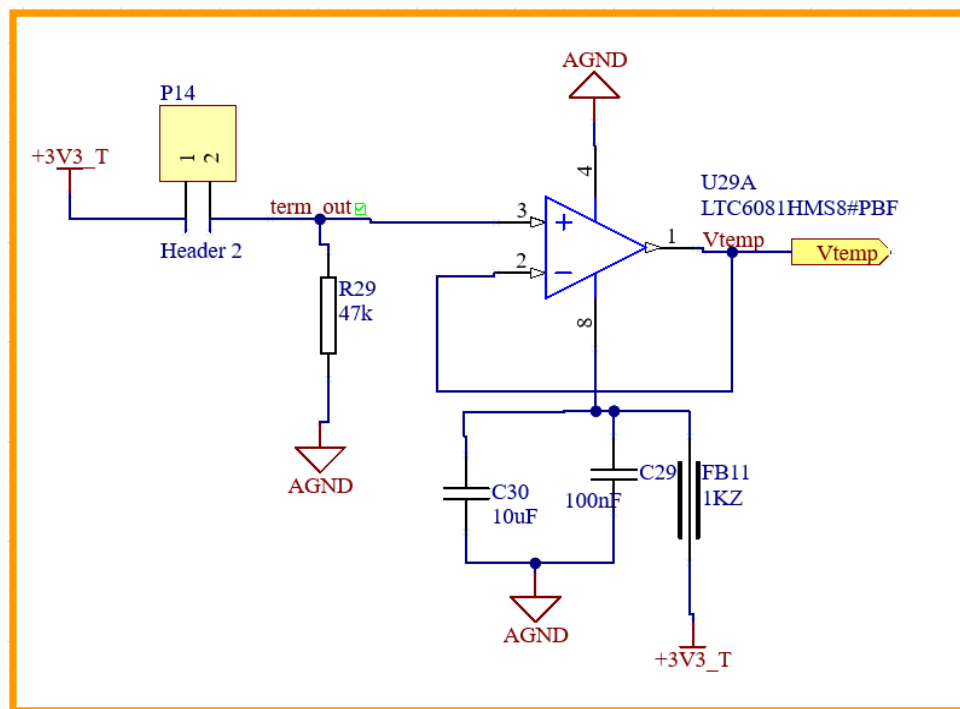


Figure B.1.6: Schematic design of temperature sensor, using Altium Designer.

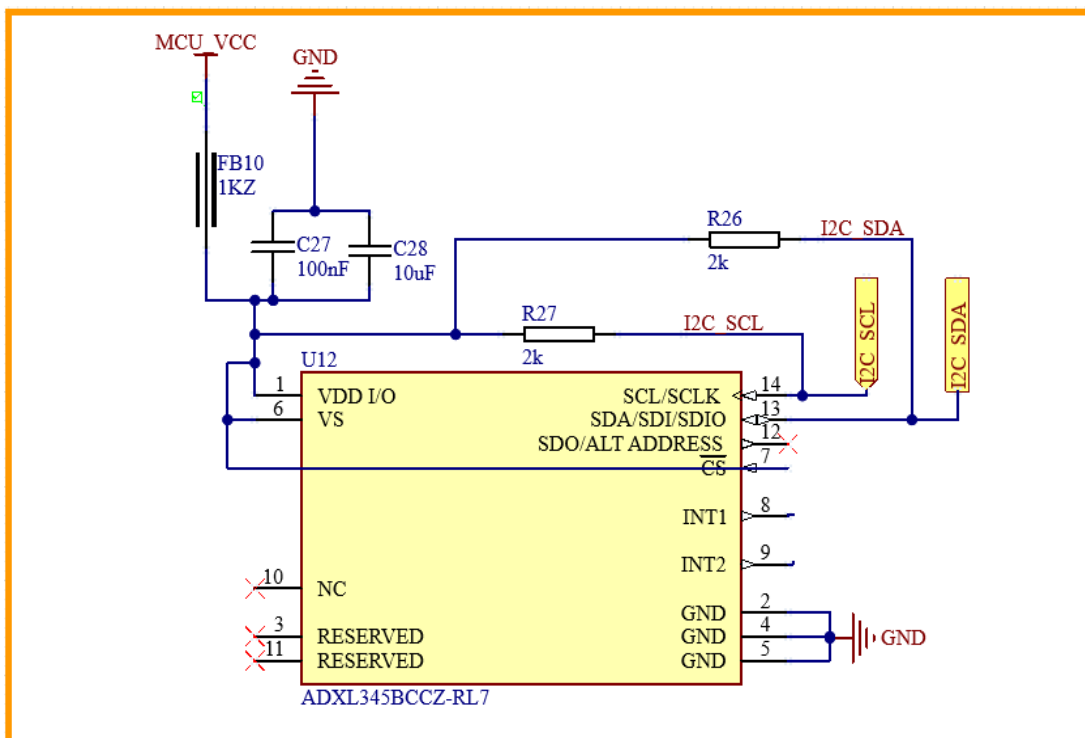


Figure B.1.7: Schematic design of motion sensor, using Altium Designer.

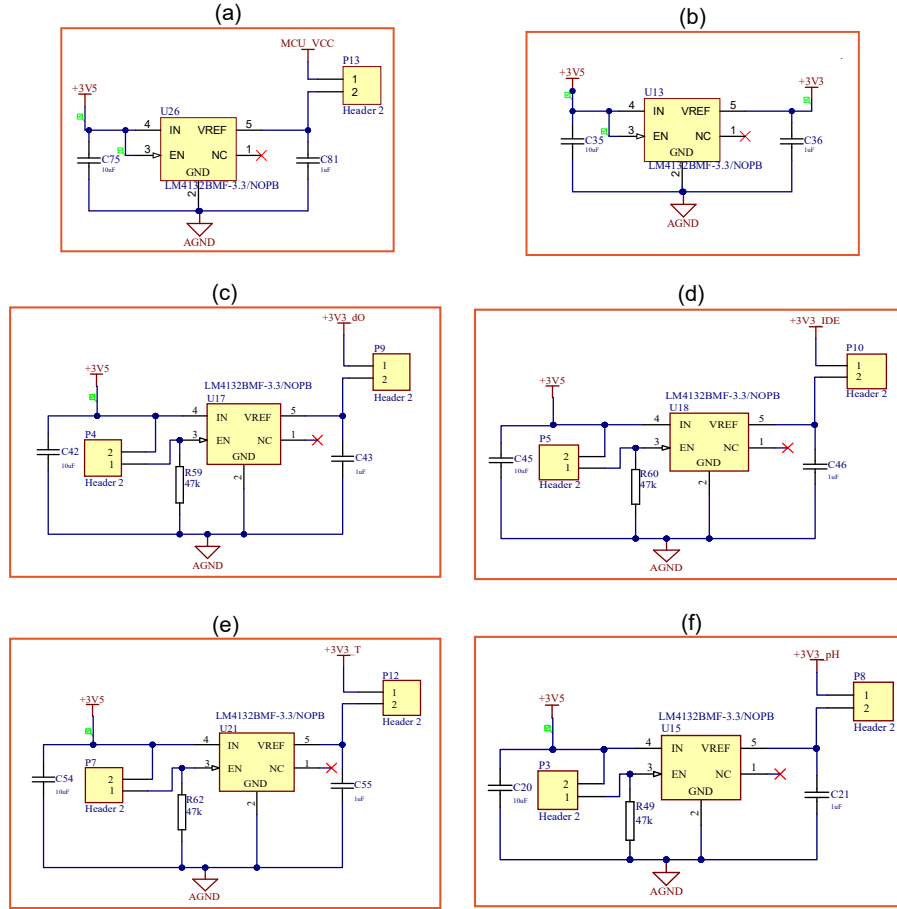


Figure B.1.8: Power supply circuit module for (a) the MCU, (b) the MCU and communication circuit, (c) the voltammetric AFE circuit, (d) the impedimetric AFE circuit, (e) the temperature circuit, and (f) the potentiometric AFE circuit.

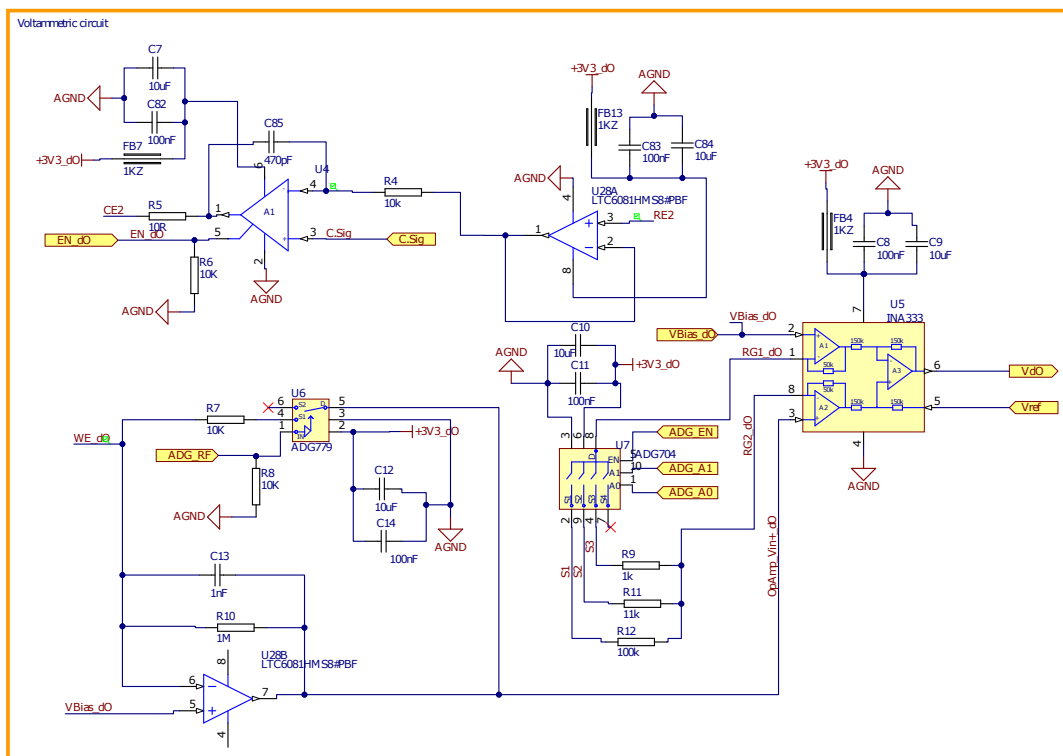


Figure B.1.9: Schematic circuit of the voltammetric AFE in Altium Designer.

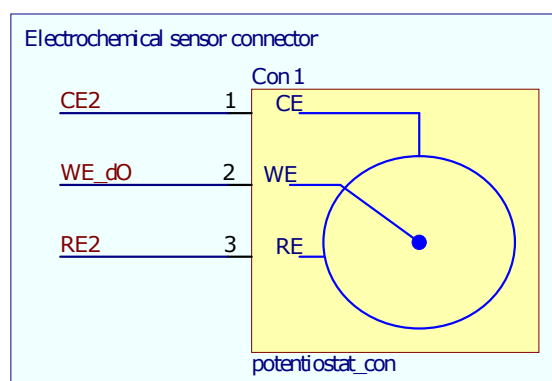


Figure B.1.10: Electrochemical sensor connector with 3-electrodes: CE2 - counter electrode, WE_d0 -working electrode, and RE2 - reference electrode.

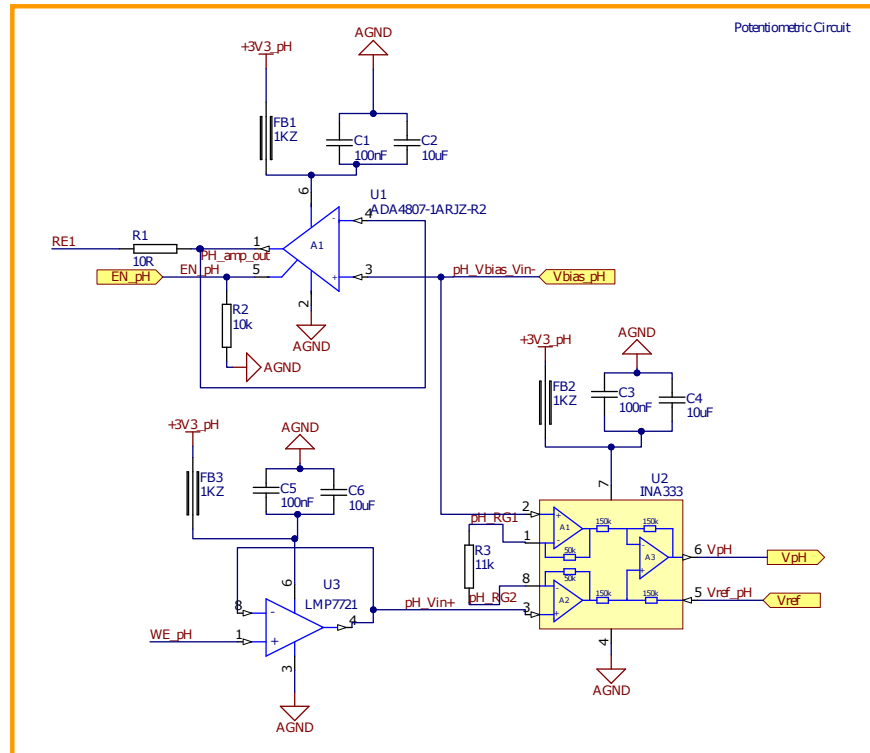
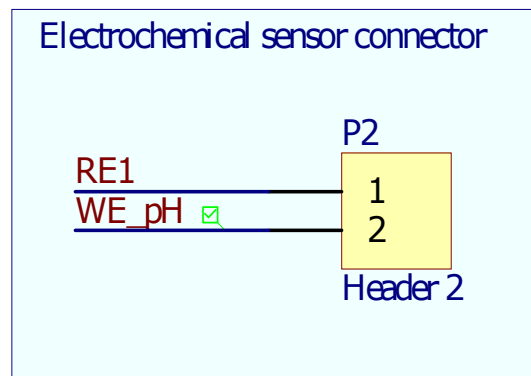


Figure B.1.11: Schematic circuit of the potentiometric AFE in Altium Designer.



Electrochemical sensor connector with 2-electrodes: WE_pH -working electrode, and RE1 - reference electrode.

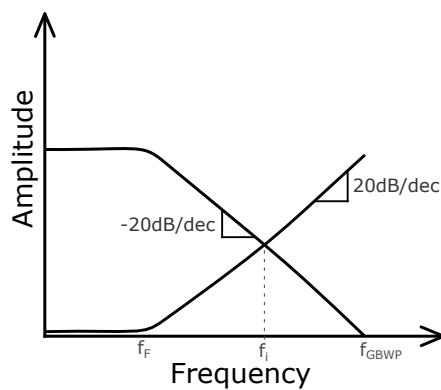


Figure B.1.12: Open loop gain and the reciprocal of feedback loop versus frequency.

B.2 Calibration of ADC for Voltammetric AFE

This calibration process was fundamental to ensure the accuracy and reliability of the measurements obtained from the developed device. To achieve this, a systematic calibration procedure was meticulously conducted, employing a benchtop voltage generator (Rigol DP1308A - Beijing, China) that can precisely sweep voltage from -1.5 V to 1.5 V, spanning the operational voltage range of the electrochemical cell under investigation.

During the calibration process, the voltage levels were annotated. This annotation included the recorded voltage values obtained from two distinct sources: firstly, the voltage measurements directly acquired using a high-precision multimeter (Fluke 289 True-RMS Data Logging Multimeter - Washington, U.S.), which served as a reference for the real voltage values; and secondly, the voltage values received through the PULSE’s serial port. The use of the multimeter provided means to verify and validate the accuracy of the electrochemical sensing platform’s measurements. Finally, a linear regression analysis was conducted, using the values in Table B.2.1.

Table B.2.1: Calibration data for the voltammetric circuit, with V_{serial} as the serial port voltage, and V_{real} is the real voltage value.

Vapplied (V)	Vserial (V)	Vreal (V)
-1.5	2.7222	2.7677
-1.25	2.46956	2.515
-1	2.47016	2.5153
-0.75	2.20685	2.253
-0.5	1.96227	2.0094
-0.25	1.70883	1.7407
0	1.45438	1.503
0.25	1.20073	1.2495
0.5	0.946484	0.9962
0.75	0.692033	0.7421
1	0.43677	0.4875
1.25	0.18152	0.2332
1.5	0	0.002

This dual-source annotation process ensured that the calibration dataset encompassed a wide range of voltage values, facilitating the creation of a robust and comprehensive calibration equation. Additionally, a graphical representation of these results is provided in Figure B.2.1. This plot visually illustrated the calibration curve, showcasing the correlation between the recorded voltage readings and the real-world value. The linear regression analysis, yielded Equation B.2.1, with a R^2 of 0.999, further affirming the robustness and precision of the calibration.

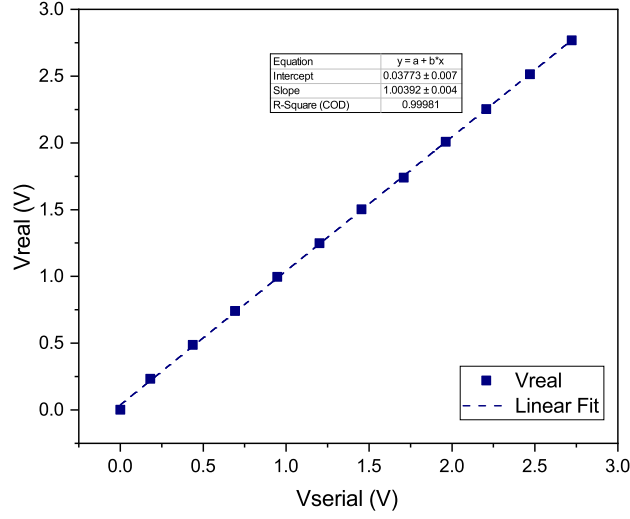


Figure B.2.1: Calibration curve for the voltammetric circuit.

$$V_{real} = 1.003919 \times V_{serial} + 0.037731 \quad (\text{B.2.1})$$

B.3 Calibration of ADC for Potentiometric AFE

The calibration process was undertaken for ADC within the potentiometric AFE. This step is required to ensure that the recorded voltage readings are aligned with their corresponding real-world values, enhancing the reliability of the measurements. To achieve this calibration, a direct connection was established between a benchtop voltage supply (Rigol DP1308A - Beijing, China) and the desired MCU pin, utilizing the designated test point (TP15). This configuration allowed to simulate a controlled voltage input (V_{real}) to the ADC. The voltage values received by the serial port (V_{serial}) of the electrochemical sensing platform were meticulously documented and organized in Table B.3.1. The meticulous collection of this data is fundamental to establishing a reliable calibration curve.

Additionally, a graphical representation of the calibration results can be found in Figure B.3.1. This plot vividly illustrates the calibration curve, demonstrating the inherent correlation between the recorded voltage readings (V_{serial}) and the real values (V_{real}). The calibration process employed linear regression analysis, resulting in the generation of Equation B.3.1, which described the relationship between the V_{serial} , and the V_{real} . The R^2 of 1 attested to the high degree of linearity and accuracy achieved through this calibration process.

$$V_{real} = 1.004436 \times V_{serial} - 0.0527213 \quad (\text{B.3.1})$$

Table B.3.1: Calibration data for the potentiometric circuit, with V_{serial} as the serial port voltage, and V_{real} the real voltage value.

Vreal (V)	Vserial (V)
0.098	0.04593
0.197	0.14586
0.297	0.24659
0.397	0.34571
0.497	0.44564
0.596	0.54557
0.696	0.64711
0.796	0.74703
0.895	0.84615
0.995	0.94608
1.095	1.04601
1.194	1.14674
1.294	1.24747
1.394	1.34659
1.493	1.44733
1.593	1.54725
1.692	1.64718
1.792	1.74711
1.892	1.84703
1.992	1.94777
2.091	2.04769
2.191	2.14842
2.291	2.24755
2.39	2.34828
2.49	2.44821
2.59	2.54894
2.689	2.64806
2.789	2.7496
2.889	2.84872
2.988	2.94945
3.088	3.04938
3.188	3.1493
3.288	3.24923

This comprehensive calibration process ensured that the data collected and processed by the developed device was both reliable and accurately representative of the electrochemical phenomena under investigation.

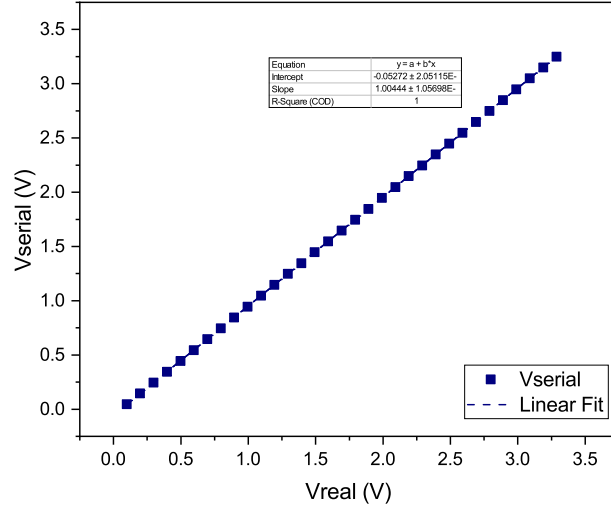


Figure B.3.1: Calibration curve for the potentiometric circuit.

B.4 External DAC

In the design of the electrochemical sensing platform, precise control over voltage outputs was critical to ensure accurate and reliable measurements. To achieve this level of control, the DAC8568ICPW, a high-performance, 16-bit DAC manufactured by Texas Instruments, was incorporated. This component offered a range of features that made it an ideal choice for the application:

- High Resolution - provided a 16-bit resolution, allowing for fine-grained control over the output voltage levels.
- Multiple Channels - included a total of eight output channels, making it suitable for versatile applications. In our design, five of these channels were utilized for specific functions.
- SPI Interface - communication with the DAC was facilitated through a Serial Peripheral Interface, ensuring reliable and efficient data transfer.
- Low Voltage Operation - operated at a low voltage, aligning with the requirements of our electrochemical sensing platform.

The DAC was interfaced with the MCU to facilitate precise control over analog output voltages. The following pin connections were established between the DAC8568 and the MCU:

- DAC_CLK (DAC Clock) - PD8 (SPI2_SCK): The DAC_CLK pin is responsible for clock synchronization, was connected to pin PD8 (SPI2_SCK)

on the MCU. This connection enables the MCU to provide the necessary clock signals for synchronous data transmission.

- DAC_SYNK (DAC Sync) - PB9 (GPIO_Output): The DAC_SYNK pin, which serves as a synchronization signal, was linked to pin PB9 on the MCU configured as a GPIO output. This connection ensures proper synchronization between both components during data transmission.
- DAC_DIN (DAC Data Input) - PC3 (SPI2_MOSI): DAC_DIN, responsible for receiving digital data, was connected to pin PC3 (SPI2_MOSI) on the MCU. This connection allows the MCU to transmit digital data to the DAC for conversion into analog signals.
- LDAC (Load DAC) - PA7 (GPIO_Output): The LDAC pin, used to load DAC input data, was connected to pin PA7 on the MCU, configured as a GPIO output. This connection enables the MCU to control when data should be loaded into the DAC, ensuring precise timing of signal updates.
- DAC_CLR (DAC Clear) - PA9 (GPIO_Output): DAC_CLR, responsible for clearing the DAC registers, was connected to pin PA9 on the MCU, configured as a GPIO output. This connection allows the MCU to initiate a clear operation when necessary, ensuring a clean state for subsequent conversions.

These pin connections established a vital link between the DAC and the MCU, enabling seamless data transmission, synchronisation, and control over the analog output signals. This robust interface ensures the accuracy and reliability of the electrochemical measurements conducted by the developed sensing platform. The above-mentioned signals, as well as the channels outputs are outlined in Figure B.1.4. Each channel served a specific purpose in controlling voltages crucial to the functionality of the PULSE:

- Channel A (V_{Bias1}): This channel was responsible for generating the bias voltage V_{Bias1} , which was used in the voltammetric AFE.
- Channel B (V_{ref}): Channel B was dedicated to providing a stable reference voltage, V_{ref} , serving as a reference point for other voltage levels in the system.
- Channel C (V_{Bias2}): Similar to Channel A, Channel C generated the bias voltage V_{Bias2} , playing a crucial role in maintaining the desired conditions within the electrochemical cell at the potentiometric AFE.

- Channel D (C.Sig): Channel D was utilized to control the voltage applied to the CE (C.Sig), contributing to precise electrochemical measurements at the voltammetric AFE.
- Channel E: Although not used in practice, it remained available for potential future use.

The relationship between the digital input code (D_{in}) and the DAC output voltage, denoted as V_{out} can be expressed using the equation:

$$V_{out} = \frac{D_{in}}{2^n} \times V_{REF} \times Gain \quad (B.4.1)$$

, where V_{REF} is 2.5 V and $Gain$ is 2, and n signifies the resolution of the DAC. In this case, 2^n is 65536, resulting in highly precise control over the output voltage levels. Therefore, the equation can be simplified as:

$$V_{out} = \frac{5 \times D_{in}}{65536} \quad (B.4.2)$$

To ensure the accuracy and reliability of the DAC output, a calibration process was undertaken. This calibration aimed to establish a relationship between the D_{in} , and the real output voltage produced by the component.

Multiple data points were collected by applying various D_{in} values, spanning from 0 to the maximum reading. For each D_{in} value, both the output voltage at each channel and the corresponding signal voltage were measured. It's important to note that a discrepancy exists between the channel output voltage and the signal voltage due to a resistance at the output causing a voltage drop. Table B.4.1 showed the values taken from Channel A, while Figure B.4.1 illustrated the signal voltage according to D_{in} . The signal voltage peaked at 3.47 V, amplified by a factor of 2 due to the internal buffer. The output buffer amplifier is capable of generating rail-to-rail voltages on its output, giving a maximum output range of 0 V to AVDD=3.47 V.

To generate the calibration curve, a linear regression approach was employed. By fitting a linear equation to this section, the calibration equation for each channel was derived, ensuring accurate translation of digital input codes into precise output voltages. Thus Equation B.4.3 represented the calibration for the channels, with R^2 of 0.9999.

$$V_{out} = 0.000076 \times D_{in} + 0.005498 \quad (B.4.3)$$

This calibration process played a critical role in maintaining the accuracy and effectiveness of the system, enabling it to adapt to various experimental conditions and requirements with confidence.

Table B.4.1: Calibration table for DAC Channel A, with the digital input in decimal (Din(dec)) and hexadecimal (Din(hex)), along the output voltage (VoutA), and the corresponding signal voltage (V_{Bias1}).

