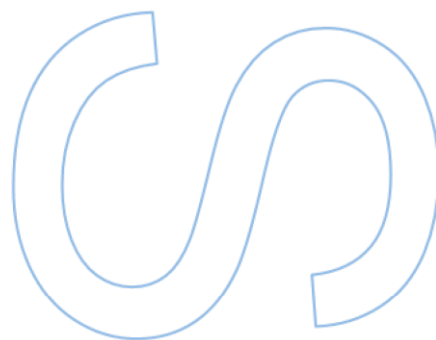
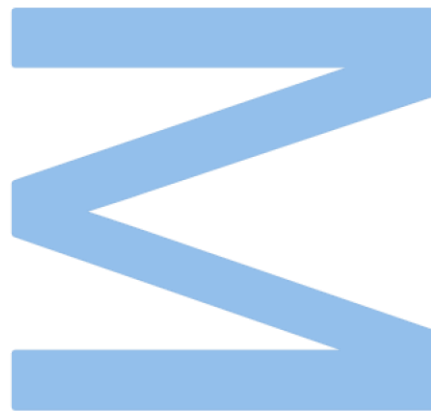


Development of an optical module system for monitoring dissolved CO₂ in macroalgae production tanks within the framework of the European project INNOAQUA

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Master in Engineering Physics
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Resumo

Foi desenvolvido um protótipo de sensor colorimétrico para monitorizar o CO₂ dissolvido, com base em pesquisas estabelecidas e modelos teóricos existentes. Embora o sensor tenha sido concebido para aplicações em aquacultura, demonstrou potencial de aplicação para além deste domínio, dada a ampla necessidade de monitorização fiável de CO₂ em diversas áreas.

Este trabalho foi desenvolvido como parte integrante do projeto INNOAQUA, financiado pela União Europeia. O objetivo deste projeto é demonstrar e implementar sistemas de aquacultura sustentáveis com foco em sistemas baseados em algas no contexto de sistemas multitróficos integrados.

O princípio de funcionamento do sensor baseia-se na medição da absorção de luz de uma membrana colorimétrica através de métodos óticos. Este sensor foi especificamente projetado para ser mais compacto, económico, estável e prático do que os sensores comerciais existentes. A melhoria do desempenho do sensor foi investigada através da otimização e aperfeiçoamento do processo de fabrico da membrana sensível ao CO₂, ajustando-se a concentração dos reagentes, resultando numa duplicação da sua sensibilidade inicial.

O sensor demonstrou uma elevada precisão, e tempos de resposta curtos, necessitando de 6 minutos em água estagnada e apenas alguns segundos em água corrente, alcançando um limite de deteção de 1,7 mg/L num intervalo de concentração entre 1 e 100 mg/L. Além disso, o protótipo foi validado através da medição de CO₂ dissolvido em água marinha, onde o seu desempenho foi comparado diretamente com o de um sensor comercial de CO₂.

Este sensor superou significativamente os modelos anteriormente investigados, apesar do potencial para melhorias adicionais. O sensor opera dentro da gama ideal para aplicações em aquacultura, tornando-o uma ferramenta altamente adequada para a área de produção alimentos de origem marinha.

Palavras-chave: Sensor Ótico, Sensor Colorimétrico, CO₂ Dissolvido, Aquacultura, Algas

Abstract

A prototype colorimetric sensor for detecting and monitoring dissolved CO₂ has been developed, drawing upon established research and existing theoretical models. While the sensor was conceived with aquaculture applications in mind, its potential utility extends beyond this domain, given the broad demand for reliable CO₂ monitoring across various fields.

This work was carried out as part of the INNOAQUA project, funded by the European Union. The project's goal is to demonstrate and implement sustainable, eco-friendly inland aquaculture systems, with a focus on algae-based solutions in the context of integrated multi-trophic systems.

The operating principle of the sensor is based on measuring the light absorption of a colorimetric membrane through optical methods. This sensor was specifically engineered to be more compact, cost-effective, stable, and practical than existing commercial sensors. The sensor's performance enhancement was investigated by optimizing and refining the CO₂-sensitive membrane fabrication process, adjusting the concentration of reagents, resulting in a twofold increase in its initial sensitivity.

The sensor exhibited high accuracy and demonstrated short response times, requiring 6 minutes in stagnant water and only seconds in flowing water, achieving a detection limit of 1.7 mg/L in a concentration interval ranging from 1 to 100 mg/L. Furthermore, the prototype was validated by measuring dissolved CO₂ in natural marine water, where its performance was directly compared with that of a commercially available CO₂ sensor.

This sensor significantly outperformed previously investigated models despite the potential for further enhancements. It operates within the optimal range required for aquaculture applications, making it a highly suitable tool for this field.

Keywords: Optical Sensor, Colorimetric sensor, Dissolved CO₂, Aquaculture, Algae

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List of Abbreviations

RAS Recirculating aquaculture system

IMTA Integrated Multi-Trophic Aquaculture

NDIR Non-Dispersive Infrared

ISFET Ion Selective Field Effect Transistor

LED Light-Emitting Diode

RGB Red, Green and Blue

PCB Printed Circuit Board

OPAMP Operational Amplifier

SNR Signal-to-Noise Ratio

pNPh p-nitrophenol

TOA-OH tetraoctylammonium hydroxide

BminBf₄ 1-Butyl-3-methyl-imidazolium tetrafluoroborate

IL Ionic Liquid

LoD Limit of Detection

1. Introduction

Sensing and monitoring carbon dioxide (CO₂) levels has multiple applications in various fields, from the well-known environmental impact to the food industry. Different industries require sensors with specialized capabilities such as very high sensitivity or fast response time. In this case, a CO₂ sensor was designed to respond to the demands of the aquaculture industry.

To address those challenges, the sensor must be accurate, fast, and, most importantly, capable of measuring dissolved CO₂ in aquatic environments such as recirculating water systems. This work was based on previous research on developing an optical sensor for aquaculture applications. It is based on a colorimetric membrane that reacts with dissolved CO₂ resulting in a color change that enables the measurement of CO₂ concentration after a proper optical analysis.

In this chapter, a general insight into the current applications of CO₂ sensors will be given, as well as the capabilities of the currently available CO₂ sensors. Afterward, a detailed explanation of the materials and methods employed to conduct and develop the sensor will be presented. Next, the significant experiments conducted will be presented and discussed. Finally, to conclude this thesis, possible improvements and future work will be presented.

1.1. Motivation

The demand for sea food has been increasing primarily due to population growth, which increases the demand for food supply. Aquatic ecosystems cannot meet consumer needs since wild fish populations do not accompany consumer demand. To solve this issue, aquaculture production has been exponentially increasing.

However, this raises environmental-related questions such as waste streams, wild fisheries for aquafeed, and invasive species escaping to the environment. To address these issues, the INNOAQUA project is under development, and this work is an important component of it. The project's goal is to demonstrate and implement sustainable and eco-friendly in-land aquaculture systems, with focus on algae-based solutions [1]. Monitoring dissolved CO₂ is crucial in aquaculture systems, as its concentration significantly influences fish and algae growth.

Even though this sensor was developed for aquaculture purposes, it can be used in various applications, as monitoring this gas is important in multiple fields and industries.

1.2. Applications of CO₂ Sensors

Carbon dioxide is a gas composed of two oxygen atoms joined by a covalent bond with a carbon atom. It is also classified as a greenhouse gas since it absorbs infrared radiation, which harms the ecosystem. The phenomenon is caused by the characteristic vibrational frequency of the CO₂ molecule in the Infrared range, thus absorbing that specific radiation and turning it into vibrational energy. For this reason, CO₂ monitoring is increasingly important and necessary. Currently, atmospheric CO₂ levels are over 420 ppm, an increase of 30% since the start of the industrial revolution [2].

Furthermore, carbon dioxide is a crucial part of living beings. Plants, algae, and other photosynthetic beings consume CO₂, while animals and other consumers generate CO₂ during respiration. For example, if concentrations of CO₂ in the air are high enough, it might difficult or block the respiration process. This critical role of CO₂ explains the necessity of monitoring this gas in multiple industries, such as the food industry, medical applications, and air quality measurement.

1.2.1 Environmental Impact of CO₂

The ability to measure and control the CO₂ levels in the environment is crucial as it has exerted substantial control on the climate. This gas significantly affects the Earth's radiation budget and contributes to climate change. Numerous significant changes in CO₂ concentration in the atmosphere throughout history are present in the geological record [3].

