Faculdade de Engenharia da Universidade do Porto

A Wearable Cortisol Monitoring Microsystem

Mariana Alves Queirós Santos

Dissertation submitted in partial fulfillment
of the requirements for the degree of
Master in Biomedical Engineering

Supervisor: José Machado da Silva, PhD

July 2021

Abstract

Neurological diseases deserve, nowadays, significant attention from the scientific community, being often considered "the diseases of modern times".

Epilepsy is a neurological disease that affects about 50 million people of all ages, killing more than 100 000 people worldwide, every year.

Cortisol is a glucocorticoid hormone, known as the “stress hormone”, whose release is controlled by the HPA (hypothalamic-pituitary-adrenal) axis of the central nervous system. Due to its involvement in so many physiological processes and its essential functions in our body, the correct balance of cortisol levels is essential for human health.

The continuous and non-invasive monitoring of biomolecules associated with chronic health conditions, such as cancer, diabetes, epilepsy, stress, has received special attention in last decades.

The relationship between physiological stress conditions and cortisol levels in the blood, sweat and saliva has also been studied, and the potential of cortisol to be used as a biomarker of a sudden and unexpected death in epilepsy has already been demonstrated.

On the other hand, the enormous technological evolution in terms of microelectronics and biocompatible nanomaterials has allowed the development of implantable, portable, and wearable diagnostic or point-of-care (PoC) devices. These can be used to monitor cortisol levels in daily activities and, thus, allow to control the health condition of an individual and to early diagnose and prevent several pathologies or more acute illness status.

This dissertation project aims to develop a wearable and low-power microsystem capable of monitoring, continuously and in real-time, cortisol levels to prevent sudden and unexpected death in epilepsy.

The monitoring device includes a microcontroller-based system that receives, and processes cortisol values measured by the sensor and a mobile application developed for the Android operating system. This application is able to receive the data obtained by Bluetooth Low Energy (BLE) and evaluate them, deciding when an alert should be issued due to the existence of a high risk of sudden death in epilepsy.

Since it is still a prototype, the development was guided considering its use as a test and concept validation tool.

Resumo

Atualmente, as doenças do foro neurológico merecem atenção significativa por parte da comunidade científica, sendo muitas vezes consideradas "as doenças dos tempos modernos".

A epilepsia é uma doença neurológica que afeta, em todo o mundo, cerca de 50 milhões de pessoas de todas as idades, matando por ano mais de 100 000 pessoas.

O cortisol é uma hormona glicocorticóide, conhecida como a "hormona do stress", cuja libertação é controlada pelo eixo HPA (hipotálamo-pituitária adrenal) do sistema nervoso central. Devido ao seu envolvimento em tantos processos fisiológicos e às suas funções essenciais no nosso organismo, o equilíbrio correto dos níveis de cortisol é essencial para a saúde humana.

A monitorização contínua e não invasiva de biomoléculas associadas a condições crónicas de saúde, como cancro, diabetes, epilepsia, stress, tem recebido especial atenção nas últimas décadas.

A relação entre diversas condições de stress fisiológico e os níveis de cortisol no sangue, saliva e suor já foi estudada, e o potencial do cortisol para ser usado como biomarcador de uma morte súbita e inesperada na epilepsia já foi demonstrado.

Por outro lado, a enorme evolução tecnológica em termos de microeletrónica e nanomateriais biocompatíveis permitiu o desenvolvimento de dispositivos implantáveis, portáteis e vestíveis de diagnóstico ou point-of-care (Poc). Estes dispositivos podem ser utilizados para monitorizar os níveis de cortisol nas atividades diárias e desta forma, permitem controlar o estado de saúde de um indivíduo e diagnosticar e prevenir precocemente diversas patologias ou estados de doença mais graves.

Este projeto de dissertação visa desenvolver um microssistema vestível e de baixa potência capaz de monitorizar, de forma contínua e em tempo real, os níveis de cortisol para prevenir a morte súbita por epilepsia.

O dispositivo de monitorização inclui um sistema baseado em microcontrolador que recebe e processa os valores de cortisol medidos pelo sensor e uma aplicação móvel desenvolvida para o sistema operativo Android. Esta aplicação é capaz de receber os dados obtidos, por Bluetooth Low Energy (BLE) e avaliá-los, decidindo quando deve ser emitido um alerta devido à existência de um risco elevado de morte súbita por epilepsia.

Por se tratar ainda de um protótipo, o desenvolvimento foi orientado considerando a sua utilização como uma ferramenta de teste e de validação de conceitos.

Acknowledgements

Na realização desta dissertação, contei com o apoio direto e indireto de múltiplas pessoas e instituições às quais estou profundamente grata. Correndo o risco de injustamente não mencionar algum dos contributos, quero deixar expressos os meus agradecimentos:

Ao meu Orientador, Professor José Machado da Silva, pela orientação, disponibilidade, espírito crítico e exigência sempre demonstradas. Deixo aqui expressa a minha gratidão.

Às minhas colegas e amigas pela paciência, apoio e incentivo que, em muitas horas difíceis, foram o alento necessário para continuar.

Aos técnicos do laboratório I001 do Departamento de Engenharia Eletrotécnica e de Computadores da FEUP e também aos colegas Telmo Soares e Pedro Carneiro pela enorme ajuda prestada na realização de tantos e tantos testes laboratoriais efetuados. Obrigada ainda pela simpatia, paciência e disponibilidade, sem vocês certamente não teria conseguido.

Ao meu grande amigo Márcio pela preciosa ajuda, pelas horas perdidas e pela paciência que teve comigo nos meus momentos de desespero.

Quero agradecer também ao João Pedro, provavelmente aquele que mais lidou com as minhas dúvidas e incertezas durante todo este processo e que "celebrou" os meus sucessos e "chorou" as minhas derrotas como se fossem as dele. Obrigada pela ajuda, pela compreensão, pela imensa paciência que tens comigo, mas essencialmente obrigada por nunca desistires de mim. Foste indispensável nesta fase do meu percurso.

Por fim e em lugar de destaque, não poderia deixar de agradecer à minha Família: à minha mãe, ao meu pai, ao meu irmão… Não me é possível exprimir em palavras a enorme gratidão que tenho para com eles, não conseguindo por vezes segurar a emoção quando penso em tudo o que fizeram e continuam a fazer por mim. Obrigado pelo apoio, ajuda e dedicação incondicionais. Obrigada por me permitirem ter a oportunidade de tentar, garantindo sempre que se eu caísse, estariam lá para me ajudar a levantar. Mas agradeço-lhes acima de tudo pelo exemplo, são para mim um modelo de amor, coragem e determinação. A vocês dedico este trabalho.

A todos, o meu sincero e sentido Muito Obrigada!

Mariana Santos

Contents

List of figures

List of Tables

Acronims, abbreviations and Symbols

AC	Alternating Current
ACTH	Adrenocorticotropic Hormone
ADC	Analog to Digital Converter
AFE	Analog Front-End
API	Application Programming Interface
AuNPs	Gold Nanoparticles
BLE	Bluetooth Low Energy
CCI	Current Conveyor Circuit
CE	Counter Electrode
CLIA	Chemiluminescence Immunoassay
CL-LFIA	Chemiluminescence Lateral Flow Immunoassay
c-Mab	Cortisol Monoclonal antibodies
CRH	Corticotrophin Hormone
CV	Cyclic Voltammetry
DAC	Digital to Analog Converter
d-BSA	Denatured Bovine Serum Albumin
EIA	Enzyme Immunoassay
EIS	Electrochemical Impedance Spectroscopy
ELISA	Enzyme-Linked Immunosorbent Assay
Fe_2O_3	Iron (III) oxide
HPA	Hypothalamic-Pituitary-Adrenal
HTML	Hypertext Mark-up Language
ISF	Interstitial Fluid
I2C	Inter-Integrated Circuit
LFIA	Lateral Flow Immunoassay
LMR	Lossy Mode Resonance
LOD	Limit of Detection
LPF	Low-Pass Filter
MCU	Microcontroller
MIP	Molecularly Imprinted Polymer
MoS_2	Molybdenum disulfide

PCB	Printed Circuit Board
PDMS	Polydimethylsiloxane
PoC	Point-of-Care
PPy	Polypyrrole
RE	Reference Electrode
rGO	Reduced graphene oxide
RIA	Radioimmunoassay
SNR	Signal-to-Noise Ratio
SPCE	Screen-Pinted Carbon Electrodes
SPEs	Screen-Printed Electrodes
SPI	Serial Peripheral Interface
SUDEP	Sudden and Unexpected Death in Epilepsy
SWCNT	Single-Walled Carbon Nanotubes
SWV	Square Wave Voltammetry
TIA	Transimpedance Amplifier
UART	Universal Asynchronous Receiver-Transmitter
UFC	Urinary Free Cortisol
WE	Working Electrode
WHO	World Health Organization
ZnO	Zinc Oxide

List of symbols

dB	Decibel
Hz/nA	Hertz per nanoampere
mA	Miliampere
mM	Milimolar
mV	MiliVolts
ng/mL	Nanogram per milliliter
nM	Nanomolar
pg/mL	Picogram per milliliter
pM	Picomolar
μA	Microampere
μg/mL	Microgram per milliliter
μW	MicroWatt

Chapter 1

Introduction

Physical inactivity plays a role in several chronic health conditions ranging from type-2 diabetes, stress, heart disease, and cancer. Monitoring biomolecules associated with these chronic health conditions in a dynamic and non-invasive manner helps the diagnosis, the supervision of on-going treatments, and prevents more acute illness status. The relationship between those physiological stress states and cortisol levels in blood, saliva and sweat has been studied and is already well established [1][2]. It has also been demonstrated the potential of cortisol as a biomarker of a sudden unexpected death in epilepsy [3].

Cortisol functions to increase blood sugar through gluconeogenesis, suppress the immune system, and aid in the metabolism of fat, protein, and carbohydrates while also decreasing bone formation [4].

This chapter presents a contextualization of the work, the objectives to be accomplished, the reasons that motivated this work, as well as the structure how this report is organized.

1.1 – Context

The development of portable and intelligent devices for the detection of cortisol in daily life has received special importance concerning improving health care effectiveness and the diagnosis and prevention of several serious pathologies.

This dissertation project aims to develop a wearable, low-power microsystem capable of monitoring, continuously and in real-time, cortisol levels to prevent sudden and unexpected death in epilepsy and trigger the delivery of an emergency drug in case of a life-threatening seizure.

1.2 - Objectives

Point-of-care devices have been developed to promote and facilitate the monitoring of diverse biomarkers and detection of acute pathological situations, by the patient himself, in the physician office or at small clinics. There are already some portable or point-of-care diagnostic devices proper for detecting cortisol [5]. Electroanalytical techniques are desired over other transduction methods due to increased accuracy and sensitivity with minimal instrumentation

that utilize low power sources. The use of portable electroanalytical techniques in sweat-based wearables ranging from amperometry, cyclic voltammetry, chronoamperometry, and electrochemical impedance spectroscopy (EIS) has been investigated in recent years [6] but these devices are not dedicated to monitor an epilepsy risk.

The present dissertation was developed in the framework of a project aiming at developing a wearable, low-power, cortisol monitor to be integrated in a system, capable of continuously monitor sudden unexpected death in epilepsy risk and trigger the delivery of an emergency drug in case of a life-threatening seizure.

The main objectives of this dissertation are, in addition to conducting a literature review on the main topics of the subject, with the aim of strengthening knowledge about the area, the fulfillment of three essential points for the development of the intended system:

- Design of the front-end circuit to operate with the sensor;
- Development of the microcontroller-based system to acquire cortisol data and send it, via Bluetooth Low Energy, to a smartphone;
- Design of the mobile application that evaluates the received cortisol data and decides whether an alarm should be issued due to an elevated risk of sudden and unexpected death in epilepsy.

1.3 - Motivation

According to the World Health Organization (WHO), neurological disorders have become a significant and growing issue. These diseases are already responsible for 9 million deaths [7] and affect about 1 billion (american nomenclature) people worldwide [8].

Epilepsy is one of the most common neurological diseases, affecting, in 2019, about 50 million people worldwide [9], with about 1,16 cases of sudden and unexpected death in epilepsy (SUDEP) in every 1000 people with epilepsy, per year [10].

Based on these worrisome values, it is clear that more efforts must be made to improve the quality of life of people with these diseases and, in some cases, even save their lives.

Thus, the reasons that motivated the choice of this dissertation are the possibility of developing a technology that aims to save thousands of human lives and improve the living conditions of millions of people affected by a disease such as epilepsy and the natural impulse to technological evolution and innovation.

1.4 - Document Structure

This document is structured in six more independent chapters, followed by annexes and bibliographical references. In this first chapter, in addition to the contextualization and presentation of the project's objectives, it is also possible to find the motivations that led to the accomplishment of this work, as well as the structure of the document. The summary of the contents for each chapter are shown bellow.

Chapter 2 provides a background on cortisol, its functions in the body and the importance of monitoring it to diagnose and/or detect different pathologies. It is also shown the crucial role of cortisol in preventing sudden and unexpected death in epilepsy and the conventional methods of measurement in different biofluids.

Chapter 3 describes the state of the art in terms of portable cortisol sensors and circuits that operate with these sensors. This study aimed in first place to acquire specific knowledge that will serve as a basis for the development of the dissertation. This study allowed us to identify the different solutions available on the market in terms of sensors and electronic interface circuits and to conclude which is the best approach to be used in the dissertation project.

Regarding chapter 4, this describes the proposed system architecture for the proposed problem, as well as the adopted solution, a technical exposition of all the components involved in this device and the function and application of each one of them.

Chapter 5 is dedicated to the validation of the developed system, in which the obtained results are exposed and discussed.

Finally, chapter 6 addresses the main conclusions of this work, as well as points out some ideas for future work within the scope of this dissertation theme.

Chapter 2

Cortisol, a Key Molecule for Health

This chapter addresses the presence of the cortisol hormone in the human body and the importance of monitoring it for diagnosis and prevention purposes in various pathologies. The different biofluids in which cortisol can be detected are presented, as well as the conventional methods used to measure its concentration.

2.1 - Cortisol

Cortisol is a glucocorticoid hormone, known as the “stress hormone”, whose release is controlled by the HPA (hypothalamic-pituitary-adrenal) axis of the central nervous system. This axis, consisting of these three organs and the interactions between them, is responsible for the regulation of several body and physiological processes related to the cardiovascular, reproductive, metabolic, immunological, and central nervous system [11].

The process of synthesis and release of the cortisol begins in the hypothalamus with the release of the corticotrophin hormone (CRH) as a response to stress and other stimuli. This hormone will stimulate the pituitary gland to release the adrenocorticotropic hormone (ACTH), in greater or lesser amounts, according to the needs. In turn, ACTH stimulates the adrenal gland to synthesize and release cortisol [11]. The quantity of cortisol produced and released diffuses into the bloodstream and, therefore, to other parts of the body, being possible to detect it, in different amounts, in blood, sweat, saliva, urine, as it was more recently found, in the dermal interstitial fluid (ISF). Thus, it can be found in the body in two forms: free, in sweat, saliva and urine, allowing connections to specific receptors, and linked to the globulin in the blood, which constitutes most of the cortisol existing in the body [12].

Cortisol is the hormone responsible for regulating various physiological processes [13], whether at the cardiovascular, musculoskeletal, respiratory, reproductive, and nervous levels. This hormone acts in the regulation of glucose concentrations, in the activation of the inflammatory response when there is an infection, regulates the activity of the immune system, interrupting or stimulating the multiplication of the responsible cells [14], maintains blood pressure, also plays a fundamental role in nutrition [12] and in metabolism, providing the necessary nutrients to the body, acts in bone formation, fetal development during pregnancy, kidney activity, among many others [14].

2.2 - The importance of the cortisol monitoring

The continuous and non-invasive monitoring of biomolecules associated with chronic health conditions, such as cancer, diabetes, epilepsy, stress, has received special attention in last decades.

The relationship between physiological stress conditions and cortisol levels in the blood, sweat and saliva has been studied, and the potential of cortisol to be used as a biomarker of a sudden and unexpected death in epilepsy has already been demonstrated.

Normally, cortisol levels vary over 24 hours, following the circadian rhythm that ensures the balance necessary for the body to function properly. During the morning, the levels are higher, decreasing throughout the afternoon and night [15].

However, with exposure to physical and psychological stressors, such as diets, physical activity, infections [12], failure of the adrenal cortex [5], stress, psychological disorders (depression) [16] or other diseases such as cancer, abnormal levels of cortisol are produced and released [12].

Due to being a hormone involved in so many processes, disorders in its levels can cause symptoms such as stomach pain, headaches, heartburn, nausea, irritability and in more severe situations, can affect physiological processes regulated by cortisol such as metabolism and growth [12] and lead to cardiovascular diseases [6], causing heart attacks by increasing heart rate and hypertension [12]; neurological like strokes [14]; psychological issues such as anxiety and depression [6]; metabolic disorders such as obesity and diabetes due to insulin resistance [14], these being the most important ones.

When at extremely high levels, cortisol can cause diseases such as Cushing's syndrome, which manifests itself in the form of obesity of the trunk and face, hypertension [14], weak muscles, bone loss and fractures [12], among other signs. On the other hand, reduced levels give rise to other diseases such as Addison's disease that results in weight loss, reduced blood pressure, muscle weakness [12], vomiting, tiredness [14] and excessive pigmentation [13].

Thus, the importance of daily and non-invasive monitoring of cortisol levels as a form of prevention, supervision and diagnosis should be emphasized, since too high or too low levels of cortisol provide important information about physiological conditions [16] and they also warn of diseases such as Addison's disease, Cushing's syndrome, and epilepsy [17].

This monitoring proved to be very advantageous since it allows to obtain results in real-time and there is no need for specialized staff and travels to clinics and medical posts, when done with portable devices [18].

2.2.1 - Pathologies associated to abnormal cortisol levels

Many problems are caused by abnormal cortisol secretion. However, there are two diseases that stand out for being directly related to cortisol: Addison's disease and Cushing's syndrome.

Addison's disease, also known as adrenal insufficiency, is a rare and chronic disease that can appear at all ages [19] and affects between 40 and 60 people per million worldwide [20].

It results from the slow failure of the adrenal glands caused by genetic problems, autoimmune reactions, cancer, infections, or some other diseases, which leads to the low production of hormones such as cortisol and aldosterone, essential for the regulation of the activity of all organs and tissues [11].

The symptoms of this disease vary from individual to individual and usually appear gradually in the form of fatigue, muscle weakness, hyperpigmentation, nausea, vomiting, diarrhea, weight loss, low blood pressure and muscle pain. These crises usually appear when individuals

with this disease are under stress and must be properly controlled because they can cause fatal problems, if left untreated [20].

The Addison's disease is diagnosed through symptoms evaluation, X-Rays examination and performing blood, urine and saliva analizes that reveal abnormal levels of cortisol and of other elements such as potassium and sodium.

Treatment requires the administration of drugs that replace cortisol and aldosterone, which are missing in the organism [19].

On the other hand, Cushing's syndrome is another rare disease characterized by a set of symptoms and clinical abnormalities caused by prolonged exposure to extremely high cortisol concentrations [21].

This syndrome is more common in male adults between the ages of 25 and 40 and is caused by several conditions that increase the body cortisol concentration, such as treatments with cortisol-like drugs to treat other diseases, e.g., tumors, asthma, arthritis, lung diseases, among others.

The most common symptoms include weight gain on the face and neck, obesity on the trunk, very thin arms and legs, fragile and difficult to heal skin, muscle wasting and osteoporosis and hypertension [22].

The diagnosis of this disease is based on the performance of imaging exames and hormonal laboratory tests that assess the concentration of cortisol in the urine, saliva and blood. The treatment can be done through the administration and ingestion of proteins, potassium and adrenal inhibitors that block cortisol secretion or through surgery or radiotherapy to remove adrenal tumors that are the basis of this disease [21].

2.3 - Cortisol in Epilepsy

Epilepsy, the focus of this study, is considered one of the most common neurological pathologies, with more than 50 million people worldwide suffering from this disease [9]. About 1,16 out of 1000 people with epilepsy die from Sudden and Unexpected Death in Epilepsy (SUDEP) every year [10].

This disease manifests itself in the form of involuntary and unexpected crises, which can have neurological, psychological, cognitive, and social repercussions, such as loss of memory and senses, anxiety, and depression [3].

Several studies show a relationship between episodes of extreme stress and cortisol levels in different body fluids (blood, saliva and sweat) and the potential of cortisol as a biomarker for the prevention of SUDEP [1].

Although few recent studies have been done in this area and some of the results obtained are different from study to study, most of them show that people with epilepsy have higher basal cortisol levels than healthy people and that exposure to acute and constant stress leads to hypersecretion of glucocorticoids and, consequently, to higher cortisol levels than normal, which may be the cause of seizures [3] and, in some more severe cases, of SUDEP.

To obtain more reliable results when monitoring this hormone in people with epilepsy, it is crucial to keep in mind that cortisol levels are dependent on several factors that can influence cortisol levels and monitoring results [12]. In addition to the circadian cycle, in which the values are higher in the morning and lower throughout the afternoon and evening [3], several other factors such as the physical habits, physical activity, diets, addictions, the individual's sex and age [23], etc., can lead to abnormal cortisol secretions, affecting the results obtained and leading to incorrect diagnoses [2].

2.4 - Cortisol in Biofluids

The monitoring of cortisol is often done from plasma, saliva, urine and sweat samples and more recently it has also been done from dermal interstitial fluid (ISF) [24][25], and for each one of them, there are different reference ranges for cortisol concentrations. The cortisol concentration ranges that can be found in the different biofluids of healthy individuals are listed in Table 2.1.

Table 2.1 - Cortisol healthy ranges in different biofluids.

Sample	Cortisol concentration ranges	Ref.
Urine	10–100 µg/24 h	[12]
Sweat	8,16 – 141,7 ng/ml	[12]
Saliva	1 – 12 ng/mL (morning), 0,1 – 3 ng/mL (evening)	[14]
Plasma (Blood)	25 µg/mL (morning), 2 µg/mL (midnight)	[14]
ISF	12 – 34 ng/mL (morning), 9 – 13 ng/mL (evening)	[14]

The cortisol concentration ranges presented above are characteristic of a healthy individual who does not have any pathological condition [14]. Considering the circadian rhythm, varying quantities of cortisol are recorded during the day. In this way, there are higher cortisol ranges when the collection is made in the morning and smaller cortisol ranges when the collection is made in the evening and night.

Levels referring to urine cortisol are presented in micrograms per 24 hours (µg/24h), because this test allows to measure the total amount of cortisol expelled by the subject in one day, independently of the amount of urine collected, which varies from individual to individual.

The analysis and measure of cortisol concentration is typically made by means of laboratory conventional methods based on the analysis of the collected samples, using methods that vary with the type of biofluid being analysed.

2.4.1 - Blood (plasma)

Blood analysis is the oldest and most widely used method for measuring cortisol. The collection of this sample is done through the veins/capillaries puncture, usually 2 times in the same day, once in the morning and once in the afternoon, since the levels vary according to the circadian rhythm and takes a few minutes [12].

Cortisol measurement is generally done using immunochemical methods such as ELISA (Enzyme-Linked Immunosorbent Assay) and CLIA (Chemiluminescence Immunoassay) [14] and the results are usually available in 24 h to 48 h [26].

Although it is the most reliable method of analysis, the collection process is invasive and can cause anxiety and discomfort in patients.

In addition, it uses specialized equipment and personnel, and the storage of samples is more expensive [12].

2.4.2 - Urine

Concerning the collection of urine for the analysis of cortisol levels, traditionally the 24 hours UFC test (urinary free cortisol) [14] is used, which allows a painless and non-invasive sample collection.

However, this method can be considered unpleasant and inconvenient for the patient, since the sample is collected and stored for 24 hours [12].

The concentration of urinary cortisol can be determined using analytical methods such as RIA, mass spectrometry and chromatography [14]. The results of this exam take a few days to be obtained [27].

2.4.3 - Saliva

The collection of saliva samples is simple, non-invasive and represents a minimum effort for the patient [14].

In addition, this collection does not require large logistical resources, since it is done with a cotton swab for 2 to 3 minutes [28]. For these reasons, in recent years, this biofluid has received significant attention.

However, samples may show errors due to infections in the mouth and some instability at temperature [12].

The analysis of salive for cortisol detection purposes is made by means of analytical techniques such as mass spectrometry and ELISA [14] and it takes a few days or a few weeks to obtain results [29].

2.4.4 - Sweat

As for the collection of sweat for analysis, the process is non-invasive, it is easy to collect, being possible to perform it in different parts of the body and the analyses process is simple [14].

However, sensors that quantify cortisol after sweat can be contaminated due to the accumulation of sweat and are not very reusable, since sweat destroys surfaces [12].

The measurement of cortisol concentration in sweat is normally done by ELISA and chromatography [14] and can take a few days, or even a few weeks in some cases, when done in the laboratory [29].

2.4.5 - Dermal interstitial fluid (ISF)

The collection of dermal ISF is still a very recent process and needs more investigation, but it has been shown to be a promising alternative to the collection of blood samples, since this fluid has an identical composition to that of blood.

For this fluid, the collection is made with microneedles that allow to perforate the superficial layers of the skin in a painless and minimally invasive way.

Because it is such a recent technology, many studies have been done to test the biodegradability and biocompatibility of these microneedles.

The continued use of these needles can become a problem due to infections in the skin collection area [12].

The measurement of cortisol in this fluid can be done using conventional analytical methods such as ELISA.

2.5 - Conventional methods of measuring cortisol

Since the importance of measuring and controlling cortisol for the diagnosis and prevention of various diseases has been proven, several methods have emerged to quantify and monitor cortisol levels in the body.

The conventional methods used for the diagnosis and measurement of cortisol levels are common to practically all types of samples to be examined and have different advantages and disadvantages. These methods include laboratory analytical techniques such as immunoassays, chromatography techniques and mass spectrometry [12].

2.5.1 - Immunoassays

Immunoassays are analytical methods that use specific antigen-antibody binding to detect and measure the concentration of a particular analyte [13].

These methods are the most commonly used, because they are fast and highly sensitive and selective, however they need a large amount of sample content to perform the analysis, the antibodies have high production costs and enzyme/or antibody-based techniques are environmentally unstable [30].

Of the many existing immunoassays, the most traditionally used are ELISA (Enzyme-linked immunosorbent assay) that uses enzyme-labeled antibodies and chromogenic substrates to measure the analyte [31]; CLIA, which produces a light response when antigen-antibody binding occurs [32] and RIA (radioimmunoassay), identical to ELISA, but which uses radioisotopes to mark antigens. The latter has fallen into disuse due to the harmful effects of radioisotopes.

ELISA and CLIA are commonly used to measure cortisol in blood, saliva and sweat, while RIA is most commonly used to measure concentrations in urine and blood [13].