Din(dec)	Din(hex)	VoutA (V)	V_{Bias1} (V)
0	0	0	0
15	000F	0.0001	0.0001
111	006F	0.0073	0.0074
255	00FF	0.0184	0.0185
1791	06FF	0.1357	0.1355
6553	1999	0.4991	0.4987
13106	3332	0.999	0.9983
19659	4CCB	1.4991	1.4978
26212	6664	1.9992	1.9976
32765	7FFD	2.496	2.495
39318	9996	2.996	2.994
45871	B32F	3.465	3.462
52424	CCC8	3.465	3.463
58977	E661	3.465	3.463
65530	FFFA	3.465	3.463
65535	FFFF	3.465	3.465

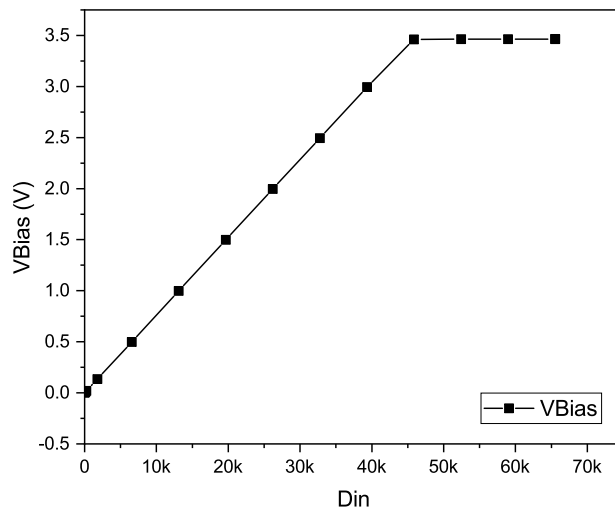


Figure B.4.1: The output voltage in channel A (V_{outA}) according to the digital input in decimal (Din).

In the FW architecture, DAC plays a crucial role in translating user-defined voltage settings into analog signals that can be accurately applied to the specified channels. This process involves several steps to ensure precision and reliability in voltage control. At the APP level, the user sets the desired voltage values for each respective channel. These values are then transmitted to the MCU for further

processing and conversion into a format that the DAC can understand. Upon receiving the message from the APP, the MCU plays a pivotal role in processing and decoding the message to extract the necessary information. It must identify the channel identifier, retrieve the Din value, and perform any necessary error-checking using the provided checksum.

Once the MCU has successfully decoded the message, it proceeds to interface with the DAC. It sends the extracted Din value to the DAC, which then translates this digital input into an analog voltage signal. This voltage signal is precisely applied to the specified channel, allowing the system to maintain accurate and user-defined settings. The above mentioned was incorporated at the FW level in the "*dac.c*" file, which is responsible for managing the DAC functionalities. This file has been successfully completed and encompasses various essential functions for DAC control and data transmission including:

- DAC Initialisation: This function, `Dac_Init(void)`, was responsible for initialising the DAC module. During this initialization process, various HW settings of the DAC were configured to ensure its proper operation. Specifically, the pins associated with `DAC_SYNK_Pin` and `LDAC_Pin` were set to a logic high state. The `DAC_CLR_Pin`, on the other hand, was toggled between low and high states. Additionally, the internal reference was enabled.
- Selecting a DAC Channel: This function - `Dac_Select_Ch(uint8_t ch)` - enabled the specification of the target channel without requiring direct interaction. It accepted a channel identifier *ch* as an argument, and called the `Dac_Set_Data(ch)` function to set up data for the selected channel.
- Set up Data: `Dac_Set_Data (uint8_t ch)` - was responsible for configuring the data to be transmitted by the DAC. It accepted *ch* as the channel identifier, and processed the data accordingly before transmitting it. This function prepared the data to be sent to the DAC by combining values and then transmitted the prepared data.
- Preparing Data for Transmission: This function - `Dac_PrepareToWrite()` - was responsible for configuring the DAC before data transmission. It ensured that the specified channel was powered up, stored the provided value in the `BiasDataA` array, and selected the channel for data transmission.
- Selecting Power-Down DAC Channel: `Dac_PowerDown_Ch (uint8_t ch)` - in scenarios necessitating power conservation or deactivation of a particular channel, this function was used. It accepted a channel identifier as input and then passed it to the `Dac_PowDown_Set` function.

- Powering Down DAC Channel: This function - `Dac_PowDown_Set (uint8_t dacNum)` - initiated the power-down sequence for the specified channel by taking the channel identifier as an input.
- Selecting Power-Up DAC Channel: Conversely, when it was necessary to activate a channel, this function - `Dac_PowerUp_Ch (uint8_t ch)` - was employed. It accepted the channel identifier and passed it to the `Dac_PowUp_Set` function.
- Powering Up DAC channel: Responsible for configuring the power-up mode of the desired channel, this function - `Dac_PowUp_Set (uint8_t dacNum)` - used the *dacNum* input to power up the selected channel.
- Transmite to DAC: This function - `Dac_TransmitU32()` - was responsible for efficiently transmitting data to the DAC. It took a 32-bit dataTx argument and converted it into four 8-bit bytes for precise data handling. This function managed the synchronization signal by toggling the DAC_SYNK pin, ensuring proper communication with the module. Using the Hardware Abstraction Layer (HAL) library, it transmitted the prepared data via Serial Peripheral Interface (SPI) interface, maintaining synchronized data transfer. Finally, it toggled the DAC_SYNK pin back to its original state to complete the data transmission process, facilitating accurate communication between the MCU and the DAC module.

These functions collectively provided comprehensive control over the DAC, from initialisation to channel selection data preparation, and transmission. By utilizing these functions within the FW, it was possible to control the outputs of the DAC channels, making it a crucial part of the embedded system.

B.5 Communication Protocol

B.5.1 From the User to MCU

The protocol defines a set of commands that can be sent to the MCU from the user device for controlling the AFE and performing various measurements. Each command serves as a directive for the MCU to execute specific tasks or measurements, contributing to the overall functionality of the system. It is important to highlight that in all subsequent commands, the `<parameterXOR>` signifies the 2-bit XOR-checksum parameter. The commands encompass a range of functionalities including:

- Configure voltammetric AFE: This command allows the user to send configuration parameters to set up the voltammetric AFE.
 - Command: '\$H<parameter1><parameter2><parameterXOR>\n'
 - Parameter 1: INA333 amplification
 - Parameter 2: TIA feedback resistor value
- Define settings for CV: The user smart device can specify the parameters required for performing CV experiments, including setting the initial, maximum, and minimum voltages, scan rate, and the number of cycles.
 - Command: '\$J<parameter1><parameter2><parameter3><parameter4><parameter5><parameterXOR>\n'
 - Parameter 1: initial voltage to start CV
 - Parameter 2: maximum voltage
 - Parameter 3: minimum voltage
 - Parameter 4: slope/ scan rate
 - Parameter 5: number of cycles
- Start CV measurements: Upon receiving this command, the MCU initiates CV measurements based on the previously defined settings.
 - Command: '\$V<parameterXOR>\n'
- Stop CV measurements: This command instructs the MCU to halt the ongoing CV measurements and return any acquired data or results to the user device.
 - Command: '\$N<parameterXOR>\n'
- Set electrochemical cell voltage for CA: use this command to set the voltage applied to the electrochemical cell. It allows precise control over the cell's potential during experiments.
 - Command: '\$D<parameter1><parameterXOR>\n'
 - Parameter 1: voltage value
- Start Chronoamperometry: When this command is received, the MCU starts CA measurements, recording the current response over time.
 - Command: '\$P<parameterXOR>\n'

- Stop Chronoamperometry: To conclude amperometric measurements, the APP sends this command, prompting the MCU to cease data acquisition and provide the results.
 - Command: '\$S<parameterXOR>\n'
- Start Potentiometry: This command initiates potentiometric measurements, enabling the MCU to monitor the voltage at the electrochemical cell.
 - Command: '\$U<parameterXOR>\n'
- Stop Potentiometry: To end potentiometric measurements, the APP sends this command, and the MCU cease data acquisition.
 - Command: '\$W<parameterXOR>\n'
- Define settings for LSV: The user can specify the parameters required for performing LSV experiments, including setting the initial, stop, and minimum voltages, and scan rate.
 - Command: '\$L<parameter1><parameter2><parameter3><parameter4><parameterXOR>\n'
 - Parameter 1: initial voltage
 - Parameter 2: stop voltage
 - Parameter 3: minimum voltage
 - Parameter 4: slope/ scan rate
- Start LSV: This command initiates LSV measurements, enabling the MCU to monitor the current at the WE.
 - Command: '\$E<parameterXOR>\n'
- Stop LSV: To end LSV measurements, the APP sends this command, and the MCU cease data acquisition.
 - Command: '\$F<parameterXOR>\n'
- Define settings for SWV: The user can specify the parameters required for performing SWV experiments, including setting the initial, stop, minimum, and step voltages.
 - Command: '\$K<parameter1><parameter2><parameter3><parameter4><parameterXOR>\n'

- Parameter 1: initial voltage
- Parameter 2: stop voltage
- Parameter 3: minimum voltage
- Parameter 4: step voltage
- Start SWV: This command initiates SWV measurements, enabling the MCU to monitor the current at the WE.
 - Command: '\$G<parameterXOR>\n'
- Stop SWV: To end SWV measurements, the APP sends this command, and the MCU cease data acquisition.
 - Command: '\$I<parameterXOR>\n'

B.5.2 From the MCU to the User

In the bidirectional communication protocol, instructions are also transmitted from the MCU to the user interface, establishing a seamless interaction between the system and the user. This communication channel is vital for real-time data collection, ensuring efficient monitoring and control. In all transmitted commands, the `<parameterXOR>` represents the 2-bit XOR-checksum parameter. These commands sent encompass the following:

- Internal temperature: This command retrieves temperature measurements from the MCU's internal sensor.
 - Command: '\$I<data1><parameterXOR>\n'
 - Data 1: temperature data
- External temperature: This command involves the measurement of the external temperature sensor.
 - Command: '\$E<data1><parameterXOR>\n'
 - Data 1: External temperature data
- Battery monitoring: This command retrieves voltage measurements at the power line using the MCU's internal battery monitoring.
 - Command: '\$B<data1><parameterXOR>\n'
 - Data 1: battery voltage data

- Power consumption: This command transmits power consumption measurements obtained through an MAX44284 from Analog Devices Inc.
 - Command: '\$P<data1><parameterXOR>\n'
 - Data 1: power consumption data
- Accelerometer data: This command sends the data from the ADXL345 from Analog Devices Inc., providing information on acceleration along the x, y, and z axes.
 - Command: '\$M<data1><data2><data3><parameterXOR>\n'
 - Data 1: acceleration data on the x-axis
 - Data 2: acceleration data on the y-axis
 - Data 3: acceleration data on the z-axis
- Chronoamperometry data: This command sends the data obtained performing CA measurements.
 - Command: '\$A<data1><parameterXOR>\n'
 - Data 1: CA data
- Cyclic Voltammetry data: This command sends the data acquired from performing CV measurements.
 - Command: '\$V<data1><parameterXOR>\n'
 - Data 1: CV data
- Cyclic Voltammetry finish command: This command informs the user that the measurement is completed.
 - Command: '\$Y<parameterXOR>\n'
- Linear Sweep Voltammetry data: This command sends the data acquired from performing LSV measurements.
 - Command: '\$L<data1><parameterXOR>\n'
 - Data 1: LSV data
- Square Wave Voltammetry data: This command sends the data acquired from performing SWV measurements.

- Command: '\$S<data1><data2><parameterXOR>\n'
 - Data 1: SWV data regarding forward current
 - Data 2: SWV data regarding reverse current
- Open Circuit Potential data: This command transmits data obtained from performing OCP measurements.
 - Command: '\$H<data1><parameterXOR>\n'
 - Data 1: OCP data

B.5.3 Error Handling

Error handling in a communication protocol is crucial for ensuring the integrity and reliability of data transmission between devices. In the described system, multiple error-handling methods have been implemented to address potential issues and enhance the overall robustness of the communication protocol.

The **XOR-checksums** at both the MCU and APP levels, serve as a form of error detection by calculating a checksum based on the transmitted data. By comparing the calculated checksum with the received checksum, discrepancies can be identified, indicating a potential error during transmission. This method helps verify the accuracy of the data and provides a means to detect errors caused by noise or other communication issues.

Another aspect of error handling was incorporated at the APP level, where specific **error messages** are generated. For instance, error messages are triggered when attempting to start a measurement without sending the required parameters in advance or when the serial port is not opened. These messages serve as informative alerts, guiding the user or developer to rectify the issue and ensure proper communication.

Furthermore, the system employed a mechanism of **fragmented message handling**, meaning it handles incomplete messages at the APP level. In cases where a message is received in fragments, the incomplete data is temporarily stored in a buffer. Subsequently, when the missing parts of the message are received, they are appended to the incomplete data, reconstructing the complete message. This approach minimizes the impact of message fragmentation and contributes to the overall robustness of the communication protocol.

Overall, these error-handling methods collectively contribute to the reliability and effectiveness of the communication protocol by detecting, reporting, and mitigating errors at various stages of data transmission.

B.6 APP Instructions

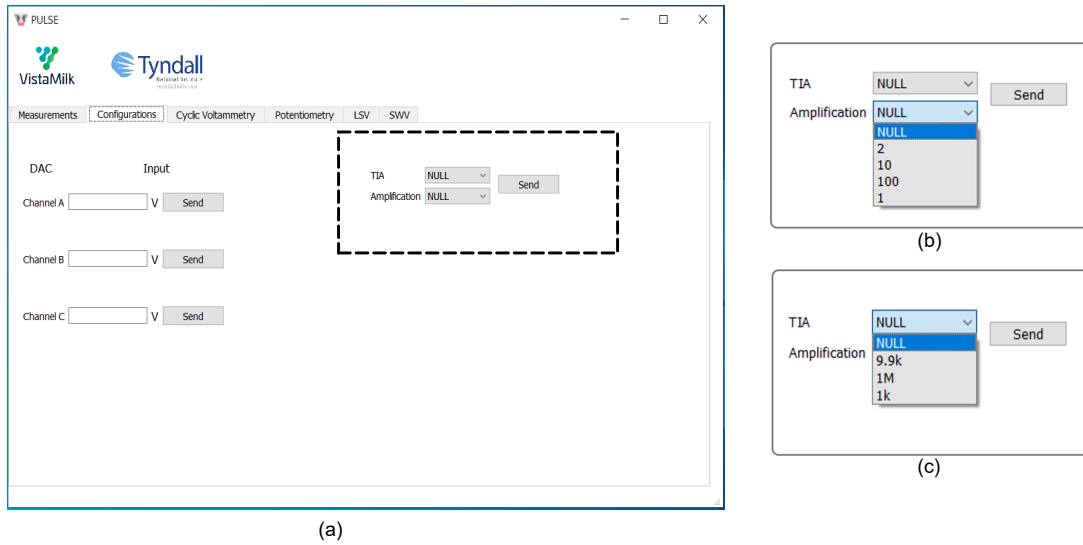


Figure B.6.1: Application: (a) configuration tab with green dashed square highlighting the voltammetric settings; (b) amplification options; and (c) TIA feedback resistor values.

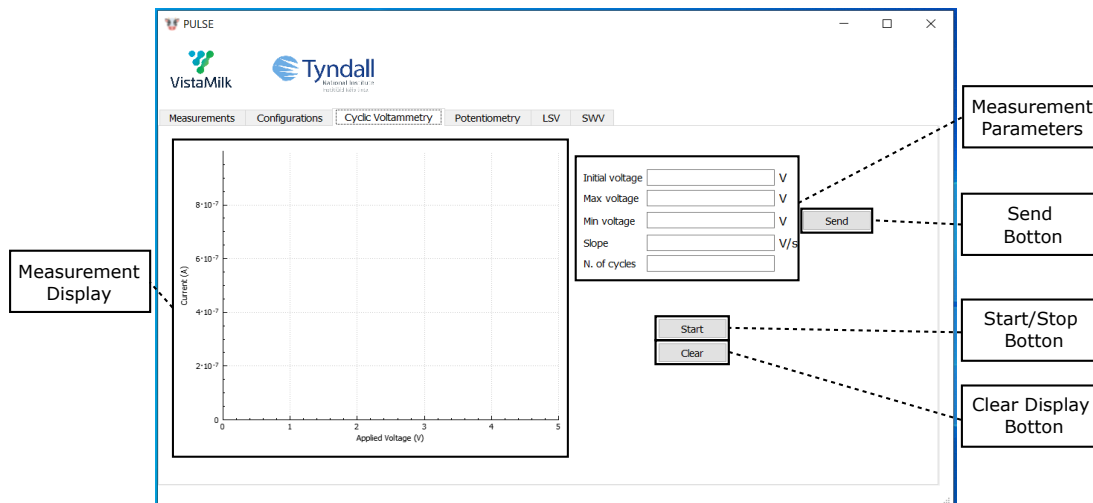


Figure B.6.2: Overview of the APP's "Cyclic Voltammetry" tab. Before starting a measurement, the user must input the measurement parameters and press the 'Send' button. The measurement is then initiated by pressing the 'Start' button, which also serves to stop the process once it has begun. Between measurements, the display can be cleared using the 'Clear' button.

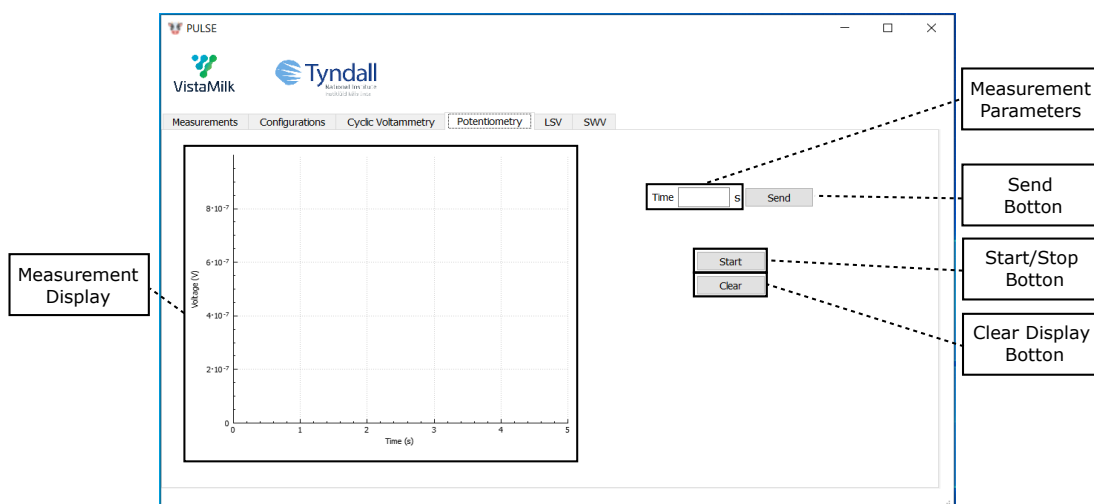


Figure B.6.3: Overview of the APP's "Potentiometry" tab. Before starting a measurement, the user can input a time stamp for the measurement. The measurement is initiated by pressing the 'Start' button, which also serves to stop the process once it has begun. Between measurements, the display can be cleared using the 'Clear' button.

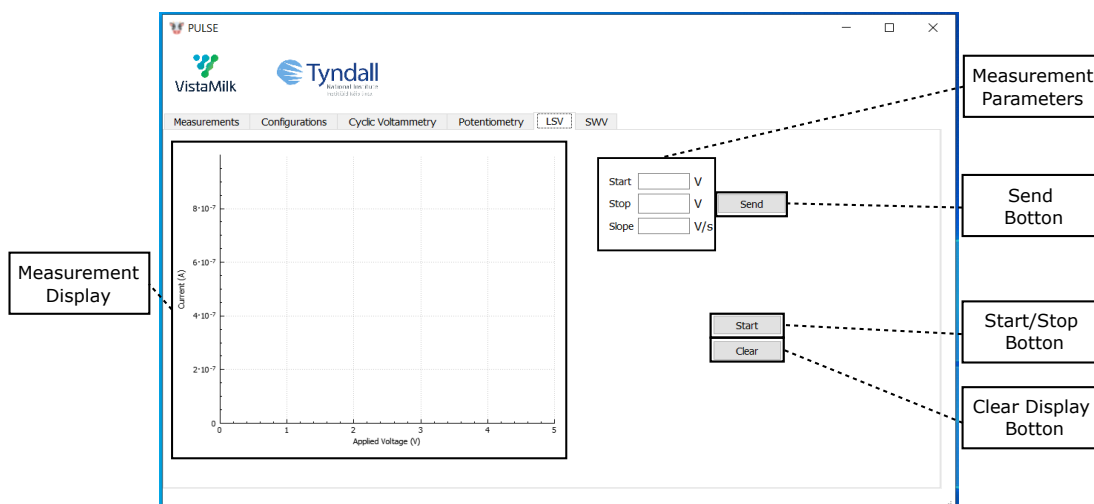


Figure B.6.4: Overview of the APP's "LSV" tab. Before starting a measurement, the user must input the measurement parameters and press the 'Send' button. The measurement is then initiated by pressing the 'Start' button, which also serves to stop the process once it has begun. Between measurements, the display can be cleared using the 'Clear' button.

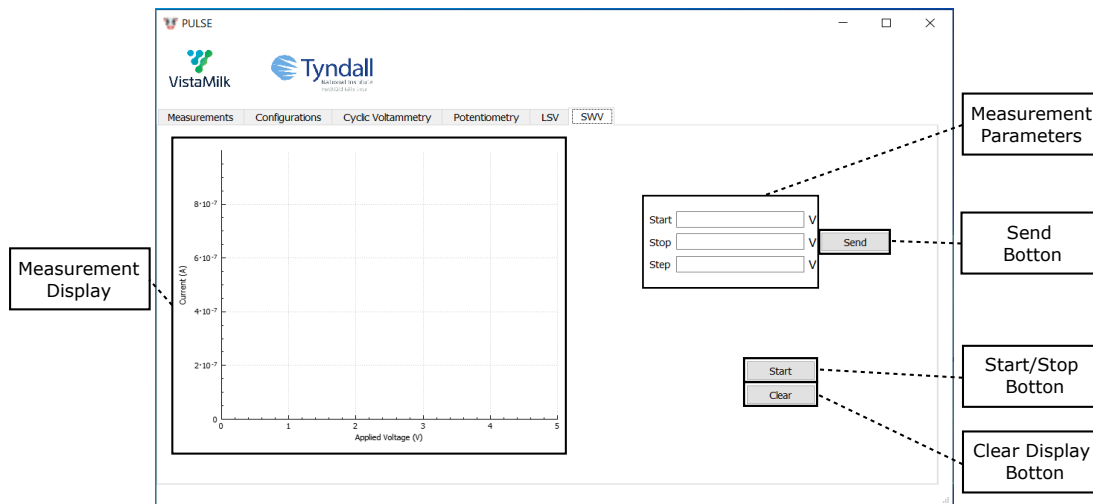


Figure B.6.5: Overview of the APP's "SWV" tab. Before starting a measurement, the user must input the measurement parameters and press the 'Send' button. The measurement is then initiated by pressing the 'Start' button, which also serves to stop the process once it has begun. Between measurements, the display can be cleared using the 'Clear' button.

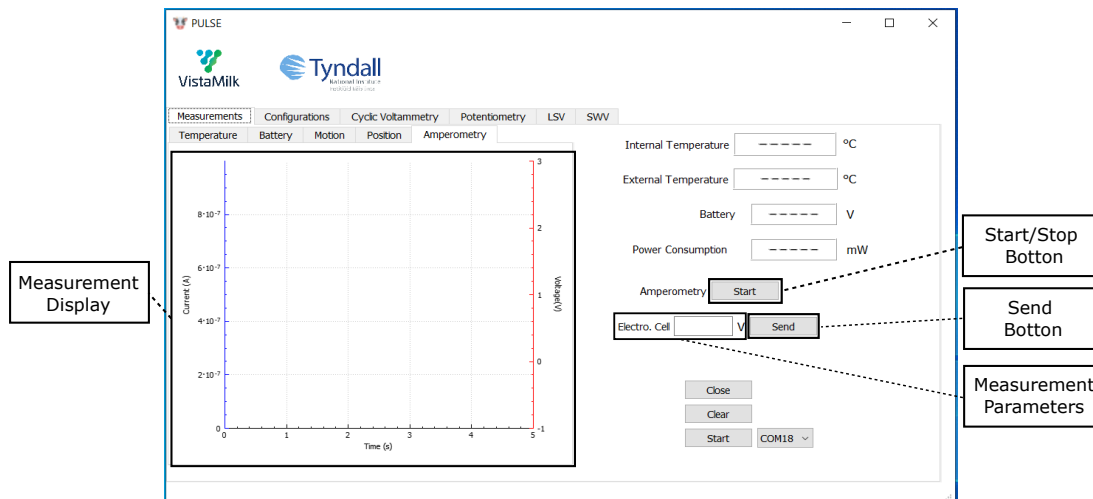
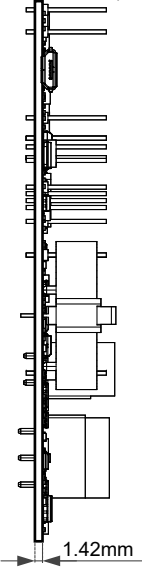


Figure B.6.6: Overview of the APP's subtab "Amperometry" under the "Measurements" tab. Before starting a measurement, the user must input the measurement parameters and press the 'Send' button. The measurement is then initiated by pressing the 'Start' button, which also serves to stop the process once it has begun. Between measurements, the display can be cleared using the 'Clear' button.