Current levels of CO₂ caused by anthropogenic sources were last seen 3 million years ago and a reduction of CO₂ emissions is necessary to prevent the return to CO₂ levels of the Miocene in the next century [3].

Henry's law states that the concentration of a gas dissolved in a liquid is proportional to its partial pressure in the air. As such, an increase in the concentration of CO₂ in the atmosphere will cause an increase in dissolved CO₂ in the Ocean [4]. In mathematical terms it follows:

$$C_s = HP \quad (1.1)$$

where C_s is the concentration of the CO₂ in the aqueous phase, P is the partial pressure of the gas in the gas phase, and H is Henry's constant.

An increased dissolved CO₂ in the Ocean will cause lower pH levels as dissolved CO₂ reacts with water molecules, increasing the amount of H⁺ [5]. Ocean acidification can bring negative consequences to the environment and living organisms.

This phenomenon's most well-known and understood consequence is the reduction of carbonate ions (CO₃⁻²) due to its reaction with H⁺ [6]. It is expected that the pH levels should decrease by 0.3-0.4 this century, corresponding to an increase of 150% in H⁺ and a decrease of 50% in CO₃⁻².

Many marine organisms need relatively high concentrations of carbonate ions to produce calcium carbonate, which is fundamental to the calcification of shells and skeleton formation for many marine species. Therefore, many species, such as corals and mollusks, cannot naturally form their shells [7].

1.2.2 Indoor Air Quality Monitoring

Guaranteeing acceptable levels of CO₂ is crucial in order to ensure a healthy indoor environment, as high CO₂ levels can result in serious health risks, especially in industrial environments in which fluctuations in CO₂ levels are more likely to occur. It is also a prominent health concern in modern lifestyles since people spend 90% of their time indoors. Several standards and guidelines consider a concentration of CO₂ under 800 ppm a suitable threshold for air quality [8].

In office environments, poor air ventilation can lead to increased CO₂ concentration from the people inside the room, which can cause discomfort, reduce productivity, and impair decision-making ability. For example, it has been shown that in a room with a shortage of fresh air, the heart-beat intensity increases by about 20% compared with a room with a proper flow of fresh air [9].

For this reason, CO₂ sensors and other gas sensors are necessary to monitor acceptable and healthy air concentrations to ensure air quality [10].

1.2.3 Medical Applications

The primary use of CO₂ sensor systems in medical applications is on the technique called capnography. It works by measuring the concentration of CO₂ during the process of respiration through time. When air is breathed in, oxygen is distributed into the bloodstream to be used in the metabolic process, generating CO₂ as one of the byproducts. This gas is then exhaled, and the respiratory cycle is finished. It is possible to measure and monitor this respiratory cycle using this technique.

This way, by plotting the partial pressure of CO₂ in the air through time, it is possible to detect and identify anomalies in the patient's respiratory system [11]. For instance, this technique is widely applied during tracheal intubation and is crucial in ensuring proper airway and ventilator management [12].

There are two main types of capnography: mainstream and sidestream. Mainstream consists of connecting breathed gas to a cell in which the concentration of CO₂ is accessed. Sidestream consists of aspirating gas close to the patient's face and transferring the gas through a tube to the gas analyzer[13].

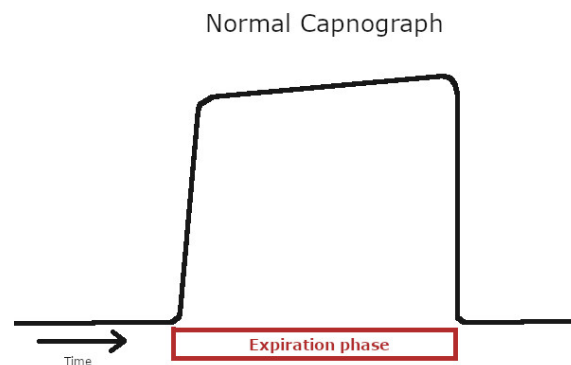


FIGURE 1.1: Typical capnography cycle of an healthy individual. Adapted from [12]

Another technique is the measure of dissolved gases in arterial blood. Dissolved carbon dioxide and oxygen are crucial parameters as they give insight into the adequacy of the gas exchange in the respiratory system. In addition, the measurement of dissolved CO₂ and pH values can provide insight into the bicarbonate concentration present in the blood [14].

1.2.4 Food Storage

A modified atmosphere composed of nitrogen, carbon dioxide, and oxygen is often used to prevent microbial spoilage in grain, meat, fruit, and vegetables. Maintaining CO₂ concentrations above a certain threshold prevents metabolic activities from bacteria or other living beings that would otherwise contaminate the food. In order to maintain appropriate concentrations, the environment needs to be continuously monitored. For example, high concentrations of CO₂ are an effective method of preventing grain deterioration from external agents such as some types of insects [15]. Beyond food storage, a modified atmosphere can also be used in food packaging to prevent deterioration. Besides, a decrease in CO₂ concentration in the package can be used as a sign of leakage since CO₂ concentrations should be high inside [16].

Measuring CO₂ levels can also be a valuable method to measure food deterioration since the growth of insects, bugs, and fungi will increase the CO₂ concentration in the environment, thus indicating that the food has been spoiled. For instance, a sensor with a resolution of 0.1% can detect grain deterioration by spoilage organisms [17].

1.2.5 Aquaculture

Aquaculture is the controlled cultivation and breeding of aquatic organisms such as fish, mollusks, and/or algae. This practice can be performed in freshwater as well as marine water.

Aquaculture production has steadily increased in the past decades and is vital to the human food supply. In the case of fish as a food source, a large portion of its supply was, for many years, captured from the wild instead of obtained by farming [18]. However, the worldwide increase in population, which has increased fish consumption, requires alternatives to supply the demand for food. In that way, aquaculture emerges as a viable food source on a large scale [19]. Nevertheless, aquaculture cannot support significant increases in supply on a sustainable basis. As a substantial increase in aquaculture production is expected in the near future, efforts need to be made to use aquaculture sustainably.

An emerging aquaculture technique to overcome those issues is the recirculating aquaculture systems (RAS). It consists of reusing the same water in a closed system, allowing a high degree of control over the water quality and products produced. This technique is also environmentally friendly since it is produced indoors and infinitely expandable.

This RAS requires more control and monitoring of key water quality parameters. Unlike offshore aquaculture, where the environment constantly renews water, some parameters must be controlled and measured in RAS systems as they can change over time. These critical parameters include dissolved CO₂, oxygen, turbidity, pH, temperature, nitrites, and nitrates [20].

The levels of dissolved CO₂ in the water significantly impact production and efficiency. For example, it has been shown that Atlantic salmon raised in aquaculture systems with increased concentrations of CO₂ have exhibited significantly lower body weight and growth [21]. Therefore, monitoring CO₂ levels in aquaculture systems is essential to maximize production and improve cost-effectiveness.

Another important technique that can be employed in conjunction with RAS is Integrated Multi-Trophic Aquaculture (IMTA). In this technique, different species are raised in proximity so that the products and wastes of one species are recycled and consumed by other cultured species and vice-versa (for example, fish and algae). This technique substantially reduces the environmental cost of aquaculture, and it also has high economic potential [22]. With this in mind, it is easy to see that algae play a critical role in IMTA as they can be used to increase sustainability and as a direct food source.

Besides fish, multiple species of algae show increased growth at very high CO₂ concentrations, sometimes of over 20%(v/v) [23], which shows that maintaining high concentrations of CO₂ is critical to achieving high production efficiency.

It is thus easy to conclude that monitoring levels of dissolved carbon dioxide, particularly in RAS and other aquaculture contexts, is crucial to maximize production value and efficiency. However, commercially available sensors for dissolved CO₂ measurement today cannot meet the requirements imposed by aquaculture systems as they are generally expensive, slow, and inaccurate. This work aims to develop a sensor that can satisfy the needs of sustainable aquaculture systems.

1.3. CO₂ Sensing Techniques

A sensor is a device that responds to a particular physical or chemical quantity and produces a measurable output. It can be divided into 4 four parts [16]: i) a sampling area that makes contact with the environment; ii), a transducer that transforms the physical, chemical, or biological change into an electronic signal; iii) electronics to process the received signal; iv) a signal display.

In general, CO₂ sensors can be distinguished and categorized by the transduction technique employed. Various types of CO₂ sensors will be presented in this section, including their respective characteristics, advantages, and disadvantages.