2.5.2 - Chromatography techniques

Known for being the oldest and the pioneers in the detection of cortisol [13], chromatography techniques allow to separate the components of a mixture based on the distribution of the components between the stationary phase and the mobile phase [33].

These techniques, traditionally used to measure cortisol in urine and sweat [14], are considered more accurate compared to other techniques and, contrary to immunoassays based tests, small amounts of sample are sufficient for analysis.

On the other hand, these methods require expensive instrumentation and specialized personnel [34].

2.5.3 - Mass spectrometry

Often used for cortisol measurement in urine and saliva [14], mass spectrometry is a sensitive, accurate and selective technique, usually combined with chromatography techniques, which allows to quantify a molecule by separating the sample ions, based on their mass-to-charge ratio, and recording its relative abundance.

This technique has as disadvantages, the need to use specialized and expensive personnel and equipment, which do not allow for portable and wearable monitoring [35][36].

2.6 - Conclusion

The monitoring of biomolecules, such as cortisol, associated with various chronic health conditions, helps in the diagnosis, control and prevention of the effects of various diseases, including epilepsy.

In this way, the extreme importance of monitoring is evident, and it is crucial to optimize this process.

The methods conventionally used for cortisol analysis presented in this section (2.5) are usually implemented in clinical laboratories and require the use of bulky equipment, being the results obtained tipically after several days or even weeks.

To circumvent this disadvantage, some of these techniques were implemented in portable and wearable sensors and are presented in subsection 3.1.1 of the following chapter.

Chapter 3

Cortisol Sensing: A Review

Given the need for fast, accurate and non-invasive monitoring of cortisol in the daily life of an individual with epilepsy [6][37], some portable and wearable or point-of-care (PoC) diagnostic devices for cortisol monitoring have already been developed.

This chapter provides a background of the portable cortisol sensors available on the market, as well as the proposed interface circuits for electrochemical sensors.

3.1 - Sensors of Cortisol

The sensor is the main element of the process of collection and detection of the analyte and can act using analytical or electrochemical techniques [6].

3.1.1 - Analytical techniques sensors

Analytical techniques are a class of techniques used in analytical chemistry and in clinical analysis, which allows the detection, identification and quantification of a specific analyte based on physical and chemical properties through classical analysis or instrumental methods [38].

Typically, to detect and quantify the concentration of analytes based on these techniques, chemical and/or enzymatic interactions, such as antigen-antibody affinity binding, and certain types of chemical reactions and instrumental methods are used [6].

There is a wide range of advanced analytical techniques used in the detection of analytes, including immunoenzymatic assays, such as ELISA (Enzyme-Linked Immunosorbent Assay), CLIA (Chemiluminescence immunoassay), LFIA (Lateral Flow Immunoassay) [39], spectroscopy, which measures the radiation emitted and absorbed by the interaction between molecules [40], chromatography, which identifies an analyte by separating a mixture based on the affinities of the particles for the mobile phase and for the stationary phase [33], and spectrometry, that uses spectral components to identify and measure particles [36], among others.

Since they are laboratory techniques and take time to obtain results, some portable sensors have been implemented using these techniques.

Based on enzyme-linked immunosorbent assays (ELISA and EIA), sensors that allow the detection of small amounts of antigen in sweat have been proposed. The antigens are immobilized on a surface and, when in contact with the sample, are linked to their specific antibodies, marked with enzymes. After antigen-antibody binding, a chromogenic substrate that reacts

with the present enzymes is added, producing a colorimetric response, when the presence of binding is detected. In this technique, the detection of cortisol is done based on an optical response given by the change of color [41].

As in the previous work, other approaches based on optical responses were developed and presented. Based on Chemiluminescence lateral flow immunoassay (CL-LFIA), a simple detection device, consisting of a lateral flow test strip inside a cartridge and an image system that includes a smartphone and an LFIA cartridge adapter for smartphone, was created to detect and measure salivary cortisol (Figure 3.1). Cortisol antibodies are immobilized on the strip and a chemiluminescent substrate is added that binds to the antigen-antibody complex and produces an optical response. The cartridge is placed in the adapter in front of the smartphone's camera to detect the optical response and quantify the level of cortisol present in the sample, through an application. Calibration curves were obtained and studied to evaluate the performance of the sensor. The results reported for this sensor show a detection range of 0,3 – 60 ng/mL, with a limit of detection (LOD) of 0,3 ng/mL and a long detection time of 30 minutes, compared to other developed sensors [42].

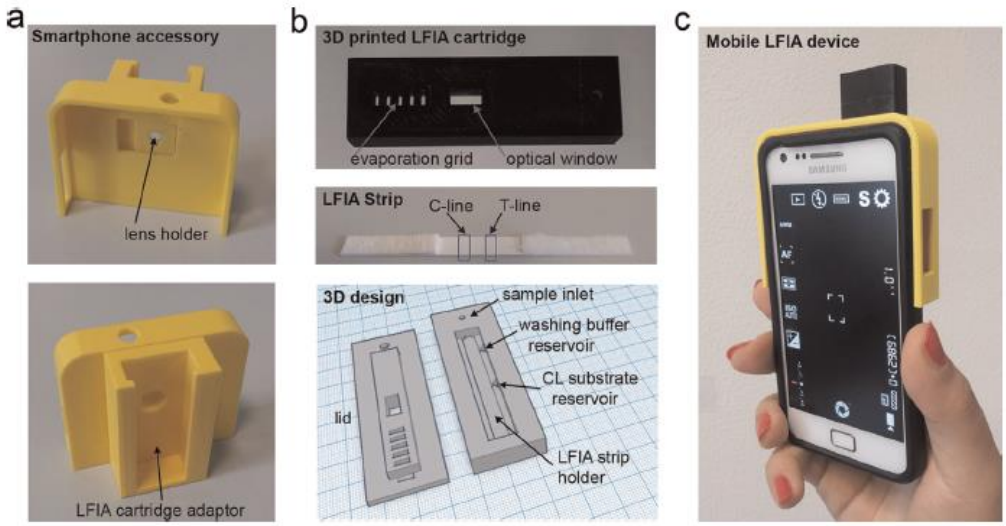


Figure 3.1 – **a)** Smartphone accessory with LFIA cartridge adaptor; **b)** LFIA cartridge comprising the LFIA strip and a 3D model of the cartridge; **c)** Cortisol LFIA smatphone-based device developted in [42].

Over the years, new techniques and more innovative technologies have emerged, allowing more sensitive, specific, and stable sensors.

Using spectroscopy, an optical fiber-based salivary cortisol sensor coated with a nanocomposite film composed of zinc oxide (ZnO) nanoparticles and molecularly imprinted polymers (MIPs) was presented [43]. The detection process starts at the molecularly imprinted polymers, where the detection of the analyte is done by binding the cortisol molecules present in the salivary sample to the corresponding imprinted sites on the polymer. The established connections, proportional to the concentration of cortisol in the sample, cause changes in the properties of the ZnO film, which generate lossy mode resonances (LMR), for each concentration of cortisol. LMR absorbance spectrum is recorded by a spectrometer and sent to a computer for cortisol quantification purposes. The sensor has been tested and evaluated for the main parameters that affect performance, presenting a high sensitivity with a detection limit of 25,9 fg/ml and a detection range from 10^{-12} g/ml to 10^{-6} g/ml and a 20s response time [43].

According to several studies, these techniques present a high sensitivity, specificity [6], reproducibility and fast detection time, are easy to manufacture and low cost, and most of them can be implemented in wearable and portable devices [41]. However, some of these

techniques, such as mass spectrometry, require specialized staff and equipment and cannot be implemented in portable devices, use fragile and changeable materials and have a long preparation time [39][41].

3.1.2 - Electrochemical sensors

In recent years, sensors based on electrochemical techniques have been suggested as a promising alternative to some of the used analytical techniques, which involve time consuming preparation and measurement processes and cannot provide real-time, portable and continuous monitoring of cortisol levels [12].

Comparing to the methods presented in the previous subsection, the techniques and methods presented here are characterized for their high sensitivity and accuracy, for allowing real-time, fast and portable monitoring and for the ease of use and manufacture using simple and inexpensive instrumentation [14].

On the other hand, electrochemical or electroanalytical techniques are another class of techniques used in analytical chemistry, which measure the potential and/or current in an electrochemical cell where redox reactions have occurred [44]. An electrochemical cell is a device used to transform energy, usually composed by electrodes and an electrolyte solution. These cells can be of two types: galvanic cells, which transform chemical energy, released by a redox reaction, into electrical energy, generating a current, and electrolytic cells, which do the reverse, converting electrical energy into chemical energy [45].

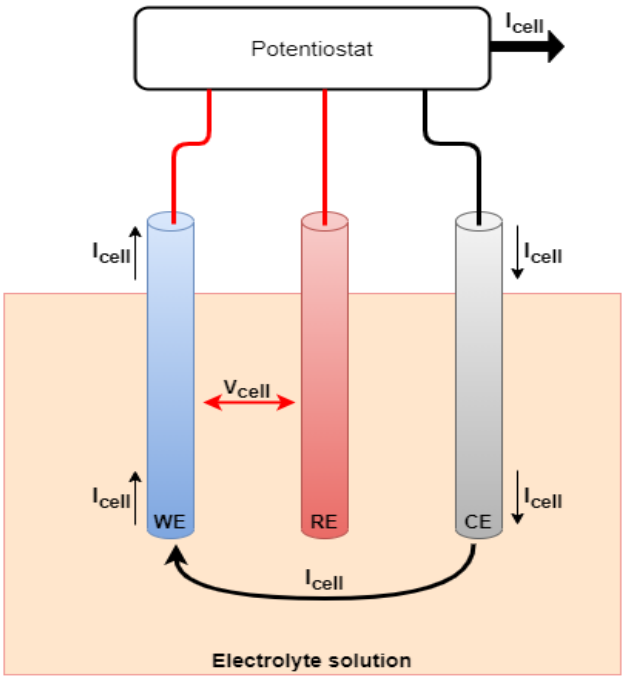


Figure 3.2 – Three-electrode electrochemical cell composed of working electrode (WE), reference electrode (RE) and counter electrode (CE). The potentiostat that maintains the potential difference between WE and RE and that measures the current generated between CE and WE is also presented.

A typical electrochemical sensor used to detect an analyte, consists of a galvanic electrochemical cell, shown in Figure 3.2, composed of three electrodes: a working electrode (WE), where the analyte redox reaction occurs; a reference electrode (RE), which establishes the reference potential to control the potential of the working electrode and a counter electrode (CE), that balances the reaction of the working electrode, doing the reverse reaction and is used along with the working electrode to provide a circuit over which current is applied or

measured [46]. These electrodes can be made of various materials such as carbon, gold, metal oxides, graphene, platinum, among others [14].

Depending on the variable to be measured, these techniques range from amperometry, voltammetry, chronoamperometry, potentiometry, coulometry to electrochemical impedance spectroscopy (EIS) and are desired over other techniques due to increased accuracy and sensitivity and minimal instrumentation [6]. Amperometry is a simple and very common technique, which measures the current resulting from the oxidation or reduction of an analyte in the WE, when a constant potential is applied [47]. On the other hand, chronoamperometry allows to measure the resulting current as a function of time after the application of a square wave potential to the working electrode [48]. Because it is considered a low selectivity technique, other techniques, such as voltammetry, are preferred [49]. Voltammetry is one of the most used and powerful detection techniques and allows the measurement of the current generated, when a variable potential is applied to the cell [50].

Another method that measures the current is Coulometry. This technique is not very common and differs from the others because it measures the current consumed by the reaction and not the current generated [51].

The unique technique that is based only on the potential difference is Potentiometry, which allows obtaining information about an analyte, after measuring the potential difference between electrodes of an electrochemical cell [52].

Finally, electrochemical impedance spectroscopy is one of the most complex and sensitive techniques, used mainly in the laboratory environment, because it has a longer data collection time, greater complexity in analyzing results and uses specific instrumentation. It allows the determination of electrical properties related to chemical transformations, such as impedance or current, in a frequency range, through the application of an AC potential [53].

Some of these techniques will be presented more indetail further ahead.

Several studies provide examples of sensors based on electrochemical detection used to monitor cortisol levels.

Kim et al. [54] developed a simple three-electrode sensor, with the working electrode (WE) made of reduced graphene oxide nanosheets, rGO, coated with a layer of denatured bovine serum albumin protein, d-BSA, to detect and monitor salivary cortisol. The d-BSA layer facilitates the immobilization of cortisol specific antibodies and serves as a block for the binding of interfering molecules, providing high selectivity to cortisol. Taking advantage of the specificity of the antigen-antibody bond, cortisol was detected, and its levels were measured by electrochemical impedance spectroscopy (EIS) using a potentiostat. The performance of the sensor was assessed by producing a calibration curve that proved the high sensitivity of the device with a wide detection range from 10 pM (picomolar) to 100 nM (nanomolar).

Still using the high electrical conductivity and biocompatibility of rGO, a reduced graphene oxide (rGO) chemiresistor sensor has been functionalized with cortisol monoclonal antibodies (c-Mab) for the detection of salivary cortisol [55]. A chemiresistor is a chemical sensor that allows direct detection and quantification of cortisol levels by measuring changes in resistance in response to the binding of the sample's cortisol to its specific antibody [56]. The sensor performance was evaluated by obtaining resistance calibration plots of the I-V measurement, which showed a good correlation between the results obtained and the ELISA results, and excellent sensitivity of the sensor with a limit of detection of 10 pg/mL (picogram per milliliter) [55].

Kinnamon et al. [11] presented the first portable, wearable and non-invasive biosensor, composed by a parallel electrodes system separated by a polyamide membrane with Molybdenum disulfide (MoS_2) nanosheets functionalized with antibodies, for cortisol monitoring in

low volumes of sweat. Sensing was made by recording the impedance changes, associated with the modulation of the surface charge of MoS_2 caused by the antigen-antibody binding, by electrochemical impedance spectroscopy (EIS). After the bench tests, the sensor was implemented with a potentiostatic electronic circuit, and the results obtained showed a correlation value of 0,998. The device was studied for its mechanical properties by cyclic bending, demonstrating a small variation in the impedance response in relation to the applied stress and it was tested for its specificity, sensitivity, and response time, having obtained a dynamic range of 1 – 500 ng/mL, with a limit of detection (LOD) of 1 ng/mL and a fast detection time of three minutes.

More recently, the "CortiWatch" device (Figure 3.3 c)) was proposed that, as the name suggests, consists in a watch that monitors cortisol levels on the wrist. This device is organized in a cartridge system, which contains the three-gold-electrodes sensor, functionalized with alpha-cortisol antibodies (3.3 a)) and a portable and miniaturized potentiostatic circuit printed on a circuit board (PCB), that detects and quantifies, by chronoamperometry, the concentration of cortisol in low volumes of sweat. The sensor was studied and tested on the human wrist, showing high sensitivity to variations in cortisol concentrations over 9 hours and a detection range of 1 pg/ml to 150 ng/ml [5].

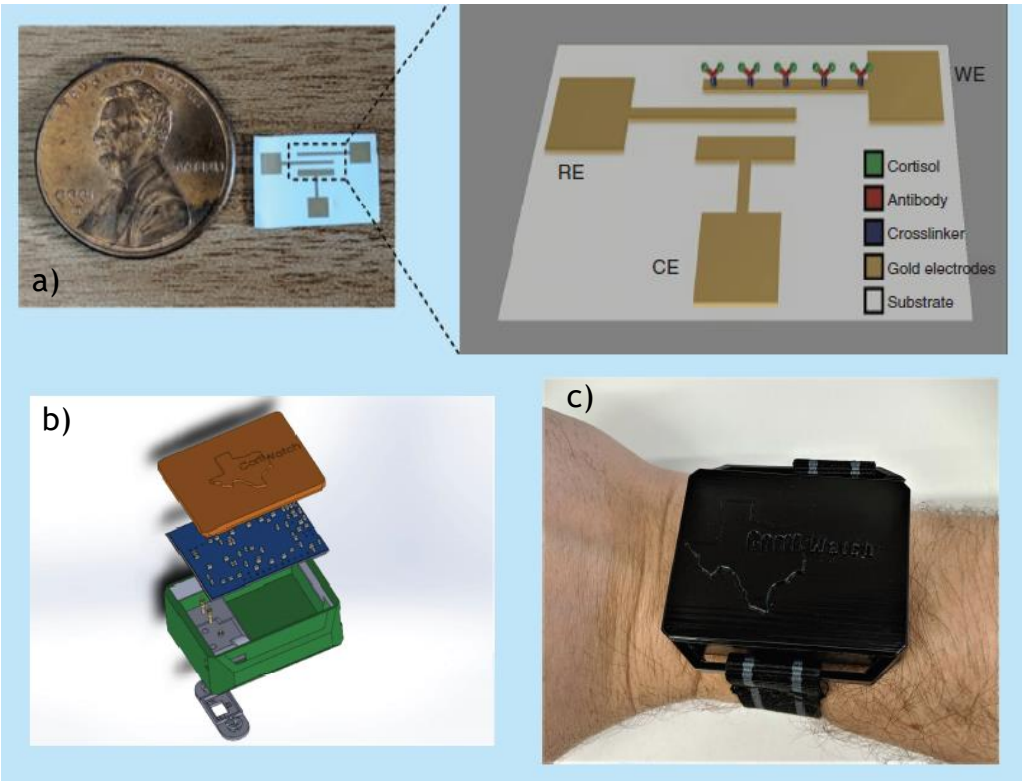


Figure 3.3 – **a)** Image of the CortiWatch sensor and the three-eletcrodes functionalization schematic; **b)** Different components of CortiWatch inside the cartridge system; **c)** CortiWatch wearable device on a human's wrist [5].

To overcome the instability and irreproducibility of the antibody-antigen binding, some authors have presented alternatives to antibodies. Sanghavi et al. [57] presented a microfluidic device that integrates a graphene-modified carbon three-electrode sensor functionalized with aptamer nanoparticles, cortisol marker, which allows the fast and specific monitoring of salivary cortisol levels by square wave voltammetry (SWV), a fast and sensitive voltammetry technique that applies a linear potential combined with a square wave potential, to the working electrode [58].

Mugo and Alberkant [16], on the other hand, developed a microneedle cortisol sensor, composed of a three-electrode system encased in a micropillar structure of polydimethylsiloxane (PDMS) and conductive carbon nanotubes coated with molecularly imprinted polymers (MIPs) that function as cortisol receptors. The sweat sample is collected through the microneedle matrix, the sample's cortisol molecules bind to the specific locations printed on the polymer and the cortisol levels are quantified by cyclic voltammetry using a wireless potentiostat connected to a smartphone. This sensor presented a good performance with a dynamic range of 10 ng/mL to 66 ng/mL, a detection limit of 2,0 ng/mL ± 0,4 ng/mL, high specificity to cortisol and fast detetion time of three minutes.

Also Sekar et al. [39] presented a flexible electrochemical sensor in highly conductive and resistant carbon fiber (CCY), coated with ellipsoidal Fe_2O_3 to immobilize specific antibodies that allows the monitoring of cortisol in sweat, by cyclic voltammetry. The developed sensor was tested with samples of human sweat, showing excellent specificity and sensibility with a limit of detection of 0,005 fg/mL and a wide detection range from 1 fg to 1 µg. The results obtained were validated through the commercial chemiluminescence immunoassay (CLIA) technique.

In the last year, a new all-in-one, fully integrated, flexible, and wearable monitoring device was presented, composed of a microfluidic three-working-electrodes sensor made of graphene and functionalized with antibodies, and a potentiostatic circuit with Bluetooth module, for real-time monitoring of cortisol levels in human sweat (Figure 3.4). The current generated due to redox reactions is measured by the electronic circuit through amperometry and these values are sent to the user's device via Bluetooth. This technology allows continuous evaluations and fast detections in 1 minute exhibiting high sensitivity and specificity to cortisol and low-cost production. One of the main advantages of this system is its flexibility, since it can be adapted to any part of the body in a very comfortable way [15].

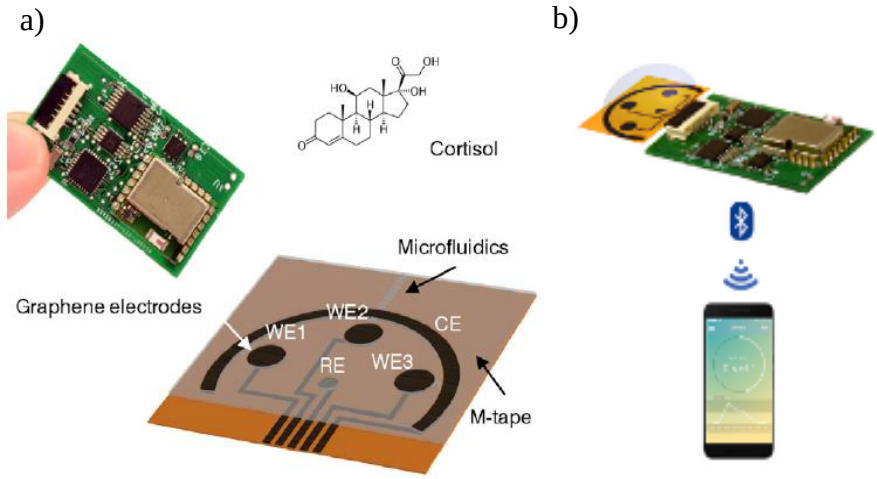


Figure 3.4 – **a)** Flexible microfluidic three-electrodes sensor and interface circuit for cortisol monitoring; **b)** All-in-one device developed in [15], composed of the microfluid sensor and the printed circuit board interface for processing and sending data to a smatphone, via Bluetooth.

All the sensors presented above were validated with commercial techniques such as ELISA and CLIA, or with measurements done in the benchtop instrumentation, having obtained high performance and good correlation of results.

The electrical equivalent is intended to help understand and discover important information about the characteristics of a given process, as it produces a response similar to that of the real system.

An equivalent electrical schematic of a system can be represented by resistors, inductors and capacitors and is commonly used for simulation purposes.

The electrical equivalent circuit most used to represent a three-electrode electrochemical cell, shown in Figure 3.5, is known as the Randle's equivalent circuit [59].

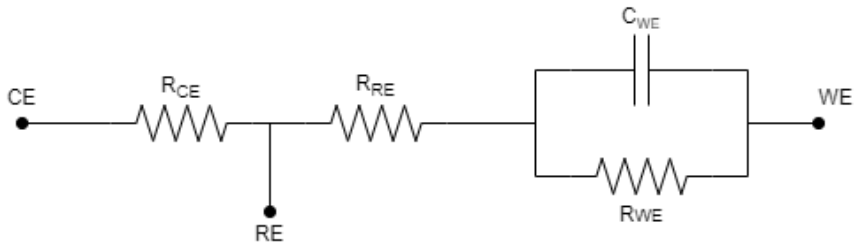


Figure 3.5 – Randle's Equivalent Circuit representative of a three-electrode electrochemical cell.

The series resistor, R_{CE}, represents the resistance between the counter electrode and the reference electrode, R_{RE} represents the resistance between the reference electrode and the working electrode, and the resistor $R_{WE,}$ which is in parallel with the capacitor (C_{WE}), is related to the oxidation-reduction current limited by the charge-carrying mechanism [60].

3.1.2.1 - Electrochemical techniques

Of the various existing electrochemical techniques, there are two that stand out for this work, as they are the best known and most widely used: Cyclic Voltammetry and Square Wave Voltammetry.

Cyclic voltammetry (CV) is one of the most complex electrochemical techniques, but at the same time the most powerful and popular. This technique is commonly used to investigate the reduction and oxidation processes of molecular species and also to study many chemical systems [61].

Typically, to implement this technique, an electrochemical cell with three electrodes is used, consisting of a counter electrode (CE), a reference electrode (RE) and a working electrode (WE), all of them immersed in an electrolytic solution (solvent + supporting electrolyte) and connected to a potentiostat [62].

The potentiostat maintains a constant potential between the reference electrode and the working electrode and applies a linear ramped potential that generates a current in the electrochemical cell.

During scanning, Figure 3.6, the potential is first scanned negatively, i.e, from the highest potential (A) to the lowest potential (D). Reverse scan occurs from the lowest potential (D) to the highest (G) and is where the potential positively sweeps [62].

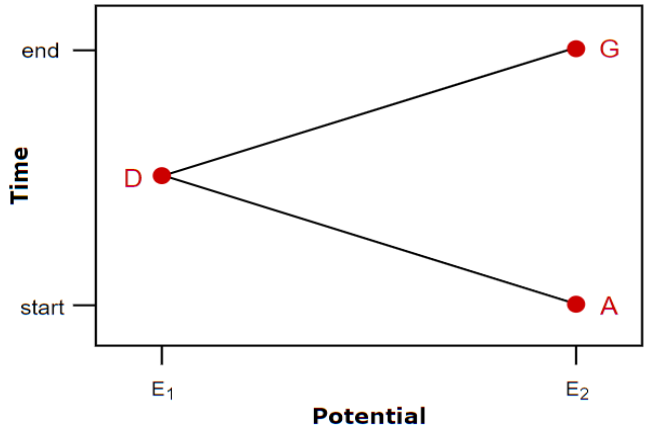


Figure 3.6 - Applied potential as a function of time.

The result of this scan is a voltammogram, a graph in which the x-axis represents the applied potential, and the y-axis represents the current resulting from the applied potential.

Figure 3.7 shows that from (A) to (D) there is a reduction and from (D) to (G) there is oxidation. This cycle can be repeated, and the scan rate can be varied.

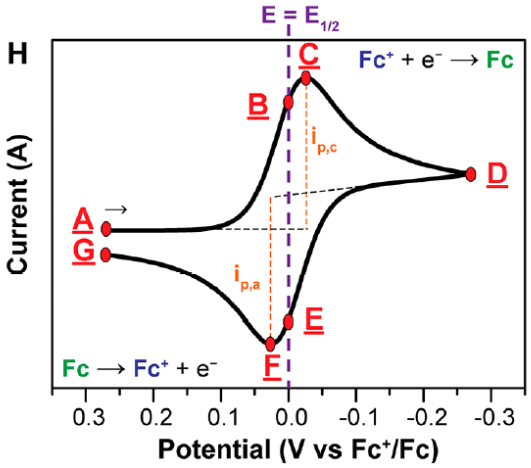


Figure 3.7 – Voltamogram of a 1 mM Fc+ solution to Fc [62].

This equilibrium is described by the Nernst equation, equation 3.1, which relates the electrochemical cell potential (E) to the standard potential of the species (E0) and the oxidation (Ox) and analyte reduction (Red) activities in the equilibrium system.

$$E = E^0 + \frac{RT}{nF} ln \frac{(Ox)}{(Red)} = E^0 + 2.3026 \frac{RT}{nF} log_{10} \frac{(Ox)}{(Red)} \quad (3.1)$$

where F is the Faraday constant, R is the universal gas constant, n is the number of electrons, and T is the temperature.