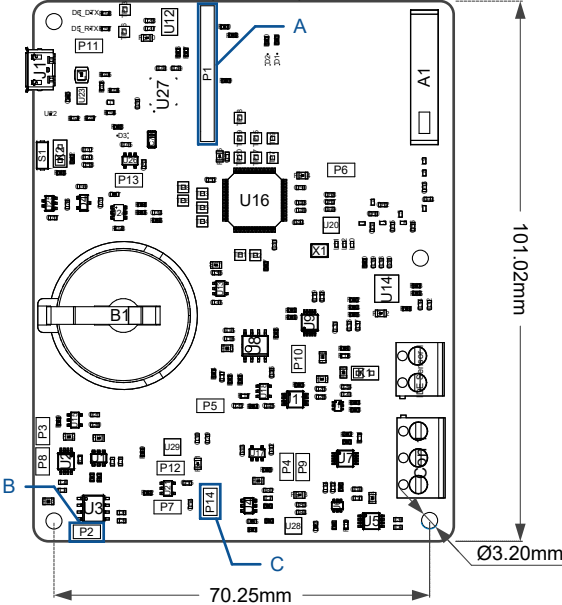
B.7 PCB Fabrication Documentation

Assembly View

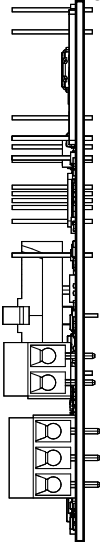
View from Left side (Scale 1:1)



View from Top side (Scale 1:1)



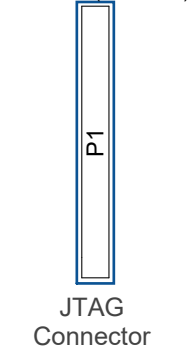
View from Right side (Scale 1:1)



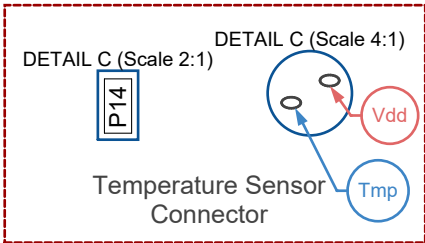
View from Front side (Scale 1:1)



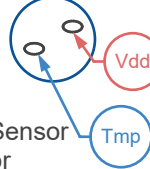
DETAIL A (Scale 2:1)



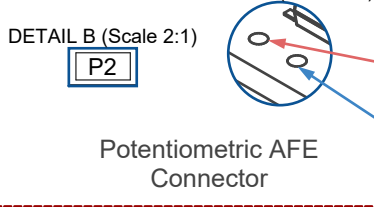
DETAIL C (Scale 2:1)



DETAIL C (Scale 4:1)



DETAIL B (Scale 4:1)



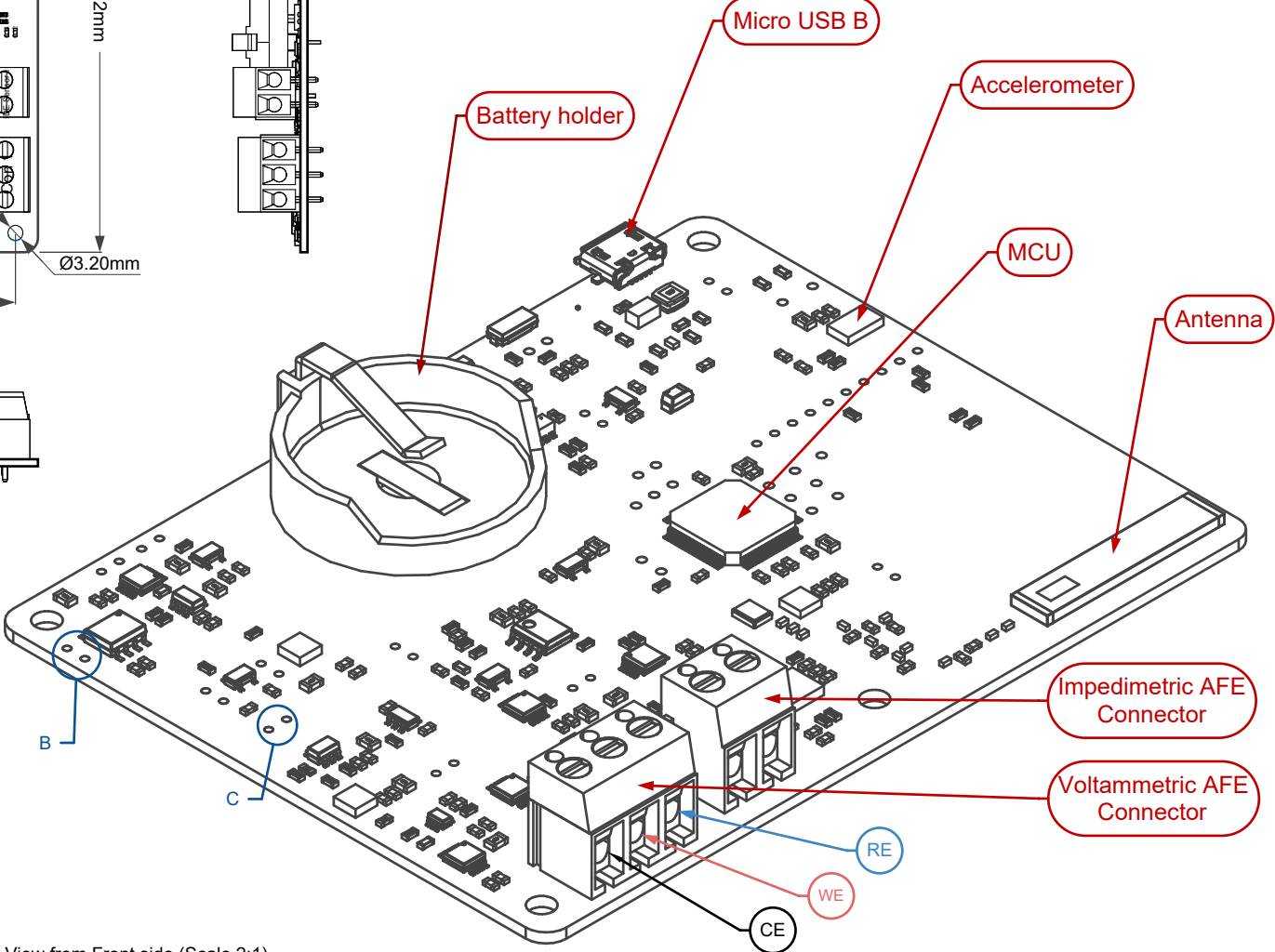
DETAIL B (Scale 2:1)



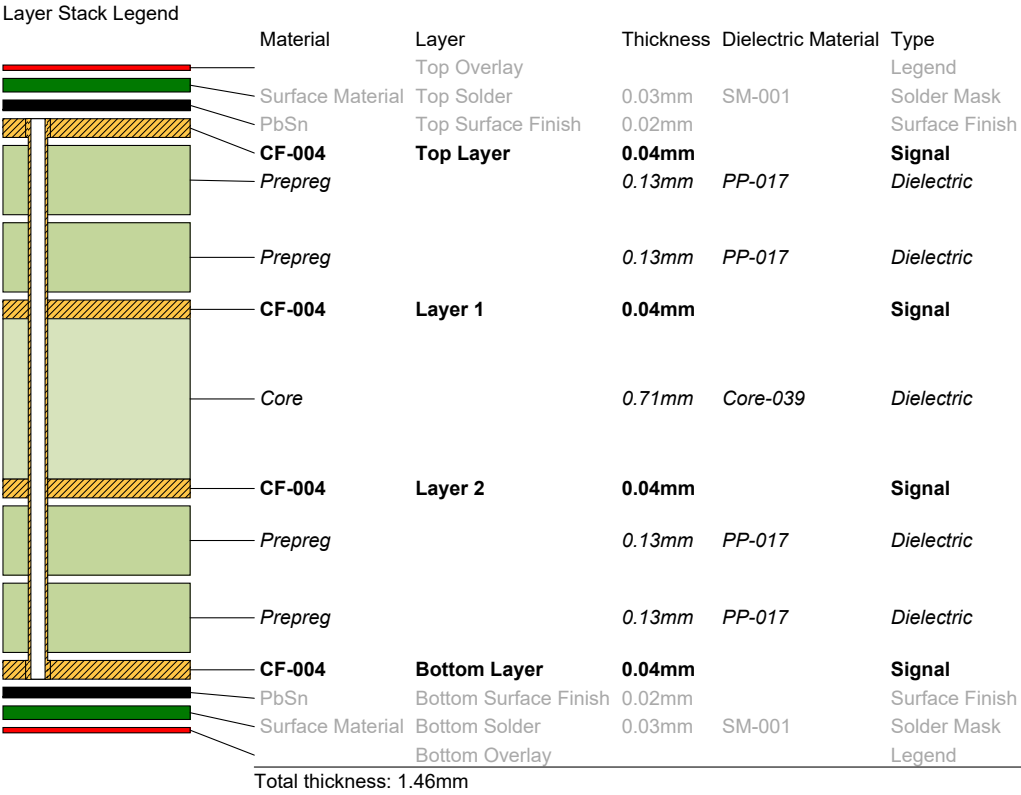
WE

RE

View from Front side (Scale 2:1)



Layer Stack



BOM

Bill Of Materials

Line #	Designator	Comment	Quantity
1	A1	0433AT62A0020	1
2	B1	BH1000G	1
3	C1, C3, C5, C8, C11, C14, C16, C17, C19, C24, C27, C29, C39, C40, C41, C44, C49, C50, C51, C52, C58, C59, C67, C71, C74, C78, C79, C80, C82, C83	100nF	30
4	C2, C4, C6, C7, C9, C10, C12, C15, C18, C20, C23, C26, C28, C30, C35, C38, C42, C45, C48, C54, C72, C73, C75, C84	10uF	24
5	C13	1nF	1
6	C21, C36, C43, C46, C55, C76, C77, C81	1uF	8
7	C22	82pF	1
8	C25	100nF	1
9	C31, C32, C33, C34, C37	10nF	5
10	C56, C57	4.7uF	2
11	C60	3p6F	1
12	C61	7p5F	1
13	C62	12pF	1
14	C63	220pF	1
15	C64, C65	10pF	2
16	C66	18pF	1
17	C68	1uF	1
18	C69	100pF	1
19	C70	T491A105K016AT	1
20	C85	470pF	1
21	Con 1	potentiostat con	1
22	D1, D2, D5 RTX, D6_DTX	LTST-C190KGKT	4
23	D3	LTST-C190KRKT	1
24	D4	BAT54CHYFH	1
25	FB1, FB2, FB3, FB4, FB5, FB6, FB7, FB8, FB9, FB10, FB11, FB12, FB13, FB14, FB15, SC1	1KZ	16
26	IDE Sensor 1	IDEsensor	1
27	J1	47346-0001	1
28	L1	47uH	1
29	L2	82nH	1
30	L3, L8	68nH	2
31	L4	270nH	1
32	L5	100nH	1
33	L6, L7	22nH	2
34	L9	39nH	1
35	L10, L11, L12	12nH	3
36	P3, P8	Header 2	2
37	Q1	NTK3134NT5G	1
38	R1, R5, R23	10R	3

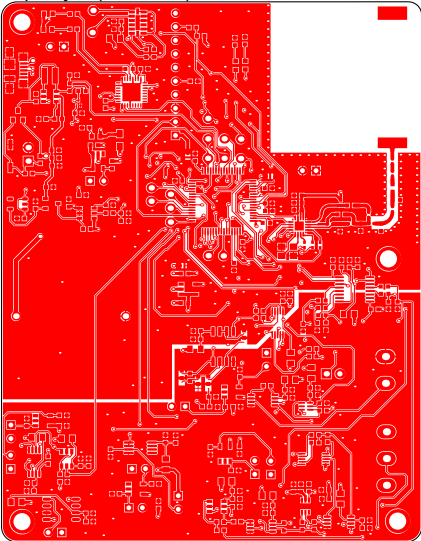
Bill Of Materials

Line #	Designator	Comment	Quantity
39	R2, R4, R6, R7, R8, R20, R24, R30, R32, R33, R34, R35, R36, R37, R38, R39, R44, R55, R56	10k	19
40	R3, R11	11k	2
41	R9, R19	1k	2
42	R10, R43, R45	1M	3
43	R12, R21, R25	100k	3
44	R13, R14, R15, R16	796R	4
45	R17	240k	1
46	R18	1R	1
47	R26, R27	2k	2
48	R29, R31, R49, R51, R59, R60, R62	47k	7
49	R40, R41, R48	100R	3
50	R42	220k	1
51	R46	806k	1
52	R47	590k	1
53	R50	0R	1
54	R52	309k	1
55	R53	0R1	1
56	R54	200k	1
57	R57, R58	1.8k	2
58	S1	MK24-B-3	1
59	U1, U4	ADA4807-1ARJZ-R2	2
60	U2, U5, U11	INA333	3
61	U3	LMP7721	1
62	U6	ADG779	1
63	U7, U9	ADG704	2
64	U8	LMC6462BIM/NOPB	1
65	U10	ADA4807-1AKSZ-R2	1
66	U12	ADXL345BCCZ-RL7	1
67	U13, U15, U17, U18, U21, U26	LM4132BMF-3.3/NOPB	6
68	U14	DAC8568ICPW	1
69	U16	STM32F373RBTX	1
70	U20	Si4455-B1A-FM	1
71	U22	SSM3J306T(Te85L,F)	1
72	U23	MCP1640B-IMC	1
73	U24	MAX44284HAUT+T	1
74	U25	NCP161ASN350T1G	1
75	U27	FT232RQ-REEL	1
76	U28, U29	LTC6081HMS8#PBF	2
77	X1	7M-30.000MEEQ-T	1

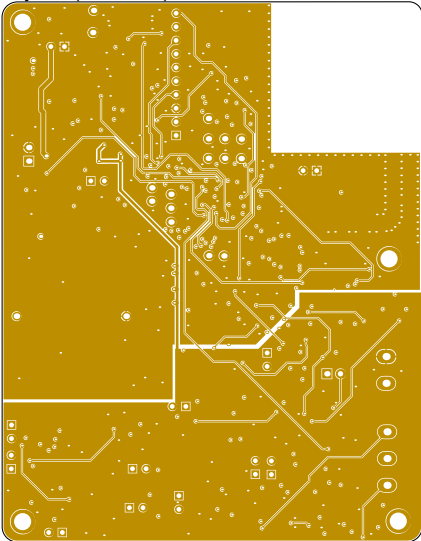
Board Fabrication View



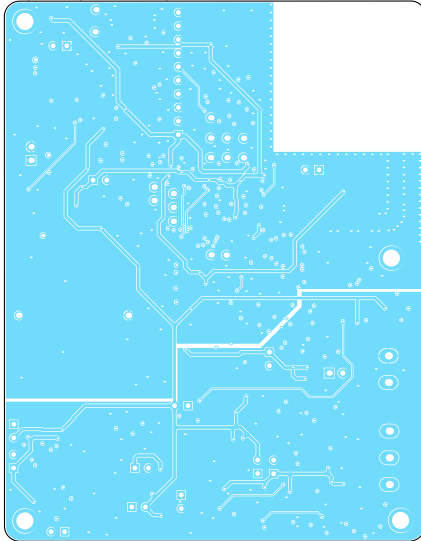
Top Layer (Scale 1:1)



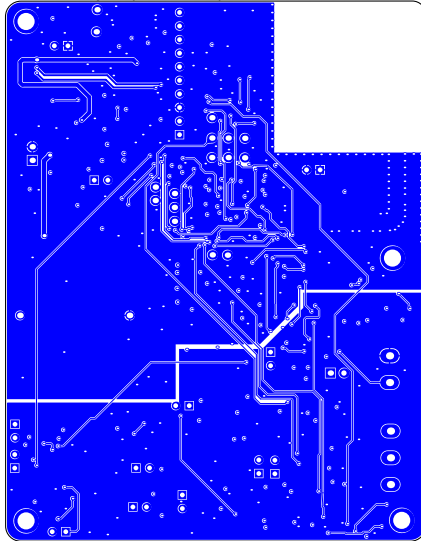
Layer 1 (Scale 1:1)



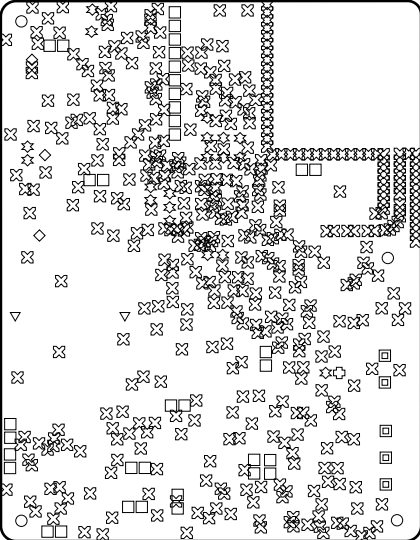
Layer 2 (Scale 1:1)



Bottom Layer (Scale 1:1)



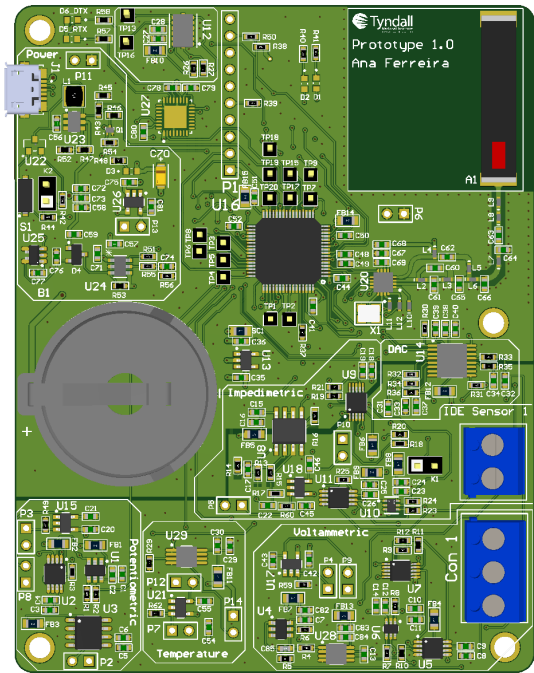
Drill Drawing View (Scale 1:1)



Drill Table

Symbol	Count	Hole Size	Plated	Hole Type
⊠	475	0.30mm	Plated	Round
◇	3	0.60mm	Plated	Round
□	36	0.90mm	Plated	Round
⊕	1	1.00mm	Plated	Round
☆	19	1.02mm	Plated	Round
▽	2	1.07mm	Plated	Round
⊞	5	1.35mm	Plated	Round
○	4	3.20mm	Plated	Round
545 Total				

Realistic View



B.8 CV Measurements Validation

Table B.8.1: CV oxidation and reduction peaks at multiple concentrations of $[\text{Fe}(\text{CN})_6]^{3-}/[\text{Fe}(\text{CN})_6]^{4-}$ in 10mM PBS using the PULSE with sensor#1.

Concentration (mM)	Oxidation Peak		Reduction Peak	
	Potential (V)	Current (μA)	Potential (V)	Current (μA)
5	0.134	143.782	0.058	-152.041
4	0.134	113.246	0.058	-118.509
3	0.137	85.026	0.063	-89.281
2	0.152	58.903	0.065	-60.816
1	0.164	32.317	0.055	-31.315

Table B.8.2: CV oxidation and reduction peaks at multiple concentrations of $[\text{Fe}(\text{CN})_6]^{3-}/[\text{Fe}(\text{CN})_6]^{4-}$ in 10mM PBS using the PULSE with sensor#2.

Concentration (mM)	Oxidation Peak		Reduction Peak	
	Potential (V)	Current (μA)	Potential (V)	Current (μA)
5	0.160	141.058	0.083	-152.205
4	0.155	113.083	0.088	-121.941
3	0.155	77.862	0.088	-82.635
2	0.152	51.902	0.083	-55.477
1	0.155	25.643	0.081	-26.276

Table B.8.3: CV oxidation and reduction peaks at multiple concentrations of $[\text{Fe}(\text{CN})_6]^{3-}/[\text{Fe}(\text{CN})_6]^{4-}$ in 10mM PBS using the PULSE with sensor#3.

Concentration (mM)	Oxidation Peak		Reduction Peak	
	Potential (V)	Current (μA)	Potential (V)	Current (μA)
5	0.221	108.615	0.335	-105.162
4	0.211	90.065	0.038	-87.240
3	0.201	69.799	0.048	-69.696
2	0.192	48.470	0.063	-49.103
1	0.197	24.200	0.083	-24.669

Table B.8.4: Average and standard deviation (SD) of multiple concentrations of $[\text{Fe}(\text{CN})_6]^{3-}/[\text{Fe}(\text{CN})_6]^{4-}$ detected using the PULSE in 10mM PBS (n=3).

Con. (mM)	Average				SD			
	Oxidation Peak		Reduction Peak		Oxidation Peak		Reduction Peak	
	Pot. (V)	I (μA)	Pot. (V)	I (μA)	Pot. (V)	I (μA)	Pot. (V)	I (μA)
5	0.172	131.152	0.159	-66.361	0.037	15.975	0.125	121.285
4	0.167	105.465	0.062	-51.070	0.033	10.889	0.020	97.810
3	0.165	77.562	0.066	-34.073	0.027	6.220	0.016	73.426
2	0.165	53.092	0.070	-22.397	0.019	4.341	0.009	50.604
1	0.172	27.387	0.073	-10.974	0.018	3.536	0.013	25.287

Table B.8.5: CV oxidation and reduction peaks at multiple concentrations of $[\text{Fe}(\text{CN})_6]^{3-}/[\text{Fe}(\text{CN})_6]^{4-}$ in 10mM PBS using the benchtop potentiostat with sensor#1.

Concentration (mM)	Oxidation Peak		Reduction Peak	
	Potential (V)	Current (μA)	Potential (V)	Current (μA)
5	0.169	155.609	0.061	-155.029
4	0.200	123.688	0.100	-123.810
3	0.193	97.076	0.105	-95.428
2	0.186	64.575	0.112	-64.484
1	0.181	33.215	0.112	-32.498

Table B.8.6: CV oxidation and reduction peaks at multiple concentrations of $[\text{Fe}(\text{CN})_6]^{3-}/[\text{Fe}(\text{CN})_6]^{4-}$ in 10mM PBS using the benchtop potentiostat with sensor#2.

Concentration (mM)	Oxidation Peak		Reduction Peak	
	Potential (V)	Current (μA)	Potential (V)	Current (μA)
5	0.154	132.629	0.054	-134.216
4	0.149	105.621	0.056	-109.711
3	0.108	78.186	0.061	-80.750
2	0.147	50.171	0.066	-51.483
1	0.137	23.914	0.061	-25.046

Table B.8.7: CV oxidation and reduction peaks at multiple concentrations of $[\text{Fe}(\text{CN})_6]^{3-}/[\text{Fe}(\text{CN})_6]^{4-}$ in 10mM PBS using the benchtop potentiostat with sensor#3.

Concentration (mM)	Oxidation Peak		Reduction Peak	
	Potential (V)	Current (μA)	Potential (V)	Current (μA)
5	0.213	105.133	0.025	-105.682
4	0.197	86.746	0.031	-81.635
3	0.191	61.890	0.037	-63.354
2	0.191	44.354	0.049	-42.322
1	0.181	21.216	0.054	-21.555

Table B.8.8: Average and standard deviation (SD) of multiple concentrations of $[\text{Fe}(\text{CN})_6]^{3-}/[\text{Fe}(\text{CN})_6]^{4-}$ detected using a benchtop potentiostat in 10mM PBS (n=3).

Con. (mM)	Average				SD			
	Oxidation Peak		Reduction Peak		Oxidation Peak		Reduction Peak	
	Pot. (V)	I (μA)	Pot. (V)	I (μA)	Pot. (V)	I (μA)	Pot. (V)	I (μA)
5	0.178	131.124	0.057	-132.803	0.026	20.634	0.027	22.759
4	0.167	105.352	0.065	-105.531	0.023	15.083	0.030	18.753
3	0.158	79.051	0.069	-79.793	0.022	14.378	0.028	13.781
2	0.157	53.033	0.076	-52.103	0.018	8.500	0.027	9.636
1	0.151	26.115	0.074	-24.804	0.018	5.140	0.028	4.964

B.9 CA Measurements Validation

Table B.9.1: CA measurements for multiple concentrations of ferri-ferro, using the PULSE system (n=3, mean $\pm$ SD).

Concentration (mM)	Current (μ A)	Standard Deviation
5	4.866	1.173×10^{-8}
4	3.820	7.512×10^{-8}
3	2.600	6.953×10^{-8}
2	1.269	7.956×10^{-8}
1	0.685	4.990×10^{-8}

Table B.9.2: CA measurements for multiple concentrations of ferri-ferro, using the benchtop instrument system (n=3, mean $\pm$ SD).

Concentration (mM)	Current (μ A)	Standard Deviation
5	4.880	3.416×10^{-8}
4	3.623	6.710×10^{-8}
3	2.580	9.305×10^{-8}
2	1.421	2.945×10^{-8}
1	0.596	4.802×10^{-8}

B.10 pH Measurements Validation

In order to make IrOx, IV chloride hydrate ($\text{IrCl}_4 \cdot \text{H}_2\text{O}$) was first dissolved in DI water with magnetic stirring for 30 minutes, then 1 mL of aqueous 30% H_2O_2 was added to the solution, which was then stirred for an additional 30 minutes. Oxalic acid ($\text{C}_2\text{H}_2\text{O}_4$) was then added in a quantity of 0.5 g, and the mixture was agitated for 30 minutes. The addition of Na_2CO_3 changed the pH of the solution to ~ 10 . After stabilising at room temperature, the solution gradually changed from bright yellow to dark blue. At the base of the solution bottle, there was no precipitation to be observed.

Table B.10.1: OCP measurements in PBS solutions at multiple pH levels (n=3), using the benchtop instrument.

pH	Voltage (V)				
	Sensor 1	Sensor 2	Sensor 3	Average	Standard Deviation
3	0.256	0.256	0.256	0.256	0.000
5	0.136	0.135	0.134	0.135	0.817m
7	-0.005	-0.005	-0.005	-0.005	0.000
9	-0.105	-0.109	-0.112	-0.109	2.867m
11	-0.231	-0.250	-0.218	-0.233	13.140m

Table B.10.2: OCP measurements in PBS solutions at multiple pH levels (n=3), using the PULSE system.

	Voltage (V)				
pH	Sensor 1	Sensor 2	Sensor 3	Average	Standard Deviation
3	0.248	0.248	0.248	0.248	50.04 μ
5	0.119	0.119	0.119	0.119	0.371m
7	-0.014	-0.014	-0.015	-0.014	86.810 μ
9	-0.133	-0.134	-0.134	-0.134	0.411m
11	-0.235	-0.230	-0.225	-0.230	4.098m

PULSE EXPERIMENTAL DATA

C.1 Electrode Characterisation

The Randles-Sevcik equation was employed to calculate the electrochemical active area of the WE in both the flexible gold-printed electrodes and the commercial SPEs. For this, CV measurements were performed using the benchtop instrument in triplicate using 5 mM solution of ferri-ferro at various scan rates, ranging from 10 to 200 mV/s, to assess the relationship between scan rate and peak current [1]. Equation 1.14 can be further simplified and expressed for area as [2]:

$$A = \frac{i_p}{2.69 \times 10^5 n^{\frac{3}{2}} C \sqrt{Dv}} \quad (\text{C.1.1})$$

Where A is the electrode area in cm^2 , n denotes the number of electrons transferred in the redox event (equal to 1), D is the diffusion coefficient in cm^2/s (given as $6.400 \times 10^{-6} \text{ cm}^2/\text{s}$ [3]), C represents the concentration in mol/cm^3 , and v stands for the scan rate in V/s . For this calculation, the peak current (i_p) corresponded to the oxidation peak measured at a scan rate of 50 mV/s (v) for a concentration of 5 mM (C). This approach enabled an accurate determination of the electrochemically active surface area.

To support the findings of the electrochemical active areas, a Scanning Electron Microscope (SEM) - Zeiss Supra 40 SEM (Oberkochen, Germany) was used to capture images and perform compositional analysis of the sensor's surface before and after modifications. Raman measurements were done with a Renishaw InVia system (England, UK) with a wavelength of 514 nm and power of 7 mW at 100%, using an objective magnification of 50.

C.1.1 Commercial SPE

To determine the electrochemical active area of the WE, CV measurements were initially performed on the control commercial SPEs ($n=3$) in 5 mM ferri-ferrocyanide across various scan rates in the range of 10 to 200 mV/s, as shown in Figure C.1.1 (a). The resulting current peaks from both oxidative and reductive reactions were plotted in Figure C.1.1 (b), demonstrating a linear correlation with the square root of the scanning rate. Subsequently, following the deposition of IrOx illustrated in Figure C.1.1 (c), a symmetrical enhancement in peak oxidation and reduction was observed, as depicted in Figure C.1.1 (d). This suggested an increase in the electrochemical active surface area. Therefore, to quantify the new electrochemical active area after the IrOx deposition, the same procedure was repeated. CV measurements were again performed in 5 mM ferri-ferrocyanide at various scan rates, as illustrated in Figure C.1.1 (e). The relationship between the peak oxidation and reduction currents, and the square root of the scan rate remained linear, as shown in Figure C.1.1 (f).

Based on the geometry of the WE, the geometrical area was calculated using the formula for the area of a circle, resulting in approximately $12.57 \times 10^{-2} \text{ cm}^2$. To calculate the electrochemical active area, Equation C.1.1 was applied. It was found that the modification of the electrode surface with IrOx resulted in a 127.8% increase in the electrochemical active area compared to the control, as supported by literature [4, 5]. Specifically, the control electrode exhibited an active area of $16.36 \times 10^{-2} \text{ cm}^2$, which increased to $37.26 \times 10^{-2} \text{ cm}^2$ following IrOx deposition. These represented a 30.1% increase from the geometrical area to the control electrochemical active area, and an increase of 196.5% after IrOx modification. Such differences between the geometrical and electrochemical active areas were consistent with findings in the literature, due to factors such as surface roughness and porosity [6].