1.3.1 Non-Dispersive Infrared Sensors

Non-dispersive infrared (NDIR) sensors work by emitting infrared radiation that is allowed to interact with the gas molecules in a chamber, and the gas concentration is obtained by the change in transmitted radiation [24]. The sensing mechanism comprises four main steps: i) emission of IR light; ii) absorption by gas molecules; iii) optical filtering; iv) detection and signal processing.

As the IR light passes through the gas sample, specific wavelengths are absorbed by the gas molecules. Each gas has a unique absorption spectrum, which absorbs IR light at specific wavelengths. The optical filter allows only the wavelengths of interest (those absorbed by the target gas) to pass through to the detector. The detector measures the intensity of the transmitted IR light, reporting that a reduction in intensity at the specific wavelength (compared to the reference or baseline intensity) is proportional to the gas concentration. The sensor's electronics process the detector's signal to determine the gas concentration. The amount of light absorbed follows Beer-Lambert's law, which relates the absorbance to the concentration of the absorbing species. A typical configuration of an NDIR CO₂ sensor is represented in Figure 1.2.

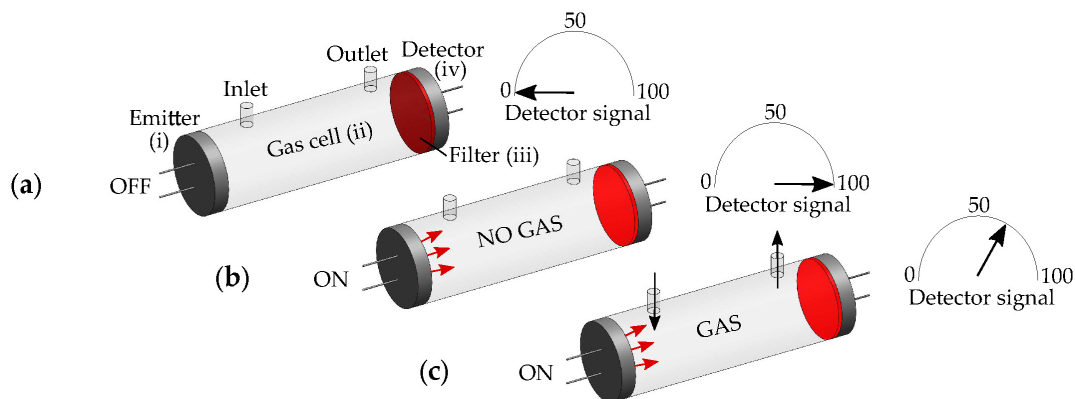


FIGURE 1.2: Configuration Scheme of an NDIR CO₂ sensor. Reproduced with permission from [25]

The Beer-Lambert Law relates the radiant power in a beam to the length of the path of the beam in an absorbing medium 1.2:

$$I(\lambda) = I_o(\lambda)e^{\alpha(\lambda)cl} \quad (1.2)$$

where $I(\lambda)$ is the intensity of the transmitted radiation, $I_o(\lambda)$ is the intensity of emitted radiation, $\alpha(\lambda)$ is the gas absorption coefficient, c is the gas concentration, and l is the path length. According to this law, controlling for the other variables makes it possible to relate CO₂ concentration with detected radiation intensity.

These sensors can be quite bulky since their accuracy is proportional to the length of the light path. However, miniaturized NDIR sensors have been developed recently [26].

This category of sensors is generally high-speed, accurate and can reach measurement precision on the order of 1ppm [27]. However, they have some significant disadvantages. These sensors are generally cross-sensitive to temperature and pressure and are relatively expensive compared to other CO₂ sensors.

Due to their particular characteristics, NDIR sensors are, perhaps, the most common devices to measure atmospheric CO₂ levels and capnography, and they are generally only used for gaseous CO₂ monitoring. They can also be used for dissolved CO₂ monitoring by using a membrane that is permeable to CO₂ but impermeable to water. However, this significantly decreases accuracy and response time, making them a poor option for aquaculture applications [28].

1.3.2 Photoacoustic Sensors

This sensor is composed of 4 different parts: i) a modulated light source, which, for the specific case of CO₂, is in a wavelength ranging from 9-11 μm ; ii) a sample cell containing the gas; iii) a microphone attached to the sample cell to detect the acoustic signal; iv) signal processing electronics to amplify and filter the signal for best results [29].

Since they are based on the photoacoustic effect, they involve the generation of sound waves after the absorption of modulated light by gas molecules. The theory behind this effect is that light absorbed by a sample excites a fraction of the gas ground-state molecular population into higher energy levels, which can return to the ground-state by or without emitting radiation. When it does not emit radiation, it generates heat and pressure waves that can be detected with an appropriate microphone.

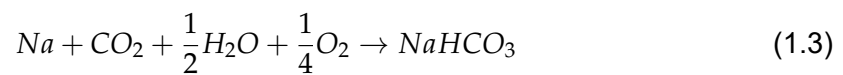
They operate by modulating an infrared source into the sample and measuring the amplitude of the pressure wave generated. This amplitude should increase with the gas concentration, thus allowing the accurate concentration measurement [30].

Besides photoacoustic sensors, acoustic sensors measure CO₂ concentration based on acoustic properties such as time of flight [31] or acoustic attenuation [32]. However, these sensors have low selectivity and are ineffective with more than two gas mixtures.

1.3.3 Electrochemical sensors

Electrochemical sensors are widely used for gas sensing due to their high sensitivity, specificity, and ability to operate in various environmental conditions. These sensors work on the principle of electrochemical reactions between the target gas and the sensor's electrode surface. The sensor's structure is divided as follows: i) a sensing electrode, commonly known as a working electrode, where the target gas undergoes a redox reaction; ii) a counter electrode that completes the circuit by providing a surface for the complementary redox reaction; iii) a reference electrode that is responsible for maintaining a stable potential to ensure accurate measurement; iv) an electrolyte to facilitate the ionic changes; v) a gas permeable membrane, allowing the target gas to reach the sensing electrode while blocking contaminants [33]. They can be classified into amperometric, conductometric, and potentiometric, depending on whether the measured signal is a current, a potential difference, or electric resistivity, respectively [16].

One of the most common chemical components used for this type of sensors is NASICON ($Na_3Zr_2Si_2PO_{11}$), which works as an ion bridge. In this type of sensors, chemical energy is converted into electrical energy through the following reaction [34]:



Moreover, the relation between the electric potential E generated on the cell and CO₂ partial pressure is given by:

$$E = E_0 + \frac{kT}{q} \ln P_{CO_2} \quad (1.4)$$

This way, the concentration of CO₂ can be determined since its logarithm is proportional to the electromotive force of the cell.

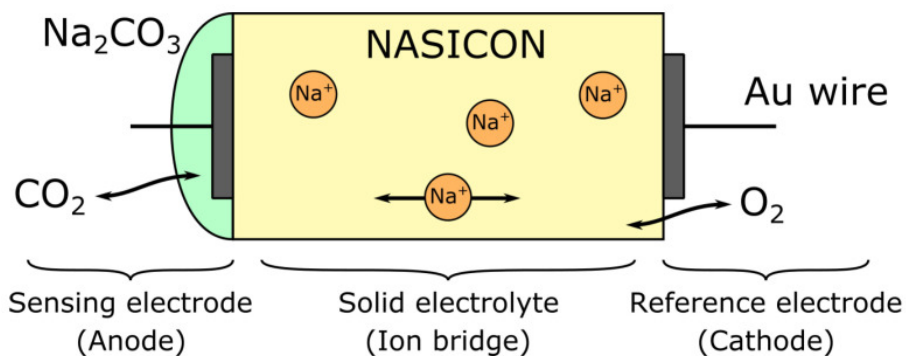


FIGURE 1.3: Schematic of a potentiometric CO₂ sensor. Adapted from [35]

Recent sensors fabricated from NASICON have been developed for a wide range of applications such as evaluating CO₂ capture materials [36].

The main advantage of these types of sensors is their small size and good accuracy at very low concentrations. However, they suffer from the inconvenience of operating at high temperatures (300-700 °C).

1.3.4 Metal Oxide Sensors

Metal oxide sensors work on the principle that adsorption consists of the adhesion of gas molecules on the surface of a metal oxide, causing a change in the conductivity of the metal oxide. The gas undergoes a redox reaction on the surface of the metal oxide film, which changes charge carrier concentration, which, in turn, changes the material's conductivity. Additionally, these sensors can be classified as n-type or p-type, depending on their semiconductor type [37].