Square wave voltammetry (SWV) is one of the fastest, most sensitive and effective pulse voltammetric techniques traditionally used in researching the mechanism of enzymatic kinetics, in the detection of contaminants and chemical species and in the development of new methods to improve the surfaces of nanomaterials.

In this technique, a square wave-shaped potential, identical to the one in the Figure 3.8, is applied, generating a varying current in agreement with the increasing potential [63].

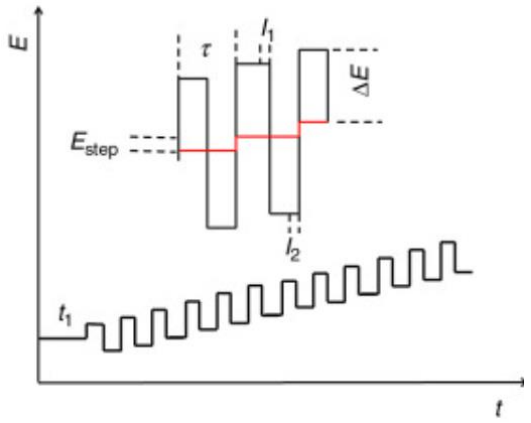


Figure 3.8 - Potential applied in SWV technique [63].

Current is measured at the end of each voltage pulse, that is, the first current is measured at the end of the direct square wave pulse and the second is measured at the end of the reverse pulse.

The resulting current signal is calculated from the difference between the currents measured from direct and reverse voltammograms and is represented by a larger peak because both individual signals have opposite signs (Figure 3.9) [61].

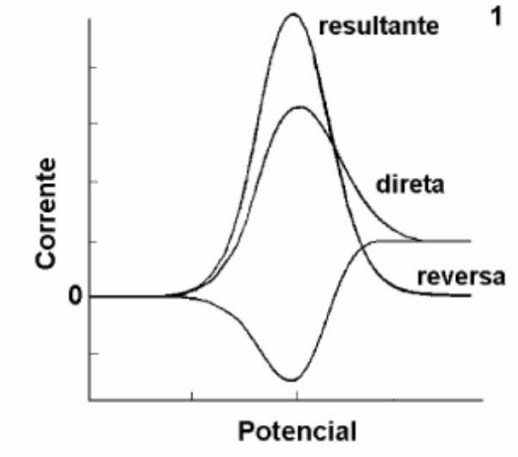


Figure 3.9 - Resulting current signal in square wave voltammetry [63].

3.2 - Interface circuits for electrochemical sensors

Portable and implantable sensors play a crucial role in health control [64], but in order to be completely independent and wireless, it is necessary to implement them with an electronic circuit [46] that provides conditioning and acquisition of the signal generated by the sensor and the transmission of the extracted values through a communication device.

The acquisition and conditioning of the signal generated by an electrochemical sensor is generally achieved through the implementation of a potentiostatic circuit and an analog front-end (AFE) that converts the analog data produced by the sensors into digital data [65].

The transmission of the captured values is done through an interface circuit, usually a microcontroller-based system integrated with a Bluetooth low energy communication device, which allows communication with another device to transmit the obtained values.

The potentiostatic is an electronic instrument essential for the proper functioning of the three-electrode electrochemical sensor, used to maintain the desired potential diference between the working and reference electrodes and to measure the current that circulates between working electrode and counter electrode [46]. In order for being used within portable sensors, it is important that it has small dimensions, low energy consumption, low-cost production and that it is easy to integrate with a signal processing circuit [64]. A potentiostatic circuit is usually implemented with operational amplifiers based circuits [59] and can follow two configurations: grounded WE and grounded CE. The grounded WE topology, presented in 3.10 a), is the most used configuration where WE is maintained at the ground potential and the cell current is controlled by an operational amplifier that maintain the desired potential at the reference electrode; The grounded CE, shown in Figure 3.10 b), is a more complex configuration, where the CE terminal is maintained at the ground potential and a two operational amplifier circuit is needed to maintain the desired potential between WE e RE electrodes and to generate the current between CE e WE [46][64].

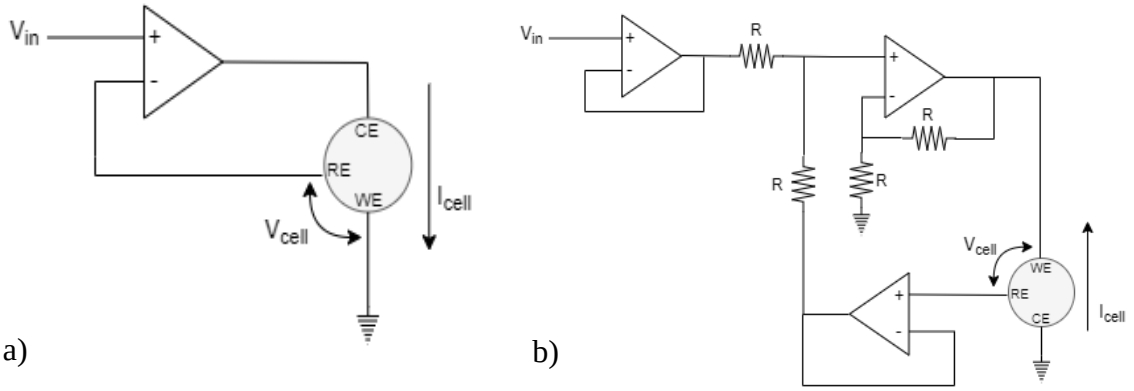


Figure 3.10 – **a)** Potentiostatic circuit using grounded WE configuration; **b)** Potentiostatic circuit using grounded CE configuration.

To measure the current generated in the electrochemical cell, the most common approach is the use of a transimpedance amplifier (TIA), shown in Figure 3.11. This amplifier connects to the working electrode and converts the current generated into voltage, proportional to the current.

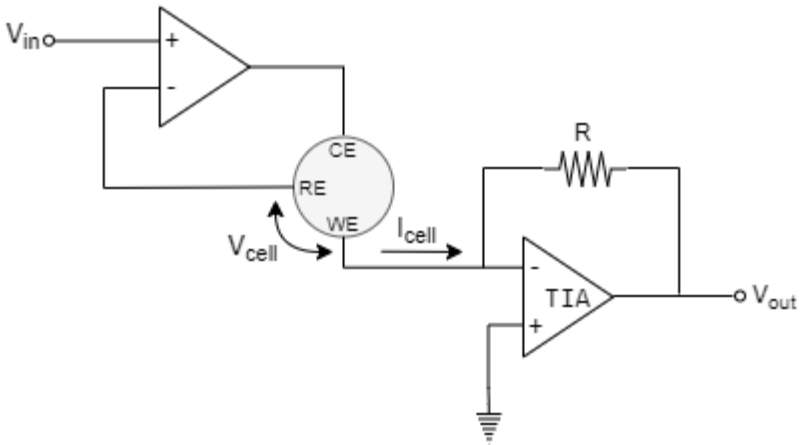


Figure 3.11 – Convencional transimpedance amplifier (TIA) for current measurement.

Electrochemical measurement and characterization are performed with one of the various electrochemical techniques [59] presented in the previous subsection.

Some eletronic circuits used for this type of applications have been developed and presented in several studies.

Islam et al. [64] proposed a fully integrated potentiostatic circuit on a chip, presented in Figure 3.12, which keeps the working and reference electrodes at a desired potential, to generate a current in the CE proportional to the concentration of analyte. This on-chip potenciostat uses a modified CE grounded configuration and is composed of four operational amplifiers, two of them, U1 e U2, working as buffers, U3 working as a differential amplifier and U4 acting as an error amplifier.

The U4 amplifier, where a fixed reference potential of 0,7 V is injected, feeds back the working electrode. As U1 and U2 are buffers, the output voltages of these amplifiers are equal to the input voltages. Thus, the voltage that enters in the inverting terminal of U3 is 0,7 V and the voltage that enters in the non-inverting terminal of U3 is $V_{WE}/2$, due to the resistive divider. The output voltage of U3 is 0,7 V and equal to the input voltage of U4.

In this way, applying Kirchhoff's law to the U3 amplifier, it is known that a constant potential difference (Vcell) of 0,7 V is maintained between the WE and the RE, even if WE and RE change their potential.

$$V_{RE} - \frac{V_{WE}}{2} = \frac{V_{WE}}{2} - 0.7\ V \Leftrightarrow V_{WE} - V_{RE} = 0.7\ V \tag{3.2}$$

This is a simple approach, with few components and with a low-power consumption of 66 µW for a power supply of 2,5 V.

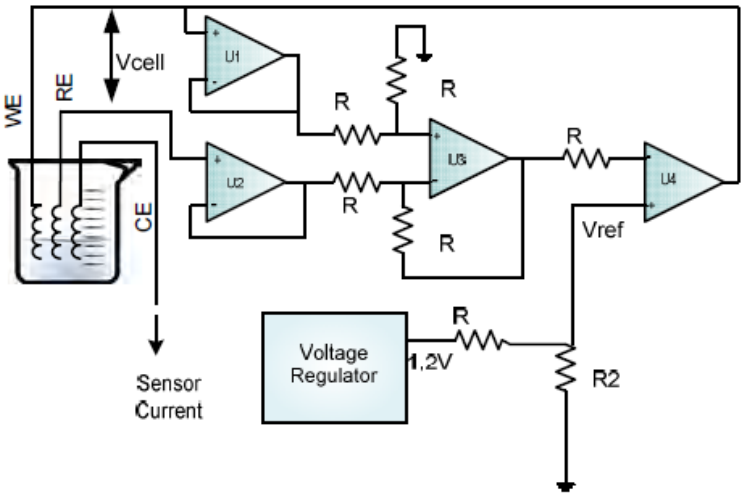


Figure 3.12 - Schematic of the on-chip potenciotast developed in [64].

Ahmadi et al. [46] presented a current-mirror-based potentiostat circuit, shown in Figure 3.13, which mirrors the sensor current in a cascode topology implemented with four transistors (M1, M2, M3 and M4).

An input voltage Vin is injected into the inverting terminal of the operational amplifier A1. To keep the reference electrode at the desired potential for making amperometric measurements, A_1 and the M_1 transistor compose the feedback loop. The grounded WE configuration is implemented, so the current generated in the electrochemical cell is measured at the counter electrode.

In this way, the current that comes out of the counter electrode, in a range from 1 nA to 1 µA, enters in the M_1 transistor, and is mirrored in M_2. The current mirrored in M_2 is mirrored again with M_3 and M_4 transistors and is then conducted to the current-to-frequency (*I*-to-*F*) converter. Here, the mirrored current is converted into a rectangular wave with frequency $1/T$ and pulsewidth D, at the output of the flip-flop. Current and frequency have an inversely proportional relationship and the pulsewidth is constant, that can be proved through the equations 3.3 and 3.4,

$$T = \frac{V_{DD} C_{INT}}{2 I_F} \tag{3.3}$$

$$D = \frac{V_{DD} C_{INT}}{2 I_{ref}} \tag{3.4}$$

where I_{ref} is the reference current, I_F is the cell current, V_{DD} is the supply voltage and C_{INT} is the capacitor value.

This approach allows low-power consumption, in the order of 50 µW from a 1,8 V supply voltage, good linearity and stability and has a current range from 1 nA to 1 µA, an accuracy of 0,1%, and reduced dimension. As it uses few active and passive components to measure the

current and the WE is grounded to a true ground, the circuit generates a low noise and is more insensitive to interference and noise [46].

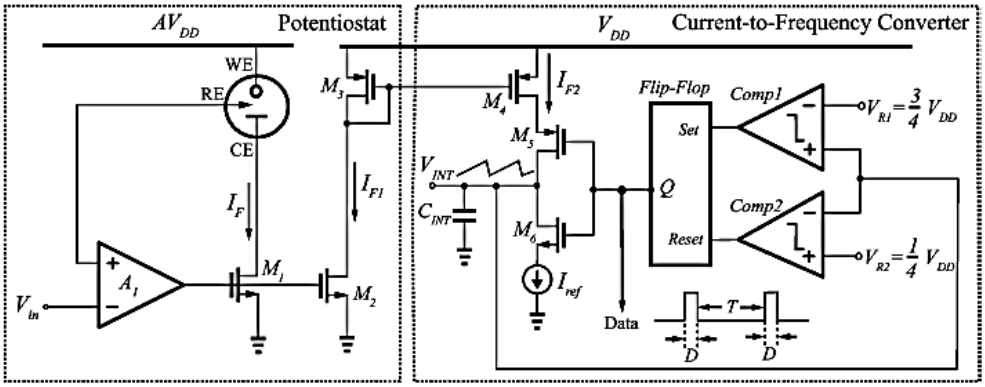


Figure 3.13 - Schematic of the potentiostatic circuit and the I-to-F converter implemented in [46].

A front-end readout circuit for electrochemical detection sensor using a current-mirror-based potenciostat scheme Identical to the previous one was developed by Chen et al. [49].

The readout circuit, presented in Figure 3.14 and 3.15, consists of a low-noise current-mirror-based potentiostat, a current to frequency (I-to-F) converter and a frequency to digital converter. This potentiostat, in addition to being current-mirror-based, is composed of a chopper stabilizer that allows to reduce the circuit noise and improve the signal-to-noise ratio (SNR) and of a constant current generator, implemented to compensate the negative current and thus allow bidirectional detection by cyclic voltammetry.

A ramp generator is used to generate an input voltage that is applied between WE and RE to perform cyclic voltammetry. The current generated by the electrochemical cell is injected into the current-mirror-based potentiostat. The mirrored current is conducted to an I-to-F converter that converts the mirrored current into frequency, which is then digitized by a frequency to digital converter.

The output frequency of the I-to-F converter was plotted for different current values, showing a linearity of 0,9928, a sensitivity of 8,45 Hz/nA, a dynamic range of 73,2 dB and a current range between -2 µA to 12,2 µA with a power consumption of 40,5 µW at maximum current.

The proposed circuit presents a complex approach, composed of many components, which can be a disadvantage for its implementation. On the other hand, it allows to minimize the circuit noise and improve the signal-to-noise ratio (SNR).

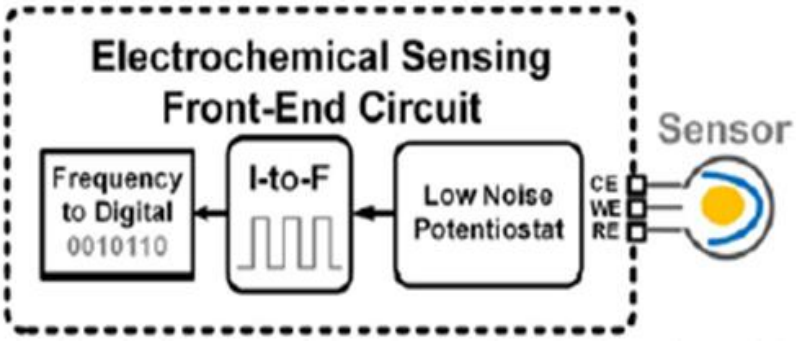


Figure 3.14 - Block diagram of the circuit implemented in [49], including the potenciostat block, the I-to-F converter block and the frequency to digital converter block.

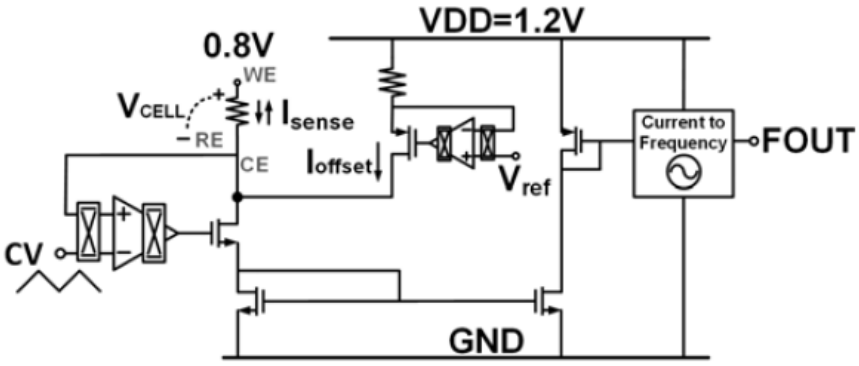


Figure 3.15 - The chopper-stabilized potentiostat circuitry proposed in [49].

More recently, Souza et al. [59] developed a grounded WE configuration potentiostatic circuit for electrochemical sensors, shown in Figure 3.16 , based on a two-stage operational amplifier (AOP) and a Current Conveyor circuit (CCI).

The AOP was designed to control the potential difference between WE and the RE through an input voltage Vin and to generate current for the CE.

Vin is applied to the AOP non-inverting terminal that corresponds to the M4 transistor. Since M3 and RE are connected, the feedback signal is applied to the AOP inverting terminal. In this way, the remaining AOP circuit transistors, which correspond to the first and second stages, amplify the potential difference between Vin and RE. The resulting signal from the second stage, transistors M7 and M8, is applied to the CE, which supplies the necessary current to the electrochemical cell.

The CCI is implemented with the objective of measuring, by cyclic voltammetry, the current that passes through the sensor. Connected to the working electrode, the current conveyor circuit receives the current generated by the electrochemical cell at its X terminal, which is transmitted to its Z output terminal. The output current in Z is converted, by the output resistance, into an output voltage proportional to the current generated and to the concentration of the analyte.

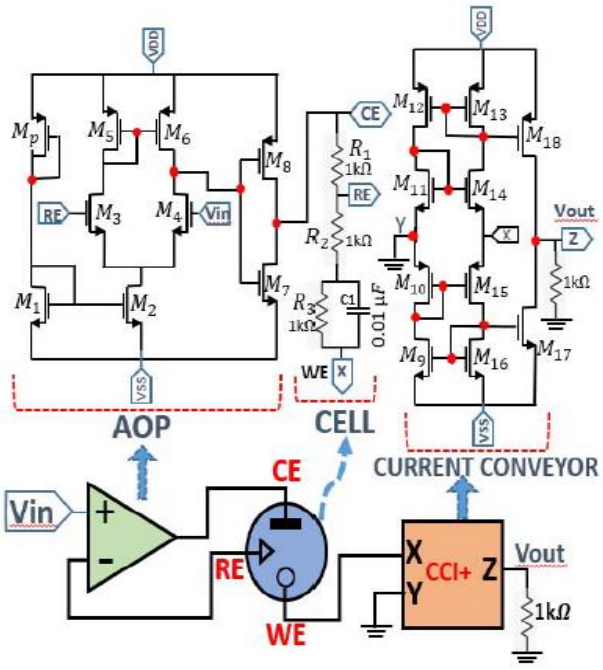


Figure 3.16 - Proposed interface circuit based on a two-stage operational amplifier and a Current Conveyor circuit (CCI) [59].

Specifically for cortisol levels monitoring, Rice et al. [5] designed, for their fully integrated and wearable device, an electronic circuit on a rigid printed circuit board (PCB) to operate with the sensor (Figure 3.17).

A step input voltage of 150 mV is applied between the WE and RE to allow measurement of the cortisol concentration by chronoamperometry. This voltage is injected, along with a negative direct current offset signal (DC offset -), in a four-channel operational amplifier, which is used as a differential amplifier. The negative DC offset signal is generated by the microcontroller digital to analog converter (DAC) by order of the processor, to fix the input signal. A sensing system, identical to a potentiostat, is implemented using the remaining channels of the operational amplifier.

In this way, the current generated by the electrochemical cell is measured with a current sense shunt resistor connected to the working electrode. This signal is positively offset, through a positive direct current offset (DC offset +) and amplified by programmable gain amplifiers, controlled by the microcontroller. The resulting analog output signal is converted to digital by the analog to digital converter (ADC) available in the microcontroller, for data recording.

Finally, the ARM microcontroller receives digital data from the ADC and processes the information received based on the guidelines stored in its internal memory.

The collected current peaks are extracted and plotted to characterize the response of the developed device to different concentrations of cortisol.

The developed electronic circuit allows an improvement in the resolution of the output signal, in addition to allowing the device to be of smaller dimensions.

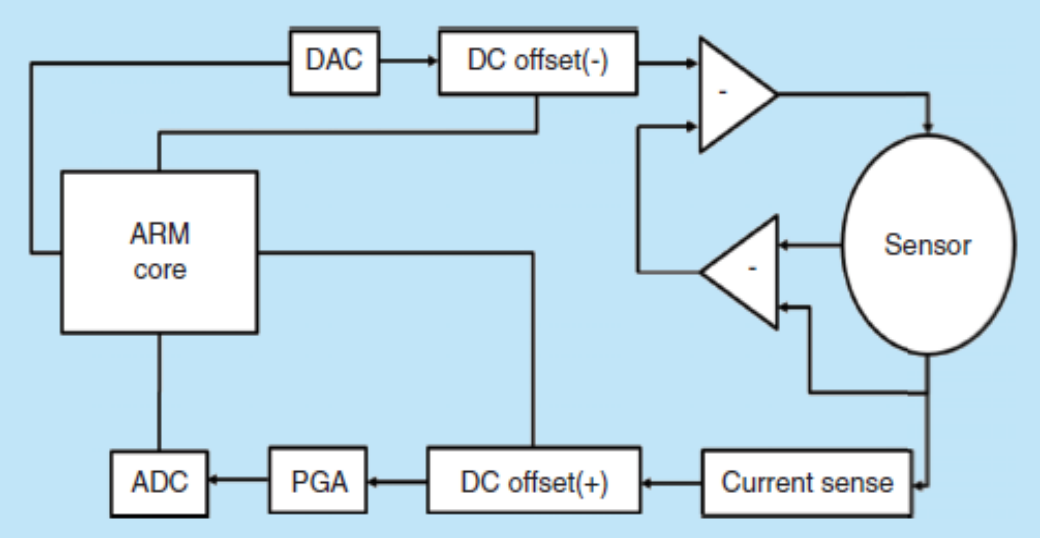


Figure 3.17 - Block diagram of the interface circuit developed to operate with CortiWacth sensor [5].

Recently, an electronic system that includes a 3,7 V lithium battery and a printed circuit board (PCB) was developed, to operate with a cortisol amperometric detection sensor.

Whenever the device user sends a command, via bluetooth, to start the measurement, the digital to analog converter (DAC) of the microcontroller (MCU) are activated to generate two input voltages, a reference voltage V_{ref} and a working voltage V_w. The V_{ref} voltage passes through a low-pass filter, where it is stabilized and is subsequently injected into the inverting terminal of the operational amplifier to set the potential of the reference electrode. This voltage polarizes the WEs in relation to the RE, maintaining a potential difference between the working electrodes and the reference electrode, which allows the current generation in the electrochemical cell. The generated current flows through each working electrode and is injected into

the transimpedance amplifiers, along with the voltage V_w, where it is amplified and converted to voltage.

The three analog to digital converters (ADCs) integrated in the MCU convert the output voltages of the amplifiers into digital data that are sent, by the Bluetooth module, to the user's device for later construction of the potential calibration plot as a function of the cortisol concentration.

This circuit enables rapid detections of less than a minute, low energy consumption of 13,3 mA x second, during a measurement, from a 3,7 V 150 mAh battery, and the creation of a small device [15].

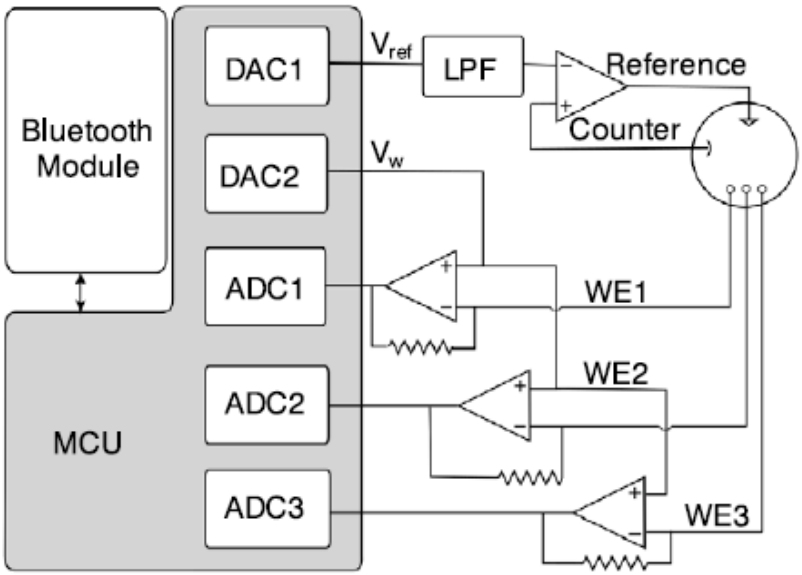


Figure 3.18 - Block diagram of the circuit presented in [15], including the microcontroller (MCU) unit, the Bluetooth module, and the low-pass filter (LPF).

For a better comparison of the interface circuits presented previously and their advantages and disadvantages, Table 3.1 is constructed.

Table 3.1 - Comparative table of interface circuits described previously.

Interface circuits	Advantages	Disadvantages
[64]	Simple approach, requires few components, low power consumption of 66 µW for a 2.5 V power supply.	----
[46]	Low energy consumption, good linearity and stability, accuracy of 0.1%, small dimension and low noise results.	Complex approach, requires the use of many components.
[49]	Allows to minimize the circuit noise and improve the signal-to-noise ratio (SNR), presents low power consumption, a linearity of 0,9928, a sensitivity of 8,45 Hz/nA and dynamic range of 73,2 dB.	Complex approach, composed of many components.
[59]	Simple approach, with small dimensions and get good results.	Uses many active components.
[5]	Best resolution output signal, small devicesl, microcontroller implementation.	----
[15]	Microcontroller and Bluetooth Module implementation, rapid detections of less than a minute, low energy consumption of 13,3 mA x second, and possibility of creating a small device.	----

3.3 - Final Remarks

In order to improve the effectiveness of healthcare, essentially through the diagnosis and prevention of serious pathologies, particular importance has been given to the development of point-of-care devices to monitor cortisol in daily life.

In this chapter some developed cortisol sensors were presented, some that use analytical detection techniques and others that use electrochemical techniques. According to some recent studies, it is concluded that electrochemical sensors are considered a promising alternative and have several advantages over analyte sensors, such as the fact that they involve rapid preparation and measurement processes, offer real-time, portable, continuous and high sensitivity monitoring that allow to obtain more accurate results.

In the final phase of this chapter, several interface circuits developed for electrochemical sensors were exposed and the functioning, advantages, and disadvantages of each one of them were described.

This chapter will serve as a basis for the development of the proposed system for solving the problem.

Chapter 4

System Architecture

The main goal of this dissertation was the development of a low-power, cortisol monitor to be integrated in a wearable system, capable of detecting abnormal cortisol levels and prevent sudden and unexpected death in epilepsy.