C.1.2 Flexible Gold Electrode

Prior to calculating the electrochemical surface area, NPG deposition is typically characterized by performing CV in 1 M NaOH, sweeping the potential from -0.7 V to 0.9 V at a scan rate of 10 mV/s. First, a scan was conducted on the unmodified planar gold electrode. Next, a gold-silver (AuAg) alloy was deposited onto the gold-printed electrode in a cyanide bath via CA at -1.19 V for 180 seconds, followed by silver removal through nitric acid etching for 30 minutes. A CV scan in 1 M NaOH was performed at each step for characterization, as illustrated in Figure C.1.2.

In 1 M NaOH, the gold surface is expected to exhibit a reduction peak at approximately 0.05 V (vs. Ag/AgCl) [7]. The CV of the planar gold electrode

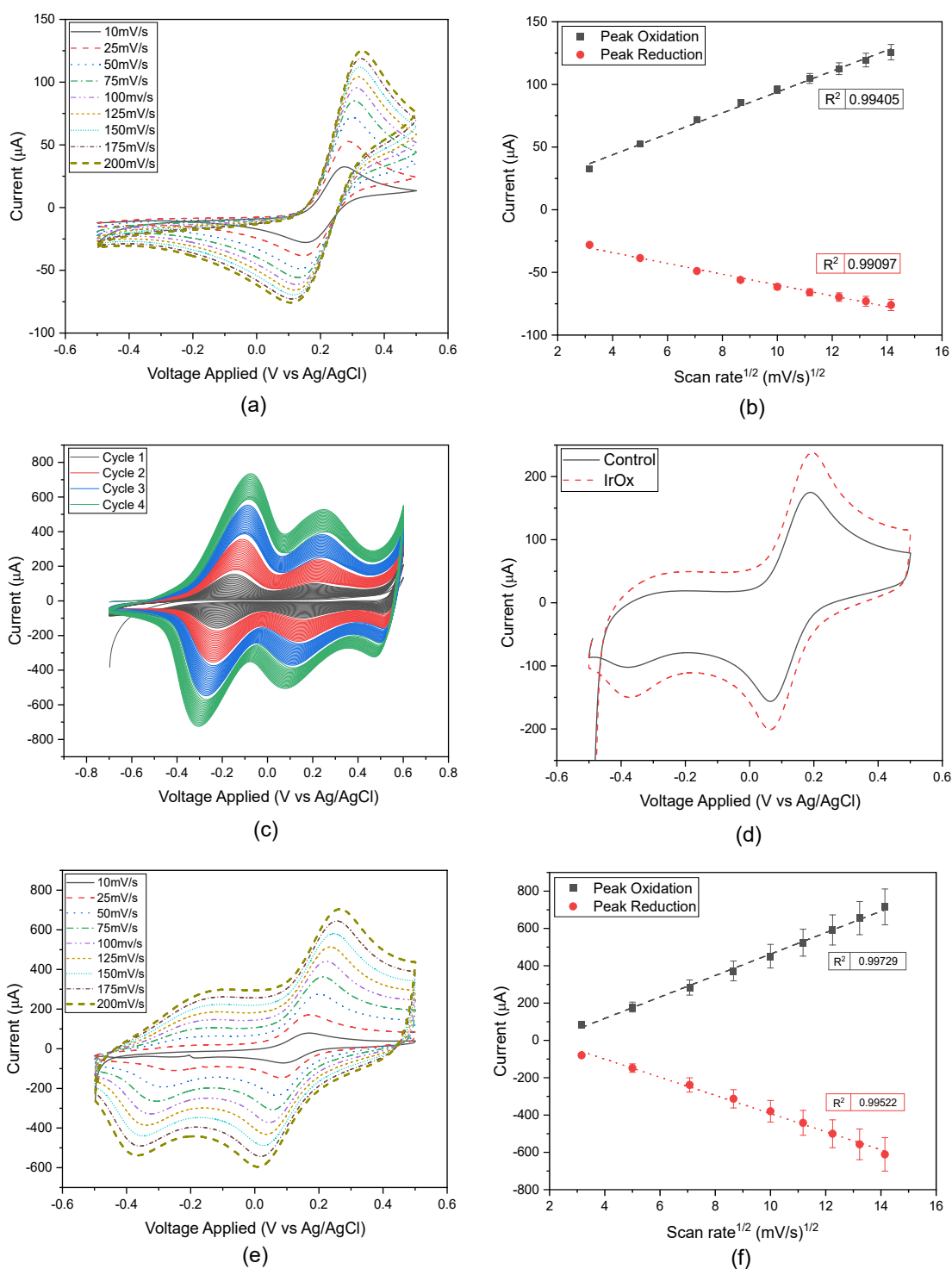


Figure C.1.1: (a) CV curves of control commercial SPEs in 5 mM Ferri-Ferro at scan rates of 10–200 mV/s; (b) corresponding redox peak currents versus square root of scan rate ($v^{1/2}$) calibration (n=3, mean $\pm$ SD); (c) CV curves of IrOx deposition (20 scans/cycle, -0.7 V to 0.6 V at 100 mV/s with 60 s rest, n=3); (d) CV curves before and after IrOx deposition in 5 mM Ferri-Ferro at 50 mV/s (n=3); (e) and (f) show the equivalent data of (a) and (b) for IrOx modified SPEs.

showed negligible current flow at this voltage. The AuAg alloy displayed distinct oxidation and reduction peaks for both gold and silver. The NPG scan confirmed

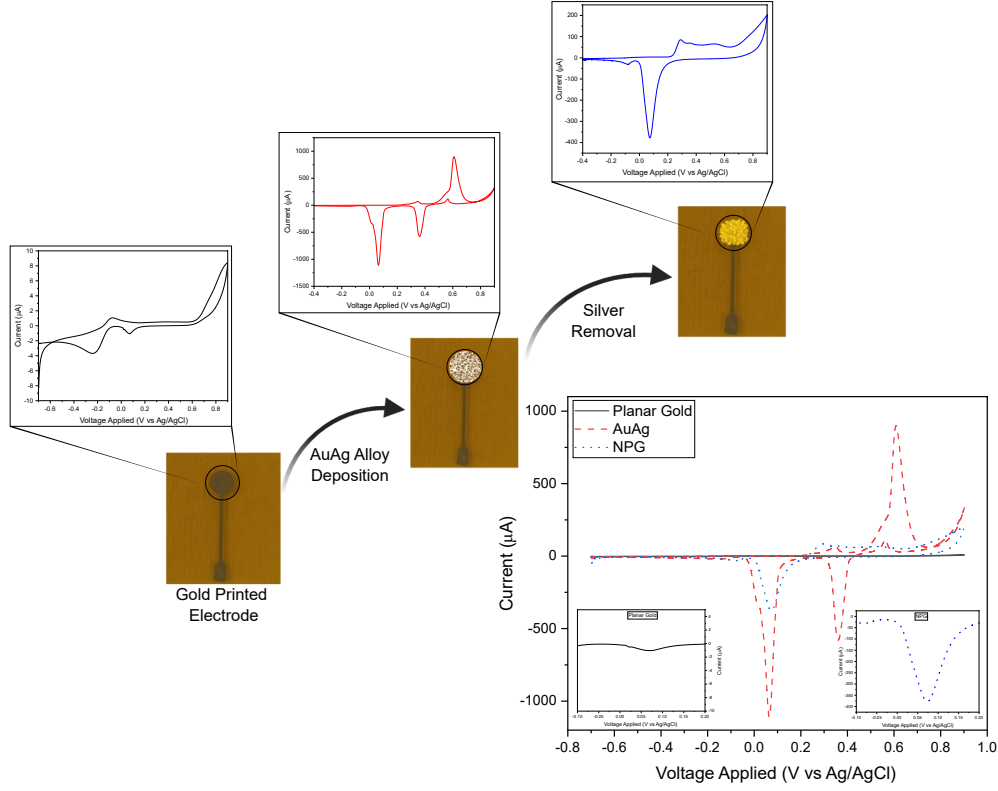


Figure C.1.2: Schematic diagram of the NPG deposition process, where the potential was swept from -0.7 V to 0.9 V , with a scan rate of 10 mV/s in 1 M NaOH .

the removal of silver from the electrode surface due to acid submersion. In this scan, the presence of the gold reduction peak, without any silver oxidation or reduction peaks, highlighted the enhanced surface area.

To evaluate the impact of NPG deposition on the sensor's active area, CV scans were conducted on both control and NPG-modified electrodes across various scan rates ranging from 10 to 200 mV/s , as shown in Figures C.1.3 (a) and (b), respectively. The resulting current peaks from both oxidative and reductive reactions exhibited a linear correlation with the square root of the scan rate, both before and after NPG functionalization, as depicted in Figures C.1.3 (c) and (d), respectively.

Further analysis, illustrated in Figure C.1.4, involved a CV scan in 1 M of NaOH , sweeping the voltage from -0.7 V to 0.9 V at 10 mV/s . After NPG deposition, a higher peak reduction current was observed, suggesting an increased active area. Calculations were made using the Randles-Sevcik equation (Equation C.1.1), revealing a 42.9% increase in the electrochemical active area after the NPG-modified electrode. The electrochemical active area of the control electrode was $9.48 \times 10^{-2}\text{ cm}^2$, while after the NPG deposition was $13.4 \times 10^{-2}\text{ cm}^2$ [8]. This further supported the detection difference between control and NPG deposition observed in Figure 4.7.

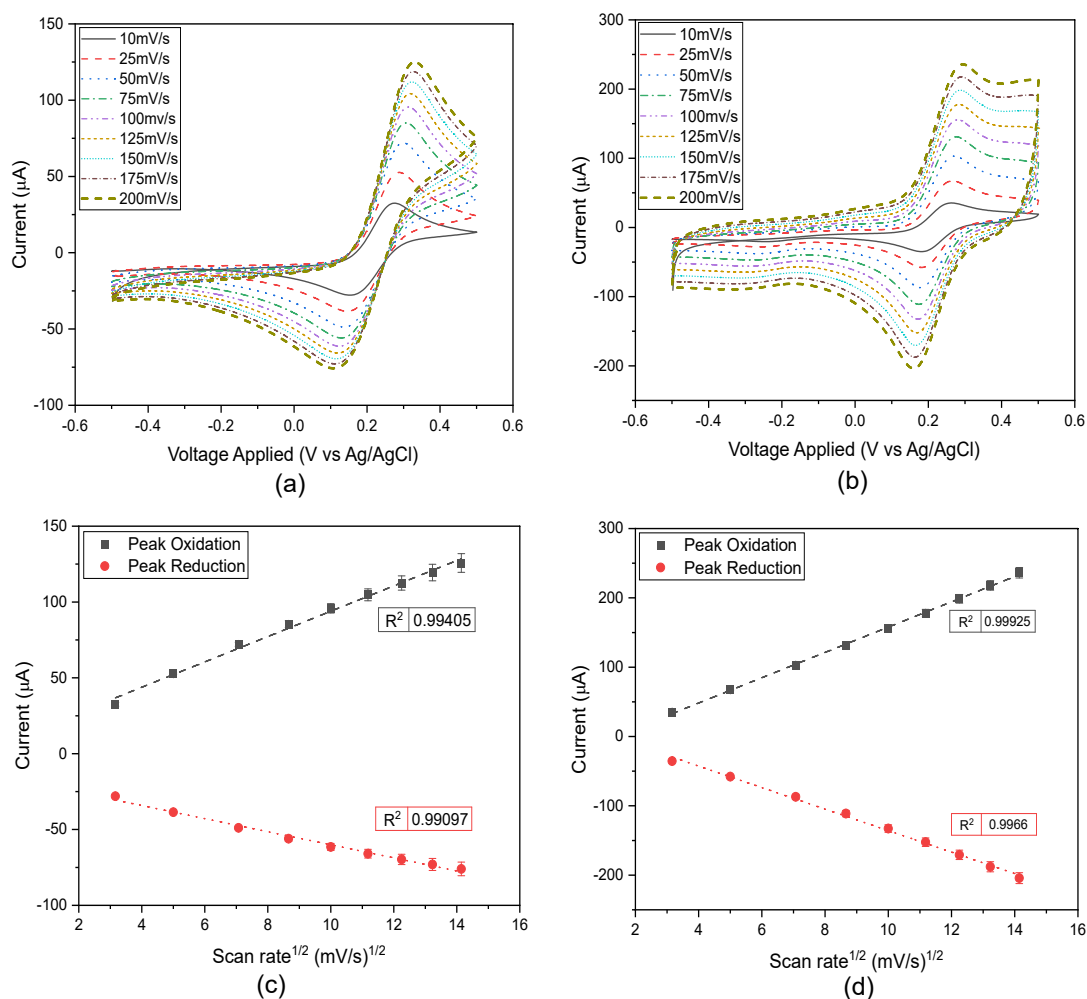


Figure C.1.3: CV curves of gold electrodes in 5 mM ferri-ferrocyanide at 10–200 mV/s: (a) control, (b) with NPG deposition, (c) and (d) corresponding redox peak currents vs. scan rate ($v^{1/2}$) calibration ($n=3$, mean $\pm$ SD).

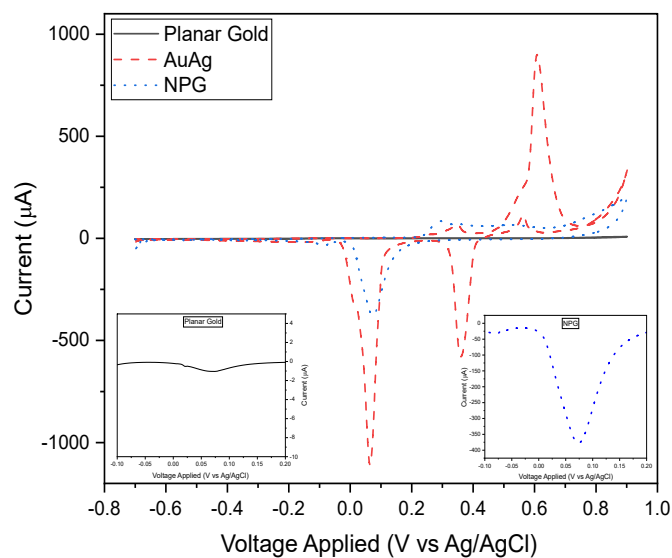


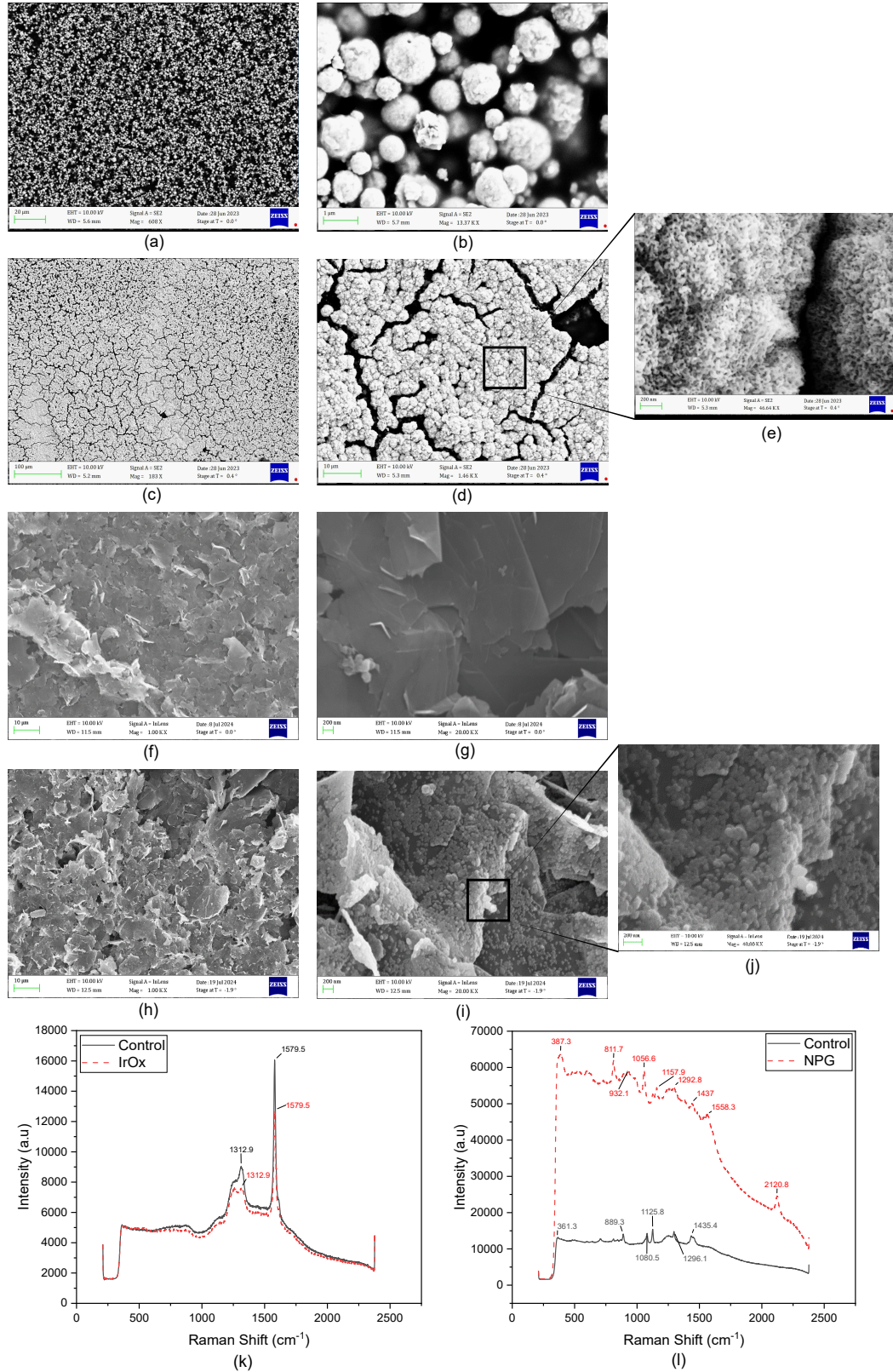
Figure C.1.4: CV curves of control, AuAg deposition, and NPG deposited in 1 M NaOH, voltage ranging from -0.7 V to 0.9 V at 10 mV/s.

C.1.2.1 SEM and Raman Analysis

SEM was employed to further characterise the surface morphology of the electrodes at higher magnifications. The gold structures of the in-house flexible electrodes are shown in Figures C.1.5 (a) and (b), while the NPG modification is illustrated in Figures C.1.5 (c)-(e). The NPG exhibited a distinct “sponge-like” 3D structure, with interconnected, tunable pores within a gold filament skeleton [9]. These pores can be adjusted in size, ranging from nanometer scale up to hundreds of nanometers, depending on fabrication conditions [10]. The NPG’s high porosity, combined with structural strength, superior catalytic properties, and high conductivity, position it as a promising addition to electrochemical sensing platforms [11]. Figures C.1.5 (f) and (g) showed SEM images of the unmodified surface of the commercial SPE, revealing a smooth surface. In contrast, the IrOx functionalization shown in Figures C.1.5 (h)-(j), displayed a rougher surface, consistent with the increased surface area introduced by the IrOx layer [12].

Raman measurements were taken before and after the deposition of IrOx and the modification with NPG to gain insight into the structural properties and potential defects of the modified layers. In Figure C.1.5 (k), two prominent signals around 1313 and 1580 cm^{-1} were observed in the spectrum of each sample. These peaks corresponded to the characteristic vibrational modes of carbon, related to the D band and G band, respectively [13]. These peaks indicate the presence of carbon material and remain visible in the presence of IrOx, though with reduced intensity [14].

The Raman spectra of the gold-printed electrode control, shown in Figure C.1.5 (l) exhibited similar behaviour to those reported in literature [15, 16]. The spectra showed an increase in intensity following the NPG structures, which is consistent with reports on the Raman signal attributed to the high surface area and unique morphology of NPG-modified electrodes [17, 18].



C.2 Peak Oxidation Comparision

This test was designed to evaluate the relative error of peak oxidation currents obtained from the PULSE in comparison to the theoretical peak oxidation values. For the purpose of comparison, the same evaluation was conducted using the benchtop potentiostat. CV was used for electrochemical characterisation of the commercial SPEs ($n=4$) in a 10 mM PBS buffer, pH 7.4, containing concentrations of ferri-ferrocyanide from 5 mM to 1 mM, sweeping the potential from -0.5 V to 0.5 V at 50 mV/s. The PULSE was subjected to different settings to evaluate the system's performance according to its configuration. The gain (G), feedback resistance (R_f), and feedback capacitor (C_f) were varied according to Table C.2.1. These settings are adjusted in the APP under the "Configurations" tab.

Table C.2.1: PULSE configuration settings variations, adjusting the feedback resistor (R_f), feedback capacitor (C_f), and gain.

System	R_f (k Ω)	Gain	C_f (nF)
A	9.9	1	10
B	1	10	10
C	1	10	1
D	9.9	1	1

The Randles-Sevcik equation was used to calculate the theoretical values of the oxidation peak current (i_p) associated with the solution concentration [19]. At a standard temperature of 25° C, Equation 1.14 can be further simplified as [2]:

$$i_p = 2.69 \times 10^5 n^{\frac{3}{2}} A C \sqrt{Dv} \quad (\text{C.2.1})$$

Where n denotes the number of electrons transferred in the redox event, A is the electrode area in cm², D is the diffusion coefficient in cm²/s, C represents the concentration in mol/cm³, and v stands for the scan rate in V/s. Since this equation does not account for capacitive currents and background noise, these effects can be mitigated by background current subtraction from the respective peak currents. The parameter values used to calculate the theoretical peak oxidation current are illustrated in Table C.2.2.

The process involved four SPE sensors along with four distinct variations of the PULSE system. The results from the PULSE are illustrated in Figure C.2.1 (a)-(d) depicting the average of four sensors across five concentrations of ferri-ferrocyanide, while Figure C.2.1 (e) depicts the results from the benchtop instrument.

Table C.2.2: Variables and corresponding values used in the Randles-Sevcik equation for theoretical peak current calculations.

Variable	Value
n	1
A^a	0.126 cm^2
D	$6.400 \times 10^{-6} \text{ cm}^2/\text{s}^b$
v	0.05 V/s
C	$1 - 5 \text{ mM}$

^a $A = \pi r^2$, where r is the radius of the WE.

^b Moldenhauer et al. [3].

The peak oxidation currents were examined and plotted against the solution concentrations to facilitate a comparative analysis among these systems, as depicted in Figure C.2.2. A summary of the linear fitting analysis is presented in Table C.2.3, including the corresponding linear equation relating the current (y) in amperes (A) to the concentration (x), and the coefficient of determination (R^2), indicating the goodness of fit for each regression model.

Table C.2.3: Summary of linear regression analyses for calibration plots from the benchtop instrument and PULSE system variations.

Device	Linear Equation	R^2
Benchtop Instrument	$y = 2.189 \times 10^{-5}x + 2.161 \times 10^{-6}$	0.9997
System A	$y = 2.046 \times 10^{-5}x + 5.993 \times 10^{-6}$	0.9934
System B	$y = 2.168 \times 10^{-5}x + 4.932 \times 10^{-6}$	0.9951
System C	$y = 2.126 \times 10^{-5}x + 6.873 \times 10^{-6}$	0.9914
System D	$y = 1.996 \times 10^{-5}x + 1.143 \times 10^{-5}$	0.9985

The subsequent linear fitting analysis revealed that all systems followed a similar trend with minor variations in coefficients. The R^2 values consistently exceed 0.99, suggesting a strong linear relationship between the current and concentration. Correlation analyses comparing the datasets from the benchtop instrument and the PULSE system demonstrated a high agreement, with correlation coefficients > 0.99 . Therefore, it is justified to conclude that the data obtained from the benchtop potentiostat and the PULSE are highly comparable.

The theoretical values associated with the oxidation peak current (i_p) across five concentrations of ferri-ferrocyanide were calculated using Equation C.2.1. As mentioned before, the calibration curve's intersection value was subtracted from

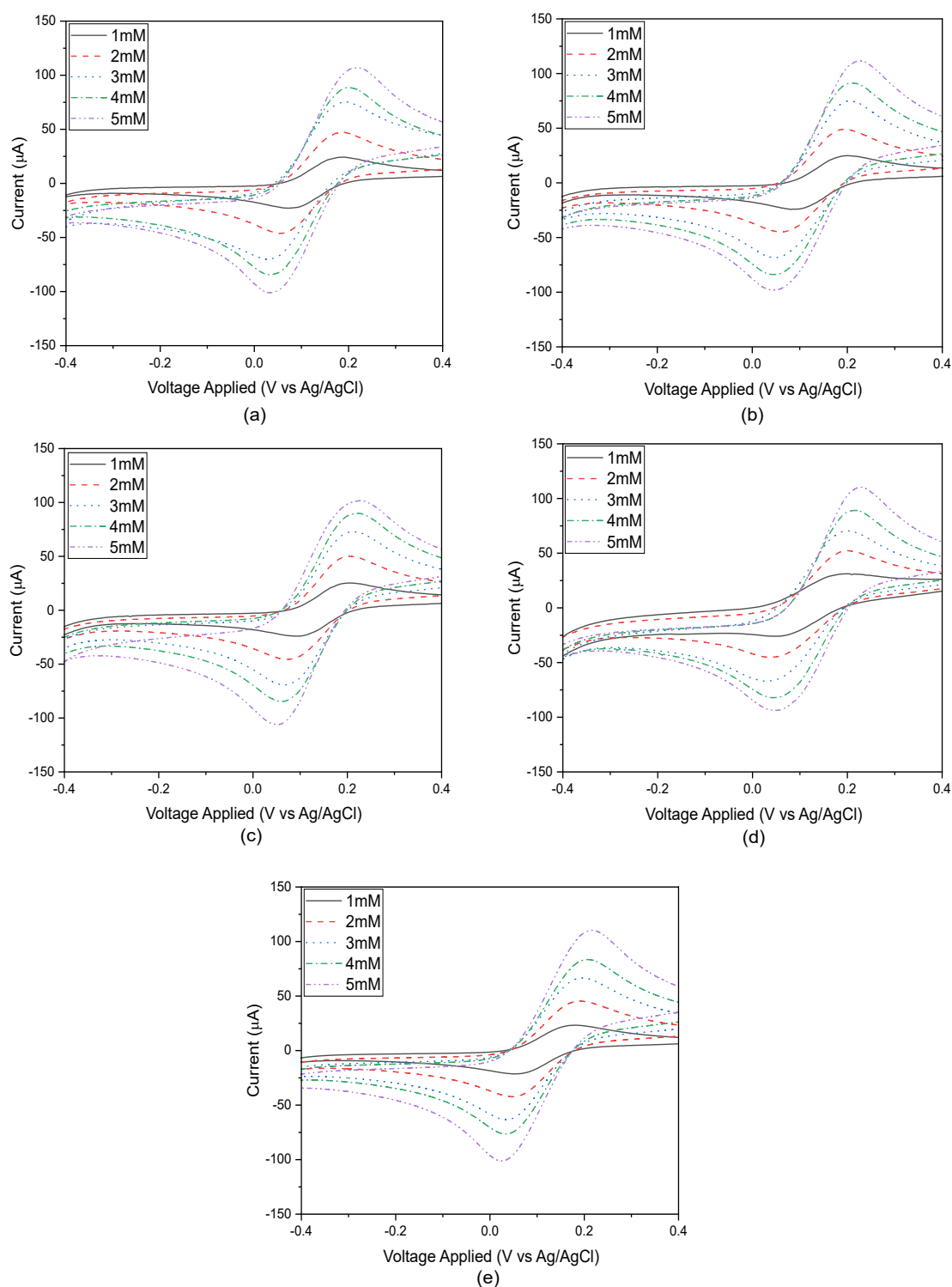


Figure C.2.1: CV scans using the PULSE System (a) A, (b) B, (c) C, and (d) D, and (e) the benchtop instrument, where the potential was swept from -0.5 V to 0.5 V at 50 mV/s with varied concentrations of ferri-ferro in 10 mM PBS ($n=4$).

the respective peak current to mitigate background noise. A detailed overview of the theoretical values alongside the peak currents after background removal is provided in Table C.2.4.