They are composed of a filter impermeable to undesirable compounds, a gas-sensitive metal-oxide layer and sensing electrodes that detect the change in the material conductivity [38].

These sensors can be divided into two types based on their materials: those that follow surface conductance effects and those that follow bulk conductance effects. The first type generally works at lower temperatures of (300K-400K) while the second group operates above 700K. Examples of materials for surface conductance are ZnO and SnO_2 ; for bulk conductance there are TiO_2 , CeO_2 , Nb_2O_5 [39].

An example of a developed sensor of this type is the one reported by Lee and Meyer (2000), where the author developed a sensitive film doped with BaTiO_3 and other materials

[40]. The sensor revealed high sensitivity to CO₂ at concentrations of around 1%. The most significant challenges were the cross-sensitivity to O₂ and temperature.

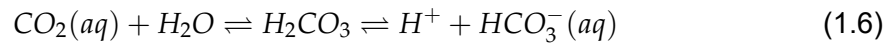
These sensors are lightweight, long-lasting, and have fast response times. It is also possible to produce them on a large scale, making them desirable and cheap. However, they have drawbacks, such as high operating temperatures and low selectivity for other gases [39].

1.3.5 Colorimetric sensors

Colorimetric sensors consist of a chemical reaction where the gas is dissolved in an aqueous solution or a thin polymer film, causing a change in the pH of the solution or film (Equation 1.5).



Once dissolved, CO₂ reacts with water or moisture inside a solid-state membrane, forming carbonic acid, thus changing the medium pH (Equation 1.6).



This pH change is associated with a particular concentration of CO₂, and it can be measured with the help of a pH-sensitive dye, which can exist in its protonated (RH) or anionic (R⁻) form. Several pH-sensitive dyes can be used, and various measuring methods can be implemented. This type of sensor can be divided into wet sensors, which use a sensitive solution encapsulated in a gas-permeable membrane where the sensitive dye is present or dry sensors that use a polymer and phase transfer agent, generally an ammonium hydroxide. Dry sensors are generally better than the wet ones.

Examples of these types of sensors include using optical fiber to measure CO₂ concentrations *in vivo* or monitoring beer's fermentation process [41, 42].

The sensor that was developed in this thesis can be classified as a colorimetric sensor. It uses a pH-sensitive colorimetric indicator, p-nitrophenol, whose light absorption at particular wavelengths can be measured, enabling the measurement of CO₂ concentration in water-based environments.

Some sensors can also be developed with a fluorescent dye instead of a colorimetric one. The working principle is very similar as this dye is also pH sensitive and exists in a protonated and anionic form. However, instead of changing its absorption spectra, it changes its fluorescence by emitting radiation with different intensities or wavelengths [43].

Instead of making use of a pH sensitivity dye, it is also possible to use Ion Selective Field-Effect Transistors (ISFETs) as they are also capable of measuring the pH of a solution [44].

Optical fiber sensors can also be employed for CO₂ sensing. They are based on the strain that works by coating the fiber with a specific hydrogel that shrinks or extends based on pH and thus CO₂ concentration [45].

While colorimetric sensors have an accuracy below 1% and response times of around 1 minute, they can be affected by humidity and temperature. However, their most significant advantage in our case is the capability of measuring dissolved CO₂ and gaseous CO₂.

Table 1.1 condensates all the essential data presented in this section.

After analyzing the most common current sensors, it can be concluded that most are not capable of measuring dissolved CO₂, and they usually come with serious disadvantages that make them an impractical solution for aquaculture applications, mostly due to slow signal response, inaccuracy, and inability to read in real-time.

Considering all the requirements, colorimetric sensors are the best option for aquaculture applications, mainly considering the response time and real-time reading needs. However, they have not been researched and developed as much as other more popular sensors. This work aims to develop a competitive colorimetric sensor capable of being used in aquaculture applications.

TABLE 1.1: Comparison of Different Types of CO₂ Sensors

Type of Sensor	Working Principle	Advantages	Disadvantages
NDIR	Beer-Lambert Law	Very accurate and fast response time	Cross-sensitivity to temperature and pressure, bad for dissolved CO ₂
Photoacoustic	Photoacoustic effect	Fast response time and good accuracy	Bulky and might have significant cross-sensitivity to humidity and temperature
Electrochemical	Electrochemical cells	Good accuracy and small size	Operates at high temperatures and is cross-sensitive to external factors
Metal Oxide	Adsorption on a Metal Oxide	Small size and good accuracy	Operation at high temperatures and low selectivity to other gases
Colorimetric	Acidification caused by dissolved CO ₂	Fast response time, small size and can measure dissolved CO ₂	Cross-sensitive to humidity

2. Materials and Methods

2.1. Membrane Fabrication

The fabrication process starts with the mixing of the reagents: p-nitrophenol (pNPh, Sigma-Aldrich ReagentPlus®, ≥99%), tetraoctylammonium hydroxide solution (TOA-OH, Sigma-Aldrich, 20% in methanol) and Hydrogel D4 solution (AdvanceSource Biochemicals). After thoroughly mixing the reagents to form a homogeneous cocktail, the liquid is spin-coated onto a Mylar foil at approximately 1250 RPM, creating the final membrane. Often, the membrane is not used immediately; thus, it is usually stored at around 5°C-8°C. The described process is represented in Figure 2.1.

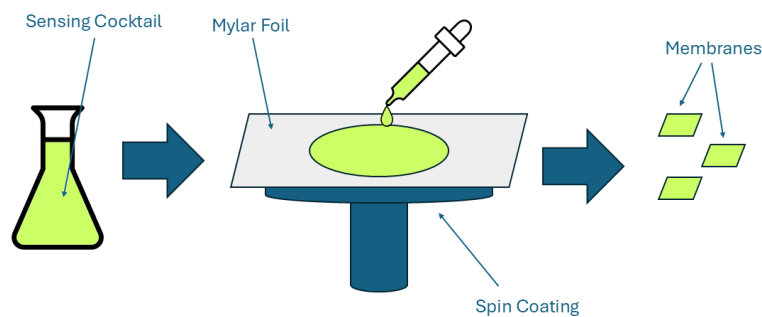


FIGURE 2.1: Representation of the membrane fabrication process. Sensing cocktail (comprising pNPh, TOA-OH and Hydrogel) is spin-coated onto a Mylar foil to produce the final product.

After fabrication, the membrane needs to be encapsulated with a protective layer of silicone elastomer (Sylgard-184). This protective layer is crucial in water environments as it prevents water from contaminating the membrane and allows gases to be diffused. To form a silicone layer, a solution of Sylgard-184 with its curing agent is prepared (10:1, w/w). Mixing will generate bubbles inside the liquid that must be removed with a vacuum pump. This liquid is then spin-coated onto a Mylar foil substrate at around 600-700 RPM. The 3D-printed pieces are attached to this layer while it cures.

The final design comprises a sensitive membrane encased between two layers of silicone and two 3D-printed components, thereby rendering it suitable for integration into the sensor. A photo of the final result is represented in 2.2.

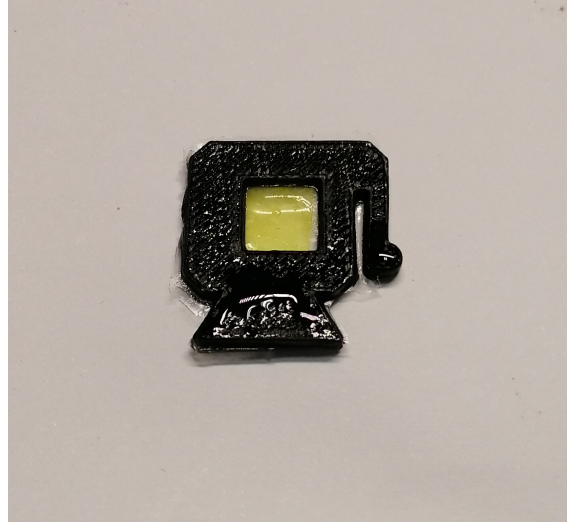
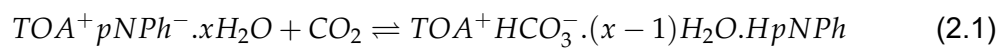


FIGURE 2.2: Photo of the final result obtained after membrane encapsulation with Sylgard-184.