As it is an engineering project, it presupposes a structuring and design phase in order to ensure that all foreseen objectives are achieved.

The first phase of a project involves collecting and enumerating the requirements associated with it, which the proposed solution will invariably have to fulfill.

In this way, a list of requirements that the system to be developed must meet was created.

In this case, it is considered that the targeted system should accomplish the following requirements:

- Be microcontroller-based;
- Have small dimensions;
- Be low-power;
- Have the ability to acquire, continuously and in real-time, cortisol data;
- Communicate this data via Bluetooth to a mobile application;
- Have a mobile app capable of evaluating received cortisol data and deciding whether an alarm should be issued to emergency contacts due to abnormal values that could lead to sudden death in epilepsy.

Taking into account that this is a system to be used by people suffering from epilepsy, the system needs to be wearable and fixed to the body to prevent its detachment during epileptic seizures; small; easy to use; intuitive; quick acting, since seizures can occur spontaneously.

At this stage, as the system to be developed will only be a prototype, some of these requirements cannot be met.

After analyzing the developed state of the art and as a result of an in-depth study of all the variables involved and bearing in mind all the work previously done, the various steps and tools necessary for carrying out the dissertation work were determined. Having in mind the overview on existing systems presented previously, this system could look like a watch or be attached to the abdomen or torax with a specific belt.

In order to develop a portable and wearable monitoring device like the one intended to be developed in this dissertation, there are four essential components: the sensor, which is the main element to detect the analyte; the electronic circuit that operates with the sensor, which

allows the acquisition of data, the microcontroller, which processes and transmits the acquired data via BLuetooth Low Energy, and the mobile application, which receives the data, checks if the values are within the normal range and decides when an alert message should be sent to emergency contacts.

In the course of this chapter, you will find the proposal for the development of the dissertation project, as well as the presentation of the final prototype, the technical exposure of the choices made and the application of all the components used in the system, materializing in the solution adopted for the proposed problem.

4.1 - Proposal

In this sense and after an analysis of the state of the art, presented in chapter 3, and the study of cortisol and the importance of its monitoring for the different pathologies, a knowledge base was created that served to support the directional decision of the project.

Section 3.1 provides knowledge of commercially available cortisol sensors and commonly used detection techniques.

Considering the advantages that electrochemical sensors have over analytical sensors, it is proposed to use an electrochemical sensor with screen-printed carbon electrodes from Metrohm DropSens [63] as the main cortisol sensor.

At this stage, the developed system is just a prototype. For this reason, the chosen sensor is not a sensor with adequate characteristics to be used as a wearable, nor is it a comfortable one to use. For a more advanced stage, the ideal sensor would be one that is permanently connected to the human body as in a clock, or based on microneedles such as those presented in [66]–[68].

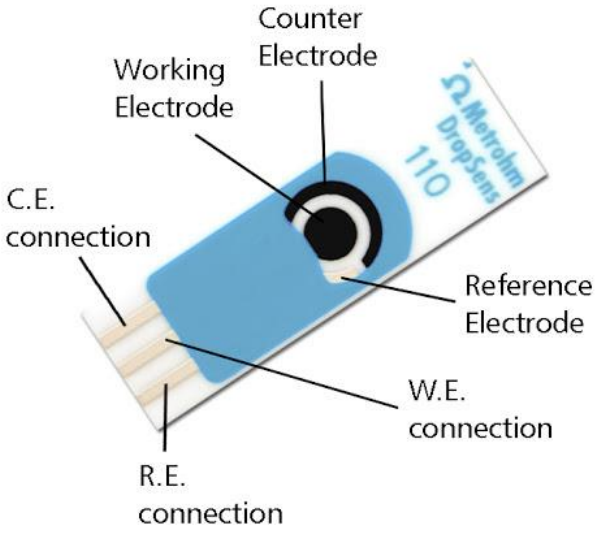


Figure 4.1 - Screen-printed and interdigitated three-electrode sensor from Metrohm DropSens chosen [69].

Figure 4.1 shows the Metrohm DropSens electrochemical sensor [69] chosen to monitor cortisol from the sweat sample.

It is important that this sensor is optimized for better sensitivity and that it is functionalized with cortisol antibodies to be able to detect cortisol in sweat.

These sensors allow the use of a wide range of electrode materials and combinations, which can be carbon, platinum, gold, palladium, modified, unmodified or customized according to the interests and needs of customers [69].

They offer several advantages over the other sensors studied, since they present small dimensions, essential for a wearable sensor, are low cost, robust and easy to use, require low maintenance, allow many experiments with low sample volumes and fast and reliable results [69][70].

In terms of the electrochemical technique to be used to characterize the redox reactions that occur in the working electrode and to measure cortisol concentrations, it is proposed to implement the Cyclic Voltammetry, as it is a powerful, widely used, simple, fast, accurate and sensitive technique which measures the current generated by the sensor when the working electrode potential is increased linearly over time [62][71] and Square Wave Voltammetry, as it is the most commonly used technique to measure this type of compound. In this way it will also be possible to characterize the sensor response using each one of these techniques.

The result obtained with these techniques is a graph of the current generated as a function of the applied potential, which allows establishing a proportional relationship between the current and the cortisol concentration [62].

Concerning the electronic circuit that operates with the sensor, it is intended that the circuit performs the acquisition, processing and continuous transmission, via Bluetooth, of the cortisol levels to a smatphone application.

Taking this into account and analyzing section 3.2 and Table 3.1, it is possible to conclude that the circuit that best serves our objective is the circuit developed by Torrente-Rodríguez et al. [15], shown in Figure 3.18.

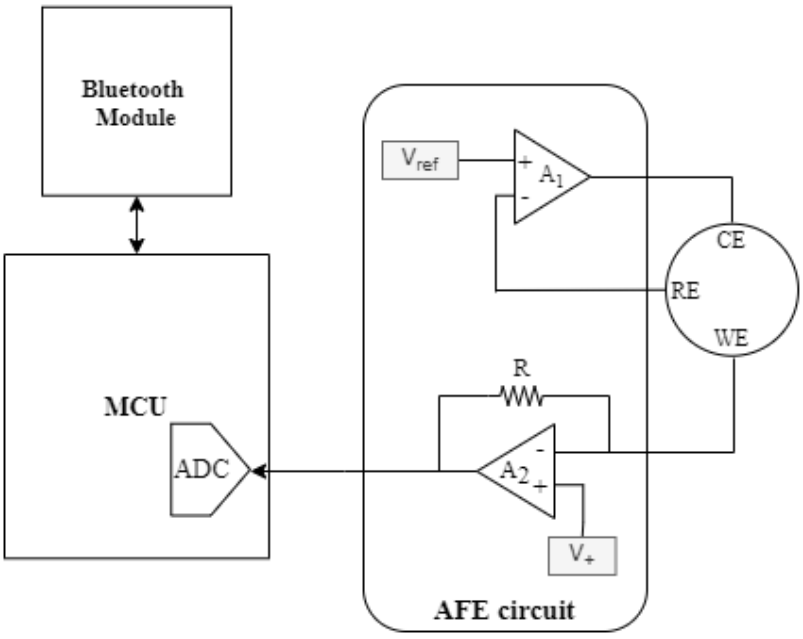


Figure 4.2 - Block diagram foreseen for the electronic interface circuit of the device composed of an Analog Front-End circuit (AFE), a microcontroller (MCU) and a Bluetooth module.

The electronic circuit must be composed of an Analog Front-End (AFE) circuit, a microcontroller (MCU) and a bluetooth module for wireless data communication.

The AFE circuit includes an operational amplifier A_1 that assumes the function of potentiostat, allowing the input of a reference voltage (V_{ref}) that controls the potential difference between RE and WE and generates a current in the sensor by cyclic voltammetry, and a

transimpedance amplifier (TIA) A_2, which converts the current generated by the sensor into a linearly proportional voltage.

This voltage is then applied to the analog to digital converter (ADC) of the microcontroller so that the analog signal is converted into digital data.

Still in the microcontroller (MCU) based system, which can be, e. g., Arduino or Bitalino based, the acquired data is analyzed and processed based on the guidelines stored in its internal memory and transmitted to an application on the user's smartphone, via the integrated Bluetooth Module.

4.2 - Developed System

To implement this system and achieve the project's objective and requirements, a hardware selection was made in order to fulfill a set of prerequisites which includes ease of development, data acquisition and processing, the use of I2C and Bluetooth Low Energy (BLE) communication to send data to an android application, low power consumption, as well as device design and cost requirements.

In an overview, the final developed system comprises four main components that interact with each other to fulfill the required operation: the sensor, used to detect the analyte; the electronic circuit that operates with the sensor, which allows data acquisition; the Arduino microcontroller, which processes and transmits the acquired data via a Bluetooth Low Energy link, and the mobile application, which receives the data and checks if the values are within the normal range and decides whether an alert message should be sent.

As shown in the flowchart in Figure 4.3, the measurement process starts in Arduino, with the configuration of the LMP91000 Potentiostat through I2C communication (step 1). This configuration is necessary to define not only the internal characteristics of the integrated, but also to program the electrochemical technique to be implemented. The connection between the Arduino and the Potentiostat is made as shown in the schematic shown in the first figure in Appendix A.

Thus, the potentiostat was programmed to make measurements according to the cyclic voltammetry technique through the application, to the sensor electrodes, of input voltages ranging between - 0,2V and 0,6 V in steps of 2 mV.

As a result of the reduction and oxidation of the compound caused by the applied voltages, the sensor returns the current generated for each one of biasing reference voltages.

Again in the potentiostat, this converts the currents generated into output voltages that are read by the Arduino through the analog pin.

In Arduino, incoming voltages are converted again into currents that are analyzed, checking to which range of currents, previously defined for each concentration, they belong.

A histogram with the distribution of currents by concentration classes is made and the concentration value with the highest frequency of observations is returned. This value is sent via the BLE link, after connection with the application and is received and shown in the application's main menu.

The algorithm developed in the application allows to decide if the received value is higher or lower than the stipulated limit. If higher, an alert message is sent, together with the local GPS coordinates, to the previously defined contacts.

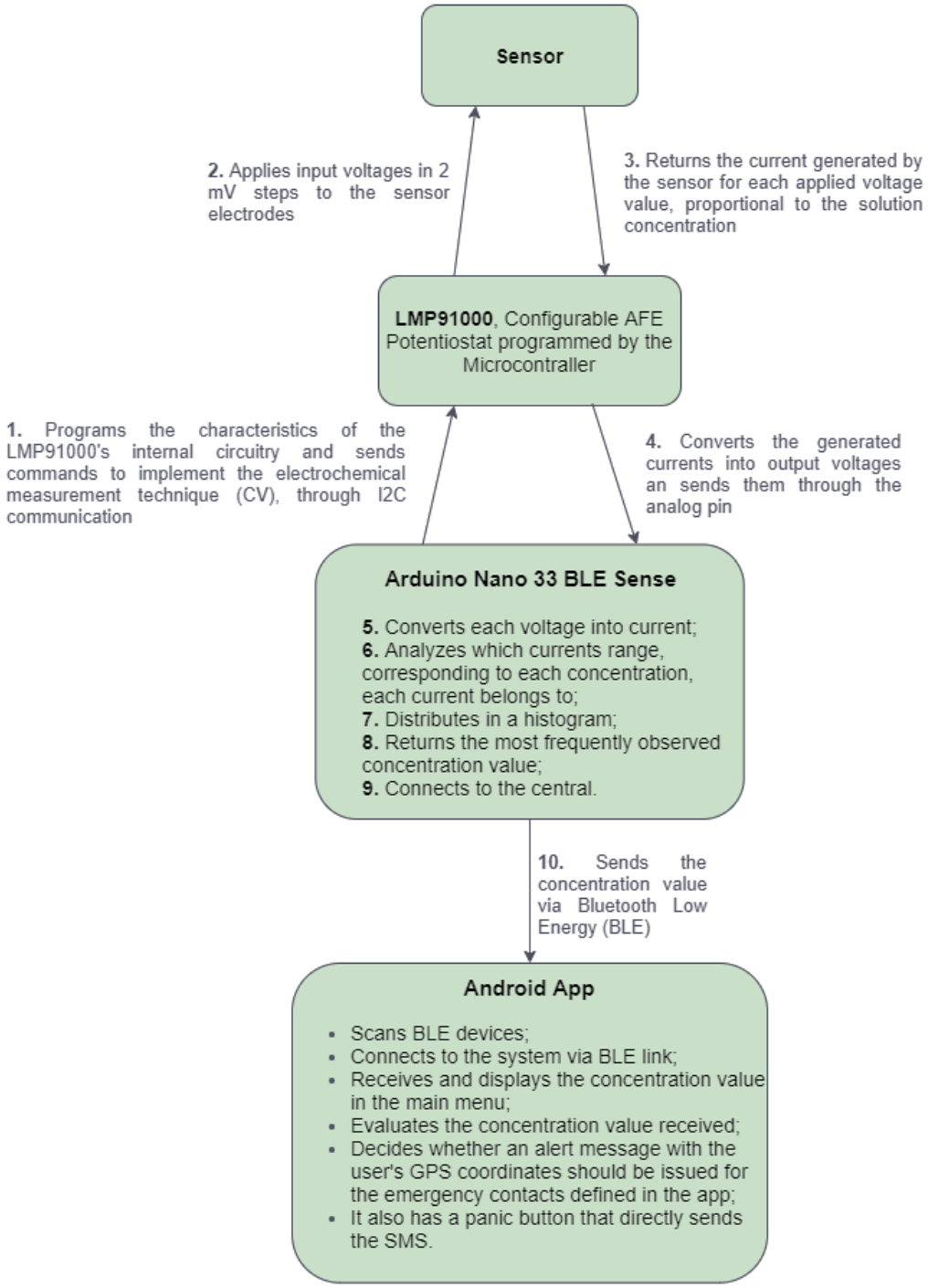


Figure 4.3 - Flowchart of the developed system functioning.

A final list of the considered and chosen devices is presented in Table 4.1. More details of each one of the components is presented in the following sections.

Table 4.1 – Final list of chosen components.

Components	Manufacturer	Ref.
Cortisol Sensor	Metrohm Dropsens	[69]
LMP91000 Sensor AFE System	Texas Instruments	[72]
Potentiostat SPE adapter	IORodeo	[73]
Arduino Nano 33 BLE Sense	Arduino	[74]

4.2.1 - Cortisol Sensor

In order to detect and measure cortisol levels, a commercial electrochemical sensor from Metrohm DropSens, made with screen-printed and interdigitated electrodes (SPEs) was chosen, with the help of colleagues from the chemistry department.

These disposable devices, normally used for electrochemical analysis in the environmental, clinical or agri-food areas, allow the use of a wide range of electrode materials and patterns, ranging from carbon, gold, platinum, palladium, and can also be personalized to the customer's interests and specifications [69].

They have several advantages over other sensors on the market such as the fact that they are low cost, easy to use, have high electrochemical activity, fast and reliable results, are suitable for working with sample microvolumes and ideal for developing specific (bio)sensors , require low maintenance and have small dimensions (3,4 x 1,0 x 0,05 cm) [70].

The chosen electrochemical cell, presented in Figure 4.4, consists of the working electrode (WE) printed with carbon in which the oxidation-reduction reaction occurs, resulting in the electrical current of interest; the counter electrode (CE) also printed with carbon; and the reference electrode printed with silver (Ag/AgCl), necessary to control the potential applied to the WE. The external interface electrical contacts are also made of silver [75].

Figure 4.4 - Screen-printed electrodes sensor from Metrohm Dropsens [69].

As it was not possible to functionalize the sensor with cortisol, all tests were performed with a solution made with the oxidation reduction pair potassium hexacyanoferrate (II) trihydrate and potassium hexacyanoferrate (III).

4.2.2 - LMP91000 Sensor AFE System: Configurable AFE Potentiostat

The LMP91000 is a programmable analog front-end (AFE) system, suitable for use with portable and wearable devices, that provides a signal path between the sensor and a microcontroller to identify, detect and measure electrochemically, chemical species.

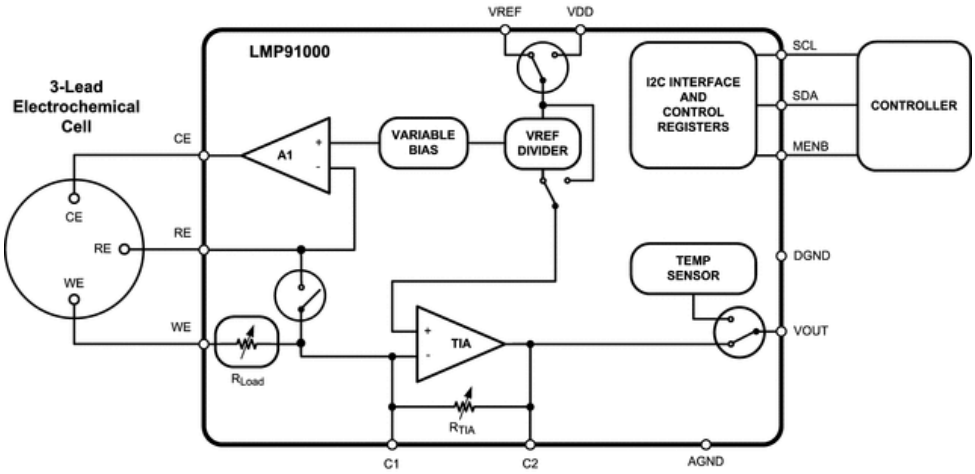


Figure 4.5 - Functional block diagram of the LMP91000 integrated circuit.

Allows a conversion of current ranges from 5 µA to 750 µA full scale and operates over a voltage range of 2,7 to 5,25 V. It is optimized for micro-power applications, so it has a total power consumption below than 10 µA at 3,3 V.

The LMP91000 IC has 6 operational modes: deep sleep, 2-lead ground referred galvanic cell, standby, 3-lead amperometric cell, temperature measurement with TIA OFF and temperature measurement with TIA ON. These modes allow to optimize the current consumption and it is possible to select them through the I2C bus, according to the user's wishes.

The programmability of the LMP91000 allows the connection with different electrochemical sensors, 3-Lead or 2-Lead Electrochemical Cell, adjust the cell polarization to 14 percentages of the source reference, program the internal voltage divider, select the gain of the transimpedance amplifier (TIA) in a set of 7 internal gain resistor values, the Rload value, the internal zero, the reference source type and the operating mode [76]. It can be programmed to perform different types of electrochemical techniques such as amperometry, chronoamperometry, cyclic voltammetry, normal wave and square wave voltammetry.

The potentiostat is configured through the microcontroller that sends specific commands to stimulate and control the analyte reaction that occurs on the WE surface. The microcontroller is connected to the LMP through the I2C interface, as shown in Figure 4.6.

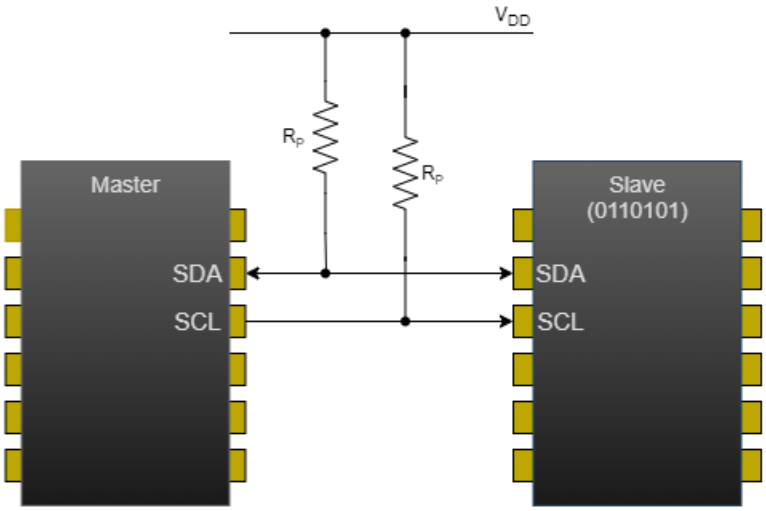


Figure 4.6 – I2C communication scheme.

Referring to Figure 4.5, amplifier A1 is the control amplifier that supplies the initial charge and bias voltage to the sensor. The Variable Bias Block is used to provide a user-configured bias potential at the positive and negative terminals of A1 to keep constant the potential between the reference and working terminals. This bias voltage can be programmed from 1% to 24% of the reference voltage.

Provided through an internal voltage divider, the internal zero is the voltage at the non-inverting pin of the TIA, which allows the counter electrode to oscillate in cases of sudden changes in the analyte concentration and can be programmed to 20%, 50% and 67% of reference voltage.

The conversion of the current flowing from CE to WE into a proportional voltage is done by the transimpedance amplifier (TIA), whose gain is dependent on a feedback resistor $R_{TIA,}$ according to equation 4.1 for 3-Lead Amperometric Cell and 2-Lead Galvanic Cell and 4.2 for 2-Lead Galvanic Cell In Ground Referred [76].

$$Gain = R_{TIA} \tag{4.1}$$

$$Gain = 1 + \frac{R_{TIA}}{R_{LOAD}} \tag{4.2}$$

This proportional voltage is connected to the V_{OUT} pin, which can be switched to output the temperature.

The calculation of the current flowing in the WE of the three electrode system (I_{WE}) is done using the following equation:

$$I_{WE} = \frac{V_{OUT} - V_{INTZ}}{R_{TIA}} \tag{4.3}$$

,with $V_{INTZ} = (20, 50\ or\ 67)\% \times V_{REF}$.

4.2.2.1 - I2C Comunication

One of the biggest advantages of this device is the I2C (Inter-Integrated Circuit) communication with the microcontroller.

The I2C communication bus is a serial interface with two bidirectional lines, Serial Data (SDA) and Serial Clock (SCL), that combines the Serial Peripheral Interface (SPI) and the Universal Asynchronous Receiver-Transmitter (UART) protocols and enables the use of multiple slaves, for a single master [77].

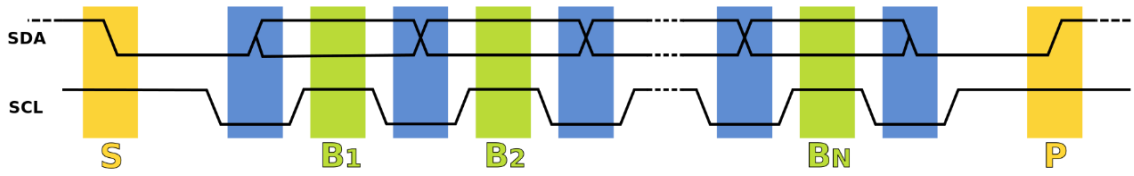


Figure 4.7 - Type communication waveforms [77].

SDA is a pin used to send or receive addresses and data. Because it is an open terminal, pull-up resistors for VCC are needed on the SDA pins to pull them high when they are not being driven low. By default, SDA changes can only occur when the SCL level is low. The only exceptions are to indicate Start and Stop conditions.

The function of the SCL line is to synchronize the data transfer to and from the device.The SCL pins also require pull-up resistors for V_{CC} [77].

To facilitate identification and communication by the master, each device that enables I2C communication has a unique 7-bit address, where the first four bits are fixed and according to device category, and the remaining three bits are programmable [77].

In our case, the LMP91000 7 bit bus fixed address is 1001 000 .

To start the I2C communication, the master generates a start condition, driving the SDA line level from high to low while keeping the SCL line high and all devices on the bus are prepared to receive incoming data. The initial condition is followed by sending a 7-bit slave address and a Read/Wwrite bit from the master to all devices, that compare the sent address with yours.

If the addresses match, the LMP91000 generates an ACK signal which is detected by the master and starts transmitting or receiving data. If the address does not match, the LMP91000 generates a NACK signal.

To transmit data from the master to the slave, the master places the first bit on the SDA line and generates a clock pulse to transmit this bit to the slave. To receive data from the slave to the master, the master releases the SDA line, allowing the slave to use it to transmit data, and generates, for each bit, a clock pulse on the SCL, reading the data.

At the end, the master changes the SDA line from low to high while keeping the SCL clock line high, generating a stop condition [76].

4.2.3 - IORodeo Potentiostat screen-printed electrode (SPE) adapter

The screen-printed electrodes adapter from IORodeo allows connecting the three electrodes (WE, RE and CE) of the sensor to a potentiostat.

This connector consists of a PCB with 3-position card readers with 0.100" spacing and R/W/C configuration, for mounting different types of carbon electrodes. They are suitable for using with screen-printed electrodes from Metrohm DropSens (Figure 4.8) and from Pine Instrumentation Inc and with Zensor screen-printed cards from CH Instruments. All versions include a ready-to-use pre-assembled PCB with a 12" long connection cable [73].

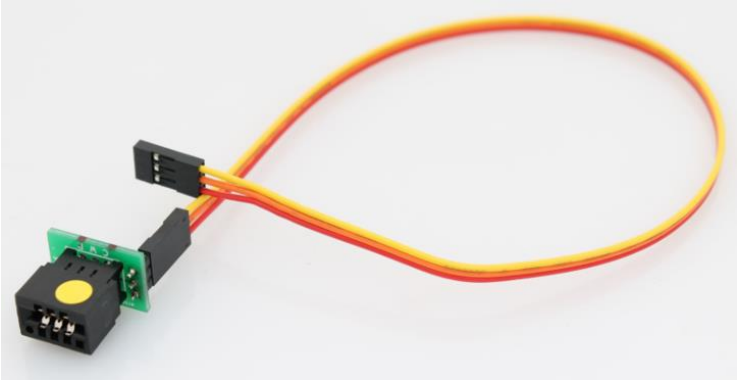


Figure 4.8 – Potentiostat adapter for SPEs from Metrohm DropSens [73].

4.2.4 - Arduino Nano 33 BLE Sense

The Arduino family systems are one of the most used fast prototyping boards worldwide, which includes a microcontroller for processing the data received by the sensors. These systems provide a programmable physical circuit board and an open source software tootlkit to program it. Sensors and/or other accessories, such as Ethernet or Bluetooth modules can be connected to this board.

The preference for a control solution for this project fell on the Arduino Nano 33 BLE Sense, presented in Figure 4.9, defining the parameters that dictated its choice by:

- **Size -** Size is a very important factor to consider. With 45x18 mm area and 5 g weigth, it presents itself as a very light solution and with an adequate size for this project;
- **Built-in Bluetooth Communication -** It was important to choose, among the options, one that had great compatibility. This device already includes a Bluetooth module that can be both a BLE and Bluetooth® client and host device. This is a significant advantage because it is not necessary to use a Bluetooth module, as it is already built-in. In terms of size it is also advantageous as the purchase of a module is avoided;
- **I2C communication -** Required to communicate with the LMP91000;

- **Better technical specifications -** It has 1 MB of program memory, 32 times bigger than Arduino Uno, which allows the creation of bigger and more variable programs due to 256 kB of SRAM, 128 times bigger than Arduino Uno.