The relative errors were calculated using the standard formula for relative

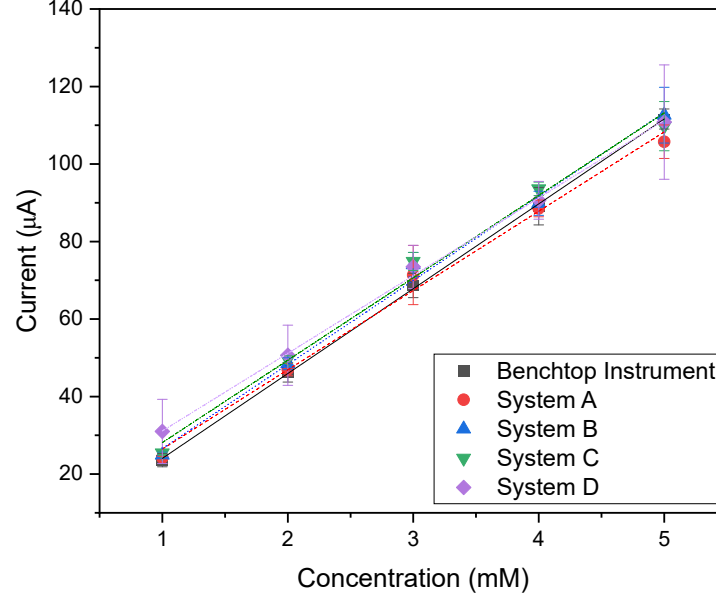


Figure C.2.2: Calibration curves of oxidation peak current versus ferri-ferrocyanide concentration measured by the PULSE system variations and benchtop instrument (n=4, mean $\pm$ SD).

Table C.2.4: Theoretical and background removed oxidation peak currents (μ A) for multiple concentrations of ferri-ferrocyanide (mM) from the benchtop instruments and the PULSE system variations.

Conc.	Theor.	Benchtop	S. A	S. B	S. C	S. D
5	95.611	109.438	99.761	107.674	102.901	110.822
4	76.489	86.935	82.535	84.920	86.721	90.637
3	57.367	66.503	65.359	69.102	67.992	73.456
2	38.244	44.069	41.005	43.543	42.757	50.656
1	19.122	21.417	18.230	19.962	18.570	31.003

error [20], and the results are displayed in Figure C.2.3.

$$\text{Relative Error} = \frac{|\text{Theoretical Value} - \text{Measured Value}|}{\text{Theoretical Value}} \quad (\text{C.2.2})$$

From the analysis of the graph, systems B and D consistently demonstrated lower relative errors than the benchtop instrument, suggesting superior accuracy in measuring oxidation peak currents during the experiment. Overall, the benchtop instrument exhibited higher relative errors and, consequently, lower accuracy in this particular experiment.

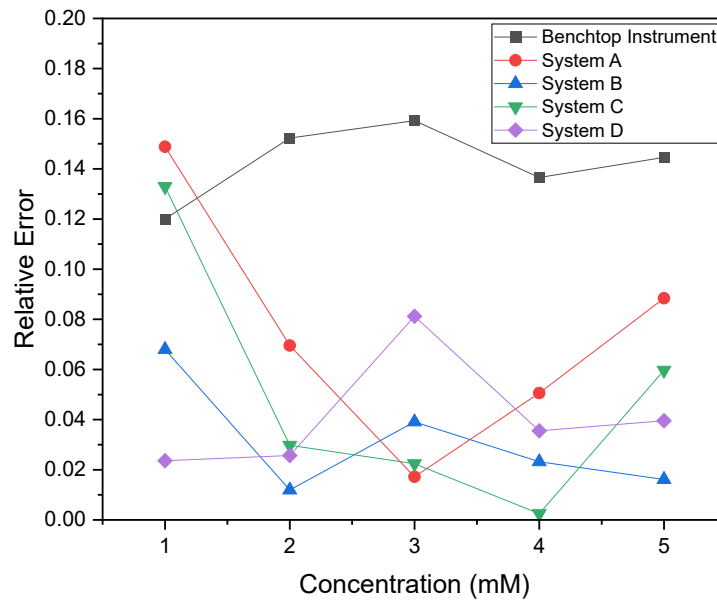


Figure C.2.3: Relative error analysis of oxidation peak current measurements against ferri-ferrocyanide concentrations for the benchtop instrument and the PULSE systems variations.

Table C.2.5: CV oxidation and reduction peaks detecting multiple concentrations of ferri-ferro using the benchtop potentiostat (n=4).

Concentration [mM]	Average				Standard Deviation			
	Oxidation Peak		Reduction Peak		Oxidation Peak		Reduction Peak	
	Potential [V]	Current [A]	Potential [V]	Current [A]	Potential [V]	Current [A]	Potential [V]	Current [A]
5	2.031E-01	1.116E-04	3.586E-02	-1.044E-04	1.983E-02	2.590E-06	9.534E-03	2.986E-06
4	1.927E-01	8.910E-05	3.647E-02	-8.285E-05	1.695E-02	4.821E-06	1.176E-02	5.201E-06
3	1.842E-01	6.866E-05	1.272E-01	-6.680E-05	1.176E-02	3.144E-06	1.265E-01	4.144E-06
2	1.805E-01	4.623E-05	5.538E-02	-4.301E-05	1.055E-02	2.498E-06	7.820E-03	1.869E-06
1	1.701E-01	2.358E-05	6.454E-02	-2.126E-05	1.014E-02	1.702E-06	8.706E-03	1.372E-06

Table C.2.6: CV oxidation and reduction peaks detecting multiple concentrations of ferri-ferro using the PULSE System A (n=4).

Concentration [mM]	Average				Standard Deviation			
	Oxidation Peak		Reduction Peak		Oxidation Peak		Reduction Peak	
	Potential [V]	Current [A]	Potential [V]	Current [A]	Potential [V]	Current [A]	Potential [V]	Current [A]
5	2.114E-01	1.058E-04	3.847E-02	-9.923E-05	1.848E-02	4.315E-06	1.159E-02	5.555E-06
4	2.003E-01	8.853E-05	4.465E-02	-8.519E-05	1.125E-02	2.152E-06	8.098E-03	4.493E-06
3	1.990E-01	7.135E-05	4.959E-02	-6.895E-05	1.077E-02	7.643E-06	5.383E-03	8.116E-06
2	1.854E-01	4.700E-05	6.441E-02	-5.200E-05	9.486E-03	1.637E-06	4.096E-03	9.676E-06
1	1.916E-01	2.422E-05	7.305E-02	-2.276E-05	6.050E-03	3.658E-07	6.986E-03	3.016E-06

Table C.2.7: CV oxidation and reduction peaks detecting multiple concentrations of ferri-ferro using the PULSE System B (n=4).

Concentration [mM]	Average				Standard Deviation			
	Oxidation Peak		Reduction Peak		Oxidation Peak		Reduction Peak	
	Potential [V]	Current [A]	Potential [V]	Current [A]	Potential [V]	Current [A]	Potential [V]	Current [A]
5	2.302E-01	1.126E-04	5.480E-02	-1.049E-04	6.273E-03	7.177E-06	3.441E-02	6.687E-06
4	2.079E-01	8.985E-05	6.221E-02	-8.945E-05	1.111E-02	3.111E-06	3.276E-02	2.747E-06
3	2.042E-01	7.403E-05	6.715E-02	-7.150E-05	7.876E-03	3.171E-06	2.979E-02	1.625E-06
2	1.956E-01	4.848E-05	7.580E-02	-4.577E-05	4.523E-03	1.522E-06	1.976E-02	1.616E-06
1	1.993E-01	2.489E-05	8.568E-02	-2.441E-05	2.226E-03	8.233E-07	1.377E-02	2.976E-07

Table C.2.8: CV oxidation and reduction peaks detecting multiple concentrations of ferri-ferro using the PULSE System C (n=4).

Concentration	Average				Standard Deviation			
	Oxidation Peak		Reduction Peak		Oxidation Peak		Reduction Peak	
	Potential	Current	Potential	Current	Potential	Current	Potential	Current
[mM]	[V]	[A]	[V]	[A]	[V]	[A]	[V]	[A]
5	2.191E-01	1.098E-04	1.549E-01	-1.069E-04	2.714E-02	6.355E-06	1.634E-01	1.227E-05
4	2.203E-01	9.359E-05	7.209E-02	-8.700E-05	1.411E-02	1.724E-06	2.805E-02	5.228E-06
3	2.129E-01	7.487E-05	7.827E-02	-7.353E-05	1.232E-02	2.334E-06	2.390E-02	2.913E-06
2	2.042E-01	4.963E-05	8.321E-02	-4.696E-05	4.142E-03	8.728E-07	2.084E-02	1.705E-06
1	2.030E-01	2.544E-05	9.062E-02	-2.447E-05	5.076E-03	4.766E-07	1.504E-02	6.233E-07

Table C.2.9: CV oxidation and reduction peaks detecting multiple concentrations of ferri-ferro using the PULSE System D (n=4).

Concentration	Average				Standard Deviation			
	Oxidation Peak		Reduction Peak		Oxidation Peak		Reduction Peak	
	Potential	Current	Potential	Current	Potential	Current	Potential	Current
{mM}	{V}	{A}	{V}	{A}	{V}	{A}	{V}	{A}
5	2.326E-01	1.108E+02	6.345E-02	-1.024E-04	6.162E-03	14.77	3.305E-02	1.357E-05
4	2.178E-01	9.064E+01	6.345E-02	-9.346E-05	1.140E-02	4.860	2.882E-02	4.958E-06
3	2.067E-01	7.346E+01	6.962E-02	-7.029E-05	2.044E-02	5.584	3.559E-02	3.571E-06
2	2.104E-01	5.066E+01	7.703E-02	-5.523E-05	4.270E-02	7.749	2.843E-02	7.630E-06
1	1.845E-01	3.100E+01	8.815E-02	-3.370E-05	3.091E-02	8.278	2.893E-02	1.035E-05

Table C.2.10: Relative error analysis of peak current measurements across ferri-ferro concentrations for the benchtop instrument and the PULSE system variations.

Concentration (mM)	Relative error				
	Benchtop Inst.	System A	System B	System C	System D
5	0.144621	0.088424	0.016123	0.059732	0.039559
4	0.136578	0.050611	0.023181	0.002464	0.035555
3	0.159258	0.017200	0.039090	0.022402	0.081238
2	0.152298	0.069529	0.011932	0.029779	0.025700
1	0.120027	0.148826	0.067953	0.132952	0.023623

Table C.2.11: Accuracy analysis of peak current measurements across Ferri-Ferro concentrations for the benchtop instrument and the PULSE system variations.

Concentration (mM)	Accuracy (%)				
	Benchtop Inst.	System A	System B	System C	System D
5	85.54	91.16	98.39	94.03	96.04
4	86.34	94.94	97.68	99.75	96.44
3	84.07	98.28	96.09	97.76	91.88
2	84.77	93.05	98.81	97.02	97.43
1	88.00	85.12	93.20	86.70	97.64

C.3 pH ISF

Table C.3.1: OCP measurements at multiple pH levels in artificial ISF, using the benchtop instrument (n=2).

pH	Voltage (V)			
	Sensor 1	Sensor 2	Average	Standard Deviation
3	0.4715	0.406333	0.438917	0.032583
5	0.308667	0.285333	0.297	0.011667
7	0.188333	0.1105	0.149417	0.038917
9	-0.002	-0.01983	-0.01092	0.008917
11	-0.13267	-0.0935	-0.11308	0.019583

Table C.3.2: OCP measurements at multiple pH levels in artificial ISF, using the PULSE (n=2).

pH	Voltage (V)			
	Sensor 1	Sensor 2	Average	Standard Deviation
3	0.405332	0.367175	0.386253	0.019079
5	0.295329	0.267872	0.2816	0.013729
7	0.141606	0.135512	0.138559	0.003047
9	0.028373	0.025668	0.027021	0.001352
11	-0.13394	-0.1209	-0.12742	0.00652

Table C.3.3: Repeatability of pH measurements (V) over 41 days for artificial ISF solutions at various pH levels.

pH	Day 1 Avg $\pm$ SD	Day 7 Avg $\pm$ SD	Day 14 Avg $\pm$ SD	Day 21 Avg $\pm$ SD	Day 41 Avg $\pm$ SD
3	0.406167 $\pm$ 0.0343	0.4015 $\pm$ 0.0166	0.385389 $\pm$ 0.0180	0.384 $\pm$ 0.0086	0.356083 $\pm$ 0.0009
5	0.2665 $\pm$ 0.0227	0.201722 $\pm$ 0.0449	0.225056 $\pm$ 0.0172	0.225778 $\pm$ 0.0061	0.221583 $\pm$ 0.0001
7	0.108611 $\pm$ 0.0197	0.102889 $\pm$ 0.0199	0.100333 $\pm$ 0.0136	0.102444 $\pm$ 0.0055	0.101917 $\pm$ 0.0006
9	-0.04039 $\pm$ 0.0160	-0.02844 $\pm$ 0.0159	-0.035 $\pm$ 0.0128	-0.03322 $\pm$ 0.0043	-0.0045 $\pm$ 0.0020

Table C.3.4: Correlation coefficient table across all days, during repeatability study.

	day 1	day7	day 14	day 21	day 41
day1	-	0.98598	0.997209	0.997348	0.997624
day 7	-	-	0.995685	0.995488	0.993946
day 14	-	-	-	0.999993	0.999417
day 21	-	-	-	-	0.999335
day 41	-	-	-	-	-

Table C.3.5: OCP measurement for the detection of interference in artificial ISF pH 3, (n=3, mean $\pm$ SD).

	pH 3	MgCl ₂	NaCl	KCl
Benchtop Instrument	0.3818 $\pm$ 0.01513	0.3937 $\pm$ 0.01963	0.3876 $\pm$ 0.02413	0.3662 $\pm$ 0.0092
PULSE System	0.3823 $\pm$ 0.0151	0.3550 $\pm$ 0.0162	0.3558 $\pm$ 0.0055	0.3507 $\pm$ 0.0077

Table C.3.6: OCP measurements in artificial ISF solutions with increasing and decreasing pH values, using the PULSE (n=3).

pH	Voltage (V)				
	Sensor 1	Sensor 2	Sensor 3	Average	Standard Deviation
3	0.31361	0.3648	0.35688	0.345097	0.022498
5	0.14732	0.245169	0.14732	0.179937	0.046126
7	0.019182	0.072992	0.057502	0.049892	0.022617
9	-0.07994	-0.04799	-0.06903	-0.06565	0.01326
11	-0.24102	-0.17316	-0.17906	-0.19775	0.030696
9	-0.12584	-0.08287	-0.09485	-0.10119	0.018108
7	0.030534	0.061506	0.047739	0.046593	0.01267
5	0.146184	0.187205	0.157925	0.163771	0.017249
3	0.342493	0.368215	0.360144	0.356951	0.010741

C.4 Nitrate Measurements

Table C.4.1: Calibration data of peak reduction currents for varying concentrations of nitrate in PBS (n=2, mean $\pm$ SD).

Concentration (ppm)	Benchtop Instrument		PULSE System	
	Current (μ A)	SD (μ A)	Current (μ A)	SD (μ A)
0	-51.6983	5.00336	-51.6983	1.61805
50	-71.54846	10.08606	-71.9238	2.42705
100	-80.03235	2.79236	-77.9914	2.02255
150	-92.43774	7.1106	-95.38535	4.8541
200	-109.22241	4.27246	-104.28455	0.809

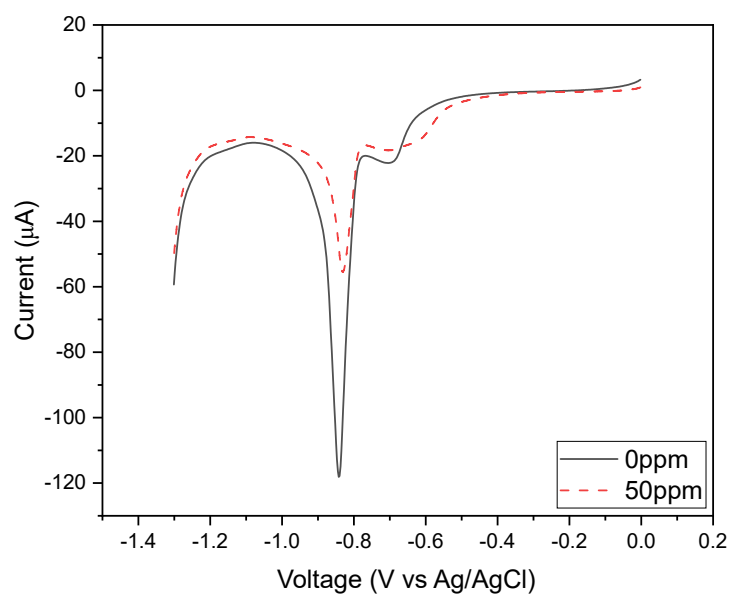


Figure C.4.1: Voltammograms detecting nitrate of 0 ppm and 50 ppm in PBS, using commercial carbon electrodes.

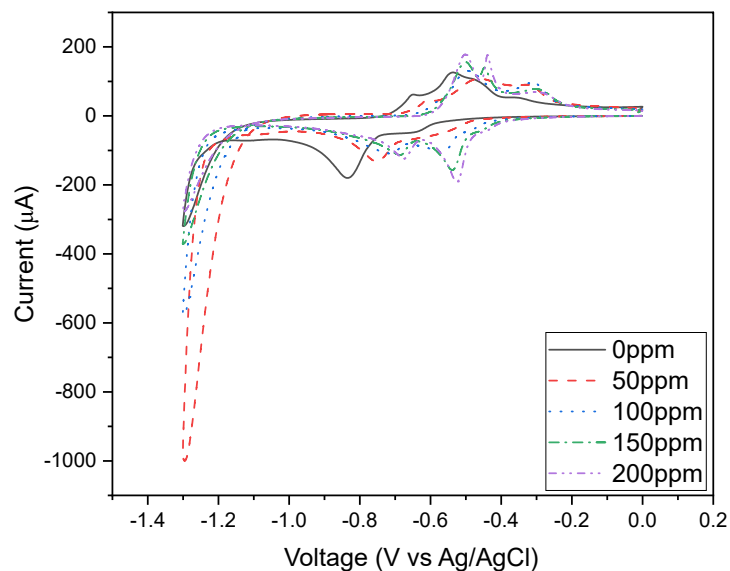


Figure C.4.2: Voltammograms scans detecting nitrate from 0 ppm to 200 ppm in PBS, using commercial gold electrodes.

C.5 Nitrite Measurements

Table C.5.1: Calibration data of the nitrite measurements, including input voltage (V_{in}), measured current (I_{real}), and serial current value (I_{serial}).

V_{in} (V)	I_{real} (A)	I_{serial} (A)
0.197	0.000095	0.001379
0.496	0.000236	0.001092
0.596	0.000287	0.000996
0.795	0.000382	0.000806
0.994	0.000477	0.000617
1.193	0.0005715	0.000427
1.393	0.000666	0.000236

Table C.5.2: Calibration data of peak oxidation currents for varying concentrations of nitrite in PBS (n=3, mean $\pm$ SD).

Concentration (ppm)	Benchtop Instrument		PULSE System	
	AVG (μ A)	SD (μ A)	AVG (μ A)	SD (μ A)
0	95.80	3.83	85.83	7.37
50	189.33	6.94	152.63	24.41
100	207.33	7.59	189.74	15.55
150	229.00	6.98	223.96	15.55
200	249.33	9.84	266.10	16.53

Table C.5.3: Calibration data of peak oxidation currents across multiple concentrations of nitrite in PBS at a fixed pH 7 (n=2, mean $\pm$ SD), along with OCP measurements (V) for pH detection.

Concentration (ppm)	Current (μ A)	SD (μ A)	Potential (V)	SD (V)
0	101.19713	7.27101	-0.11066	0.01105
50	138.35992	26.66032	-0.21773	0.04583
100	165.02015	19.38932	-0.11066	0.07687
150	201.37478	8.88644	-0.21773	0.04263
200	207.03045	46.85761	-0.18497	0.00136

Table C.5.4: pH Calibration data for in 100 ppm nitrite in PBS solution (n=2, mean $\pm$ SD), showing the average current for 100 ppm nitrite in PBS across multiple pH levels.

pH	Potential (V)	SD (V)	Current (μ A)	SD (μ A)
3	0.31563	0.08014	116.00839	13.46476
5	0.18897	0.0424	159.90355	5.11663
7	0.04274	0.04786	175.79195	0
9	-0.05776	0.05166	150.74746	2.96225

Table C.5.5: Calibration data of peak oxidation currents for varying concentrations of nitrite in ISF measured with the PULSE system (n=3, mean $\pm$ SD).

Concentration (ppm)	Current (μ A)				
	Sensor 1	Sensor 2	Sensor 3	AVG	SD
0	119.7785	127.8573	144.0151	130.5503	10.07613
50	176.3306	168	153.7097	166.0134	9.341162
100	198.9513	223	198.9513	206.9675	11.33668
150	253.8881	263.5825	252.2723	256.5809	4.994569
200	295.8972	313.6702	281.3556	296.9743	13.21439

Table C.5.6: pH calibration data for 100 ppm nitrite in artificial ISF solution (n=3, mean $\pm$ SD), including current measurements of 100 ppm nitrite across multiple pH levels, and temperature measurements.

pH	Potential (V)	SD (V)	Current (μ A)	SD (μ A)	Temperature ($^{\circ}$ C)	SD ($^{\circ}$ C)
3	0.34299	0.03499	151.55539	6.23465	20.34215	0.1363
5	0.23526	0.02795	152.27354	16.75893	19.99959	0.03977
7	0.0673	0.01851	188.71802	9.26613	19.82965	0.05215
9	-0.04305	0.01771	224.26492	7.95287	19.69944	0.06141
11	-0.21779	0.01318	228.57421	4.03124	19.65394	0.04591

Table C.5.7: Calibration data of peak oxidation currents across multiple concentrations of nitrite in ISF at a fixed pH 7 (n=3, mean $\pm$ SD), along with OCP measurements (V) for pH detection, and temperature readings.

Concentration	Current (μ A)	SD (μ A)	Potential (V)	SD (V)	Temperature ($^{\circ}$ C)	SD ($^{\circ}$ C)
0	139.16776	12.0913	0.04941	0.01781	20.62409	0.05468
50	174.17621	11.22027	0.08514	0.03332	20.66476	0.27672
100	216.72489	14.27037	0.08331	0.0356	20.37515	0.14002
150	269.50683	28.42755	0.09208	0.02809	20.57007	0.03462
200	287.27991	20.4518	0.09482	0.01356	20.83625	0.22407

C.6 Real-World Experiments

Table C.6.1: OCP measurements for multiple pH detection in ruminal fluid, using the PULSE.

pH	Voltage (V)				
	Sensor 1	Sensor 2	Sensor 3	AVG	SD
3	0.337304	0.344878	0.302284	0.328156	0.018553
5	0.17145	0.155659	0.170643	0.165917	0.007261
7	0.012042	-0.01707	-0.0149	-0.00664	0.013242
9	-0.07818	-0.04728	-0.07622	-0.06723	0.014127
11	-0.17805	-0.13028	-0.14647	-0.1516	0.019838

C.7 Water Quality Application

To demonstrate the potential of the PULSE system for water quality monitoring, an experiment was conducted using tap water from the Tyndall National Institute facilities. The water was spiked with 50, 100, and 150 ppm of nitrite and nitrate and measured using NPG-modified electrodes. The results were assessed by performing recovery calculations using the following equation:

$$Recovery(\%) = \frac{Total - Control}{Spiked} \times 100 \quad (C.7.1)$$

Where *Total* refers to the detected concentration after spiking, *Control* represents the initial concentration measured before spiking, and *Spiked* is the known added amount. These calculations provided an assessment of the device's accuracy in detecting changes in nitrate and nitrite levels under controlled experimental conditions, thereby demonstrating its potential applicability for real-world water quality applications.

Calibration curves for nitrite and nitrate were generated using solutions ranging from unaltered tap water to 200 ppm. Simultaneously, pH and temperature were recorded for each nitrite concentration. To determine the pH based on the measured voltage ($V_{measured}$), Equation C.7.2 was used, derived from Chapter 3, as a pH calibration equation in 10 mM PBS. To validate the accuracy of the pH measurements, a commercial pH probe was used before each reading.

$$pH = \frac{V_{measured} - 0.42067}{-0.06043} \quad (C.7.2)$$

Calibration curves for nitrite and nitrate, based on oxidation and reduction peaks, are shown in Figure C.7.1. The calibration curves exhibited high linearity,

with R^2 values of 0.97 for nitrite and 0.996 for nitrate. These indicated the high accuracy of the PULSE system in quantifying these analytes.

The system's reliability was further assessed through recovery tests performed at three concentration levels (50, 100, and 150 mg/kg) in triplicate for both nitrite and nitrate. For nitrite, the recovery rates ranged from 93.4% to 118.7%, while for nitrate, the recovery rates varied between 91.0% and 134.2%. These results demonstrate the PULSE system's capability to accurately detect and quantify nitrite and nitrate across a broad concentration range.

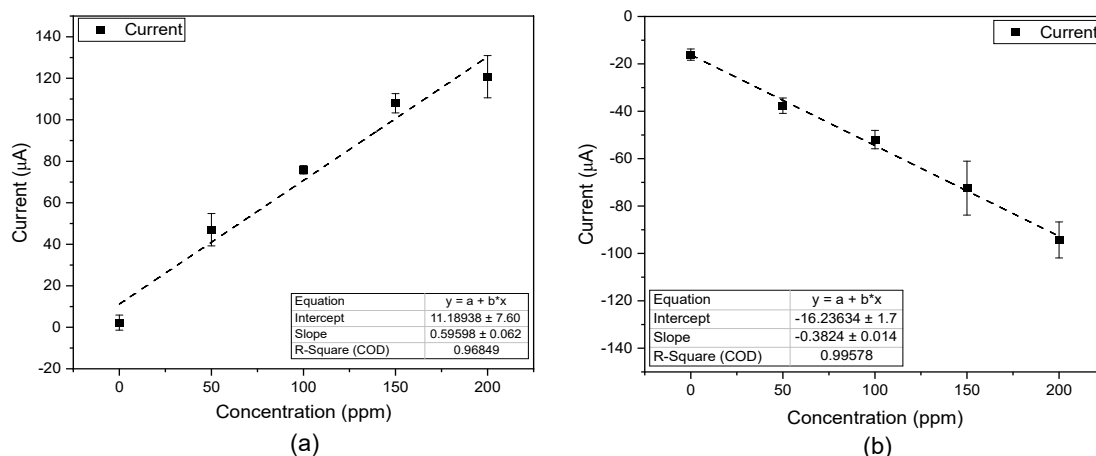


Figure C.7.1: Calibration plot of (a) peak oxidation currents across multiple concentrations of nitrite; (b) peak reduction currents across multiple concentrations of nitrate ($n=3$, mean $\pm$ SD).