2.2. Sensing Chemistry

The sensing methodology of this system is based on pH changes caused by CO₂ variations, which occur within a CO₂-sensitive membrane matrix following specific chemical modifications. The membrane fabrication relies on three primary reagents: p-nitrophenol, which is a well-known colorimetric indicator; tetraoctylammonium hydroxide solution, which is the phase transfer agent that converts pNPh into its deprotonated anionic form which reacts with CO₂, inducing changes in the pH and color of the membrane; and an ethanolic solution of Hydrogel D4, which purpose is to form a cross-linked matrix where reactions can occur. This final cocktail is then deposited on a mylar foil by spin-coating. This substrate was chosen due to its flexibility, transparency, and ability to provide good adhesion.

The membrane's sensitivity to CO₂ involves an ion-pair formation between TOA-OH and pNPh (TOA^+pNPh^-). When CO₂ is present, this ion-pair reacts with CO₂ and water converting the colorimetric indicator into its protonated form ($HpNPh$) resulting in the formation of carbonic acid following Equation 2.1:



When equilibrium is reached, the reaction stops and membrane's light absorbance will depend on the colorimetric indicator's concentration in its anionic form($pNPh^-$). When the gas concentration decreases, the equilibrium changes, and the membrane color is restored.

2.3. Optical Configuration

In the previous section, the process of membrane fabrication was presented. This section will explain the optical apparatus to measure the membrane's color change.

This membrane is yellow due to the effect of pNPh, which absorbs blue light. As the concentration of CO₂ increases and pNPh changes into its anionic form, the membrane becomes more colorless.

An optical system was employed to measure the color change, specifically the absorption of blue light, consisting of an optical emitter, collimating lenses, and an optical sensor. Two sensors were tested, one developed specifically for this thesis and designed for aquaculture applications.

The lenses were used on both sensors; their purpose was to collimate the divergent rays being emitted from the LED and converge the collimated rays onto the optical sensor (Figure 2.3).

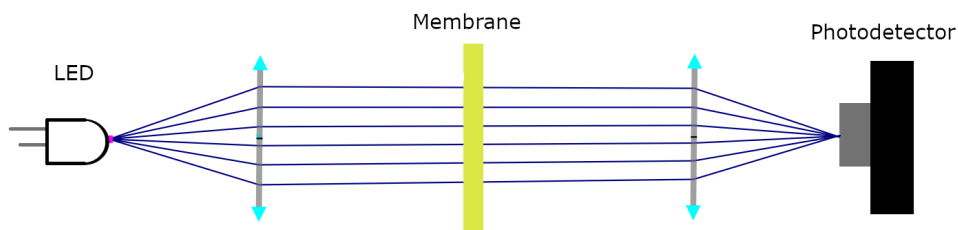


FIGURE 2.3: Optical setup to measure membrane's absorbance (implemented on both sensors)

2.3.1 RedBlue Sensor

This sensor was the most used for experiments since it had already been built from the beginning and had already been shown to have excellent capabilities (Figure 2.4).

The light is emitted by the optical emitter, which, in this case, is an RGB LED (KingBright). This LED regulated the intensity of each color channel, red, green, and blue. Only the wavelengths corresponding to blue and red were used in this case. Blue LED works as a measure signal since its absorption on the membrane is affected by CO₂ concentration, while red LED functions as a reference signal since it is unaffected by changes in CO₂.

The green LED was not used because its spectrum slightly overlaps with the blue LED, making it less effective as a reference signal.

The sensitive membrane stands between the light source and the optical sensor. It blocks the light path, absorbing a percentage of the light that goes through it, allowing the determination of color variation by measuring changes in transmittance or absorption spectra at different wavelengths. In this case, the change in the transmitted intensity of light was measured at the absorption wavelength.

The optical sensor is the component that measures the amount of light that goes through the membrane. In this case, we will use a spectrometer that receives the signal through an optical fiber (Thorlabs, FT600UMT), measuring the signal intensity by integrating the peaks corresponding to the red and blue signals provided by the spectrometer (Hamamatsu, C12880MA).

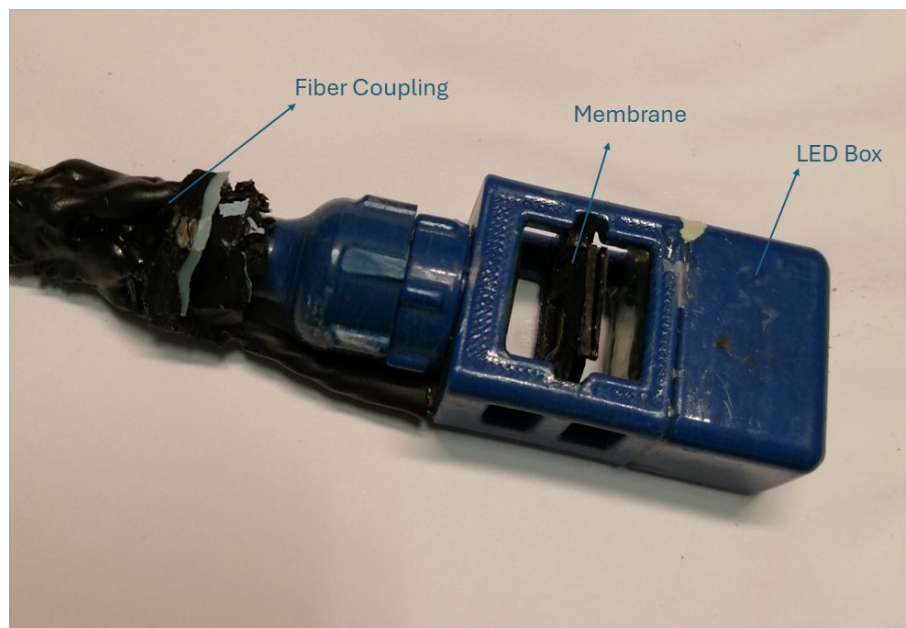


FIGURE 2.4: Photo of the RedBlue sensor, which consists of an RGB LED and a multimode optical fiber to collect the optical signal and transmit it to a spectrometer.

The data from the spectrometer shows two peaks: one corresponding to blue and the other to red. The membrane only absorbs light in the blue, so there is a change in the intensity of the light in the blue but not in the red (Figure 2.5).

The purpose of the reference signal is to reduce environmental effects on signal quality. For example, if a particle blocks the optical path, it will affect both signals equally and not interfere significantly with the CO₂ measurement.

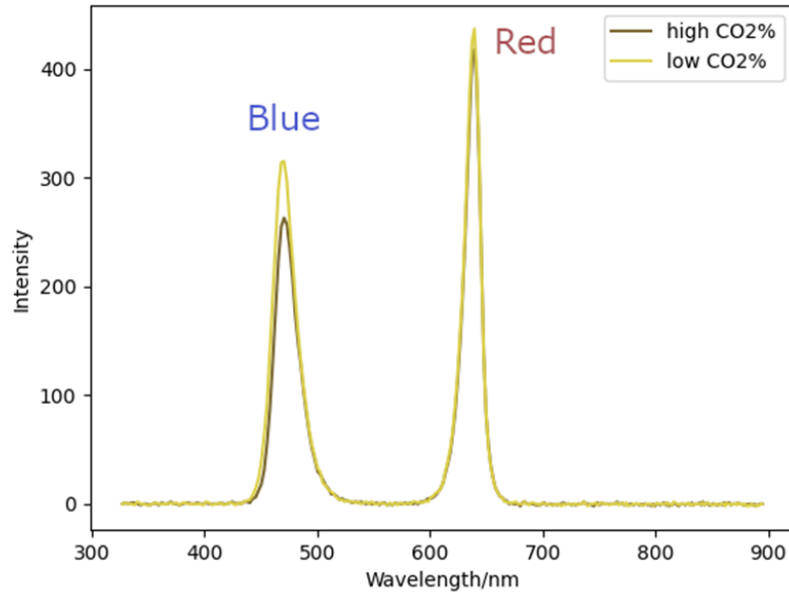


FIGURE 2.5: Signal detected by the spectrometer showing the emission spectra of both LEDs after passing through the sensing membrane at different concentrations of CO₂.