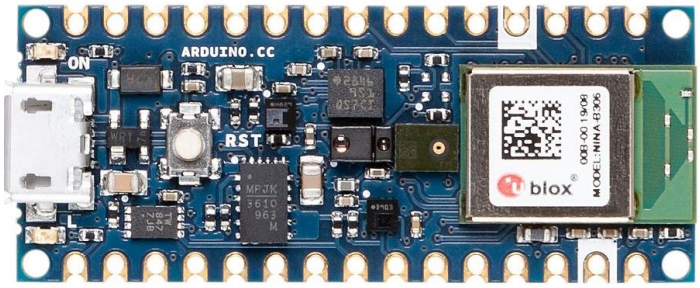


Figure 4.9 – Arduino Nano 33 BLE Sense Board [74].

The Arduino Nano 33 BLE Sense is an evolution of the traditional Arduino Nano, which features a much more powerful processor, the nRF52840 from Nordic Semiconductors, a 32-bit ARM® Cortex™ -M4 CPU running at 64 MHz and operate at 3,3 V. Includes other features like Bluetooth® pairing via NFC, the impressive selection of sensors and ultra low-power consumption modes.

The Nano 33 BLE Sense is a pin-equivalent substitute of the Arduino Nano, Figure 4.10, but the main differences to the classic Nano, besides the processor, are: the micro-USB connector and the five sensors included: LSM9DS1, IMU; MP34DT05, Microphone; APDS9960, Gesture, light, proximity; LPS22HB, Barometric pressure; HTS221, Temperature and humidity [74].

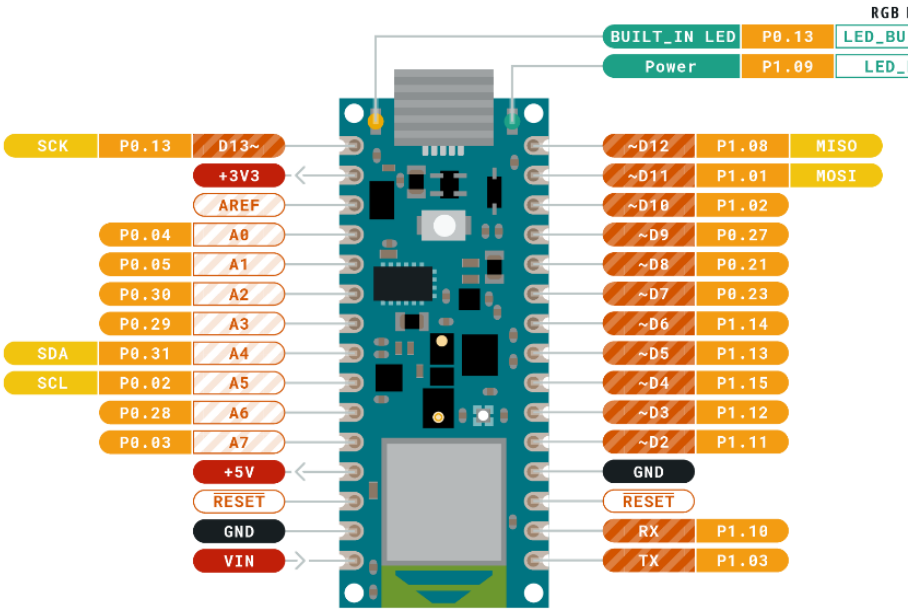


Figure 4.10 – Arduino Nano 33 BLE Senso pin-out [74].

4.2.4.1 - Arduino IDE

The programming toolkit that works with Arduino is called Arduino IDE. Arduino IDE is an open source and no cost associated software programmed in Java, which makes it easy to write code and transfer that code to the Arduino board.

Arduino IDE's programming language is C/C++ and includes many libraries that enable extremely useful functions that speed up the programming process.

Arduino programs always have the same structure. The code is divided into three parts:

- Library and variable declaration, section where variables are declared and assigned;
- Setup (), executes all the commands inside once, whenever the board is turned on or reset;
- Loop (), function that executes indefinitely all the commands inside it.

In the project, the Arduino was used to communicate through the I2C bus with the LMP91000, in order to receive and process the data coming from the sensor, and to send these data to the mobile application, via BLE. The version 1.8.15 of the Arduino IDE software and some libraries that allow executing the functions mentioned above was used in this project.

The chosen and included libraries are shown in Table 4.2.

Table 4.2 – List of libraries used in the project.

Library	Functions	Ref.
ArduinoBLE.h	Supports all Arduino boards that have the proper hardware for BLE, Bluetooth 4.0 and above;	[78]
LMP91000.h	Library used to program the LMP91000 configurable potentiostat for low power chemical detection applications;	[79]
Wire.h	Allows to establish communication with I2C devices, connecting the SDA (data line) and SCL (clock line) of the devices.	[80]

Of the 3 implemented libraries, two of them are general, ArduinoBLE.h and Wire.h, with the exception of the library responsible for programming LMP91000, LMP91000.h.

This last library serves to configure the internal characteristics of the LMP91000 configurable potentiostat and enable it to implement low power chemical detection. It is commonly used to perform electrochemical techniques such as cyclic voltammetry, chronoamperometry, square wave voltammetry, pulse voltammetry, etc [79].

This library contains specific functions used to set the transimpedance amplifier gain ("setGain"), the load resistance to compensate for voltage differences ("setRLoad"), the source for the bias voltage, the reference voltage for the transimpedance amplifier ("setIntZ"), the positive or negative bias voltage, the operating modes for the LMP91000 and even to read the output of the LMP9100 [81]. In this work, these functions are called and defined through the initLMP() command, which is called in the void setup presented in Figure 4.11.

Whenever the developed Arduino program is started and run, firstly, some hardware initialization and control routines are executed.

This developed firmware was implemented in void setup() and is shown in Figure 4.11.

This setup starts with the initialization of the Wire library to establish the I2C bus, through the function " Wire.begin() " and with the serial data transmission initialization at the baud rate of 115200 bps, through the function " Serial.begin(115200) ".

After calling these two functions, the LMP91000 IC is initialized by programming its internal characteristics via the initLMP command, explained above.

Lastly, if the BLE device is not initialized, " if (!BLE.begin()) ", a failure warning message is returned. Otherwise, the local name and service UUID are defined and the "cortisolLevel" BLECharateristic and the BLEService are added.

An initial value is defined for the added characteristic, which is automatically modified when it changes, the device starts to be advertised ("BLE.advertise()") and waits for connections.

```
void setup()
{
  Wire.begin();
  Serial.begin(115200);

//enable the potentiostat
  delay(50);
  lmp.standby();
  delay(50);
  initLMP();
  delay(2000); //warm-up time for the sensor

//initiliaze BLE
 if (!BLE.begin())
  {
    Serial.println("starting BLE failed!");
    while (1);
    }
  BLE.setLocalName("WCMM");
  BLE.setAdvertisedService(WCMMService); // add the service UUID
  WCMMService.addCharacteristic(cortisolLevel);
  BLE.addService(WCMMService);
  cortisolLevel.writeValue(oldConcentration); // set initial value for this characteristic
  BLE.advertise();

  Serial.println ("Bluetooth device active, waiting for connections...");
}
```

Figure 4.11 - Firmware developed for this project and implemented in void setup ().

4.2.4.2 - BLE

As is well known, Bluetooth is a widely used short-range wireless data communication protocol between devices and because of its reliability, Bluetooth has expanded and different standards have emerged over the past few years.

Bluetooth technology supports, since Bluetooth 4.0, two radio versions: Bluetooth Low Energy (BLE) and Bluetooth Classic [82].

In this work, the Bluetooth Low Energy version was chosen, as it is the easiest way to implement communication between small devices and because the BLE was designed for operations with low energy.

Instead of standard Bluetooth communication, based on Universal asynchronous receiver/transmitter (UART) connection, in Bluetooth LE there are two types of functions: central and peripheral.

One of the most important things is to define who is the central device and who is the peripheral device.

As a rule, the smallest and lowest power devices are set in peripheral mode and they are responsible for sending data to the central devices.

The information presented by a peripheral device is organized into services and each service is divided into characteristics. So, when there are updates, the peripheral device just needs to update each service characteristic and the central devices just need to connect to the

peripheral and read the boxes they want. It should be noted that several central devices can read services and obtain data from a peripheral device [83].

In our case, the Arduino based system are defined as peripheral device and the smartphone is defined as central device that reads data coming from Arduino.

4.2.4.3 - Measurement and data processing processes

For the purpose of validating the system without the mobile application, two codes were implemented in Arduino for data acquisition, one referring to the Cyclic Voltammetry technique and the other referring to the Square Wave Voltammetry technique.

In both, the internal characteristics of the potentiostat were defined and the electrochemical techniques were programmed according to their specificities, based on the functions available in the LMP91000.h library.

Tests were carried out with five different concentrations of the solution with the oxidation--reduction pair potassium hexacyanoferrate (II) trihydrate and potassium hexacyanoferrate (III) potassium hexacyanoferrate and the obtained currents were collected for the subsequent construction of the graphs, presented in the next chapter.

The developed algorithm for the final system measurement process is slightly different from the previous two. The measurement is made by Cyclic Voltammetry for the different concentrations, however current ranges were established for each concentration through measurements made previously.

Each current value obtained for each applied voltage value is compared with the defined current ranges and if it belongs to this range it counts as a frequency for the concentration class in question. The classes are the different concentrations, 1mM, 2.5 mM, 5 mM, 7.5 mM and 10 mM.

At the end of the measurements, which correspond to 802 final current values, a histogram is constructed with the frequencies of observations for each one of the classes and the column with the highest frequency of observations corresponds to the concentration of the solution.

This returned concentration value is sent by Bluetooth Low Energy to the mobile application.

For a better understanding and illustration of this implemented algorithm, the corresponding flowchart is presented in Figure 4.12.

The three codes described here are available on the GitHub link (CV, SWV, and CV_BLE folders) in Appendix B.

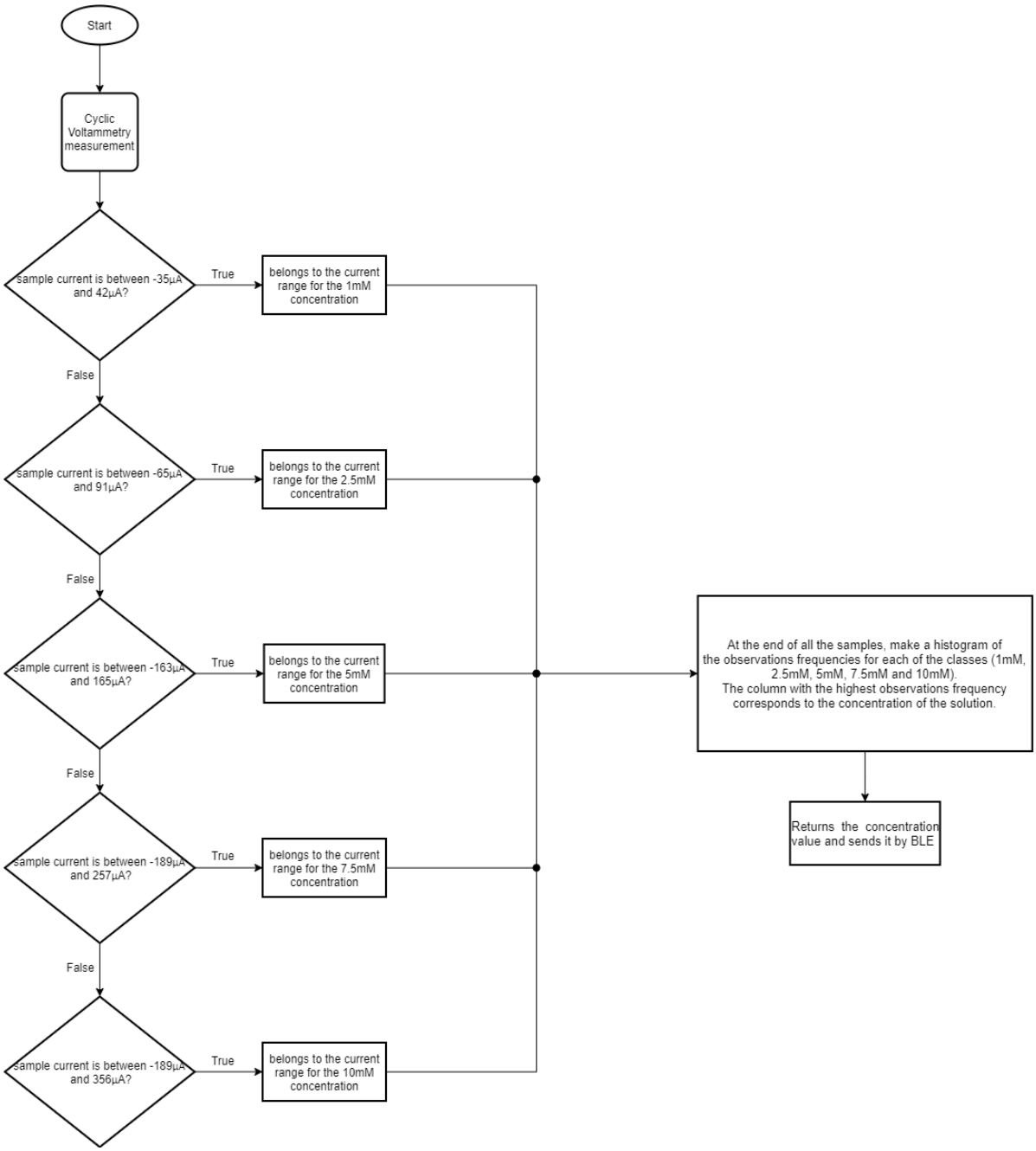


Figure 4.12 – Implemented algorithm flowchart for validation of the final and complete system.

4.3 - PCB Design

Since the LMP91000 has no protruding pins, as shown in the Figure 4.13, and does not allow breadboard implementations, it was necessary to design a printed circuit board that would include the LMP and allow connection to it.

Figure 4.13 - LMP91000 Package 14-Pin WSON [84].

To carry out this task, it was selected, from among several available options, a program for developing the printed circuit board (PCB). The program chosen to perform this procedure was Autodesk EAGLE, version 9.6.2. EAGLE is an electronic design automation (EDA) software that allows the design of printed circuit boards (PCBs). It offers libraries with vast content and several tools that speed up this process.

The choice of Autodesk EAGLE was supported due to:

- Being open source and without any associated cost;
- Being the most used program, with several tutorials that help with its use;
- The availability of numerous implemented and tested libraries;
- Including debugging tools, useful in looking for development flaws.

Firstly, the schematic was done with the desired connections, according to the schematic shown in the LMP91000 datasheet.

The serial clock line (SCL) and the serial data bus (SDA) line of the I2C bus were connected to A5 and A4 pins, which correspond to Arduino's SCL and SDA, respectively and to the 3V3 pin of the Arduino with an external pull up resistor each.

The LMP pins identified as RE, WE and CE, are connected to a generic male pin header to connect to the SPE adapter cable that communicates with the sensor.

The output voltage available on the LMP91000's, V_{OUT} pin, was routed to the Arduino's analog input 0 (A0), where it was conditioned by an internal analog-to-digital converter (ADC) for interpretation. A 1st order RC filter with a cutoff frequency of 15,4 Hz was added between V_{OUT} and the Arduino A0 pin.

The enabled module (MENB) was connected to the ground (GND) Arduino pin to signal a communication ready status to the microcontroller. The DGND and AGND pins are also connected to the Arduino ground pin.

Secondly, the positioning of the components and the routing of the board was done, and finally, a PCB with the exact dimensions of the Arduino Nano 33 BLE Sense and with the designed circuit was obtained.

The exact schematic diagrams and the PCB layout are attached in Appendix A, but images of the final PCB can be seen in Figure 4.14 a) and b).

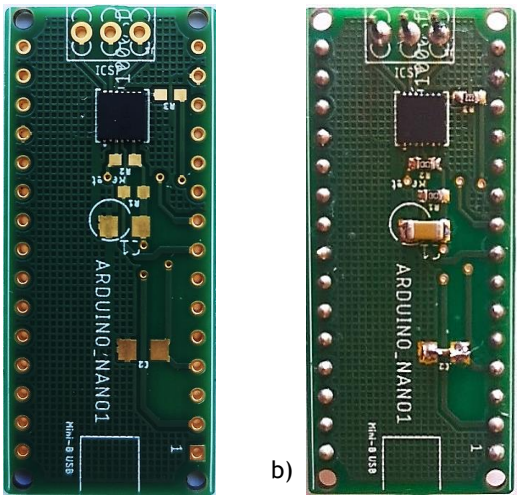


Figure 4.14 – Final PCB images. a) Before placing the components; b) After placing the components.

4.4 - Android Mobile App

The second main task of this project concerneds the development of a mobile application to operate within the Android mobile operating system.

The main requirements identified to be met are:

- Allow the connection to external devices, namely to the developed system, and receive the information collected by it;
- Checks whether the amounts received are higher than the amount stipulated as a limit.
- If the values are high, send an alert message with the user's GPS coordinates to the emergency contacts predefined by the user.

Before starting, a flowchart diagram was made, which can be seen in Figure 4.15, which guided and organized the application.

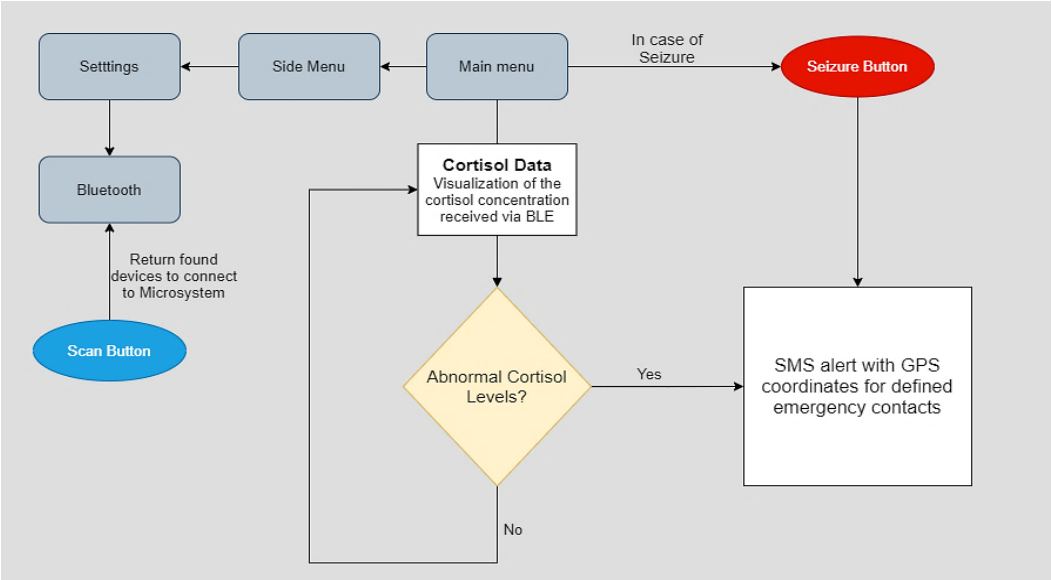


Figure 4.15 - Android application flowchart diagram.

For that, the first task of the development of the appication was to define the development program and structure of the mobile app, i.e., how many views the application should have and how the navigation between these views should be. The first thought when making the app was that it should be simple and intuitive.

The Microsoft's official code editor, Visual Studio Code, was used for application programming.

However, for Android application development, the Ionic Framework was the chosen framework. Ionic Framework is an open-source UI toolkit for developing high-quality hybrid mobile and desktop applications, using technologies such as CSS, HTML and JavaScript and integrations with popular frameworks such as Angular, React and Vue [85].

The reasons that motivated this choice were:

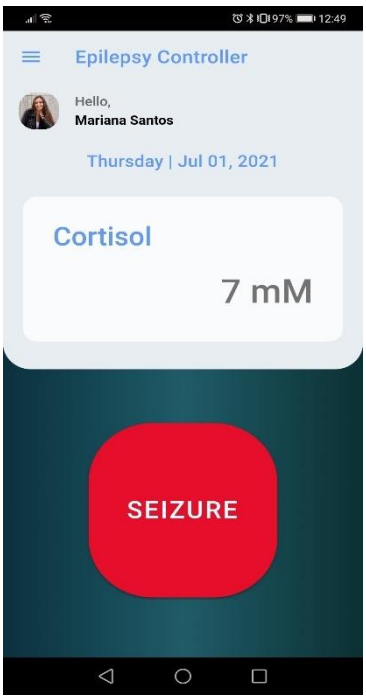
- Is an intuitive, simple, quick learning and free platform, suitable for those who do not have much experience in programming, as is the case;
- Provides a wide range of materials, libraries and UI components that enable fast and easy development and creative and interactive design;

- Is the world's leading platform for building cross-platform mobile applications with JavaScript;
- Ionic uses other structures, such as Angular, in order to integrate all the features and API's that Angular has;
- It has the ability to make different deploys to different devices and/or platforms, such as iOS, Android or Windows, with the same code, using Capacitor.

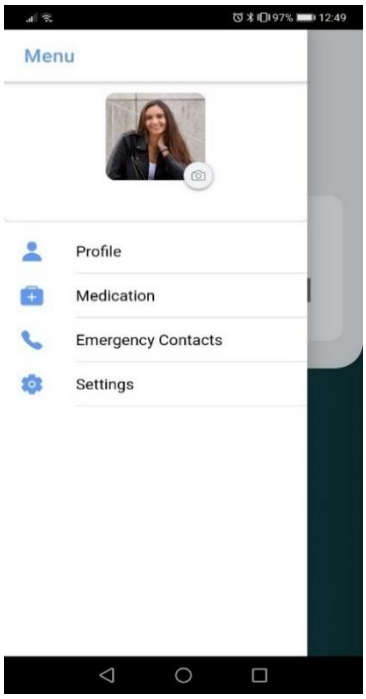
The UI structure chosen was Angular, one of the most famous and widely used frameworks and the cross-platform app runtime used was Capacitor, which includes plugins that allow access to operating system resources, such as camera, SMS, flashlight, GPS, etc.

Regarding the app structure, eight views were implemented, which include:

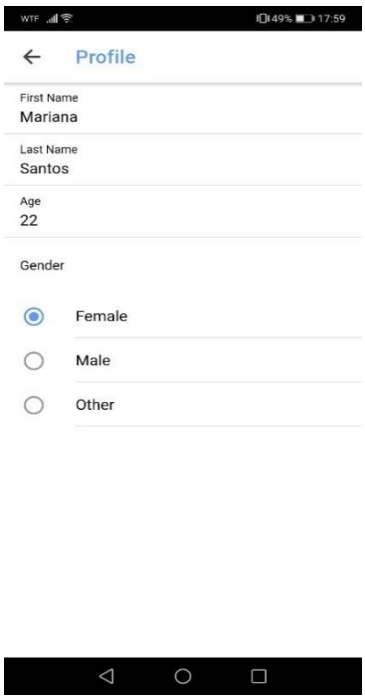
- the Main page, Figure 4.16 A, where the received concentration value are visualized, as well as a "Seizure" button, which allows the user issue the alert message whenever necessary;
- the SideMenu, Figure 4.16 B, which includes a series of other pages that allow different functionalities;
- the Profile page, Figure 4.16 C, where the user can enter their personal data;
- the page dedicated to Medication, Figure 4.16 D, in which the patient can customize the medication they take;
- the Emergency Contacts page, Figure 4.16 E, in which the emergency contacts to which alerts are sent with GPS coordinates are predefined;
- the Details BLE page, Figure 4.16 F, where the services and characteristics of the selected bluetooth device are presented;
- the Settings and Bluetooth page, Figure 4.16 G, where the available BLE devices can be scanned and the device to connect with can be selected.



A



B



C

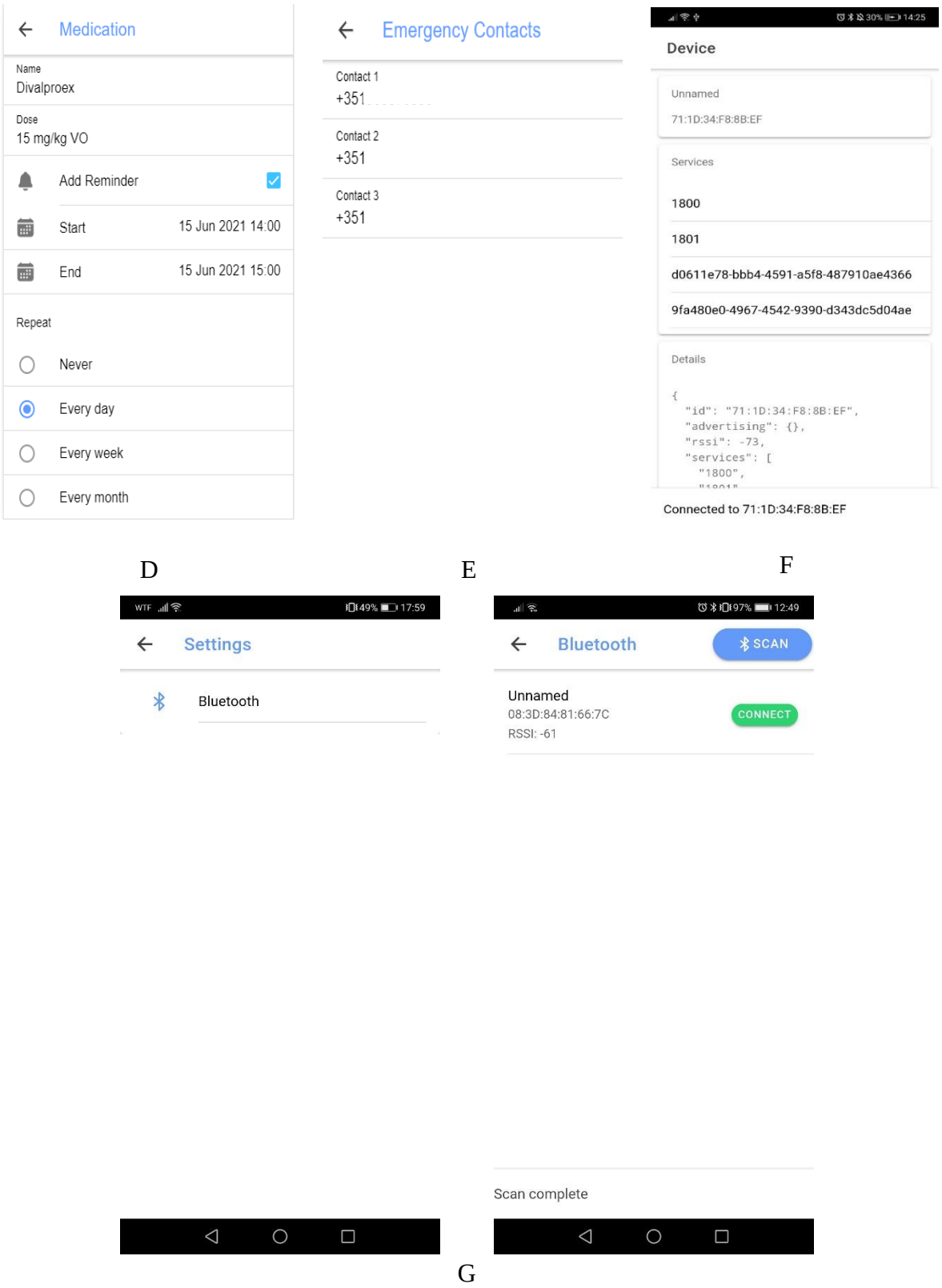


Figure 4.16 - Views implemented in the mobile application developed for Android operating system. A - Main Page Layout; B - Side Menu Layout; C - Profile Page Layout; D - Medication Page Layout; E - Emergency Contacts Page Layout; F - Details BLE Page Layout; G - Settings and Bluetooth Pages Layout.