Additionally, OCP measurements were conducted in triplicate for tap water solutions with nitrite concentrations ranging from the control (unaltered tap water) to 200 ppm. The results were compared to readings obtained from a commercial pH probe as depicted in Figure C.7.2 and Table C.7.1. The comparison revealed a strong correlation between the datasets from the two systems, with a correlation coefficient >0.99 . This high correlation, coupled with low SDs across the measurements, highlighted the consistency and reliability of the PULSE system.

Table C.7.1: pH detection in tap water spiked with nitrite, using a commercial probe and the PULSE ($n=3$).

Concentration (ppm)	Commercial Probe		PULSE System	
	pH	SD	pH	SD
0	7.343	0.0125	7.339	0.0227
50	7.403	0.0340	7.411	0.0194
100	7.337	0.0287	7.349	0.0225
150	7.253	0.0125	7.252	0.0330
200	7.220	0.0356	7.212	0.0592

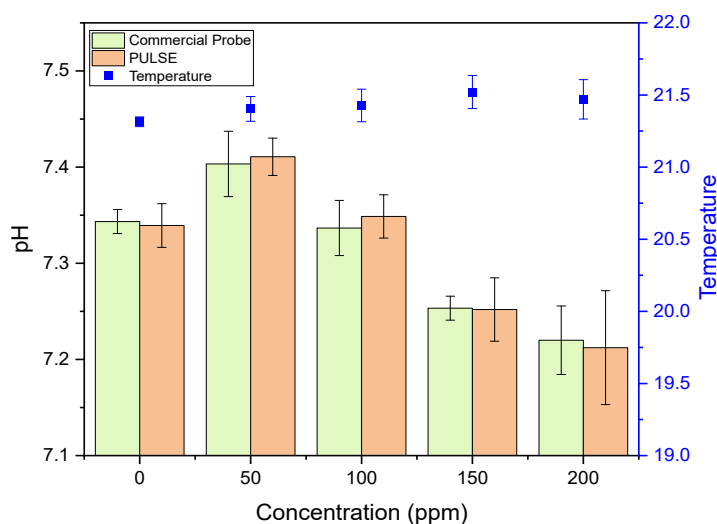


Figure C.7.2: pH measurements using a commercial probe and the PULSE, and temperature measurements, at varying concentrations of nitrite ($n=3$, mean $\pm$ SD).

C.8 Microneedle-based Application

The integration of microneedles for the detection of pH, potassium, and calcium was validated, demonstrating the device's versatility for microneedle-based applications. An holder was used to allow for the insertion of the sensors in liquid media without leakage to the electronics, as illustrated in Figure C.8.1 (a), while maintaining a connection with the PULSE as shown in Figure C.8.1 (b).

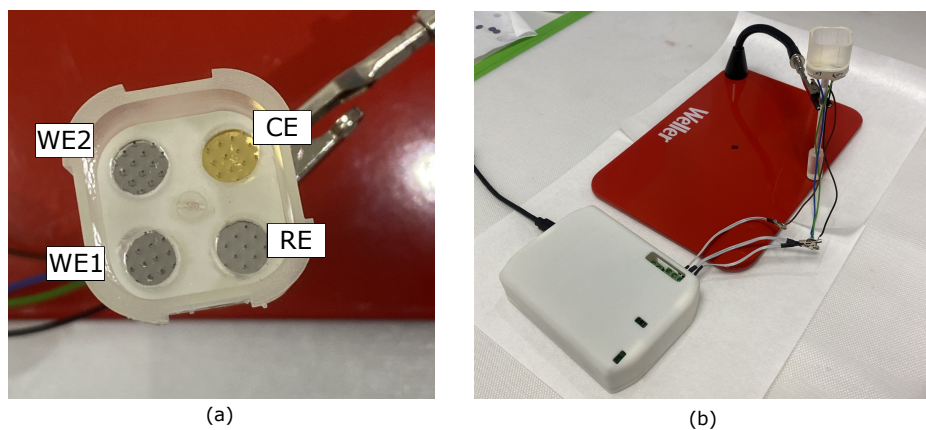


Figure C.8.1: Microneedles electrochemical setup (a) holder top view, WE1 detected potassium and WE2 detected calcium; and (b) setup used to integrate the microneedles with the PULSE.

Microneedles were passivated at the base using ArCare 7759 film (Adhesives Research, Limerick, Ireland), leaving exclusively the needles exposed. For calcium and potassium detection, PEDOT was electrochemically deposited using CA for 30 seconds at 1 V to enhance adhesion for the ISM, which was subsequently

applied by drop-casting. These microneedles were coated with ISMs specifically designed for potassium and calcium ion detection.

The ISM for potassium consisted of 2% potassium ionophore, 0.6% potassium tetrakis(4-chlorophenyl) borate, 32.7% polyvinyl chloride (PVC), and 64.7% of 2-nitrophenyloctyl ether diluted in tetrahydrofuran. For calcium detection, the ISM was composed of 32.7% PVC, 65.4% of 2-nitrophenyloctyl ether, 1.3% calcium ionophore, and 0.6% potassium tetrakis(4-chlorophenyl) borate, also diluted in tetrahydrofuran. After preparation, the membrane solution was applied to the WE surface and allowed to dry at room temperature for at least 8 hours.

SWV was used to measure potassium and calcium concentrations in triplicate ($n=3$), with concentrations ranging from 1 mM to 200 mM in DI water. The WEs were functionalised as described above, with a platinum RE and a gold CE. For potassium detection, SWV measurements utilised a potential range of -0.5 V to 0.5 V, while calcium measurements swept potential from -0.4 V to 0.4 V, both with a step size of 0.005 V and a frequency of 50 Hz. The PULSE was configured with $R_f = 9.9 \text{ k}\Omega$, $C_f = 10 \text{ nF}$, and a gain of 100 for calcium detection, yielding a current range of $\pm 1.62 \mu\text{A}$. For potassium detection, the device was configured with a gain of 10, providing a current range of $\pm 16.16 \mu\text{A}$.

For pH detection, WE microneedles were passivated with ArCare and functionalized with an IrOx sensing layer. This was achieved through 50 cycles of CV scans, performed over a potential range of -0.7 V to 0.6 V at a scan rate of 10 mV/s. OCP measurements were conducted in triplicate ($n=3$) for pH levels ranging from 3 to 11 in 10 mM of PBS.

To evaluate the reliability of the PULSE system interfaced with microneedles, its performance was benchmarked against a standard benchtop potentiostat. A correlation analysis was performed to validate the results obtained with the portable platform, demonstrating its potential for accurate and reliable detection of the target analytes.

A double-layer study was performed to obtain C_{dl} and R_{ct} , via EIS in a 200 mM calcium solution in DI water, resulting in a C_{dl} of 67 nF and R_{ct} of 8 k Ω . Calibration against an electrochemical dummy cell revealed an attenuation factor of 0.7607 for calcium, which was subsequently applied to the data to ensure accurate measurement interpretation. Similarly, EIS was conducted in a 200 mM potassium solution in DI water, yielding a C_{dl} of 495 pF and R_{ct} of 146 k Ω . Calibration using a dummy cell indicated an amplification factor of 1.0508 for potassium, which was applied to the data to maintain precision in measurements. Further details regarding the calibration data are provided in Table C.8.1.

Calcium detection was performed in triplicate across concentrations ranging

from 0 mM to 200 mM in DI water. The typical current response for calcium detection is illustrated in Figure C.8.2. From previously conducted experiments, it was determined that the peak oxidation current typically occurs at approximately -0.1 V. Based on these peak oxidation currents, a calibration curve was constructed, as shown in Figure C.8.3, demonstrating the relationship between calcium concentration and current response.

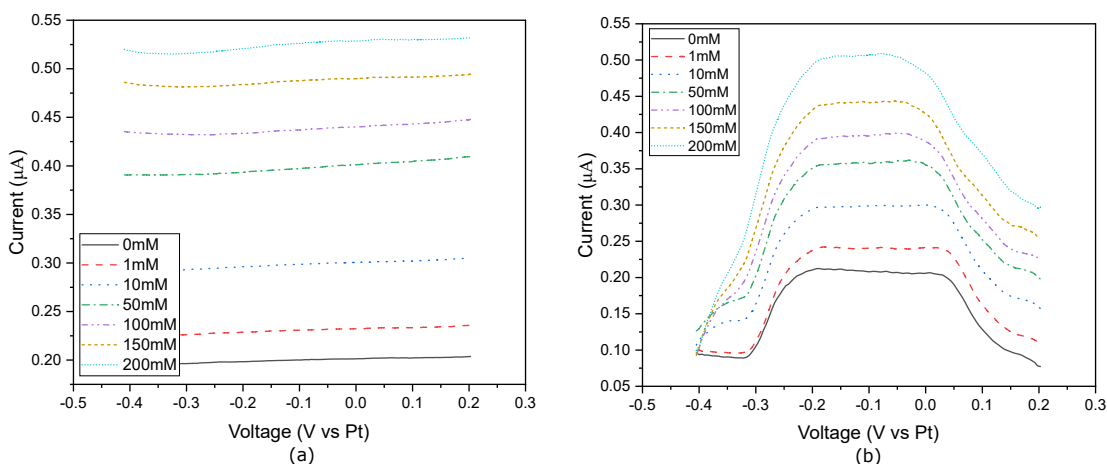


Figure C.8.2: SWV scans of calcium concentration ranging from 0 mM to 200 mM ($n=3$), using (a) the benchtop potentiostat, and (b) the PULSE.

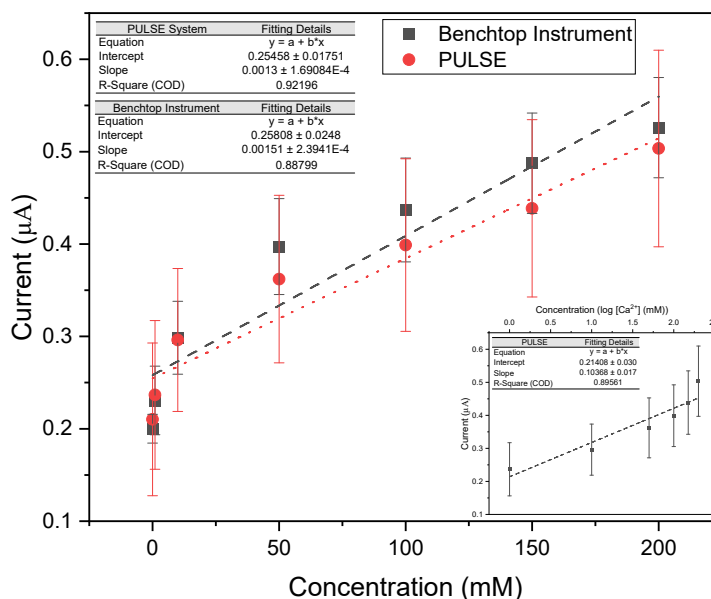


Figure C.8.3: Calcium calibration curve from 0 mM to 200 mM in DI water using the PULSE and the benchtop instrument ($n=3$, mean $\pm$ SE). Inset: represents the logarithm of calcium (Ca^{+2}) concentration and oxidation peak current using the PULSE.

The calibration curve obtained using the PULSE demonstrated a higher R^2 value of 0.92 than the benchtop instrument, with a slope of $0.0013 \mu A/mM$. The sensitivity was calculated to be $8.12 nA/mM/mm^2$, based on an electrochemical

area of 0.16 mm^2 . A correlation analysis between the data from the benchtop potentiostat and the PULSE revealed a coefficient of 0.994, indicating a high degree of agreement between the two systems. While the strong correlation demonstrated the potential of the PULSE device, the high SE must be addressed. This was observed in both systems, suggesting potential issues with the stability and reliability of the microneedles. Previous studies have reported that the SE of ion-selective microneedles reached 18% [21]. This variability might arise from the microneedles array fabrication process, and variations in the ISM coating. Addressing these challenges will require optimising the microneedle electrode manufacturing process and investigating the long-term stability of the sensors under operational conditions.

Potassium detection was conducted similarly to calcium, with concentrations ranging from 0 mM to 200 mM in DI water ($n=3$). The current responses for potassium are presented in Figure C.8.4(a) and (b), representing the data obtained using the benchtop potentiostat and the PULSE, respectively. From previous test, it was identified that the peak oxidation current typically occurs at approximately -0.1 V , which was more evident in the PULSE scans. Utilising these peak oxidation currents, a calibration curve was generated, as shown in Figure C.8.5, to illustrate the correlation between potassium concentration and the current response.

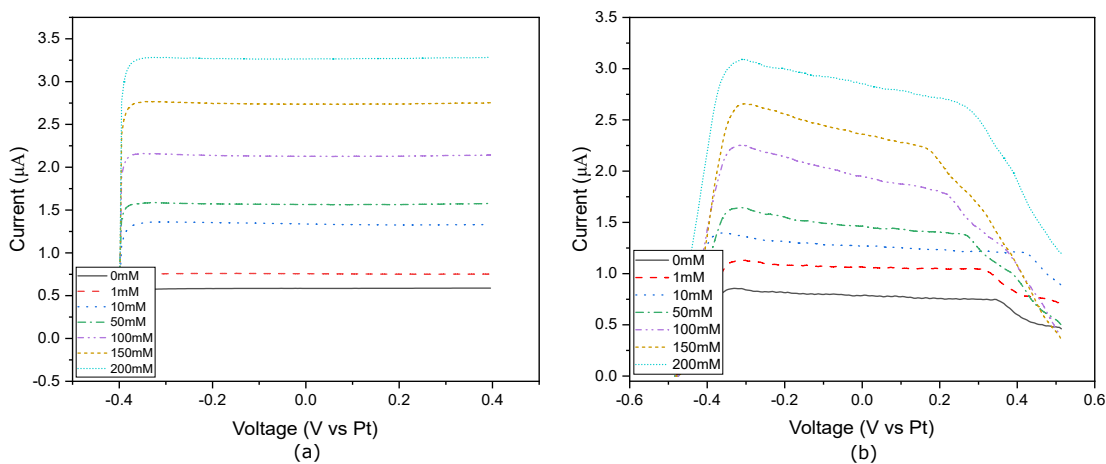


Figure C.8.4: SWV scans of potassium concentration ranging from 0 mM to 200 mM ($n=3$), using (a) the benchtop potentiostat, and (b) the PULSE.

The calibration curve obtained using the PULSE demonstrated a higher R^2 value of 0.98 than the benchtop instrument, with a slope of $0.00959 \mu\text{A}/\text{mM}$. The LOD was calculated to be 5.05 mM, with a sensitivity of $41.7 \text{ nA}/\text{mM}/\text{mm}^2$, based on an electrochemical area of 0.23 mm^2 . This sensitivity is a significant metric, reflecting the device's capability to measure low potassium concentrations, though further optimisation may reduce the LOD. A correlation analysis between

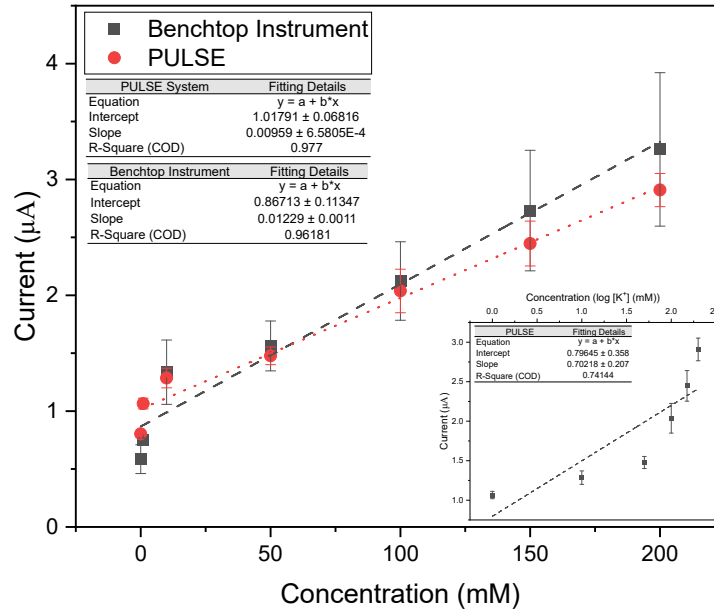


Figure C.8.5: Potassium calibration curve from 0 mM to 200 mM in DI water using the PULSE and the benchtop instrument ($n=3$, mean $\pm$ SE). Inset: represents the logarithm of potassium (K^+) concentration and oxidation peak current using the PULSE.

the data from the benchtop potentiostat and the PULSE revealed a coefficient >0.99 , indicating a strong correlation between the two datasets. Nevertheless, it is worth noting that in this experiment, the error bars associated with the dataset from the benchtop instrument were larger than those of the PULSE. Similarly to what was observed in calcium detection, this discrepancy may be attributed to the microneedle array fabrication process and variations in the ISM coating.

OCP measurements were conducted to detect multiple pH levels in 10 mM PBS, ranging from 3 to 11, with the resulting calibration curves shown in Figure C.8.6. Both systems exhibited a positive slope, with values of 10.24 mV/pH for the PULSE and 16.18 mV/pH for the benchtop, which has been previously observed in the literature [22, 23]. A correlation analysis yielded a coefficient of 0.979, indicating a strong agreement between the two datasets. While the typical pH slope is negative and ideally close to -59.1 mV/pH, the observed results indicate that the PULSE demonstrates promising consistency with the standard benchtop system [24]. Nevertheless, there is a need to continue investigation into the long-term reliability and optimisation of the microneedles for improved accuracy and consistency.

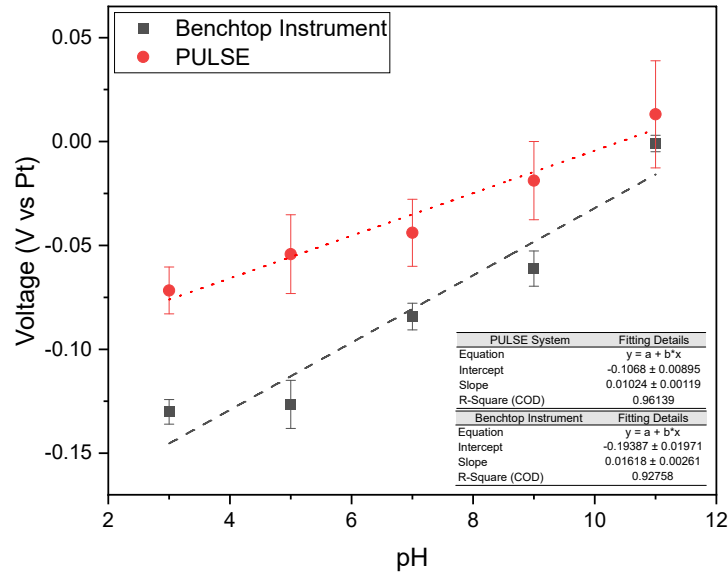


Figure C.8.6: pH calibration curves in 10 mM PBS using the PULSE and the benchtop instrument ($n=3$, mean $\pm$ SE).

Table C.8.1: Calibration data of calcium measurements, including input voltage (V_{in}), measured current (I_{real}), and serial current value (I_{serial}).

V_{in} (V)	I_{real} (A)	I_{serial} (A)
-0.5	0.00016491	8.39663E-05
-0.4	0.00016665	9.97231E-05
-0.3	0.00018033	0.000139883
-0.2	0.00018588	0.000161247
-0.1	0.00018885	0.000166504
0	0.00018428	0.000166505
0.1	0.00017896	0.000167554
0.2	0.00017362	0.000166672
0.3	0.00016824	0.000166799
0.4	0.0001568	0.00015781
0.5	0.0001445	0.000145242

Table C.8.2: Calibration data of potassium measurements, including input voltage (V_{in}), measured current (I_{real}), and serial current value (I_{serial}).

V_{in} (V)	I_{real} (A)	I_{serial} (A)
-0.5	0.00001467	1.38375E-05
-0.4	0.000014	1.32E-05
-0.3	0.00001334	1.25E-05
-0.2	0.00001264	1.18E-05
-0.1	0.000012	1.11E-05
0	0.00001136	1.05852E-05
0.1	0.00001069	9.81698E-06
0.2	0.00001002	9.08151E-06
0.3	0.00000934	8.29701E-06
0.4	0.00000866	7.56153E-06
0.5	0.00000798	6.79337E-06

Table C.8.3: Calibration data of peak oxidation currents for varying concentrations of calcium in DI water, measured with the benchtop instrument and the PULSE system (n=3, mean $\pm$ SE).

Concentration (mM)	Benchtop Instrument		PULSE System	
	Current (μ A)	SE (μ A)	Current (μ A)	SE (μ A)
0	0.20009	0.01561	0.2102	0.07813
1	0.23068	0.03708	0.2367	0.08047
10	0.2985	0.03946	0.29614	0.07741
50	0.39722	0.05189	0.36203	0.09066
100	0.43685	0.05623	0.39891	0.09346
150	0.4875	0.05434	0.43866	0.09601
200	0.52597	0.05426	0.50347	0.10637

Table C.8.4: Calibration data of peak oxidation currents for varying concentrations of potassium in DI water, measured with the benchtop instrument and the PULSE system (n=3, mean $\pm$ SE).

Concentration (mM)	Benchtop Instrument		PULSE System	
	Current (μ A)	SE (μ A)	Current (μ A)	SE (μ A)
0	0.58524	0.12432	0.80361	0.00847
1	0.75321	0.01501	1.06543	0.04681
10	1.33561	0.27773	1.28577	0.08498
50	1.56245	0.21539	1.4776	0.07655
100	2.12363	0.33871	2.03753	0.18776
150	2.73153	0.52039	2.44712	0.19331
200	3.25999	0.66229	2.90854	0.14354

Table C.8.5: Calibration data of pH in PBS, measured with the benchtop instrument and the PULSE system (n=3, mean $\pm$ SE).

pH	Benchtop Instrument		PULSE System	
	Voltage (V)	SE	Voltage (V)	SE
3	-0.13011	0.00591	-0.07167	0.01126
5	-0.1265	0.01155	-0.05417	0.01895
7	-0.08422	0.00645	-0.04391	0.01612
9	-0.06117	0.00849	-0.0188	0.01883
11	-9.44444E-4	0.00396	0.01309	0.0258

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PULSE 2.0 DEVELOPMENT AND TESTING

D.1 Schematic design

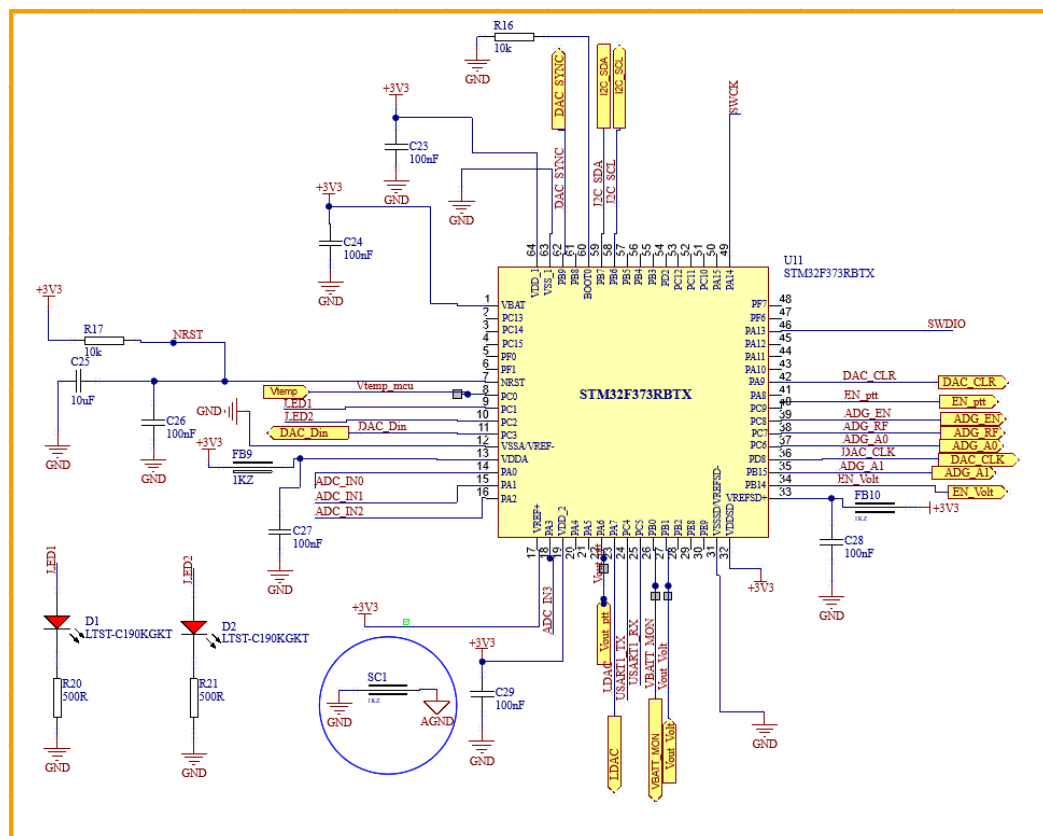


Figure D.1.1: MCU schematic design of the PULSE 2.0, using Altium Designer.

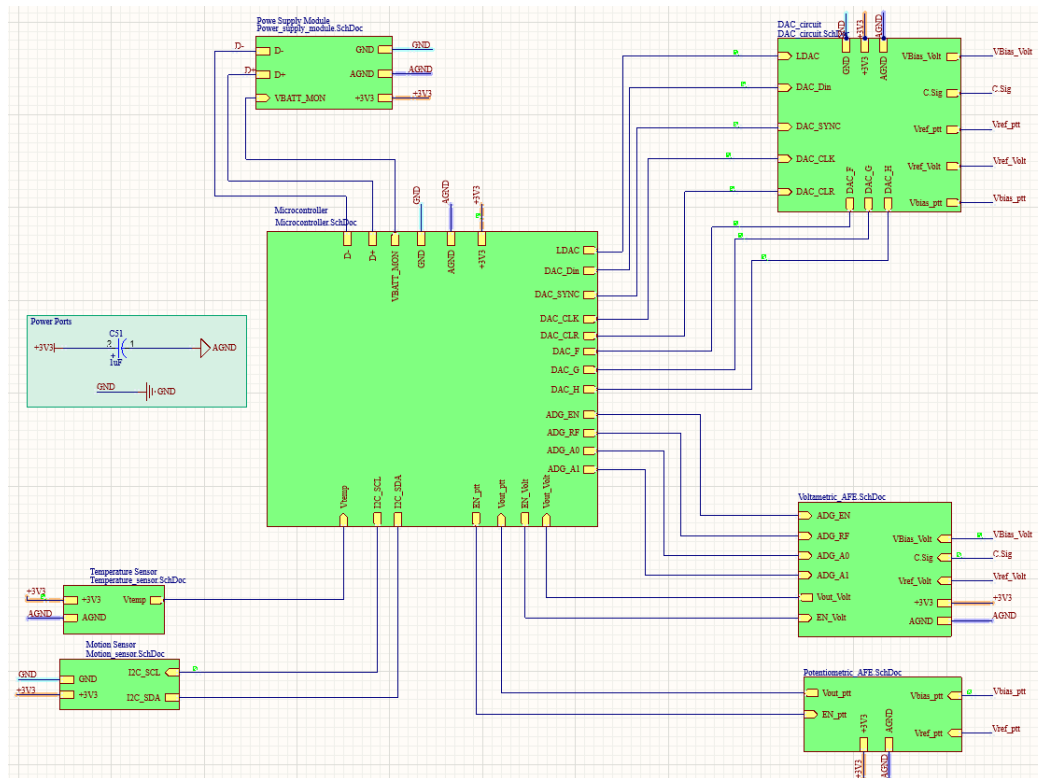


Figure D.1.2: Top-level schematic design of the PULSE 2.0, using Altium Designer.