The calculation of the signal is given by integrating the blue signal (450nm to 550nm) divided by the integration of the red signal (600nm to 680nm) (Equation 2.2).

$$Signal = \frac{\int_{550}^{450} S d\lambda}{\int_{680}^{600} S d\lambda} \quad (2.2)$$

2.3.2 White Sensor

This new sensor was developed considering aquaculture applications. It is similar to the previous version. However, it is significantly more compact and cost-effective. It was designed to minimize debris accumulation and features a straightforward calibration process (Figure 2.6).

It consists of the same composition with an optical emitter and a photodetector. However, the optical emitter is a white LED (Cree C503D-WAN), which does not allow for the emission of specific wavelengths, and the optical receptor is an RGB photodetector (Hamamatsu S9702) made up of three photodiodes that absorb light at different wavelengths (red, green, and blue) through the use of filters. This setup significantly reduces size and cost without compromising performance, allowing all components to be housed within a compact, impermeable module, simplifying the measurement process.

By comparing the intensity of the red and blue light received by the photodetector, it is possible to measure the transmittance of blue light through the membrane and thus obtain the concentration of CO₂.

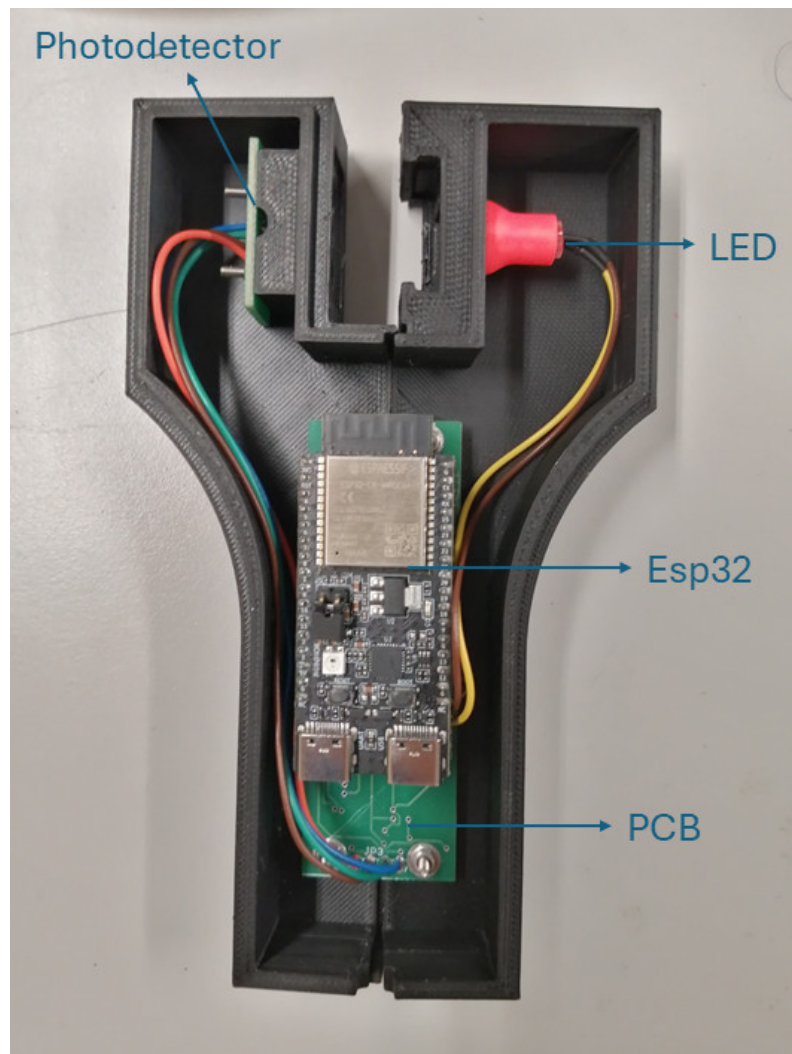


FIGURE 2.6: Photo of the interior of the White sensor where the optical and electronic components are shown.

Electronics

The electronics were developed on a PCB and can be divided into two main parts: one part to turn on the LED and another to process the data received from the photodetector.

Furthermore, an Esp32-C6-devkitc-1 was assembled on to the PCB. This programmable development kit allowed data transmission between the computer and the sensor and controlled the sensitivity of the sensor's signal.

The common cathode (that is connected to the three photodiodes each corresponding to a different color) has an input of around 2.37V in order to limit the maximum reverse voltage

applied. This potential difference is achieved with a voltage divider circuit with an Opamp (Texas Instruments OPAx340) that is divided from the source voltage VCC (3.3V provided by the Esp32). This circuit was built so that the OPAMP could provide the necessary current to the photodiode. Capacitors were used in order to filter high-frequency noise.

When the light emitted from the LED hits the photodiode, electrons jump over the electronic potential applied and a current proportional to the received light intensity is generated from the cathode to the anode. This current is then converted into voltage with a simple circuit that is connected to ground and to a negative (-2V) voltage (generated by an integrated circuit (MAX 840)) by resistors. By regulating the resistors with a digital potentiometer (AD5206), it is possible to change the output voltage when the photodetector applies no current from -60mV to 0mV. This allows the signal to be offset to light intensity. The circuit is represented in Figure 2.7.

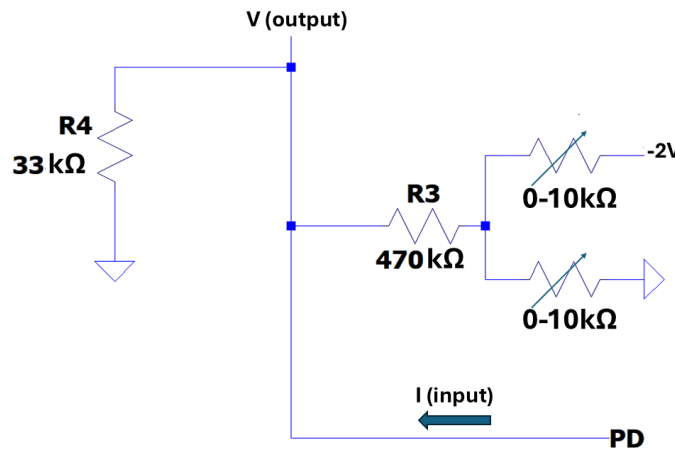


FIGURE 2.7: Circuit diagram responsible for offsetting the photocurrent signal from the photodetector.

The generated voltage is in the order of millivolts and requires amplification to be detected by the ADC, which operates within 0 to 3.3 Volts. The amplification level can be adjusted by a digital potentiometer that is connected to an OPAMP amplification circuit. As before, a capacitor is used to filter high-frequency noise. At the output of the amplification circuit, there are two Zener diodes, one connected to the ground and another to VCC (0V-3.3V), to ensure the output voltage remains within the ADC's operating range (0V-3.3V).

The equation associated with an OPAMP amplification circuit is given by:

$$V_{out} = \left(1 + \frac{R2}{R1}\right) V_{in} \quad (2.3)$$

By changing the value of R1 with the help of the digital potentiometer it is possible to change the gain and, thus, the sensitivity of the photodetector.

This circuit was reproduced strictly for each color channel (red, green, and blue), so each channel was connected to a different ADC, and each color channel was read simultaneously.

The circuit was developed using Eagle software and simulated using LTspice. The simulation mainly consisted of plotting the voltage the ADC received with the current the photodetector received. Different positions of the potentiometer were tested to check if the desired effect was achieved.

Figure 2.8 presents the output voltage as a function of varying current intensities, obtained from the simulation software.

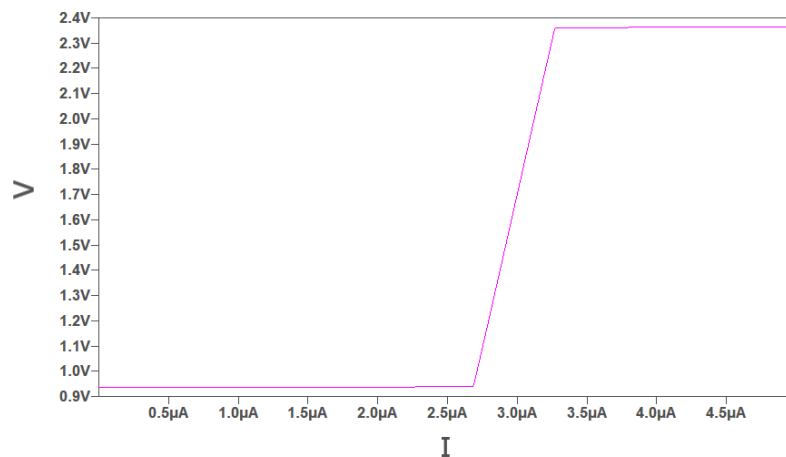


FIGURE 2.8: Graph representing the value of the electric potential received at the ADC relative to the photocurrent generated by the photodiode. Represented with LTspice.