To establish and program some necessary functions, such as the BLE wireless connection between the developed system and the Android application, it was necessary to use plugins that allow JavaScript to interface directly with the native APIs.

The main plugins installed that allowed to fulfill the established requirements were: the SMS, the BLE and the Geolocation plugin.

To access each plugin, it is necessary to initialize it through its installation, import and declare in the typescript file's constructor. The import and declaration instructions of each installed plugin can be seen in Figure 4.17.

```
import { BLE } from '@ionic-native/ble/ngx';

constructor( private ble:BLE) {}
```

a)

```
import { SMS } from '@ionic-native/sms/ngx';

constructor(
private sms: SMS,
```

b)

```
import { Geolocation } from '@ionic-native/geolocation/ngx';

constructor(
  private geolocation: Geolocation,
```

c)

Figure 4.17 - Code regarding the import and declaration of the a) BLE plugin; b) SMS plugin; c) Geolocation plugin.

4.4.1 - BLE Plugin

Ionic BLE is a plugin offered by Ionic that allows BLE communication between a smartphone and Bluetooth Low Energy (BLE) peripherals, such as, in this case, the Arduino Nano 33 BLE Sense board.

This plugin allows you to perform a series of functions, ranging from searching for nearby peripheral devices available for connection, connecting to those devices, receiving and sending information to the connected device and notification when characteristic's values change. It also allows simultaneous connection to multiple peripherals [86].

In the developed application, by clicking on the "Scan" button (Figure 4.16 G), the nearby devices available for connection are searched, the device you want to connect to can be selected and the information is received and displayed on the Main page.

4.4.2 - SMS Plugin

Cross-platform plugin that allows to easily send an SMS by defining the phone number and the message to be sent. This plugin is available for Android, iOS, Windows Phone 8 and Windows 10 Universal [87].

In this case, this plugin was used to send an alert SMS with the user's GPS location to the predefined contacts, whenever high values of solution concentration were measured.

The message sent by the system in these situations is shown in Figure 4.18.

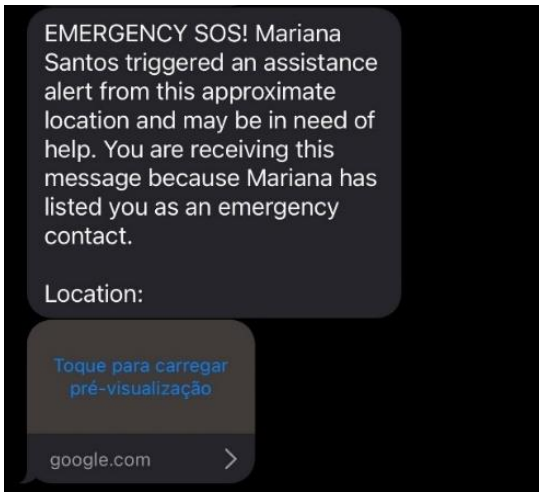


Figure 4.18 - Message with GPS location sent by the system to a previously defined contact, when a concentration above the limit concentration stipulated in the app was measured.

4.4.3 - Geolocation Plugin

This plugin accesses common location information sources such as Global Positioning System (GPS) and inferred location from network signals to provide device location information such as latitude, altitude, longitude and speed [88].

In this app, not only the latitude and longitude information is given, but also the device location address to open in browser or in Google Maps app, as shown in the Figure 4.19.

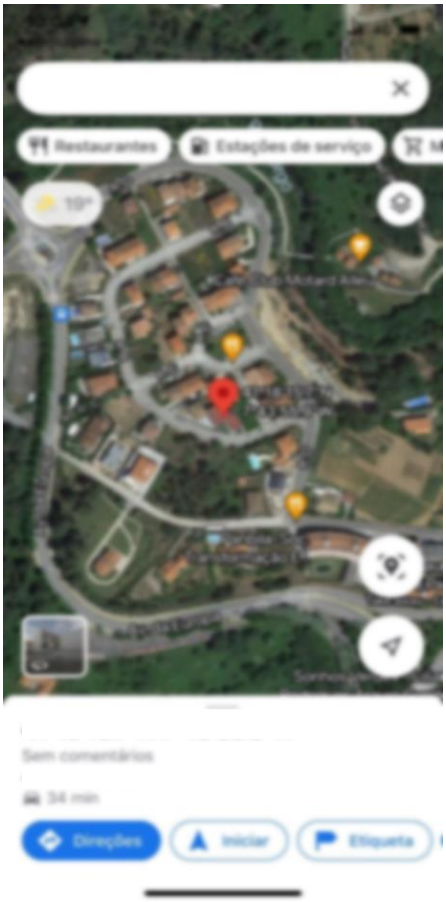


Figure 4.19 – Pacient location opened in the Google Maps application after sending an alert message. Figure intentionally blurred for privacy reasons.

4.5 - Final Remarks

The final system obtained includes the use of a carbon screen-printed electrodes sensor which is connected by a connector, to the developed PCB board that contains an LMP91000 AFE Potentiostat programmable by I2C communication. This potentiostat, which allows obtaining cortisol values, in turn, is connected to the Arduino Nano 33 BLE Sense, where the data is processed and sent by BLE, to the developed mobile application.

This app receives the collected data and decides whether an alert message with the user's GPS coordinates should be sent to emergency contacts, in case the values received are classified as abnormal and there is a risk of sudden death in epilepsy.

Arduino and mobile app developed softwares can be found on the GitHub link in Appendix B and Appendix C, respectively.

Chapter 5

System Validation and Results

This chapter presents, the tests carried out with the developed system with the purpose of validating its implementation and presenting and analyzing the results obtained.

All the results obtained were compared with an Autolab potentiostat from Metrohm Dropsens, which allows to meet all requirements in electrochemical research, make measurements with various electrochemical techniques and has a wide measurable range of current and potential.

5.1 - LMP91000 Operation Validation

In order to prove the good functioning of the LMP9100 and if the component was assembled correctly, two tests were performed.

The first test consisted of measuring the ambient temperature using the temperature sensor built into the LMP91000.

The temperature measurement is programmed by the Arduino microcontroller, via I2C communication, as shown in the Figure 5.1 and the sensor output is measured on the V_{OUT} pin.

```
#include <LMP91000.h>
#include <Wire.h>

LMP91000 lmp = LMP91000();

void setup() {
    Wire.begin();
  Serial.begin(115200);

   lmp.measureCell();
}
void loop() {
analogReadResolution(10);

 double voltage = analogRead(A0)*(3.3 / 1023);
 double temp = (voltage-1.5622)/(-0.00816);
  Serial.print(voltage);
  Serial.print(" V ; ");

  Serial.print(temp);
  Serial.print(" C \n");
  delay(1000);
}
```

Figure 5.1 - Code made for temperature measurement with the LMP91000 internal temperature sensor.

The output signal of the temperature sensor is a voltage and this voltage can be converted to temperature values in Celsius degrees by the following equation, presented in the component datasheet.

$$T = \frac{(V_{out} - 1562.2\ mV)}{-8.16\ mV} \quad (5.1)$$

, where *T* is the temperature in Celsius degrees and V_{OUT} is the output voltage in Volts.

The obtained result and displayed on the serial monitor can be seen in Figure 5.2.

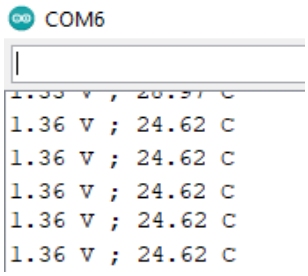


Figure 5.2 – Image of the obtained results from the temperature test on the serial monitor.

After this test, it was found that the LMP9100 was operating correctly and that the connections to the Arduino were well made.

The second test served to check if the system output voltage (V_{OUT}) varied when a variable current was forced to flow.

To carry out this test, the LMP91000 was programmed in 3-Lead Operation Mode, with FET disable, gain at 3,5 kΩ and load resistance (R_{Load}) at 10 Ω. The bias has been programmed at 20% * V_{DD}, which is 3,3 V and the internal zero (IntZ) at default, 50% * V_{DD}.

For this purpose, a 100 kΩ potentiometer was connected between the working electrode (WE), red thread, and the counter electrode (CE), black thread, as shown in the Figure 5.3, and the position of the cursor along the axis was varied. By doing this, as the obtained resistance will be different and an input voltage is applied, by Ohm's Law, the current produced will vary.

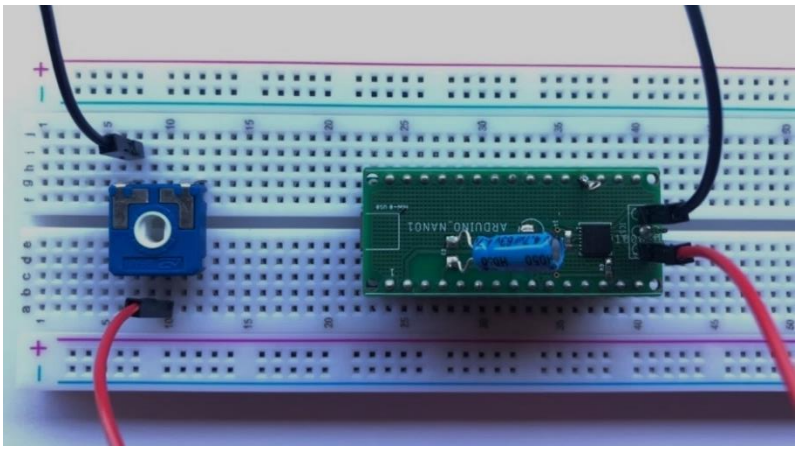


Figure 5.3 - Representative image of the assembly made with the potentiometer.

Thus, the obtained result is the output voltage corresponding to the current generated in the potentiometer (Figure 5.4).

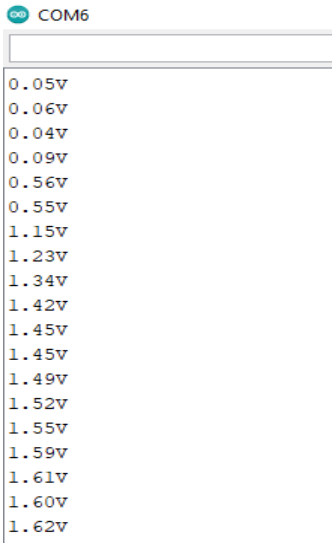


Figure 5.4 – Obtained results when the potentiometer cursor was varied counterclockwise.

The code regarding the first test can be found on Appendix B GitHub link, TempSensor folder, and the implemented code for this second test can be found on the same link in the LMP_Val folder.

5.2 - Developed System Validation Tests

The tests to validate the sensor operation were carried out using the developed system that includes the sensor, the connector, the PCB shield connected to the Arduino and the software developed for electrochemical techniques: Cyclic and Square Wave Voltammetry, as shown in Figure 5.5.

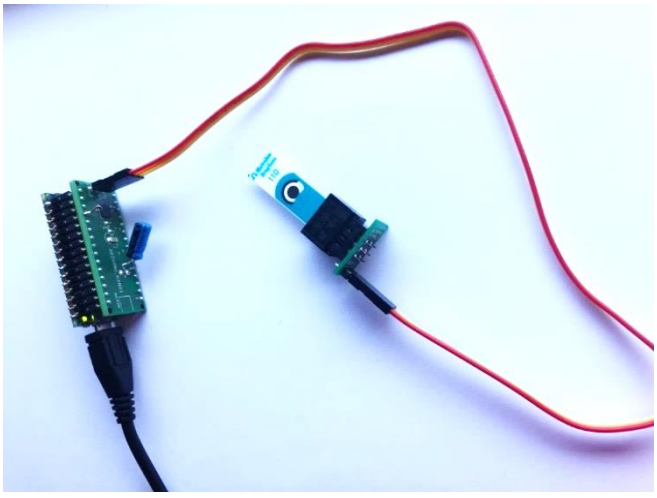


Figure 5.5 - Setup of the System validation tests.

Unfortunately, it was not possible to make measurements with cortisol, so the tests were done with five solutions with different concentrations of the oxidation reduction pair potassium hexacyanoferrate (II) trihydrate and potassium hexacyanoferrate (III): 1 mM, 2.5 mM, 5 mM, 7.5 mM e 10 mM, in order to observe if the system measured the different concentrations and to compare the results obtained by the system with the results obtained by Metrohn Dropsens Autolab PGSTAT128N [89].

In this way, a 40 µL drop of solution, to measure each concentration, was placed so as to cover the screen-printed carbon three electrodes (SPCE) of the sensor.

As far as software is concerned, two codes were implemented in Arduino IDE.

In both codes, the LMP was set to 3-Lead operating mode, gain to 3,5 kΩ, R_{Load} to 10 Ω, FET disable, and internal zero to 20% * V_{DD}.

5.2.1 - Cyclic Voltammetry Technique

In the code that implements the Cyclic Voltammetry, link in Appendix B, the LMP91000 chip was programmed to apply an input voltage, identical to that shown in Figure 5.6, varying between 0,6 V and -0,2 V and the reverse, in steps of 2 mV and frequency of 25 Hz.

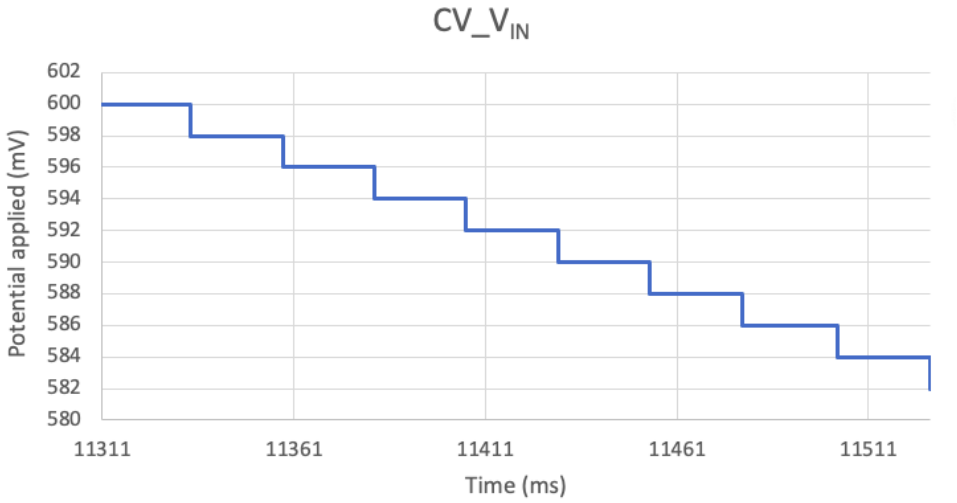


Figure 5.6 - Input voltage (V_{IN}) applied in measurement with the Cyclic Voltammetry technique.

After running the code referring to this technique, for each of the concentrations, the graph represented in Figure 5.7 was obtained.

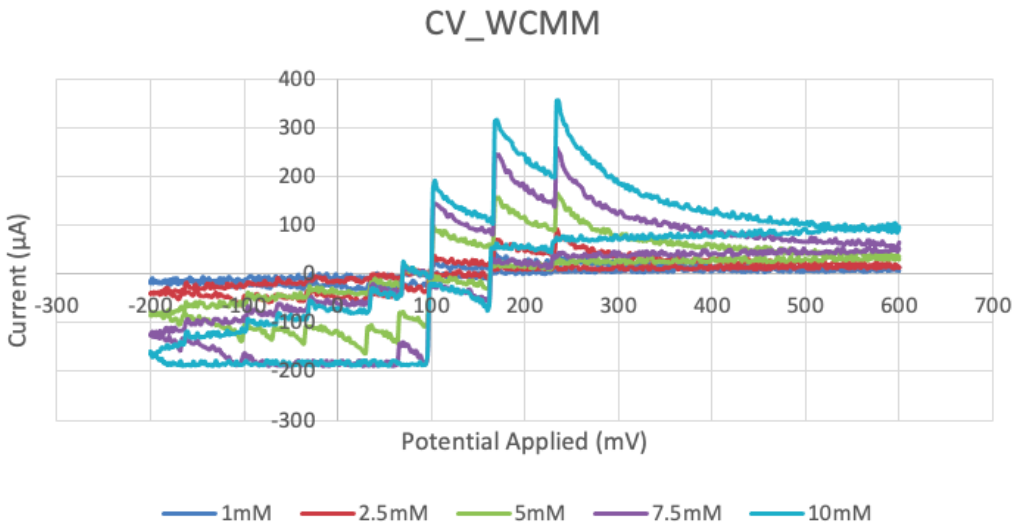


Figure 5.7 – Graph obtained with the proposed System using the CV technique, of the current as a function of the input potential applied for the concentrations of 1 mM, 2.5 mM, 5 mM, 7.5 mM and 10 mM.

Since the graph constructed with the raw obtained values, Figure 5.7, presents high background noise, a digital filtering was carried out, Figure 5.8, averaging with a moving window of 30 measured values, which significantly improved the graphs and their visualization.

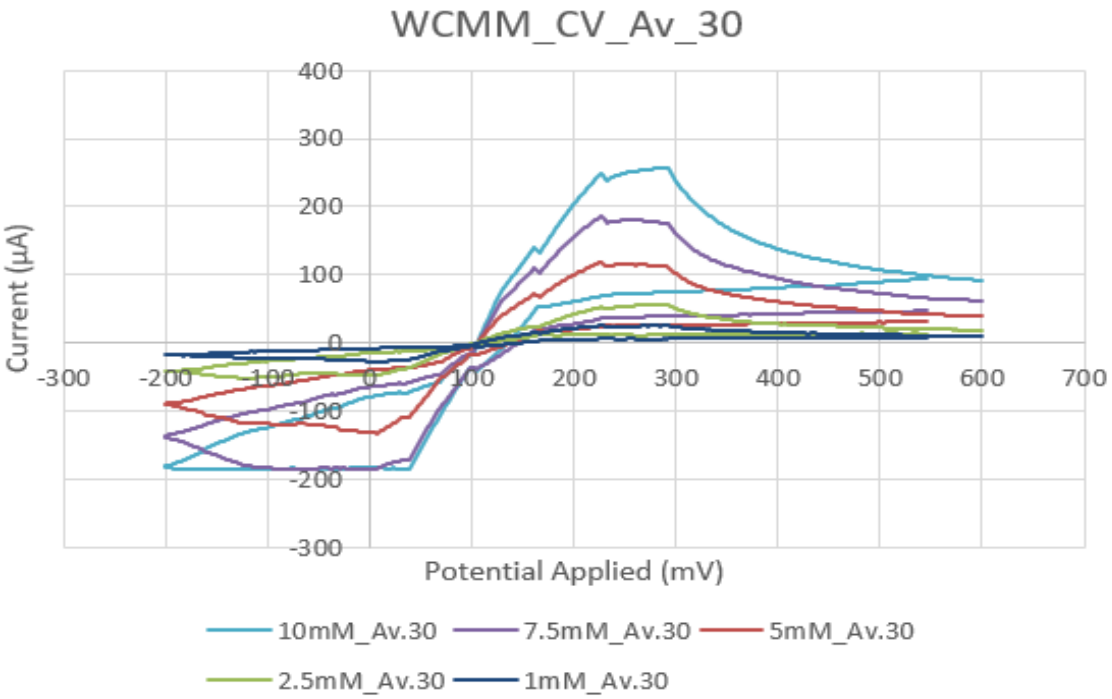


Figure 5.8 – Graph obtained in Figure 5.7 after digital filtering averaging with a moving window of 30 values.

Comparing the graph presented in Figure 5.8 with the one in Figure 5.9, obtained with Autolab, it can be concluded that, despite a slight difference in the maximum values, the system, as expected, shows that with increasing solution concentrations, increased current values are also obtained, which means that the obtained graphs present a curve identical to the expected one and that the correct and predicted relationship between the different concentrations is verified.

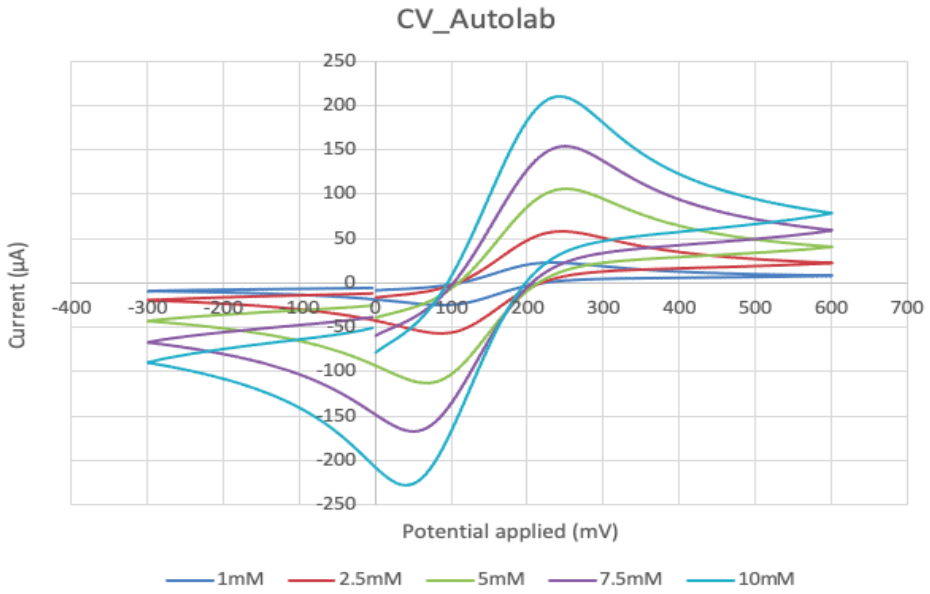


Figure 5.9 - Graph obtained with the Autolab System using the CV technique, of the current as a function of the input potential applied for the concentrations of 1 mM, 2.5 mM, 5 mM, 7.5 mM and 10 mM.

Still using this technique, another test was carried out, which consisted of measuring current values varying only the constitution of the electrodes and keeping the solution concentration at 2,5 mM.

To perform this test, the following sensors were used: an unmodified screen-printed carbon electrodes (SPCE) sensor, as purchased, without any functionalization and with two carbon electrodes [75]; a carbon nanotube electrode (SWCNT) sensor, that is, carbon composite electrodes coated with single-wall carbon nanotubes [90]; and a gold nanoparticle electrode (AuNP) sensor, in which gold nanoparticles were immobilized on the surface of the electrodes [91].

With the SPCE without any modification, the system provided values close to those that would be expected, Figure 5.11, i. e., current values between -60 µA and 60 µA, as it can be seen in the Figure 5.10.

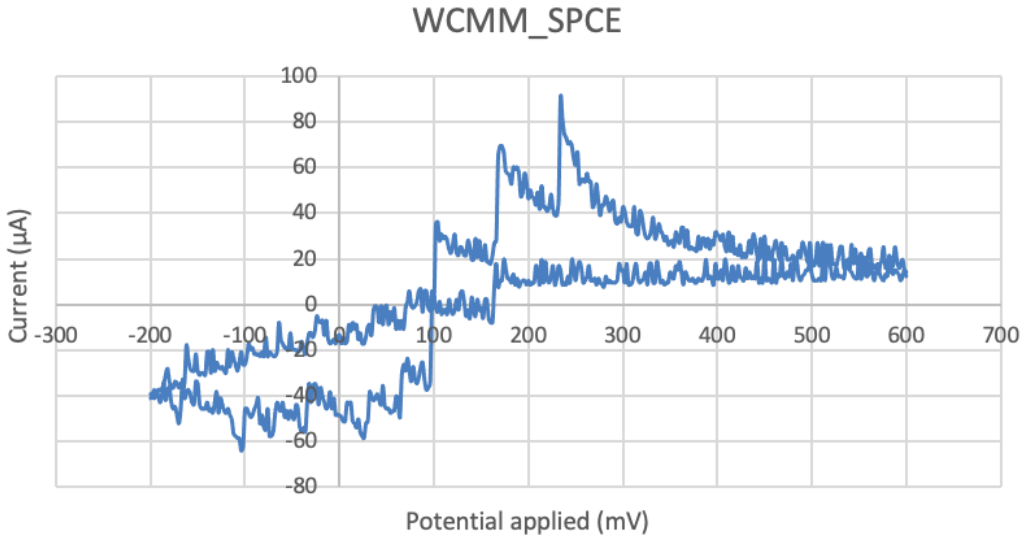


Figure 5.10 - Values obtained by the system, with the CV technique, for SPCE without any modification.

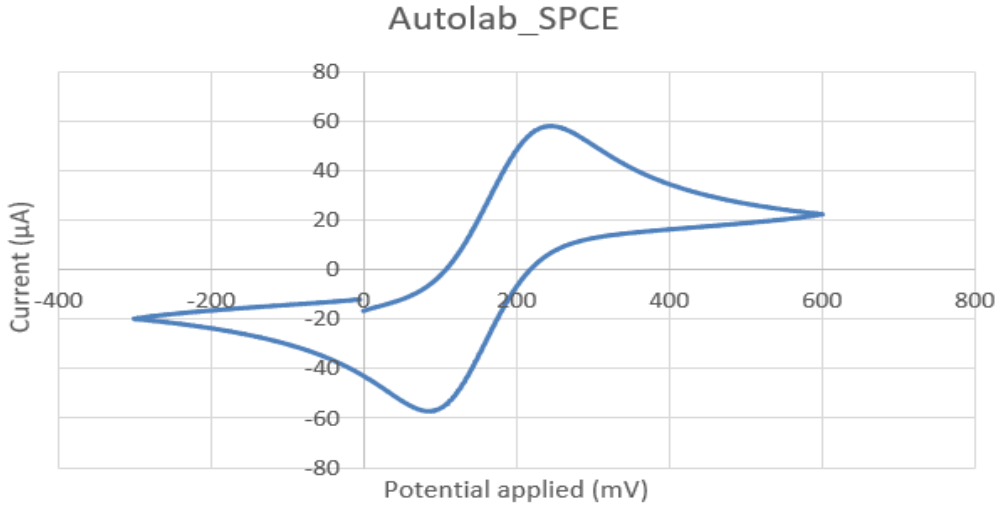


Figure 5.11 - Values obtained by the Autolab, with the CV technique, for SPCE without any modification.