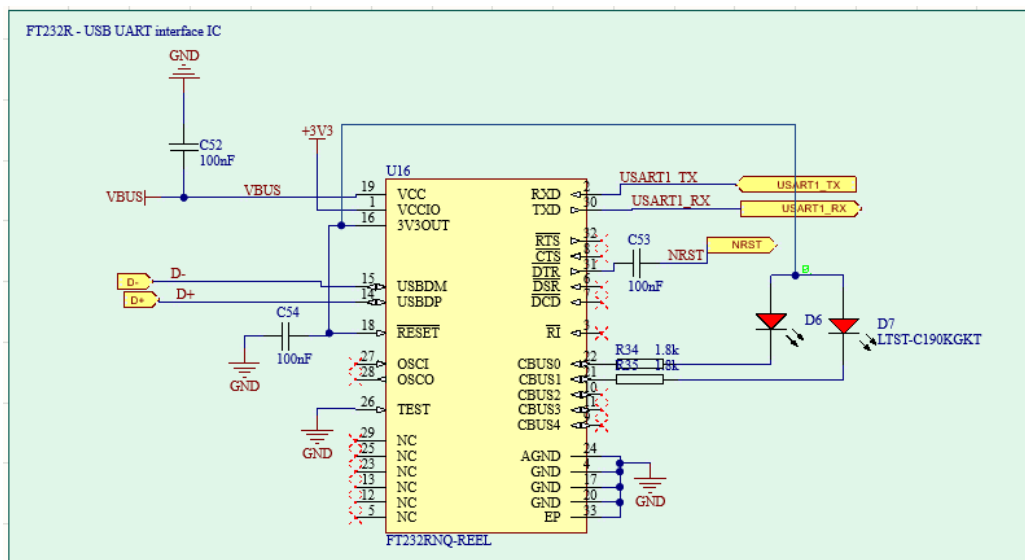


Figure D.1.3: Schematic design of USART-to-USB converter on the PULSE 2.0, using Altium Designer.

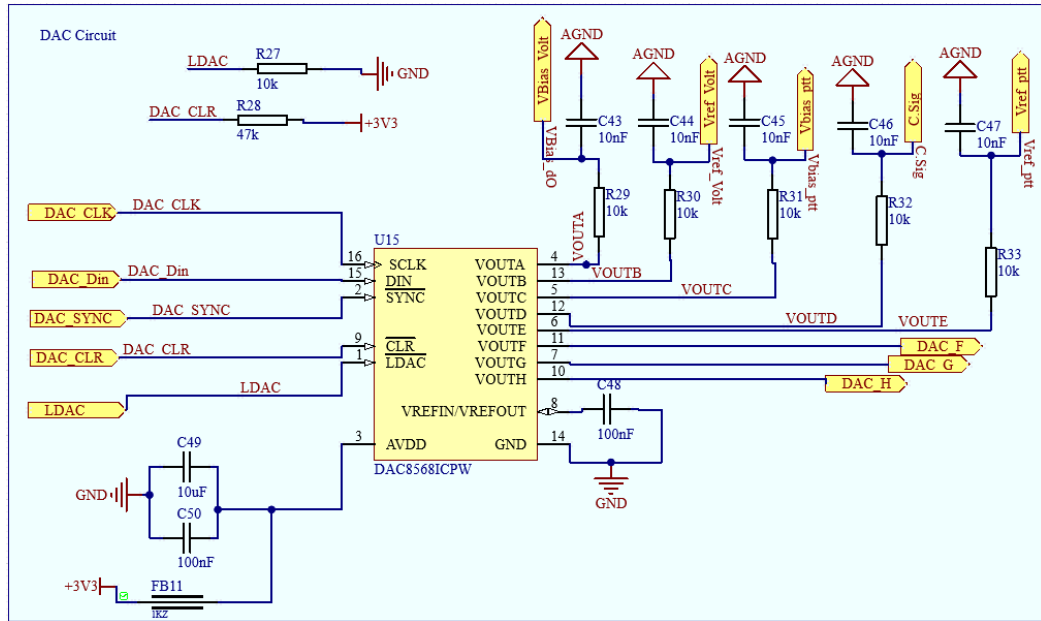


Figure D.1.4: Schematic design of external DAC circuit, using Altium Designer.

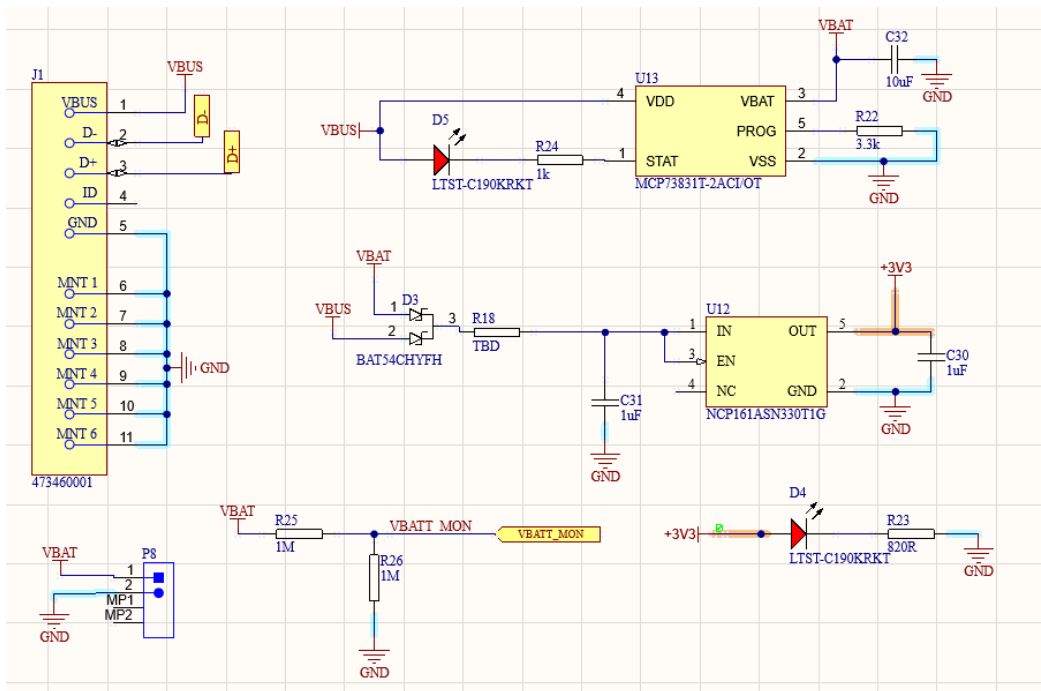


Figure D.1.5: Schematic design of power supply circuit, using Altium Designer.

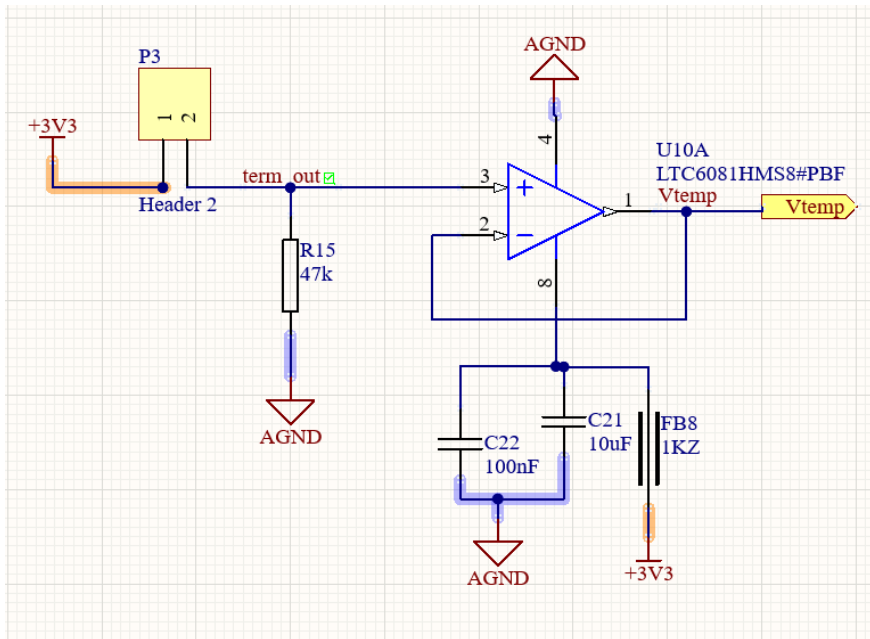


Figure D.1.6: Schematic design of temperature sensor, using Altium Designer.

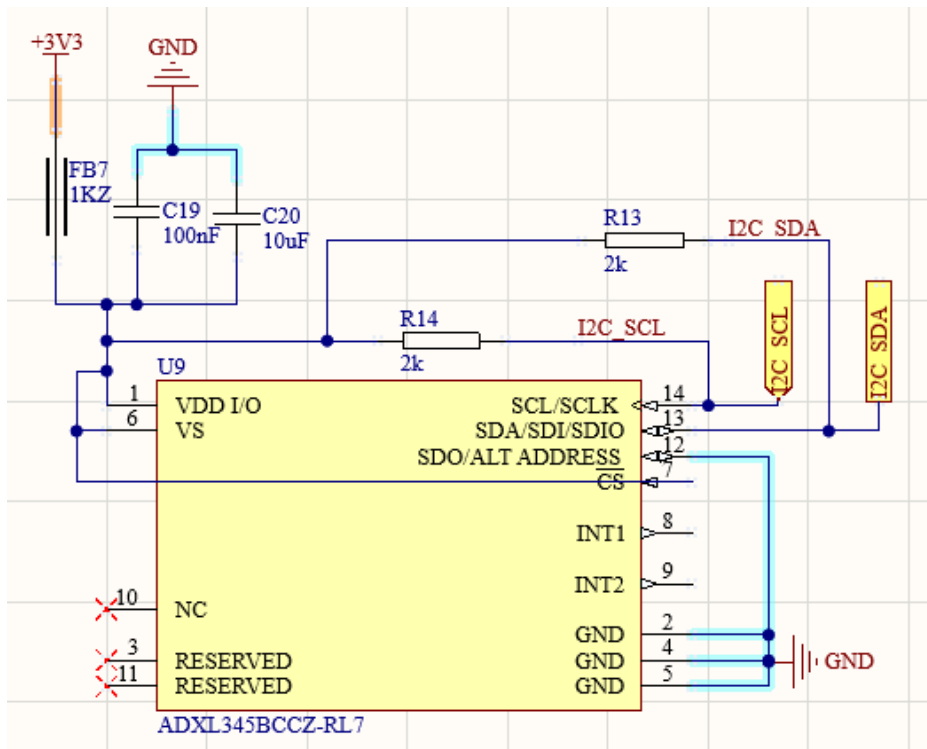


Figure D.1.7: Schematic design of motion sensor, using Altium Designer.

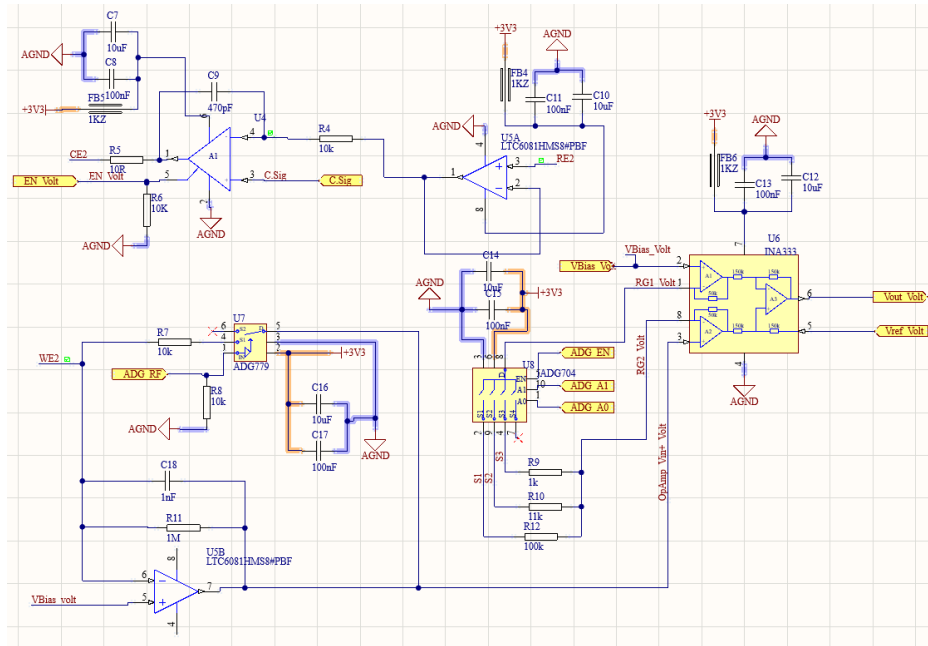


Figure D.1.8: Schematic circuit of the voltammetric AFE in Altium Designer.

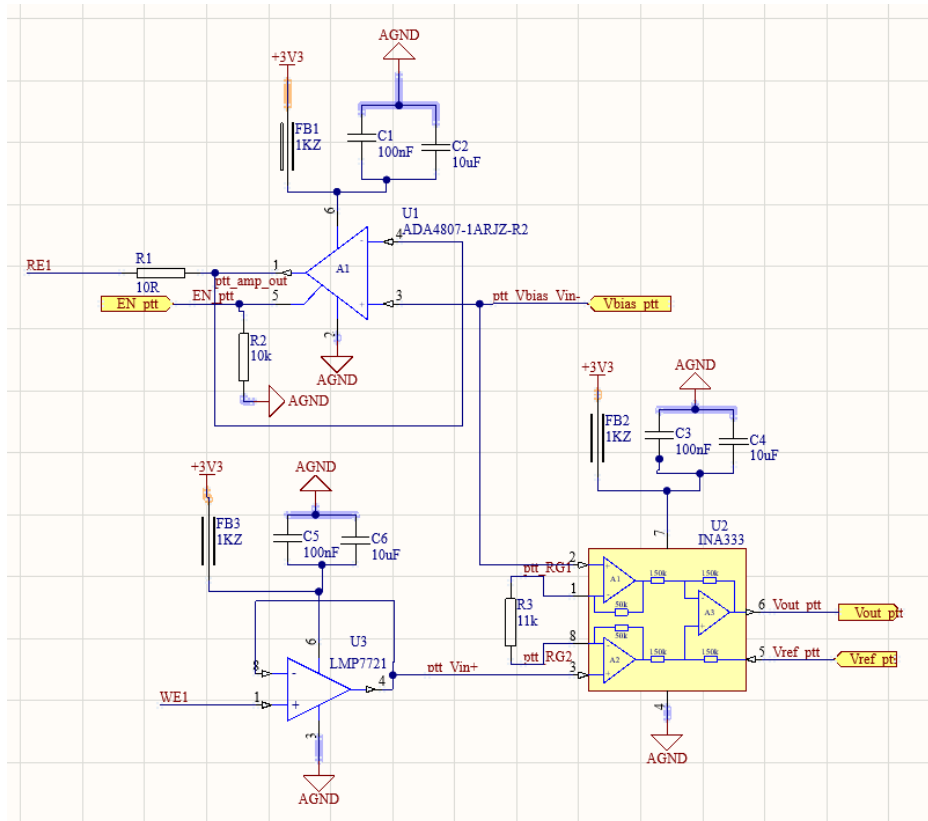


Figure D.1.9: Schematic circuit of the potentiometric AFE in Altium Designer.

D.2 PCB Fabrication Documentation

Assembly View

View from Front side (Scale 4:1)

Engineer	Ana Ferreira	Altium
Project		

Micro USB B

MCU

Accelerometer

Bluetooth Connector

P7

DETAIL C (Scale 4:1)

TX RX GND Vdd

Voltammetric AFE Connector

DETAIL D (Scale 4:1)

P2

DETAIL D (Scale 4:1)

RE WE CE

DETAIL B (Scale 4:1)

P1

RE WE

Potentiometric AFE Connector

Temperature Sensor Connector

P3

DETAIL A (Scale 4:1)

Vdd

Tmp

View from Bottom side (Scale 2:1)

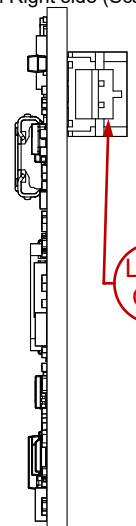
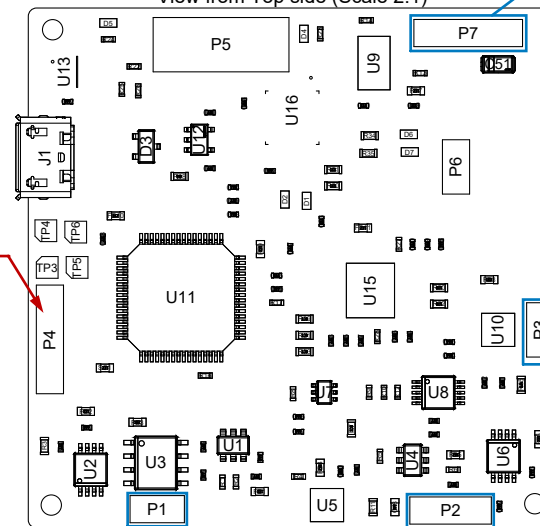
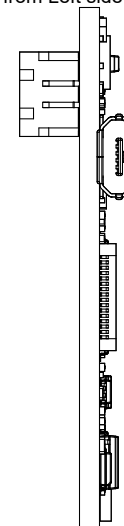
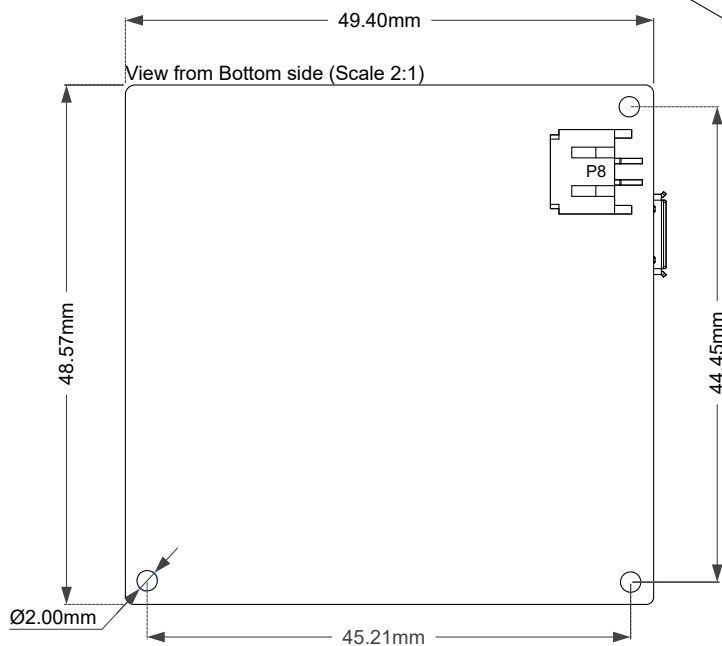
View from Left side (Scale 2:1)

View from Top side (Scale 2:1)

View from Right side (Scale 2:1)

SWD Connector

LiPo Battery Connector



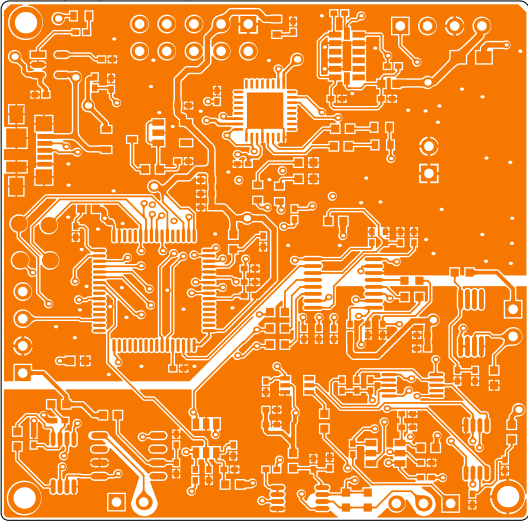
Layer Stack & BOM

Layer Stack Legend					
Material	Layer	Thickness	Dielectric Material	Type	
	Top Overlay			Legend	
	Surface Material	0.03mm	SM-001	Solder Mask	
	PbSn	0.02mm		Surface Finish	
CF-004	Top Layer	0.04mm		Signal	
Prepreg		0.13mm	PP-017	Dielectric	
CF-004	Layer 2	0.04mm		Signal	
Core		0.61mm	Core-038	Dielectric	
CF-004	Layer 3	0.04mm		Signal	
Prepreg		0.13mm	PP-017	Dielectric	
CF-004	Layer 4	0.04mm		Signal	
Core		0.61mm	Core-038	Dielectric	
CF-004	Layer 5	0.04mm		Signal	
Prepreg		0.13mm	PP-017	Dielectric	
CF-004	Bottom Layer	0.04mm		Signal	
PbSn	Bottom Surface Finish	0.02mm		Surface Finish	
Surface Material	Bottom Solder	0.03mm	SM-001	Solder Mask	
	Bottom Overlay			Legend	
Total thickness: 1.91mm					

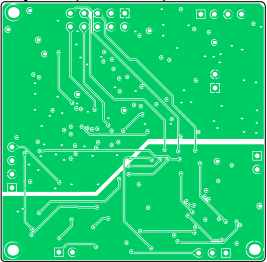
Bill Of Materials			
Line #	Designator	Comment	Quantity
1	C1, C3, C5, C8, C11, C13, C15, C17, C19, C22, C23, C24, C26, C27, C28, C29, C48, C50, C52, C53, C54, C2, C4, C6, C7, C10, C12, C14, C16, C20, C21, C25, C32, C49	100nF	21
2		10uF	13
3	C9	470pF	1
4	C18	1nF	1
5	C30, C31	1uF	2
6	C43, C44, C45, C46, C47	10nF	5
7	C51	1uF	1
8	D1, D2, D4, D5, D6, D7	LTST-C190KGKT, LTST-C190KRKT	6
9	D3	BAT54CHYFH	1
10	FB1, FB2, FB3, FB4, FB5, FB6, FB7, FB8, FB9, FB10, FB11, SC1	1KZ	12
11	J1	473460001	1
12	P8	S2B-PH-SM4-TB(LF)(SN)	1
13	R1, R5	10R	2
14	R2, R4, R8, R16, R17, R27	10k	6
15	R3	11k	1
16	R6	10K	1
17	R7, R29, R30, R31, R32, R33	10k	6
18	R9, R24	1k	2
19	R10	11k	1
20	R11	1M	1
21	R12	100k	1
22	R13, R14	2k	2
23	R15	47k	1
24	R20, R21	500R	2
25	R22	3.3k	1
26	R23	820R	1
27	R25, R26	1M	2
28	R28	47k	1
29	R34, R35	1.8k	2
30	U1, U4	ADA4807-1ARJZ-R2	2
31	U2, U6	INA333	2
32	U3	LMP7721	1
33	U5, U10	LTC6081HMS8#PBF	2
34	U7	ADG779	1
35	U8	ADG704	1
36	U9	ADXL345BCCZ-RL7	1
37	U11	STM32F373RBTX	1
38	U12	NCP161ASN330T1G	1
39	U13	MCP73831T-2ACI/OT	1
40	U15	DAC8568ICPW	1
41	U16	FT232RNQ-REEL	1

Board Fabrication View

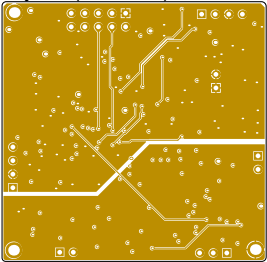
Top Layer (Scale 2:1)



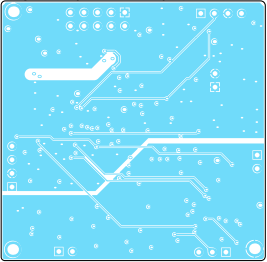
Layer 2 (Scale 1:1)



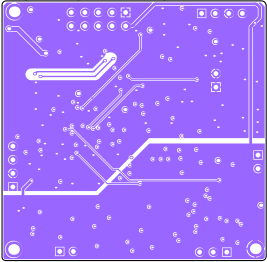
Layer 3 (Scale 1:1)



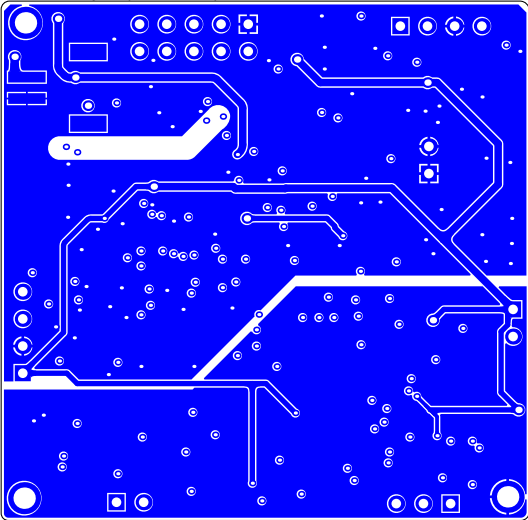
Layer 4 (Scale 1:1)



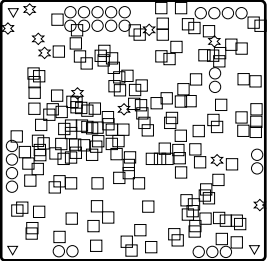
Layer 5 (Scale 1:1)



Bottom Layer (Scale 2:1)

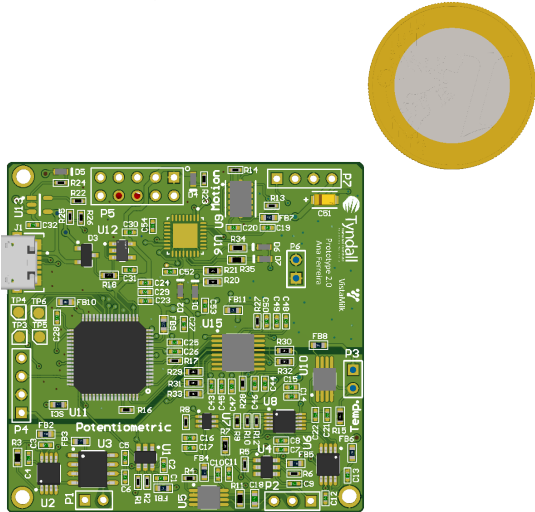


Drill Drawing View (Scale 1:1)

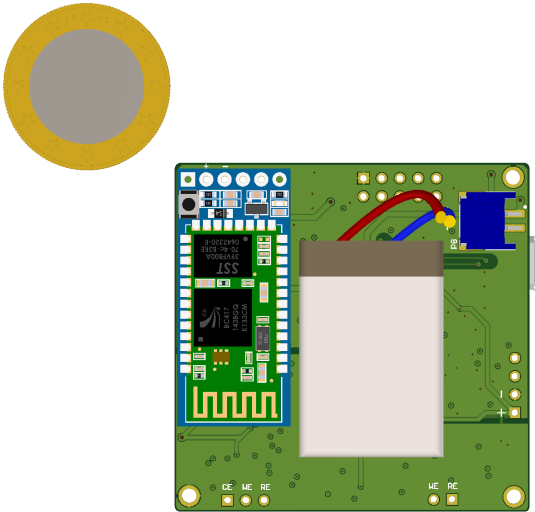


Drill Table			
Symbol	Count	Hole Size	Plated
□	165	0.30mm	Plated
☆	10	0.60mm	Plated
○	27	0.90mm	Plated
▽	3	2.00mm	Plated
205 Total			

Realistic View Top



Realistic View Bottom



D.3 Flexible Gold Electrode Characterisation

CV measurements were carried out at each stage of the flexible electrode modification, as shown in Figure D.3.1. The observed reduction in peak oxidation and reduction currents indicated a decrease in the electrochemical surface area, which was further analysed.