Enclosure

The electronics of the sensor need to be encapsulated in an impermeable case so that the integrity of the electronic systems is preserved. The case was 3D printed, its surface was treated with acetone to impermeabilize the material, and the different components were glued (Figure 2.9).

Some components were specifically designed to be inserted inside the sensor to ensure proper alignment of the optical components. The lenses were not fixed entirely, so they were glued with epoxy (Araldite) to maintain signal stability and alignment.

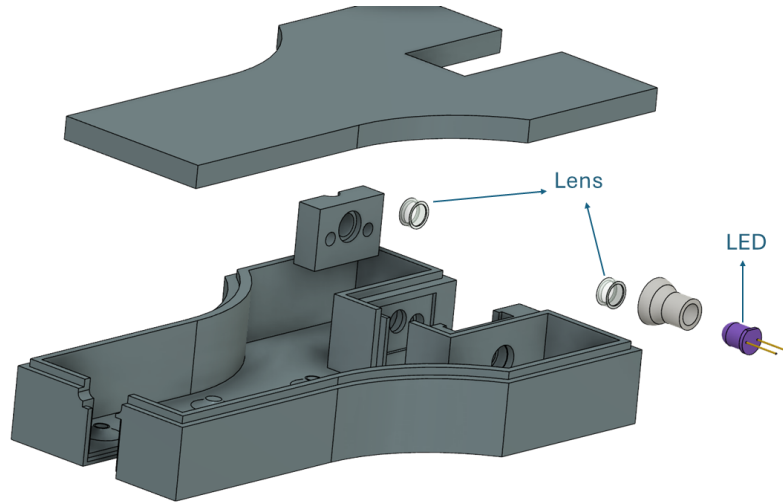


FIGURE 2.9: Image of the 3D-printed sensor enclosure. Developed using Fusion software.

The sensor was designed to be compact, user-friendly, and minimize particle accumulation while allowing for easy insertion and removal of new membranes.

Calibration

The microcontroller on the esp32 development board was programmed to control the digital potentiometers and the LED while transmitting data from the ADC, corresponding to each color channel, to the PC via UART communication through a USB cable.

In simple terms, the program begins by setting the values for each digital potentiometer. Then, the LED is turned on in the main loop, and the ADC data is sent through the serial port.

Six potentiometers were used to calibrate each received signal with its specific amplification settings. This ensures that the ADC's resolution does not limit the sensor's accuracy.

The CO₂ concentration is calculated from the ratio between the Blue and Red signals. To achieve accurate and stable results, two conditions must be met from both signals: they must have appropriate and equal sensitivity to changes in light intensity, and they must operate within the proper light intensity range to prevent the signal from reaching the maximum or minimum of the allowed range.

The calibration for the change in light intensity was done with the help of a microscope glass slide, inserting the piece of glass between the optical path, and observing the

changes in both signals. The resistance values were then adjusted so that the intensity of the signal reduction was similar on both channels. As expected, this was also proportional to the amplitude of the noise detected.

Calibrating the offset is a straightforward process that involves checking whether the signal is received at the range at the lower or maximum end and adjusting the resistance value accordingly. The correct result is typically achieved through trial and error. This calibration is necessary for every different membrane that is inserted.

PC Data Processing

Data processing was done using custom software built in Python. The Pyserial library was used to read and process the data sent from the esp32.

The data processing involves continuously reading the data received line by line in an infinite loop. As the data consists of a string with a value for the red and another for the blue separated by a comma, several processing steps must be implemented. First, a time stamp was appended to each line, which was then written in a text file. Finally, all the data is retrieved from the text file and represented on a continuously updating graph. After dividing the blue signal from the red one, a moving average with a window size of ten points (or about 2 seconds) is applied to smooth out fluctuations.

2.4. Experimental Setup

A controlled environment was achieved using a gas system to evaluate the performance of the sensors or the sensing membrane.

This gas system consists of two gas cylinders, one containing CO₂ and another N₂. Two flow controllers (Brooks, SLA5800 series) were operated to monitor and adjust the gas flow, allowing for precise control of the gas concentration by adjusting the flow ratios of N₂ and CO₂.

The gases from both cylinders were mixed and injected into an intermediary container filled with water to humidify the gas. The humidified gas was then introduced into a final chamber where the sensor was positioned. The setup is represented in Figure 2.10.

The volume of the final chamber used with the RedBlue sensor was 0.34 L while the volume of the chamber used with the White sensor was 1.5 L.

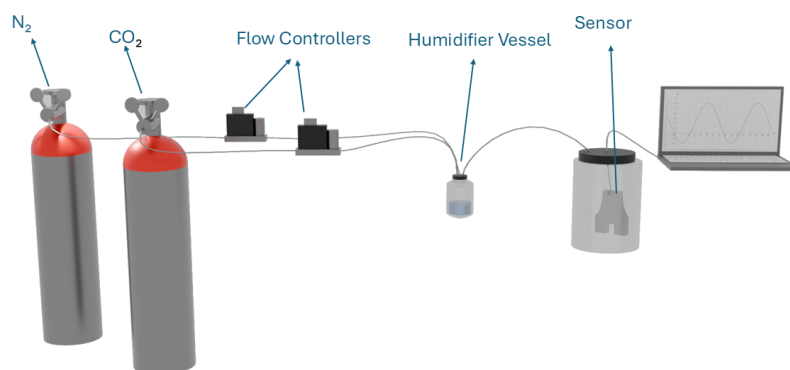


FIGURE 2.10: Figure illustrating the experimental setup used for experiments with injected gas. It consists of two gas bottles, two flow controllers and a humidifier vessel.

A similar setup was implemented to measure dissolved gas. However, this time, the final chamber was filled with deionized water, where the sensor was submerged along with the sensing membrane. The gas was injected into the water, forming gas bubbles to maximize the speed of gas dissolution. Caution was taken to prevent the bubbles from reaching the sensing membrane as it would disrupt the optical path.

2.4.1 Experimental Methods

Considering RedBlue sensor, the total gas flow rate was maintained constant in most experiments at around 700 mL/min, while the flow rate was kept at 1000 mL/min for the White sensor's experiments. This flow rate provided an excellent approximation of the desired concentration.

Temperature and pressure were not rigorously controlled. However, temperature was kept constant at room temperature (around 22 °C) and pressure at around 1 atm.

The experiments were divided into two main phases: cycles and calibration. During the cycles phase, the concentration of CO₂ and N₂ were alternated, typically between 1% CO₂ and 10%. The signal was smoothed using a moving average of 50 points, equivalent to 10 seconds. In the calibration phase the concentration of CO₂ was adjusted in small steps, most often from 0.75% to 5.25% in intervals of 0.5%. The time interval between each concentration change was fixed, and it only depended on how long it would take for the signal to stabilize.

The signal level for each concentration was determined by calculating the average from the latest 300 points (or one minute) before the subsequent concentration adjustment.

2.4.2 Henry's Law

In most experiments, the desired concentration of CO₂ was injected into a chamber. In gas environments, the concentration applied is straightforward and given by a percentage of CO₂ present compared with other gases in volume (in this case N₂). However, when it comes to water environments, the concentration of CO₂ dissolved in water has to be calculated from the partial pressure of CO₂ in the chamber. One method to do it is by applying the Henry's Law (Equation 2.4):

$$C_s = HP \quad (2.4)$$

The concentration of dissolved CO₂, C_s , is given by the product of Henry's constant, H , for CO₂ and the partial pressure, P of this gas in the chamber.

In the case of CO₂ in water environments, the Henry's constant is equal to $3.4 \times 10^{-2} \text{ mol}(L.atm)^{-1}$ at 298K [46]. Assuming that the gases in the chamber are at atmospheric pressure, it is easy to calculate CO₂ concentration in water. Table 2.1 presents the conversion of %CO₂ in milligrams per liter (mg/L) units of dissolved CO₂ in water. Intermediate values can be calculated through linear interpolation.