With the SWCNT electrodes, higher values were obtained compared to the results with the SPCE, between -152 µA and 175 µA, as shown in Figure 5.12, values somewhat distant from those that would be expected, that change between -70 µA and 65 µA (Figure 5.13).

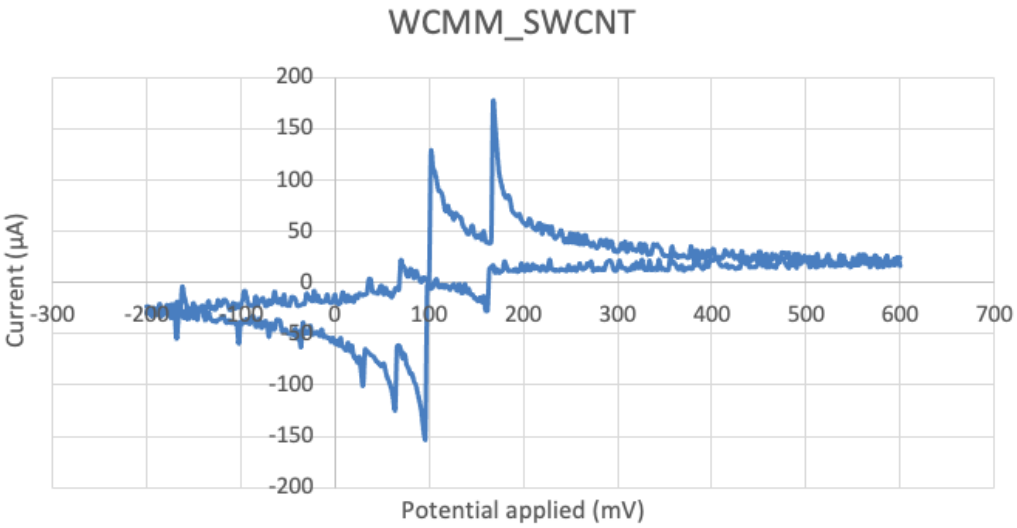


Figure 5.12 - Values obtained by the system, with the CV technique, for SWCNT electrodes.

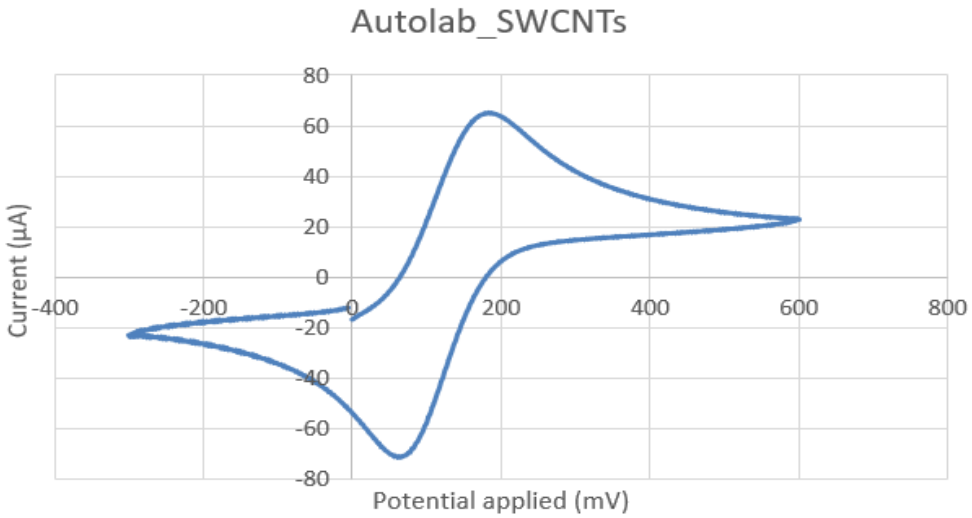


Figure 5.13 - Values obtained by the Autolab, with the CV technique, for SWCNT eletrodes.

With the AuNP electrodes, the obtained results show even higher currents than those obtained with the other electrodes, values between -167 µA and 260 µA, as presented in Figure 5.14, which are also different from the expected ones, that vary between -79 µA and 77 µA (Figure 5.15).

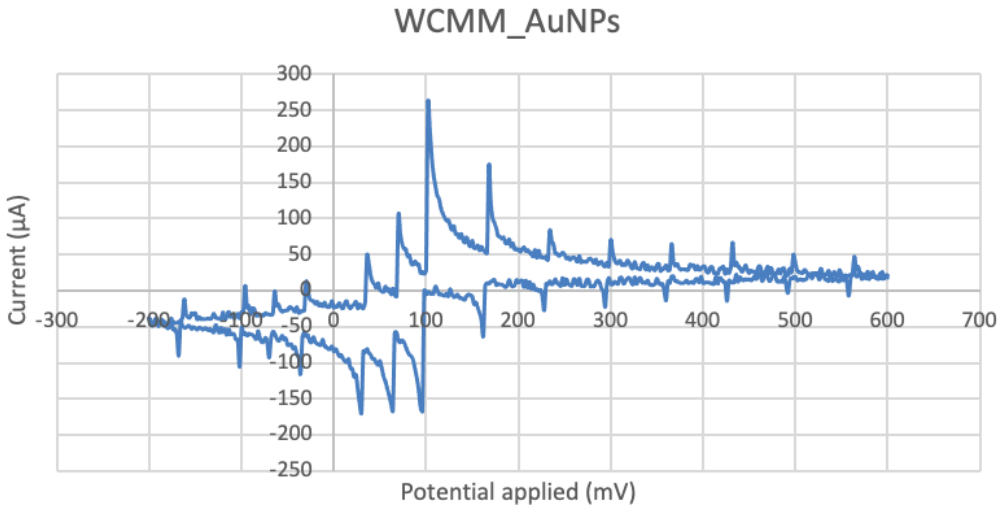


Figure 5.14 - Values obtained by the system, with the CV technique, for AuNP eletrodes.

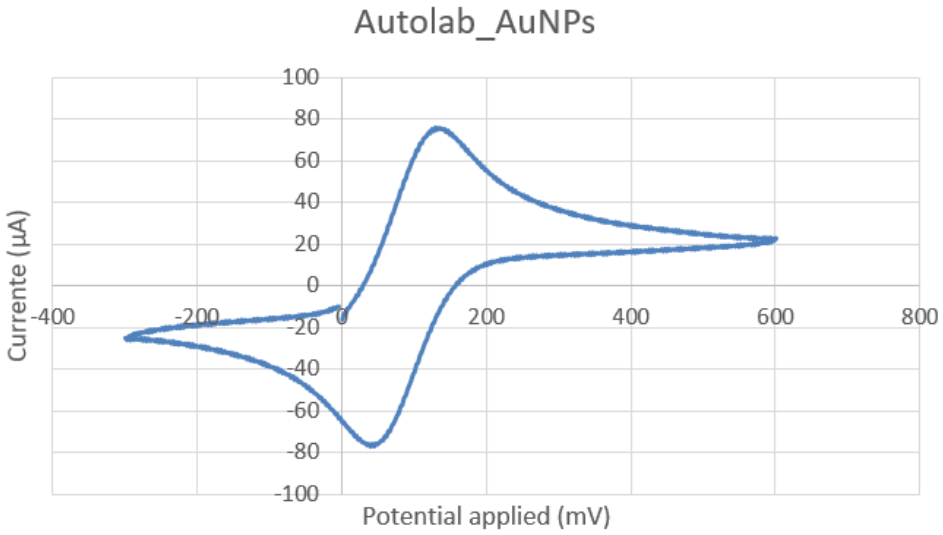


Figure 5.15 - Values obtained by the Autolab, with the CV technique, for AuNP eletrodes.

Although the results obtained with the system differ from those obtained with Autolab, they still show, that the generated current in the carbon nanotube electrodes is higher than that generated by the carbon electrodes without modification and, in turn, the current generated by the electrodes of gold nanoparticles is higher than that generated by the electrodes of carbon nanotubes. It is thus concluded that the system is working correctly, since this relationship between the different types of electrodes has been proven.

5.2.2 - Square Wave Voltammetry

To implement the square wave voltammetry technique, the LMP was programmed to apply an input voltage identical to that shown in Figure 5.16, which varies between the value 0,6 V and - 0,2 V and the reverse, with a pulse amplitude of 40 mV, in steps of 2 mV and frequency of 25 Hz.

The code for this technique can be found on GitHub link in Appendix B.

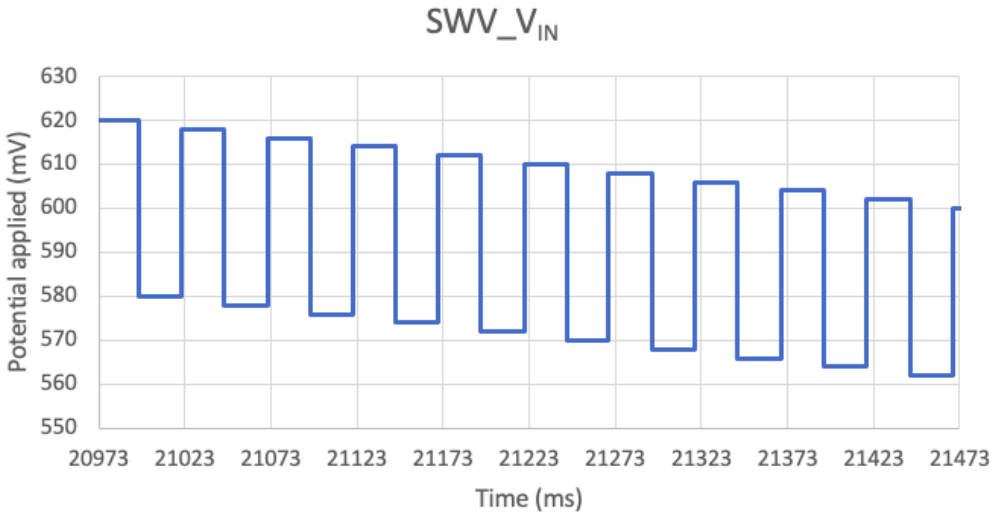


Figure 5.16 - Input voltage (V_{IN}) applied in measurement with the Square Wave Voltammetry technique.

After running the SWV code for each of the concentrations, the graph represented in Figure 5.17 was obtained.

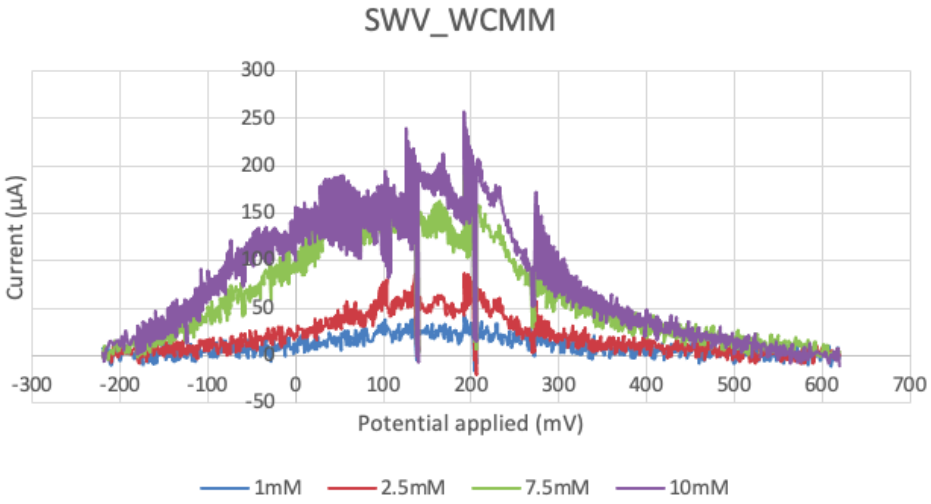


Figure 5.17 - System obtained graph with the SWV technique, of the current as a function of the input potential applied for the concentrations of 1 mM, 2.5 mM, 7.5 mM and 10 mM.

Since the graph constructed with the raw values obtained, Figure 5.17, presents high background noise, a digital filtering was carried out, averaging with a moving window of 50 measured values, Figure 5.18 a) and making a 5th order polynomial interpolation, Figure 5.18 b).

This filtering significantly improved the graphics and their visualization, and it is possible to conclude that the filtering with polynomial interpolation presents a more perfect and identical curve to the expected one.

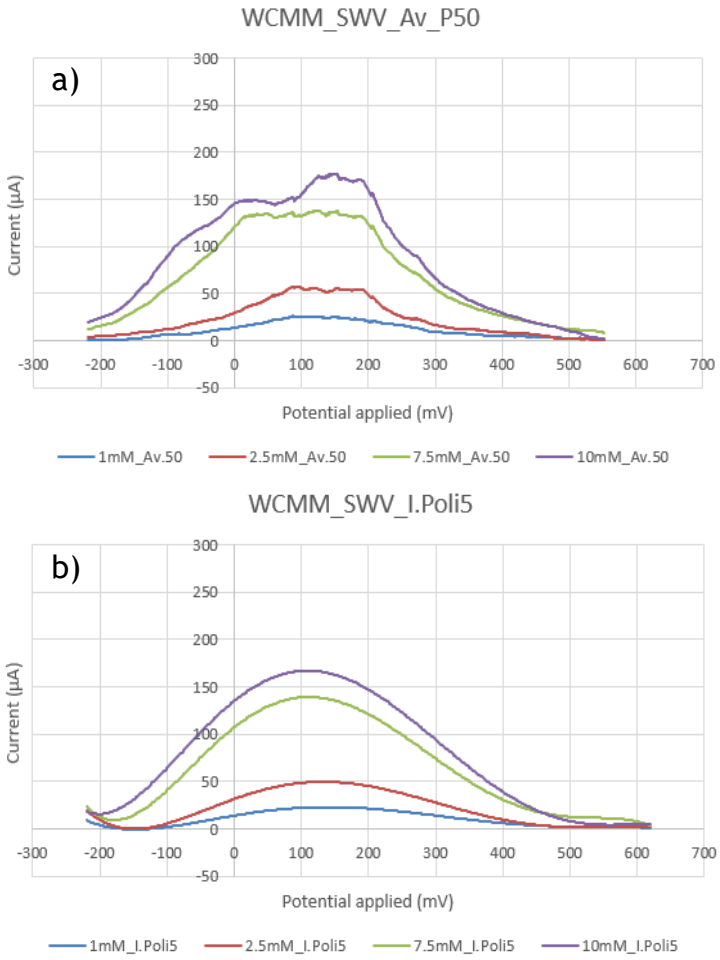


Figure 5.18 - Graph obtained in Figure 5.17 after digital filtering a) averaging with a moving window of 50 values; b) making a 5th order polynomial interpolation.

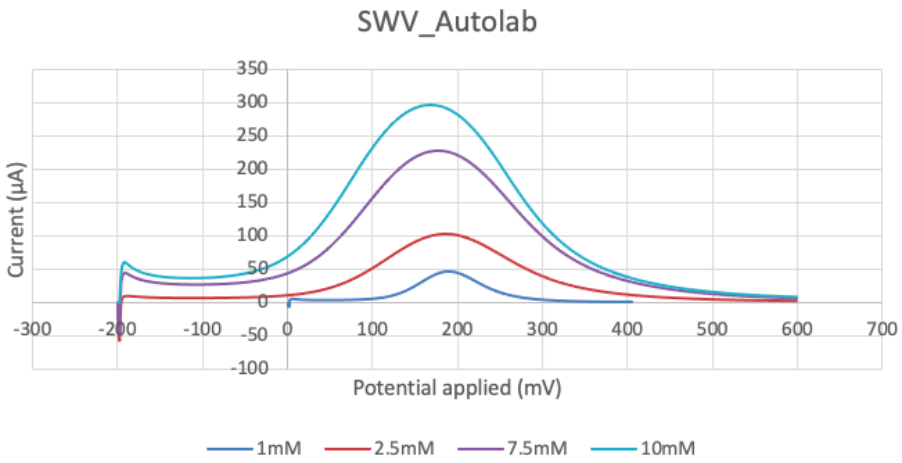


Figure 5.19 - Autolab obtained graph with the SWV technique, of the current as a function of the input potential applied for the concentrations of 1 mM, 2.5 mM, 7.5 mM and 10 mM.

Comparing the graphs obtained with the proposed system after digital filtering with that of Figure 5.19, obtained with Autolab, it is concluded that despite a slight difference in the maximum values, the system shows that the obtained graphs present a curve identical to the expected one, Figure 5.19 and that the correct and predicted relationship between the different concentrations is verified.

In short, through the tests carried out, it was possible to verify and validate the operation of the system, since the obtained results show trends similar to those presented by the values obtained with the Autolab system and the correct and predicted relationship between the different concentrations is verified.

5.2.3 - Full system validation

Regarding the tests performed with all components to prove the operation of the complete system, the code developed for Arduino described in the flowchart of Figure 4.12 was used to perform the measurements and data processing. This code is presented in the Appendix B link (CV_BLE file).

A higher concentration (7,5 mM) and a lower concentration (1 mM) than 2,5 mM (value stipulated as the threshold for the alert to occur) were applied.

It was verified that when the Arduino returned and sent to the app, a concentration value of 1mM, Figure 5.20, an alert SMS was not sent.

```
Time(ms): 24197, Voltage(mV): -170, Vout(V): 0.67, Current(uA): 4.06
Time(ms): 24233, Voltage(mV): -172, Vout(V): 0.65, Current(uA): -1.47
Time(ms): 24262, Voltage(mV): -174, Vout(V): 0.66, Current(uA): -0.55
Time(ms): 24300, Voltage(mV): -176, Vout(V): 0.66, Current(uA): 0.37
Time(ms): 24330, Voltage(mV): -178, Vout(V): 0.68, Current(uA): 4.98
Time(ms): 24362, Voltage(mV): -180, Vout(V): 0.66, Current(uA): 1.29
Time(ms): 24391, Voltage(mV): -182, Vout(V): 0.65, Current(uA): -1.47
Time(ms): 24425, Voltage(mV): -184, Vout(V): 0.66, Current(uA): -0.55
Time(ms): 24454, Voltage(mV): -186, Vout(V): 0.68, Current(uA): 5.90
Time(ms): 24483, Voltage(mV): -188, Vout(V): 0.67, Current(uA): 2.21
Time(ms): 24516, Voltage(mV): -190, Vout(V): 0.65, Current(uA): -3.32
Time(ms): 24545, Voltage(mV): -192, Vout(V): 0.67, Current(uA): 2.21
Time(ms): 24578, Voltage(mV): -194, Vout(V): 0.67, Current(uA): 4.06
Time(ms): 24605, Voltage(mV): -196, Vout(V): 0.66, Current(uA): -0.55
Time(ms): 24633, Voltage(mV): -198, Vout(V): 0.68, Current(uA): 4.98
Time(ms): 24663, Voltage(mV): -200, Vout(V): 0.66, Current(uA): -0.55
1 mM
Connected to central: 7c:4e:50:dc:17:ad
```

Figure 5.20 - Arduino serial monitor when measuring for 1mM concentration and returning and sending by BLE the 1mM concentration value.

However, after placing a drop of 7,5 mM concentration solution, the Arduino detected (Figure 5.21) and sent, to the app (Figure 5.22), the obtained concentration value, greater than 2,5 mM, that is, 7,5 mM and an alert message, similar to the one in Figure 4.18, was sent to the emergency contacts defined in the app's Emergency Contacts Page. The mobile application code can be found on the GitHub link in Appendix C.

```
Time(ms): 22791, Voltage(mV): -174, Vout(V): 0.56, Current(uA): -27.28
Time(ms): 22829, Voltage(mV): -176, Vout(V): 0.55, Current(uA): -32.81
Time(ms): 22858, Voltage(mV): -178, Vout(V): 0.55, Current(uA): -30.97
Time(ms): 22889, Voltage(mV): -180, Vout(V): 0.59, Current(uA): -20.83
Time(ms): 22921, Voltage(mV): -182, Vout(V): 0.58, Current(uA): -23.59
Time(ms): 22954, Voltage(mV): -184, Vout(V): 0.59, Current(uA): -20.83
Time(ms): 22978, Voltage(mV): -186, Vout(V): 0.56, Current(uA): -27.28
Time(ms): 23005, Voltage(mV): -188, Vout(V): 0.57, Current(uA): -26.36
Time(ms): 23033, Voltage(mV): -190, Vout(V): 0.58, Current(uA): -22.67
Time(ms): 23059, Voltage(mV): -192, Vout(V): 0.57, Current(uA): -26.36
Time(ms): 23084, Voltage(mV): -194, Vout(V): 0.57, Current(uA): -25.44
Time(ms): 23110, Voltage(mV): -196, Vout(V): 0.57, Current(uA): -25.44
Time(ms): 23136, Voltage(mV): -198, Vout(V): 0.59, Current(uA): -18.99
Time(ms): 23160, Voltage(mV): -200, Vout(V): 0.59, Current(uA): -19.91
7.5 mM
Connected to central: 5b:af:be:18:3a:24
```

Figure 5.21 - Arduino serial monitor when measuring for 7,5 mM concentration and returning and sending by BLE the 7,5 mM concentration value.

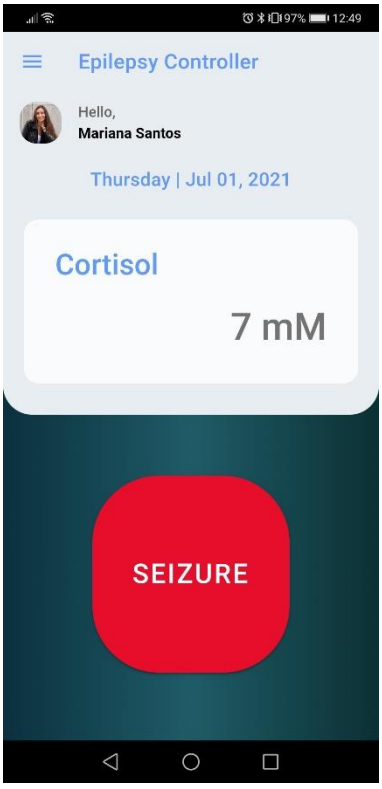


Figure 5.22 - Concentration value received by the system being displayed on the Main Page.

5.3 - Final Remarks

During the development and testing phases, some difficulties were encountered with regard to obtaining good and correct results and the development and implementation of the mobile application.

However, everything was done to overcome these difficulties and it was possible to develop a system capable of detecting and measuring correctly the different concentrations of solution applied in the two electrochemical techniques, Cyclic and Square Wave Voltammtry, as shown in this chapter, and which also allows sending, via BLE, the values detected for the developed mobile application and issue an alert message if necessary.

Chapter 6

Conclusions and Future Work

This section presents the main conclusions, as well as the importance of the study carried out for scientific development. Finally, some future work intentions are also described.

6.1 - Conclusions

The area addressed in this dissertation is a dynamic research area, characterized by constant progress in the medical field and, consequently, in the technological solutions developed.

When carrying out a survey of existing devices on the market, it was possible to verify that, compared to other systems, the developed system has the advantage of being adaptable to any type of disease, simply modifying the analyte that the sensor measures and adapting the software to the disease in question.

Recently, studies have proven the advantages of using continuous monitoring systems at various points in the treatment of all types of diseases.

In this case, since epilepsy is a potentially deadly disease, these advantages gain even greater preponderance.

This dissertation project aims to develop a low-power, wearable cortisol monitor to be integrated into a system capable of continuously monitoring the risk of sudden unexpected death in epilepsy. Thus, it can be said that the objectives of this dissertation were fulfilled, taking into account the execution of three essential points:

- a front-end circuit was created to operate with the sensor;
- a system based on a microcontroller was implemented to acquire cortisol data and send it, via a Bluetooth link, to a smartphone;
- the mobile app was developed that evaluates the received cortisol data and decides whether an alarm should be issued due to an unexpected sudden death high at risk of epilepsy.

The system created allows the collection and processing of data, in real time, and sending them to a mobile application capable of issuing an alert message when checking abnormal values.

During the dissertation work, for various reasons, there were some unforeseen events, which made measurements with a functionalized and specific sensor to measure cortisol

impossible. Either way, the system functioning was verified and validated, using the measurement of another compound at different concentrations.

Some of these impossibilities and some suggestions for improvement are presented in the next section on future work.

The biggest difficulties founded in the development of the prototype are related to the lack of information and valid examples in terms of software programming. Another unexpected challenge was the pandemic situation that befell us all and that presented us with challenges for which no one was prepared, having severely conditioned some phases of the work, largely due to the various confinements imposed.

On a personal level, in this project, I developed numerous skills in the most diverse areas, but mainly in the area related to programming in new programs and new programming languages and in the area related to electronics and instrumentation, since the entire project is focuses more on these areas.

In short, it is possible to say that the work was carried out successfully, not only because the objectives were met and a functional system was created, capable of adding value in combating the effects of epilepsy, and in extreme situations, saving human lives, but also because it enriched me personally and professionally.

6.2 - Future Work

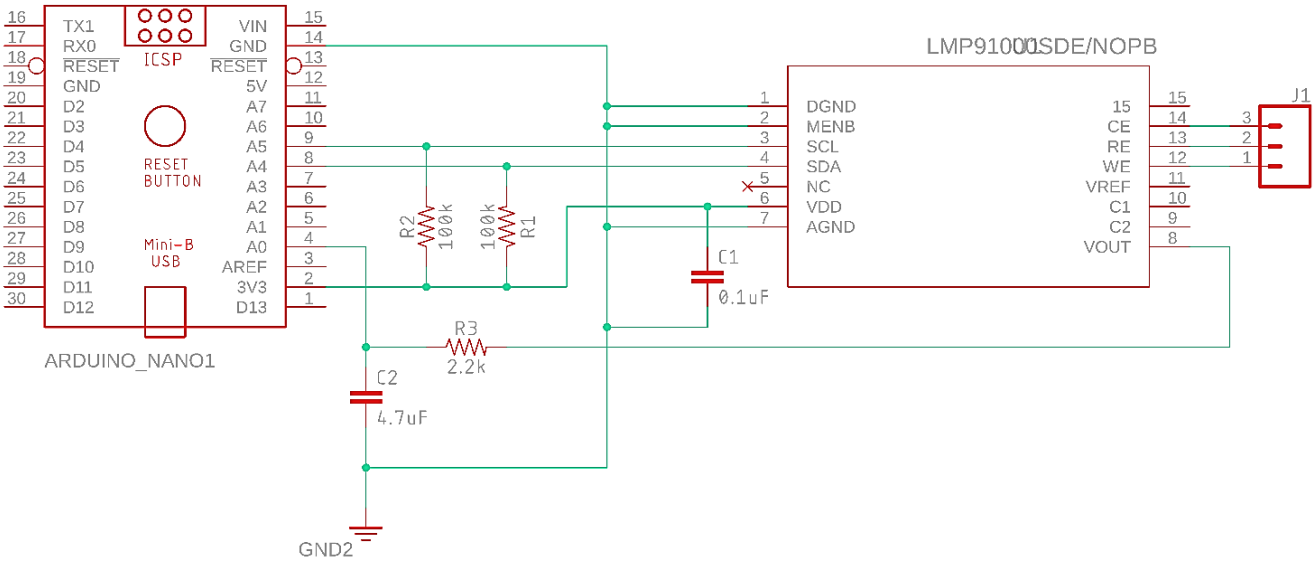
Although there are already significant advances in the development of disease monitoring systems, there is still a long way to go, so, the need for improvement is evident and still requires a lot of work.