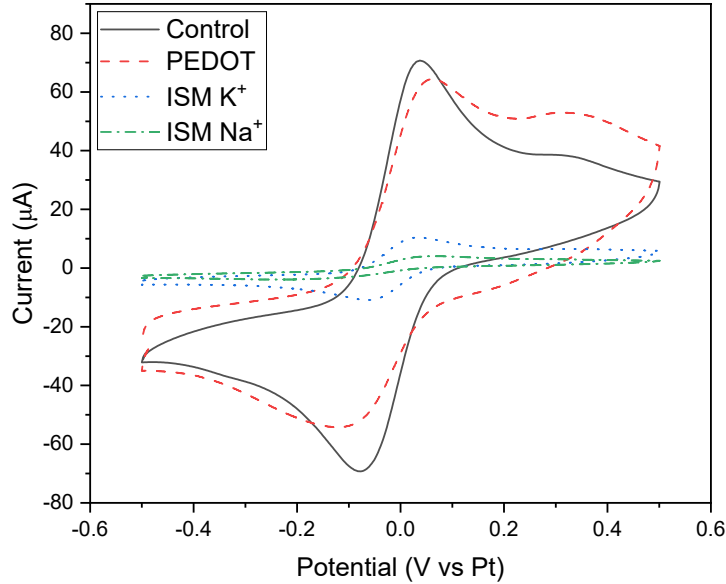


Figure D.3.1: CV curves corresponding to the control electrode, after PEDOT deposition, and ISMs for potassium (K^+) and sodium (Na^+), from -0.5 V to 0.5 V at 50 mV/s ($n=3$).

To determine the electrochemical active area of the WE, CV measurements were initially performed on the control flexible sensor ($n=3$) in 5 mM Ferri-Ferro solution across various scan rates from 10 to 200 mV/s, as shown in Figure D.3.2 (a). The resulting current peaks from both oxidative and reductive reactions were plotted in Figure D.3.2 (b), demonstrating a linear correlation with the square root of the scanning rate. The WE was designed with a geometrical area of $3.14 \times 10^{-2} \text{ cm}^2$, while the calculated electrochemical active area for the control electrode was $9.36 \times 10^{-2} \text{ cm}^2$, reflecting a 198% increase relative to the geometrical area [1]. This difference arises because the geometrical area represents the flat surface area of a circle, while the electrochemical active area is often significantly larger due to surface roughness [2].

Following PEDOT deposition, the same procedure was repeated, as illustrated in Figures D.3.2 (c), and (d). Although the linear correlation with the square root of the scan rate was maintained, the peak currents decreased. This was confirmed by the calculated electrochemical area of $8.51 \times 10^{-2} \text{ cm}^2$, representing a 9.06% reduction compared to the control. Subsequently, the ISMs were deposited, and the process was repeated for potassium and sodium membranes (Figure D.3.2 (e)-(h)).

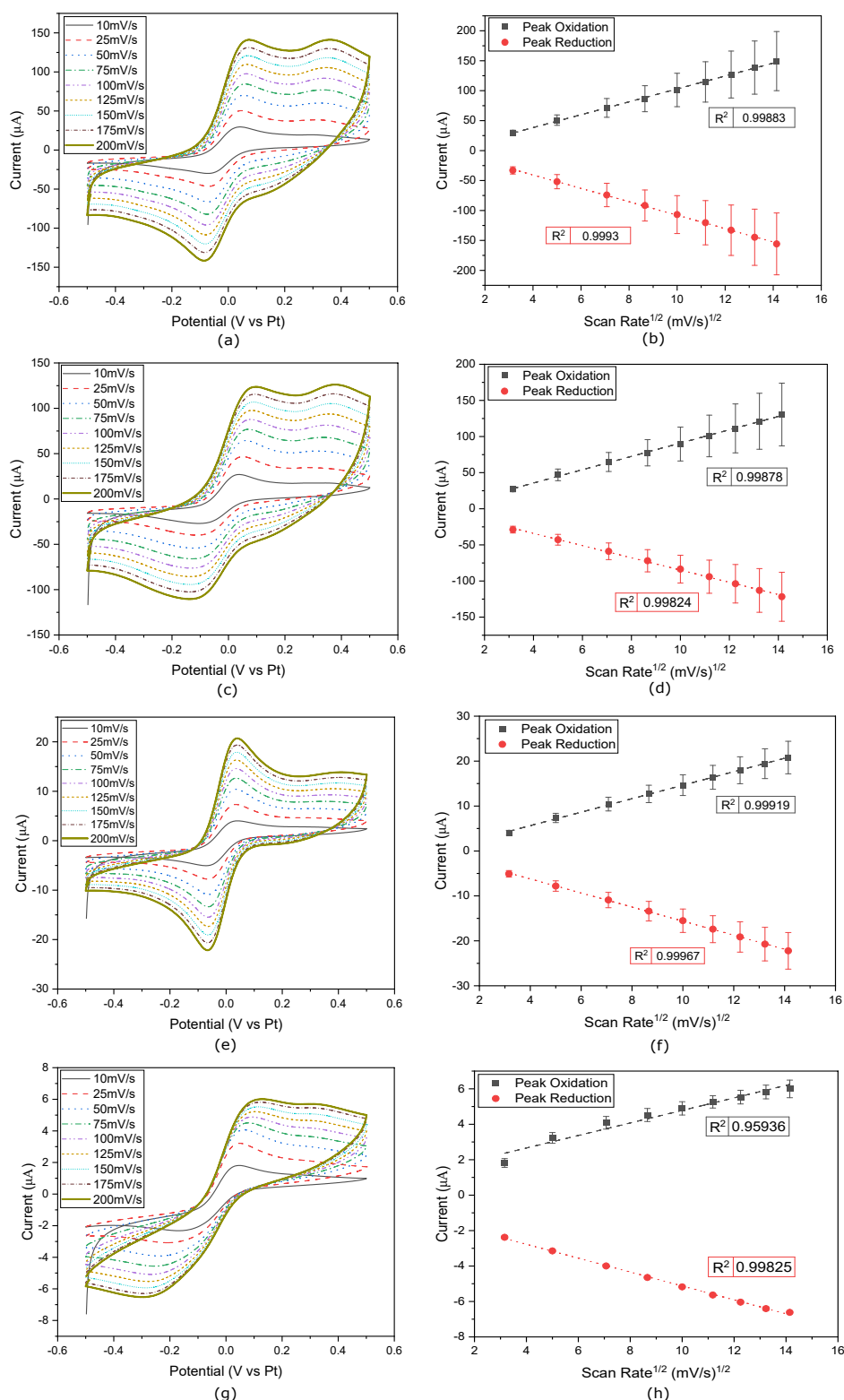


Figure D.3.2: CV curves of 5 mM Ferri-Ferro at different scan rates from 10 mV/s to 200 mV/s (n=3) (a) control, (c) PEDOT, (d) ISM Na^+ , and (e) ISM K^+ ; and corresponding redox peak currents versus the square root of scan rate ($v^{1/2}$) calibration curve, with error bars corresponding to the standard deviation SD for (b) control, (d) PEDOT, (f) ISM K^+ , and (h) ISM Na^+ .

In both cases, peak currents decreased, indicating a further reduction in the electrochemical active area, supported by previous findings in literature [3]. For the potassium ISM, the electrochemical active area was calculated as $1.37 \times 10^{-2} \text{ cm}^2$, corresponding to an 85.35% reduction from the control. Similarly, the electrochemical active area after sodium ISM deposition was $0.536 \times 10^{-2} \text{ cm}^2$, representing a 94.27% reduction relative to the control. The reduction in the observed area is linked to the use of PVC on the membrane, resulting in the formation of a membrane with multiple ion-selective channels on the electrode's surface, which is evident in the SEM images [3].

SEM (Zeiss Supra 40 SEM from Oberkochen, Germany) was utilised to further characterise the surface morphology of the electrodes at higher magnifications. The gold structures of the in-house flexible electrodes are shown in Figures D.3.3 (a)-(c). This exhibited distinct patterns consistent with those previously observed in Figure C.1.5. As expected, following the deposition of PEDOT, the electrode surface was covered in a coating, while retaining its characteristic globular structure, as shown in Figures D.3.3 (d)-(f) [4]. Subsequently, the deposition of ISM introduced a "net-shaped" morphology, similar to that reported in the literature [5]. This morphology featured micropores with diameters of approximately $2.74 \mu\text{m}$ for potassium-selective membranes and $3.64 \mu\text{m}$ for sodium-selective membranes. These micropores exhibited a uniform distribution across the electrode surface. The larger voids observed are attributed to the time-dependent factors involved in sample preparation, further emphasising the influence of preparation parameters on the final surface structure.

Contact angle measurements were taken (from Contact Angle System OCA-20 from DataPhysics Instruments GmbH, Filderstadt, Germany), as illustrated in Figure D.3.4, to evaluate the surface hydrophilicity of the electrodes at various stages of modification. A $1 \mu\text{L}$ drop of water was placed on the WE surface, and the angle between the droplet and the electrode surface was measured in triplicate. A summary of the calculated contact angles under different conditions of the electrode surface is presented in Table D.3.1.

The control electrode (Figure D.3.4 (a)) exhibited an average contact angle of 83.76° , consistent with previously reported values in the literature [6]. This moderate hydrophobicity suggested a clean gold surface, aligning with the SEM analysis that revealed the characteristic gold structures of the in-house printed electrodes.

After the deposition of PEDOT (Figure D.3.4 (b)), the contact angle decreased significantly to an average of 55.98° , suggesting increased hydrophilicity, which has been associated with PEDOT coating in previous studies [7–9]. The PEDOT layer promoted stronger adhesion and uniform deposition of subsequent ISM, and

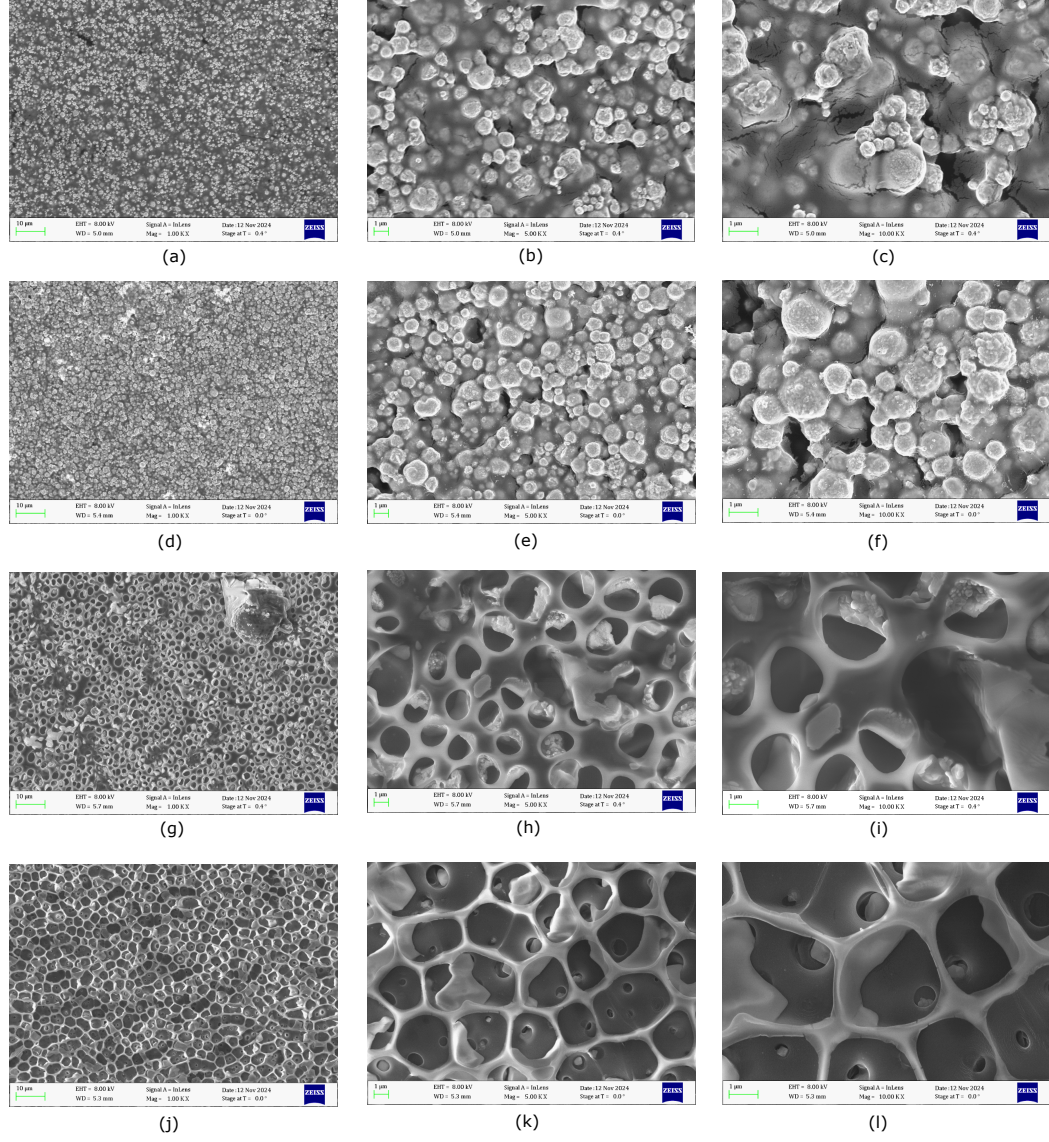


Figure D.3.3: SEM images of (a)-(c) flexible gold-printed electrode control, (d)-(f) after PEDOT deposition, (g)-(i) ISM K⁺, and (j)-(l) ISM Na⁺ at increasing magnifications from 1 k $\times$ to 10 k $\times$.

Table D.3.1: Contact angle measurements ($^{\circ}$) on the electrode surfaces at different modification stages: control, PEDOT, potassium ISM, and sodium ISM, including average (AVG) and standard deviation (SD).

Sensor	Control	PEDOT	K ⁺ ISM	Na ⁺ ISM
1	86.04	50.56	81.37	125.69
2	80.52	58.15	81.33	123.27
3	84.72	59.23	96.38	124.61
AVG	83.76	55.98	86.36	124.52
SD	2.35	3.86	7.09	0.99

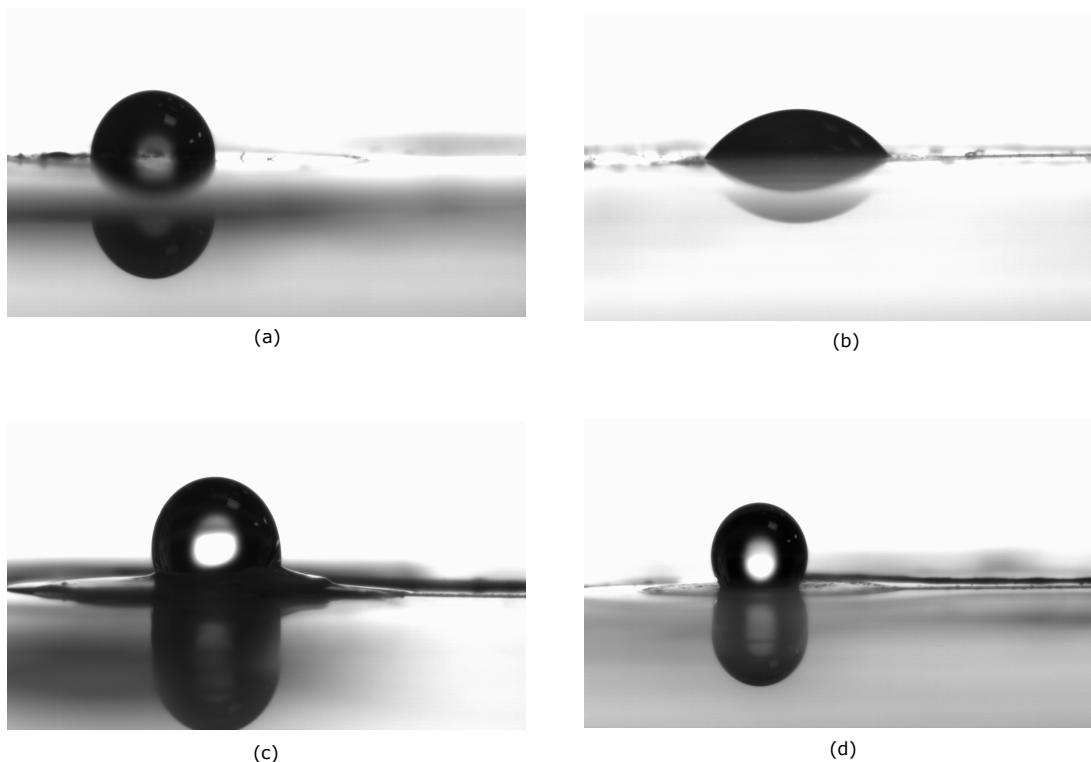


Figure D.3.4: Contact angle images of the electrode surface at (a) control, (b) PEDOT deposition, (c) potassium ISM, and (d) sodium ISM.

it has been extensively used in the literature [10]. The hydrophobic nature of the membrane can create a barrier that inhibits the diffusion of target ions to the electrode surface, potentially leading to slower ion transport [11]. The extent of this inhibition depends on several factors, including the membrane composition [12]. To address this issue, thorough characterisation of the sensors is necessary before assuming any limitations. Optimisation should be performed only as necessary, based on the obtained results and the specific criteria of its application.

Following the deposition of the potassium-selective membrane (Figure D.3.4 (c)), the contact angle increased to an average of 86.36° . This transition indicated a return to a more hydrophobic surface due to the membrane's composition, which includes hydrophobic components, as PVC and 2-nitrophenyloctyl ether [13, 14]. The sodium-selective membrane (Figure D.3.4 (d)) produced the highest contact angle, with an average of 124.52° . This increase in hydrophobicity corresponded to the membrane's composition, including high percentages of PVC and 2-nitrophenyloctyl ether [12]. This is advantageous for preventing interference from water and other hydrophilic substances, ensuring that only the target ions can pass through the membrane [15], thus enhancing its selectivity [16].

D.4 Ferri-ferro Detection

Table D.4.1: CV oxidation and reduction peaks results of ferri-ferro concentrations, using the PULSE 2.0 (n=3).

Concentration	Average				Standard Deviation			
	Oxidation Peak		Reduction Peak		Oxidation Peak		Reduction Peak	
	Voltage	Current	Voltage	Current	Voltage	Current	Voltage	Current
5	0.171319	1.231E-04	0.070873	-0.00012	0.002329	9.37E-07	0.002329	1.57E-06
4	0.171319	9.855E-05	0.084046	-8.4E-05	0.002329	9.67E-07	0.004033	1.21E-06
3	0.172966	8.194E-05	0.092279	-6.8E-05	2.78E-17	1.85E-06	0.004657	3.03E-07
2	0.171319	6.395E-05	0.092279	-5.1E-05	0.002329	6.05E-07	0.004657	4.87E-07
1	0.172966	4.451E-05	0.098866	-3.3E-05	2.78E-17	7.74E-07	0	3.03E-07

Table D.4.2: CV oxidation and reduction peaks results of ferri-ferro concentrations, using the benchtop instrument (n=3).

Concentration	Average				Standard Deviation			
	Oxidation Peak		Reduction Peak		Oxidation Peak		Reduction Peak	
	Voltage	Current	Voltage	Current	Voltage	Current	Voltage	Current
5	0.164805	0.000123	0.059357	-0.00013	0.003056	2.23E-06	0.002309	3.42E-06
4	0.16389	0.000102	0.070801	-9.7E-05	3.81E-05	2.8E-06	0.003453	3.89E-06
3	0.162231	8.42E-05	0.079814	-7.9E-05	0.001122	2.27E-06	0.001129	2.61E-06
2	0.161438	6.6E-05	0.08311	-6.1E-05	4.98E-05	1.5E-06	2.88E-05	1.7E-06
1	0.161407	4.66E-05	0.090454	-4.2E-05	6.59E-05	1.01E-06	2.49E-05	1.22E-06

D.5 pH Detection

Table D.5.1: OCP values measuring pH in PBS solutions using the benchtop potentiostat (n=3).

pH	Sensor 1	Sensor 2	Sensor 3	AVG	SD
3	0.367	0.3415	0.379667	0.362722	0.015872
5	0.211833	0.212	0.2005	0.208111	0.005382
7	0.073667	0.060333	0.046833	0.060278	0.010955
9	-0.00983	-0.02017	-0.03533	-0.02178	0.010472
11	-0.19883	-0.19883	-0.21717	-0.20494	0.008642

Table D.5.2: OCP values measuring pH in PBS solutions using the PULSE 2.0 (n=3).

pH	Sensor 1	Sensor 2	Sensor 3	AVG	SD
3	0.383547	0.393684	0.401559	0.39293	0.007373
5	0.214872	0.22262	0.215002	0.217498	0.003622
7	0.116787	0.114136	0.096911	0.109278	0.008812
9	0.003867	0.005519	-0.00132	0.00269	0.002912
11	-0.21196	-0.1895	-0.20902	-0.20349	0.009968

D.6 Potassium Detection

Table D.6.1: Calibration data of the potassium measurements, including input voltage (V_{in}), measured current (I_{real}), and serial current value (I_{serial}).

V_{in} (V)	I_{real} (A)	I_{serial} (A)
-0.4	0.00027765	0.000166
-0.3	0.00027019	0.000166
-0.2	0.00026266	0.000166
-0.1	0.00025534	0.000166
0	0.00024814	0.000166
0.1	0.00024085	0.000166
0.2	0.00023349	0.000166
0.3	0.00022612	0.000166
0.4	0.00021875	0.000166

Table D.6.2: SWV peak oxidation currents (A) measuring potassium levels in DI water solutions using the PULSE 2.0.

Concentration (mM)	Sensor 1	Sensor 2	Sensor 3	AVG	SE
0	7.15E-07	7.33E-07	7.01E-07	7.16E-07	7.64701E-09
5	3.07E-06	3.34E-06	3.38E-06	3.26E-06	7.79183E-08
10	4.65E-06	4.27E-06	4.19E-06	4.37E-06	1.14314E-07
15	4.94E-06	5.16E-06	5.09E-06	5.06E-06	5.33324E-08
20	5.26E-06	5.58E-06	5.18E-06	5.34E-06	9.82557E-08
25	5.72E-06	5.81E-06	5.62E-06	5.71E-06	4.54364E-08

Table D.6.3: SWV peak oxidation currents (A) measuring potassium levels in DI water solutions using the benchtop potentiostat.

Concentration (mM)	Sensor 1	Sensor 2	Sensor 3	AVG	SE
0	1.09222E-06	2.85034E-07	8.92334E-07	7.56531E-07	1.9817E-07
5	3.47134E-06	2.95715E-06	3.24982E-06	3.2261E-06	1.21582E-07
10	5.02747E-06	4.07159E-06	3.78265E-06	4.2939E-06	3.07121E-07
15	5.47302E-06	4.99695E-06	4.55414E-06	5.00804E-06	2.1663E-07
20	5.82214E-06	5.19562E-06	5.29846E-06	5.43874E-06	1.58389E-07
25	5.96832E-06	5.56854E-06	5.8313E-06	5.78939E-06	9.57699E-08

D.7 Sodium Detection

Table D.7.1: SWV peak oxidation currents (A) measuring sodium levels in DI water solutions using the PULSE 2.0.

Concentration (mM)	Sensor 1	Sensor 2	Sensor 3	AVG	SE
0	1.8753E-06	1.47312E-07	2.31841E-07	7.51484E-07	4.59228E-07
50	6.12462E-06	4.51665E-06	4.30249E-06	4.98125E-06	4.69499E-07
100	7.8369E-06	6.50551E-06	5.84274E-06	6.72838E-06	4.78754E-07
150	8.68337E-06	7.1731E-06	8.12695E-06	7.99447E-06	3.60059E-07
200	9.24359E-06	8.1146E-06	8.39787E-06	8.58535E-06	2.76894E-07

Table D.7.2: SWV peak oxidation currents (A) measuring sodium levels in DI water solutions using the benchtop potentiostat.

Concentration (mM)	Sensor 1	Sensor 2	Sensor 3	AVG	SE
0	1.46744E-06	1.8924E-07	1.22055E-07	5.92911E-07	3.57375E-07
50	6.43555E-06	4.73267E-06	4.49463E-06	5.22095E-06	4.99022E-07
100	7.62299E-06	6.09131E-06	5.86853E-06	6.52761E-06	4.50258E-07
150	8.64258E-06	6.97937E-06	6.79626E-06	7.47274E-06	4.79531E-07
200	9.552E-06	7.59888E-06	7.5592E-06	8.23669E-06	5.37054E-07

D.8 Rumen Fluid Analysis

Table D.8.1: OCP measurements of pH in ruminal solutions using the PULSE 2.0.

pH	Sensor 1	Sensor 2	Sensor 3	AVG	SD
3	0.389091	0.366984	0.398537	0.384871	0.013222
5	0.182907	0.211545	0.205756	0.200069	0.012364
7	0.004851	0.014638	0.002773	0.007421	0.005173
9	-0.12539	-0.12053	-0.09533	-0.11375	0.013176
11	-0.18834	-0.15392	-0.16578	-0.16935	0.014277

Table D.8.2: Temperature measurements in ruminal solutions using the PULSE 2.0.

pH	Sensor 1	Sensor 2	Sensor 3	AVG	SD
3	18.44715	18.57433	18.59481	18.53876	0.065318
5	18.41278	18.532	18.60181	18.51553	0.078043
7	18.34534	18.44176	18.49049	18.42586	0.060312
9	18.19363	18.35895	18.46852	18.34036	0.11299
11	18.1859	18.26003	18.34382	18.26325	0.064508

Table D.8.3: pH detection of control ruminal fluid solution using the PULSE 2.0, and a pH probe.

	PULSE 2.0	pH Probe
Sensor 1	9.227	9.2
Sensor 2	9.287	9.3
Sensor 3	9.253	9.3
AVG	9.256	9.267
SD	0.0245	0.0471

Table D.8.4: Potassium concentration (mM) using the PULSE 2.0 and the benchtop instrument in control ruminal fluid.

	Sensor 1	Sensor 2	Sensor 3	AVG	SD
PULSE 2.0	2.388	2.718	2.830	2.646	0.188
Benchtop Instrument	2.138	2.887	2.836	2.620	0.342

Table D.8.5: Sodium concentration (mM) using the PULSE 2.0 and the benchtop instrument in control ruminal fluid.

	Sensor 1	Sensor 2	Sensor 3	AVG	SD
PULSE 2.0	6.963	7.668	7.651	7.427	0.328
Benchtop Instrument	7.121	7.679	7.273	7.358	0.236

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CODE IMPLEMENTED

All the developed and implemented code for this PhD work can be assessed *here* or by visiting the following url: <https://github.com/analaufferreira/PhDThesis>.

This repository includes:

- PULSE FW: This folder contains the firmware code developed for the STM32 microcontroller using STM32CubeIDE.
- Qt PULSE APP: This folder contains the source code for the laptop application developed using Qt Creator.
- Kodular App: This folder contains the .aia file for the Android app developed using Kodular.