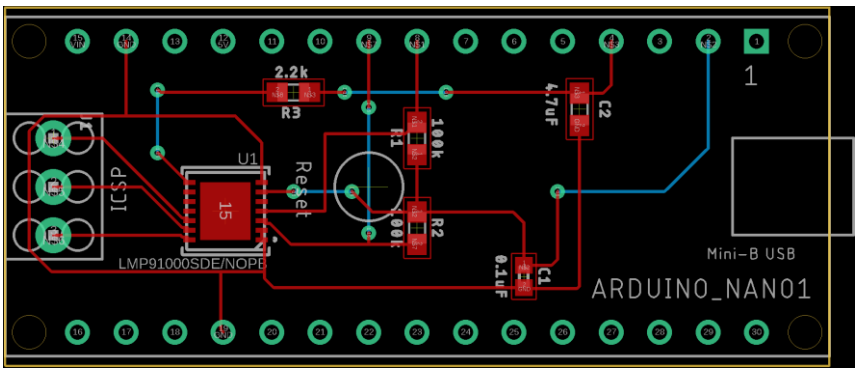
Regarding this project, as it is still a prototype, there is a whole development to be carried out and improvements to be implemented, placing the following as priorities:

- Need to carry out tests with a sensor specifically aimed at measuring cortisol levels;
- Develop an emergency drug delivery system in the event of a life-threatening seizure;
- Implement a data storage system, capable of storing daily results and subsequently sending a monthly report to the responsible healthcare professional;
- Make the system not only more comfortable and patient-friendly, but also more suitable for skin contact;
- Perform tests on epileptic patients.

The future development of this work is clearly promising, as it is an innovative project that can have a decisive contribution to significantly improve the lives of such a large number of people, who are affected by a disease, such as epilepsy, so harmful to their lives. Since it is a disease that, in extreme situations, can lead to death, this project can prove to be a preponderant tool to help medicine achieve its greatest and most important purpose, saving lives.

Appendix A


TX1
RX0
RESET
GND
D2
D3
D4
D5
D6
D7
D8
D9
D10
D11
D12
VIN
GND
RESET
5V
A7
A6
A5
A4
A3
A2
A1
A0
AREF
3V3
D13
ICSP
RESET BUTTON
Mini-B USB
ARDUINO_NANO1
LMP91000SDE/NOPB
DGND
MENB
SCL
SDA
NC
VDD
AGND
CE
RE
WE
VREF
C1
C2
VOUT
J1
R1 100k
R2 100k
R3 2.2k
C1 0.1uF
C2 4.7uF
GND2


2.2k
R3
4.7uF
C2
100k
R1
R2
C1
0.1uF
U1
Reset
ICSP
LMP91000SDE/NOPB
Mini-B USB
ARDUINO_NANO1

Appendix B – Arduino Code

Through this link you can access to the Arduino code developed to perform measurements with the CV and SWV techniques, to validate the operation of the LMP (LMP_Val and TempSensor folder) and the code developed to perform measurements, process the measured values and send the obtained concentration by Bluetooth Low Energy link.

https://github.com/MarianaAQS/WCMM_DissProj/tree/master/Arduino

Appendix C – App Code

Through the following link you can access and visualize the code of the developed mobile application where you can find the code developed for each of the pages (app, home, bluetooth, settings, contacts, medication, profile and bluetooth devices details), as well as the varSession file created to store session variables (WCMM_DissProj/WCMM1/src/providers/varSession.ts).

https://github.com/MarianaAQS/WCMM_DissProj/tree/master/WCMM1

References

[1] A. Herane Vives, V. De Angel, A. Papadopoulos, R. Strawbridge, T. Wise, A. H. Young, D. Arnone, A. J. Cleare, "The relationship between cortisol, stress and psychiatric illness: New insights using hair analysis," *J. Psychiatr. Res.*, vol. 70, pp. 38–49, 2015, doi: 10.1016/j.jpsychires.2015.08.007.

[2] S. Ouanes and J. Popp, "High Cortisol and the Risk of Dementia and Alzheimer's Disease: A Review of the Literature," *Front. Aging Neurosci.*, vol. 11, 2019, doi: 10.3389/fnagi.2019.00043.

[3] I. Cano-López and E. González-Bono, "Cortisol levels and seizures in adults with epilepsy: A systematic review," *Neurosci. Biobehav. Rev.*, vol. 103, no. May, pp. 216–229, 2019, doi: 10.1016/j.neubiorev.2019.05.023.

[4] "Cortisol and Bone Loss." https://mavendoctors.io/osteoporosis/bone-health/cortisol-and-bone-loss-nNDSjj89cUyitv21oY4X8g (accessed Jan. 20, 2021).

[5] P. Rice, S. Upasham, B. Jagannath, R. Manuel, M. Pali, and S. Prasad, "CortiWatch: Watch-based cortisol tracker," *Futur. Sci. OA*, vol. 5, no. 9, 2019, doi: 10.2144/fsoa-2019-0061.

[6] K. Hogenelst, M. Soeter, and V. Kallen, "Ambulatory measurement of cortisol: Where do we stand, and which way to follow?," *Sens. Bio-Sensing Res.*, vol. 22, no. July, 2019, doi: 10.1016/j.sbsr.2018.100249.

[7] V. L. Feigin *et al.*, "Global, regional, and national burden of neurological disorders, 1990–2016: a systematic analysis for the Global Burden of Disease Study 2016," *Lancet Neurol.*, vol. 18, no. 5, pp. 459–480, May 2019, doi: 10.1016/S1474-4422(18)30499-X.

[8] "The Five Common Neurological Disorders." https://sahyadrihospital.com/blog/The-Five-Common-Neurological-Disorders (accessed Jan. 22, 2021).

[9] World Health Organization, "Epilepsy," 2019. https://www.who.int/news-room/fact-sheets/detail/epilepsy (accessed Jan. 17, 2021).

[10] CDC, "Sudden Unexpected Death in Epilepsy (SUDEP) | CDC." https://www.cdc.gov/epilepsy/about/sudep/index.htm (accessed Dec. 21, 2020).

[11] D. Kinnamon, R. Ghanta, K. C. Lin, S. Muthukumar, and S. Prasad, "Portable biosensor for monitoring cortisol in low-volume perspired human sweat," *Sci. Rep.*, vol. 7, no. 1, pp. 1–13, 2017, doi: 10.1038/s41598-017-13684-7.

[12] M. Sekar, R. Sriramprabha, P. K. Sekhar, S. Bhansali, N. Ponpandian, M. Pandiaraj and C. Viswanathan, "Review—Towards Wearable Sensor Platforms for the Electrochemical Detection of Cortisol," *J. Electrochem. Soc.*, vol. 167, no. 6, p. 067508, 2020, doi: 10.1149/1945-7111/ab7e24.

[13] A. Kaushik, A. Vasudev, S. K. Arya, S. K. Pasha, and S. Bhansali, "Recent advances in cortisol sensing technologies for point-of-care application," *Biosens. Bioelectron.*, vol. 53, pp. 499–512, 2014, doi: 10.1016/j.bios.2013.09.060.

[14] M. Zea, F. G. Bellagambi, H. B. Halima, N. Zine, N. Jaffrezic-Renault, R. Villa, G. Gabriel, A. Errachida, "Electrochemical sensors for cortisol detections: Almost there," *TrAC - Trends Anal. Chem.*, vol. 132, p. 116058, 2020, doi: 10.1016/j.trac.2020.116058.

[15] R. M. Torrente-Rodríguez, J. Tu, Y. Yang, J. Min, M. Wang, Yu Song, You Yu, C. Xu, C. Ye, W. W. IsHak and W. Gao, "Investigation of Cortisol Dynamics in Human Sweat Using a Graphene-Based Wireless mHealth System," *Matter*, vol. 2, no. 4, pp. 921–937, 2020, doi: 10.1016/j.matt.2020.01.021.

[16] S. M. Mugo and J. Alberkant, "Flexible molecularly imprinted electrochemical sensor for cortisol monitoring in sweat," *Anal. Bioanal. Chem.*, vol. 412, no. 8, pp. 1825–1833, 2020, doi: 10.1007/s00216-020-02430-0.

[17] H. B. Lee, M. Meeseepong, T. Q. Trung, B. Y. Kim, and N. E. Lee, “A wearable lab-on-a-patch platform with stretchable nanostructured biosensor for non-invasive immunodetection of biomarker in sweat,” *Biosens. Bioelectron.*, vol. 156, no. March, p. 112133, 2020, doi: 10.1016/j.bios.2020.112133.
[18] P. R. Miller, X. Xiao, I. Brener, D. B. Burckel, R. Narayan, and R. Polsky, “Microneedle-based transdermal sensor for on-chip potentiometric determination of K+,” *Adv. Healthc. Mater.*, vol. 3, no. 6, pp. 876–881, 2014, doi: 10.1002/adhm.201300541.
[19] “Doença de Addison | CUF.” https://www.cuf.pt/saude-a-z/doenca-de-addison (accessed Jan. 12, 2021).
[20] “Addison’s Disease - NORD (National Organization for Rare Disorders).” https://rarediseases.org/rare-diseases/addisons-disease/ (accessed Jan. 13, 2021).
[21] “Síndrome de Cushing - Distúrbios endócrinos e metabólicos - Manuais MSD edição para profissionais.” https://www.msdmanuals.com/pt/profissional/distúrbios-endócrinos-e-metabólicos/distúrbios-adrenais/síndrome-de-cushing (accessed Jan. 13, 2021).
[22] “Cushing Syndrome - NORD (National Organization for Rare Disorders).” https://rarediseases.org/rare-diseases/cushing-syndrome/ (accessed Jan. 13, 2021).
[23] A. Javor, L. Zamarian, G. Ransmayr, M. Prieschl, M. Bergmann, “The role of cortisol in trust behavior: Results from an experimental study on healthy controls and patients with juvenile myoclonic epilepsy,” *Epilepsy Behav.*, vol. 110, p. 107138, 2020, doi: 10.1016/j.yebeh.2020.107138.
[24] J. Yang, X. Liu, Y. Fu, and Y. Song, “Recent advances of microneedles for biomedical applications: drug delivery and beyond,” *Acta Pharm. Sin. B*, vol. 9, no. 3, pp. 469–483, 2019, doi: 10.1016/j.apsb.2019.03.007.
[25] P. R. Miller, R. M. Taylor, B. Q. Tran, G. Boyd, T. Glaros, V. H. Chavez, “Extraction and biomolecular analysis of dermal interstitial fluid collected with hollow microneedles,” *Commun. Biol.*, vol. 1, no. 1, 2018, doi: 10.1038/s42003-018-0170-z.
[26] “Cortisol Test: Understanding High vs. Low Cortisol Levels.” https://www.webmd.com/a-to-z-guides/cortisol-test#1 (accessed Jan. 20, 2021).
[27] “Cortisol Urine Test: Purpose, Types, and Results.” https://www.healthline.com/health/cortisol-level#results (accessed Jan. 20, 2021).
[28] “How is a saliva test done to measure cortisol?” https://www.webmd.com/a-to-z-guides/qa/how-is-a-saliva-test-done-to-measure-cortisol (accessed Jan. 20, 2021).
[29] “ELISA Test Steps, Results, Types & Procedure Risks.” https://www.medicinenet.com/elisa_tests/article.htm (accessed Jan. 21, 2021).
[30] “Immunoassay - an overview | ScienceDirect Topics.” https://www.sciencedirect.com/topics/earth-and-planetary-sciences/immunoassay (accessed Jan. 14, 2021).
[31] “Enzyme-linked immunosorbent assay (ELISA) | British Society for Immunology.” https://www.immunology.org/public-information/bitesized-immunology/experimental-techniques/enzyme-linked-immunosorbent-assay (accessed Jan. 14, 2021).
[32] “Chemiluminescence Immunoassay - an overview | ScienceDirect Topics.” https://www.sciencedirect.com/topics/medicine-and-dentistry/chemiluminescence-immunoassay (accessed Jan. 14, 2021).
[33] “Chromatography - an overview | ScienceDirect Topics.” https://www.sciencedirect.com/topics/chemistry/chromatography (accessed Jan. 10, 2021).
[34] “Chrominfo: Advantages and disadvantages of Chromatography.” https://chrominfo.blogspot.com/2019/03/advantages-and-disadvantages-of.html (accessed Jan. 14, 2021).
[35] “Mass Spectrometry - an overview | ScienceDirect Topics.” https://www.sciencedirect.com/topics/materials-science/mass-spectrometry (accessed Jan. 14, 2021).
[36] “Understanding Spectrometry and Spectroscopy | ATA Scientific.” https://www.atascientific.com.au/spectrometry/ (accessed Jan. 10, 2021).
[37] H. Lee, T. K. Choi, Y. B. Lee, H. R. Cho, R. Ghaffari, L. Wang, “A graphene-based electrochemical device with thermoresponsive microneedles for diabetes monitoring and therapy,” *Nat. Nanotechnol.*, vol. 11, no. 6, pp. 566–572, 2016, doi: 10.1038/nnano.2016.38.
[38] “Analytical Techniques.” https://web.archive.org/web/20130317084149/http://www.wavesignal.com/forensics/Anlys.html (accessed Dec. 28, 2020).
[39] M. Sekar, M. Pandiaraj, S. Bhansali, N. Ponpandian, and C. Viswanathan, “Carbon fiber based electrochemical sensor for sweat cortisol measurement,” *Sci. Rep.*, vol. 9, no. 1, pp. 1–14, 2019, doi: 10.1038/s41598-018-37243-w.
[40] “Spectroscopy - an overview | ScienceDirect Topics.” https://www.sciencedirect.com/

topics/earth-and-planetary-sciences/spectroscopy (accessed Jan. 10, 2021).
[41] S. D. Gan and K. R. Patel, “Enzyme immunoassay and enzyme-linked immunosorbent assay,” *J. Invest. Dermatol.*, vol. 133, no. 9, pp. 1–3, 2013, doi: 10.1038/jid.2013.287.
[42] M. Zangheri, L. Cevenini, L. Anfossi, C. Baggiani, P. Simoni, F. DiNardo, A. Roda, “A simple and compact smartphone accessory for quantitative chemiluminescence-based lateral flow immunoassay for salivary cortisol detection,” *Biosens. Bioelectron.*, vol. 64, pp. 63–68, 2015, doi: 10.1016/j.bios.2014.08.048.
[43] S. P. Usha, A. M. Shrivastav, and B. D. Gupta, “A contemporary approach for design and characterization of fiber-optic-cortisol sensor tailoring LMR and ZnO/PPY molecularly imprinted film,” *Biosens. Bioelectron.*, vol. 87, pp. 178–186, 2017, doi: 10.1016/j.bios.2016.08.040.
[44] “Electroanalytical methods - Wikipedia.” https://en.wikipedia.org/wiki/Electroanalytical_methods (accessed Dec. 22, 2020).
[45] “Electrolytic Cells - Chemistry LibreTexts.” https://chem.libretexts.org/Bookshelves/Analytical_Chemistry/Supplemental_Modules_(Analytical_Chemistry)/Electrochemistry/Electrolytic_Cells (accessed Jan. 09, 2021).
[46] M. M. Ahmadi and G. A. Jullien, “Current-mirror-based potentiostats for three-electrode amperometric electrochemical sensors,” *IEEE Trans. Circuits Syst. I Regul. Pap.*, vol. 56, no. 7, pp. 1339–1348, 2009, doi: 10.1109/TCSI.2008.2005927.
[47] “Amperometry - an overview | ScienceDirect Topics.” https://www.sciencedirect.com/topics/chemistry/amperometry (accessed Jan. 09, 2021).
[48] “Chronoamperometry - an overview | ScienceDirect Topics.” https://www.sciencedirect.com/topics/chemistry/chronoamperometry (accessed Jan. 09, 2021).
[49] Y. C. Chen, S. Y. Lu, J. H. Tsai, and Y. Te Liao, “A power-efficient, bi-directional readout interface circuit for cyclic-voltammetry electrochemical sensors,” *2019 Int. Symp. VLSI Des. Autom. Test, VLSI-DAT 2019*, pp. 2–4, 2019, doi: 10.1109/VLSI-DAT.2019.8741529.
[50] “Voltammetry - an overview | ScienceDirect Topics.” https://www.sciencedirect.com/topics/chemistry/voltammetry (accessed Jan. 09, 2021).
[51] “Coulometry - an overview | ScienceDirect Topics.” https://www.sciencedirect.com/topics/medicine-and-dentistry/coulometry (accessed Jan. 09, 2021).
[52] “Potentiometry - an overview | ScienceDirect Topics.” https://www.sciencedirect.com/topics/chemistry/potentiometry (accessed Jan. 09, 2021).
[53] “Electrochemical Impedance Spectroscopy - an overview | ScienceDirect Topics.” https://www.sciencedirect.com/topics/engineering/electrochemical-impedance-spectroscopy (accessed Jan. 09, 2021).
[54] K. S. Kim, S. R. Lim, S. E. Kim, J. Y. Lee, C. H. Chung, W. S Choe, “Highly sensitive and selective electrochemical cortisol sensor using bifunctional protein interlayer-modified graphene electrodes,” *Sensors Actuators, B Chem.*, vol. 242, pp. 1121–1128, 2017, doi: 10.1016/j.snb.2016.09.135.
[55] Y. H. Kim, K. Lee, H. Jung, H. K. Kang, J. Jo, I. K. Park, H. H. Lee, “Direct immune-detection of cortisol by chemiresistor graphene oxide sensor,” *Biosens. Bioelectron.*, vol. 98, pp. 473–477, 2017, doi: 10.1016/j.bios.2017.07.017.
[56] “Chemiresistors - an overview | ScienceDirect Topics.” https://www.sciencedirect.com/topics/engineering/chemiresistors (accessed Jan. 09, 2021).
[57] B. J. Sanghavi, J. A. Moore, J. L. Chávez, J. A. Hagen, “Aptamer-functionalized nanoparticles for surface immobilization-free electrochemical detection of cortisol in a microfluidic device,” *Biosens. Bioelectron.*, vol. 78, pp. 244–252, 2016, doi: 10.1016/j.bios.2015.11.044.
[58] “Square Wave Voltammetry - an overview | ScienceDirect Topics.” https://www.sciencedirect.com/topics/chemistry/square-wave-voltammetry (accessed Jan. 09, 2021).
[59] A. K. P. Souza, C. A. De Moraes Cruz, G. C. Marques, L. S. O. De Castro, and T. B. Bezerra, “A compact current conveyor CMOS potentiostat circuit for electrochemical sensors,” *INSCIT 2019 - 4th Int. Symp. Instrum. Syst. Circuits Transducers*, 2019, doi: 10.1109/INSCIT.2019.8868731.
[60] S. Kumar, A. Ghosh, B. Tudu, and R. Bandyopadhyay, “An equivalent electrical network of an electronic tongue: A case study with tea samples,” in *ISOEN 2017 - ISOCS/IEEE International Symposium on Olfaction and Electronic Nose, Proceedings*, 2017, pp. 259–261, doi: 10.1109/ISOEN.2017.7968930.
[61] K. Vytřas, *Modern Electroanalytical Methods*, vol. 4, no. 6. 2000.
[62] N. Elgrishi, K. J. Rountree, B. D. McCarthy, E. S. Rountree, T. T. Eisenhart, and J. L.

Dempsey, “A Practical Beginner’s Guide to Cyclic Voltammetry,” *J. Chem. Educ.*, vol. 95, no. 2, pp. 197–206, 2018, doi: 10.1021/acs.jchemed.7b00361.
[63] D. De Souza, S. A. S. Machado, and L. A. Avaca, “Square wave voltammetry. Part I: Theoretical aspects,” *Quimica Nova*, vol. 26, no. 1. Sociedade Brasileira de Quimica, pp. 81–89, 2003, doi: 10.1590/s0100-40422003000100015.
[64] A. B. Islam, M. R. Haider, A. Atla, S. K. Islam, R. Croce, S. Vaddiraju, F. Papadimitrakopoulos, F. Jain, “A potentiostat circuit for multiple implantable electrochemical sensors,” *ICECE 2010 - 6th Int. Conf. Electr. Comput. Eng.*, no. December, pp. 314–317, 2010, doi: 10.1109/ICELCE.2010.5700691.
[65] A. Baschirotto, S. Capone, A. D’Amico, C. Di Natale, V. Ferragina, “A portable integrated wide-range gas sensing system with smart A/D front-end,” *Sensors Actuators, B Chem.*, vol. 130, no. 1, pp. 164–174, 2008, doi: 10.1016/j.snb.2007.07.144.
[66] “Blood Sugar And Cortisol | National Journal Community Of e-Experts.” http://www.njcee.org/health/blood-sugar-and-cortisol.html (accessed Jun. 16, 2021).
[67] “Apresentados sensores na pele para prevenir doenças.” https://www.dn.pt/sociedade/sensores-na-pele-para-prevenir-doencas-apresentados-hoje-em-encontro-cientifico-9201305.html (accessed Jun. 16, 2021).
[68] “Sensor ‘velcro’ pode identificar contaminação em alimentos - Olhar Digital.” https://olhardigital.com.br/2020/09/09/noticias/sensor-velcro-pode-identificar-contaminacao-em-alimentos/ (accessed Jun. 16, 2021).
[69] “Metrohm DropSens. Screen-Printed electrodes.” http://www.dropsens.com/en/screen_printed_electrodes_pag.html (accessed Oct. 26, 2020).
[70] “Electrodes for electrochemistry: screen-printed, interdigitated, and more | Metrohm.” https://www.metrohm.com/en/products/electrochemistry/electrochemistry-electrodes/ (accessed Oct. 26, 2020).
[71] “Cyclic Voltammetry and Its Applications | IntechOpen.” https://www.intechopen.com/books/voltammetry/cyclic-voltammetry-and-its-applications (accessed Jan. 18, 2021).
[72] “LMP91000 data sheet, product information and support | TI.com.” https://www.ti.com/product/LMP91000 (accessed Jun. 06, 2021).
[73] “Potentiostat screen printed electrode adapters – IO Rodeo.” https://iorodeo.com/products/cheapstat-screen-printed-electrode-adapters (accessed Apr. 10, 2021).
[74] Arduino, “Arduino Nano 33 BLE Sense | Arduino Official Store.” https://store.arduino.cc/arduino-nano-33-ble-sense (accessed Apr. 05, 2021).
[75] Metrohm Dropsens., “Screen-Printed Carbon Electrodes Electrochemical behaviour and electroanalytical performance of SPCEs for some benchmark redox systems,” pp. 75–76, 2016.
[76] T. Instruments, “LMP91002 Sensor AFE System : Configurable AFE Potentiostat for Low-Power Chemical,” 2014, [Online]. Available: https://www.ti.com/lit/ds/symlink/lmp91000.pdf?ts=1618289781550&ref_url=https%253A%252F%252Fwww.ti.com%252Fproduct%252FLMP91000.
[77] “Basics of the I2C Communication Protocol.” https://www.circuitbasics.com/basics-of-the-i2c-communication-protocol/ (accessed Jun. 06, 2021).
[78] “Arduino - ArduinoBLE.” https://www.arduino.cc/en/Reference/ArduinoBLE (accessed Apr. 10, 2021).
[79] “LMP91000 - Arduino Reference.” https://www.arduino.cc/reference/en/libraries/lmp91000/ (accessed Apr. 12, 2021).
[80] “Arduino - Wire.” https://www.arduino.cc/en/reference/wire (accessed Apr. 10, 2021).
[81] “GitHub - LinnesLab/LMP91000: Arduino library for the LMP91000 AFE Potentiostat for Electrochemical Sensing.” https://github.com/LinnesLab/LMP91000 (accessed Jul. 06, 2021).
[82] K. Townsend, “Introduction | Introduction to Bluetooth Low Energy | Adafruit Learning System,” 2014. https://learn.adafruit.com/introduction-to-bluetooth-low-energy (accessed Jun. 13, 2021).
[83] “Arduino - ArduinoBLE.” https://www.arduino.cc/en/Reference/ArduinoBLE (accessed Jun. 13, 2021).
[84] “LMP91000 data sheet, product information and support | TI.com.” https://www.ti.com/product/LMP91000 (accessed Jun. 12, 2021).
[85] “Cross-Platform Mobile App Development: Ionic Framework.” https://ionicframework.com/ (accessed Jun. 12, 2021).
[86] “BLE - Ionic Documentation.” https://ionicframework.com/docs/native/ble (accessed

Jun. 13, 2021).
[87] “SMS - Ionic Documentation.” https://ionicframework.com/docs/native/sms (accessed Jul. 07, 2021).
[88] “Geolocation | W3C Geolocator Application API Plugin.” https://ionicframework.com/docs/native/geolocation (accessed Jun. 13, 2021).
[89] “PGSTAT128N.” https://www.metrohm-autolab.com/Products/Echem/NSeriesFolder/PGSTAT128N (accessed Jun. 15, 2021).
[90] A. Aldalbahi, M. Rahaman, M. Almoiqli, A. Hamedelniel, and A. Alrehaili, “Single-walled carbon nanotube (SWCNT) loaded porous reticulated vitreous carbon (RVC) electrodes used in a capacitive deionization (CDI) cell for effective desalination,” *Nanomaterials*, vol. 8, no. 7, p. 527, Jul. 2018, doi: 10.3390/nano8070527.
[91] M. B. Kashi, Y. Wu, V. R. Gonçales, M. H. Choudhury, S. Ciampi, and J. J. Gooding, “Silicon–SAM–AuNP electrodes: Electrochemical ‘switching’ and stability,” *Electrochem. commun.*, vol. 70, pp. 28–32, Sep. 2016, doi: 10.1016/j.elecom.2016.06.014.