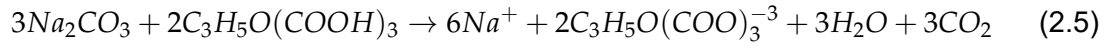
TABLE 2.1: Concentration of CO₂

Gas %	Water mg/L	Water ppm
0.05	0.75	0.31
0.1	1.5	0.61
0.5	7.5	3.1
1	15	6.1
10	150	61

2.4.3 Sodium Carbonate

While, in theory, it should be possible to calculate the dissolved CO₂ concentration using Henry's Law, it is not the most reliable method.

For this reason, an alternative experiment using sodium carbonate (Na₂CO₃) in acid media was conducted. First, a proper amount of citric acid (C₃H₅O(COOH)₃) was dissolved in water, and then precise volumes of Na₂CO₃ were progressively added to the main solution to achieve the desired CO₂ concentration. The corresponding chemical reaction is shown in Equation 2.5:



Following Equation 2.5, its stoichiometry shows that one mole of carbon dioxide forms for each mole of sodium carbonate. Therefore, as the molecular weight (Mw) ratio between both is approximately 2.4 (M_{w(Na₂CO₃)} = 106 g/mol; M_{w(CO₂)} = 44 g/mol), for each 1 mg/L of Na₂CO₃, 0.42 mg/L of CO₂ is formed. A magnetic stirrer (PCE-MSR 300) accelerates and homogenizes both liquids in the solution, considerably shortening the experiment's duration.

3. Experimental Section

This chapter presents the most significant and conclusive experiments, analyzed, and discussed.

3.1. Gas Experiments with RedBlue Sensor

Although the main goal of this project was to develop a CO₂ sensor in an aquatic environment, initial experiments were conducted in a gas environment to optimize the CO₂-sensitive membrane. Since the working principle is consistent across both environments, these preliminary experiments were run to evaluate and enhance the membrane's performance, focusing on aspects, such as sensitivity and response time.

3.1.1 Optimizing Concentration of Reagents

In this experiment, three different membranes were produced and tested. Table 3.1 exhibits the reagents and quantities used for their production with the molar ratio TOA-OH/pNPh varying across membranes M1 to M3.

TABLE 3.1: Membrane's Cocktail Composition

Comp./Memb.	M1	M2	M3
pNPh	6 mg	9 mg	6 mg
TOA-OH	500 μ L	500 μ L	750 μ L
Hydrogel	500 μ L	500 μ L	500 μ L
R TOA-OH/pNPh	19	12	30

Figure 3.1 compares the three membranes' performance, yielding similar results overall. However, a trend can be observed where the performance of M2 is higher than M1 which in turn is greater than M3. This is because M2 appears to have a higher amplitude signal, and since the noise intensity remains constant across all tests, it can be concluded that this membrane offers the highest signal quality. The signal increase over time can be explained by cross-sensitivity to humidity, which tends to increase as the injected gas is humidified.

The three linear regressions are represented in 3.2. The trend was mostly linear, although the M1 and M3 graphs showed some curvature.

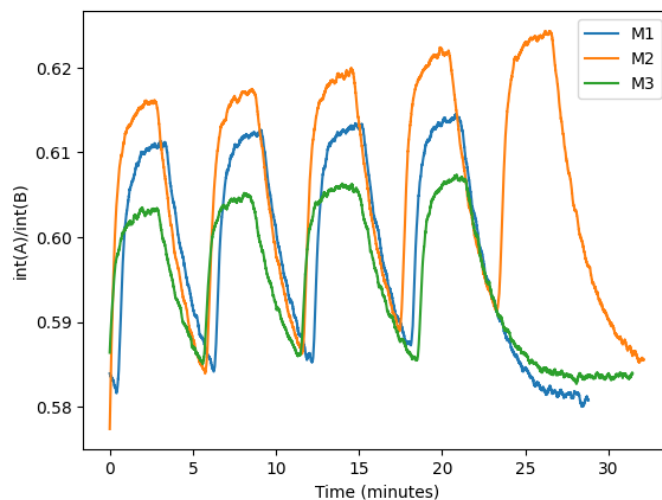


FIGURE 3.1: Graph of CO₂ cycles with membranes of different concentrations

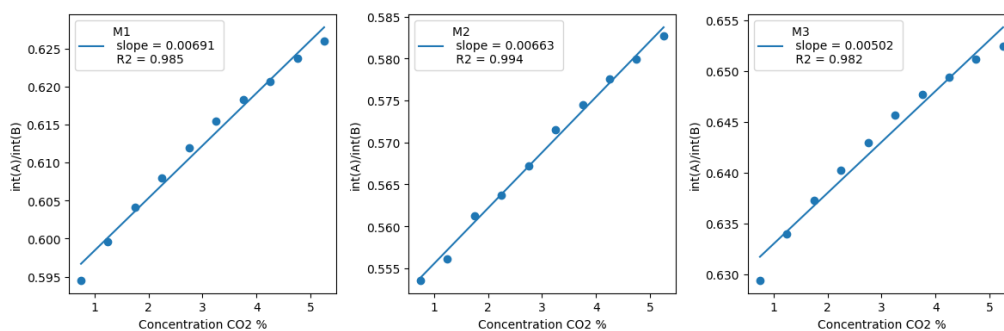


FIGURE 3.2: Linear Regression of Calibration data for each membrane

The calibration process measures the membrane's sensitivity, which is determined by the slope obtained from the linear regression. By comparing the slopes obtained for the different linear regressions, it is possible to rank the different membranes.

This results show that membrane M2 might be more sensitive. Although the molar ratio observed for membranes M1 and M3 is higher, this parameter seems inconsequential to the membrane's performance. Therefore, understanding the chemistry behind the working principle, such as ensuring that the amount of TOA-OH is sufficient to react with all pNPh, it is the concentration of the latter that dictates the control over the sensing performance. Consequently, additional experimental procedures were conducted to investigate the impact of varying the pNPh concentration in the membrane matrix, while maintaining the concentration of TOA-OH.

Optimization of P-Nitrophenol Concentration

In order to evaluate the impact of the colorimetric indicator on membrane quality, three membranes were tested, varying only the amount of pNPh indicator; the other reagents were kept constant: 500 µL of TOA-OH and 500 µL of Hydrogel. The concentrations of pNPh in the membranes were: 5mg/mL, 10mg/mL and 15 mg/mL.

The performance of the three membranes over several cycles is represented in Figure 3.3.

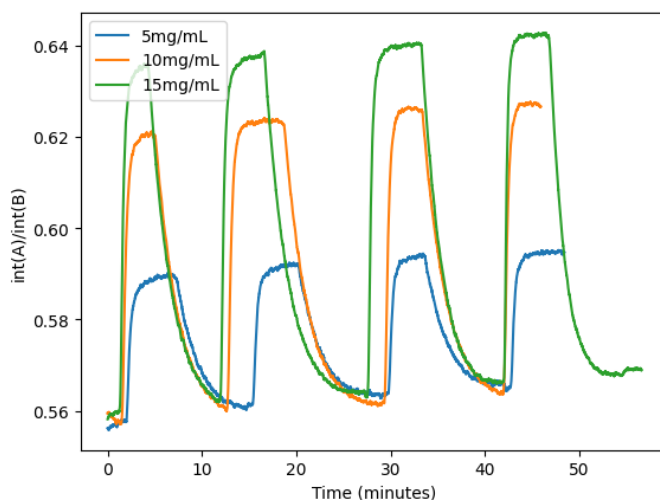


FIGURE 3.3: Cycles of 1% to 10% CO₂ for different concentrations of p-nitrophenol

Observing Figure 3.3, it is easy to see that an increase in pNPh concentration is correlated with an increase in the signal's amplitude, thus an increase in sensitivity.

In order to increase the statistical significance of these results, it was decided to replicate each calibration phase three times for each recipe. Note that all the replicates were from different membranes that followed the same fabrication process.

The data analysis consisted of doing a linear regression for each calibration process ($n = 3$). Moreover, the data was normalized so that the linear regression for each replicate had the same intercept. Finally, the mean and standard deviation of each data point was calculated. The data was normalized to eliminate the signal drift caused by environmental factors such as temperature or lightning at the measurement time. The linear regression for each membrane is represented in Figure 3.4.

Figure 3.5 shows with statistical significance that the concentration of pNPh is positively correlated with increased sensitivity within the range of 5mg/mL to 15mg/mL. The sensitivity of the membrane with 15mg/mL is approximately two times higher than that of the

5mg/mL membrane.

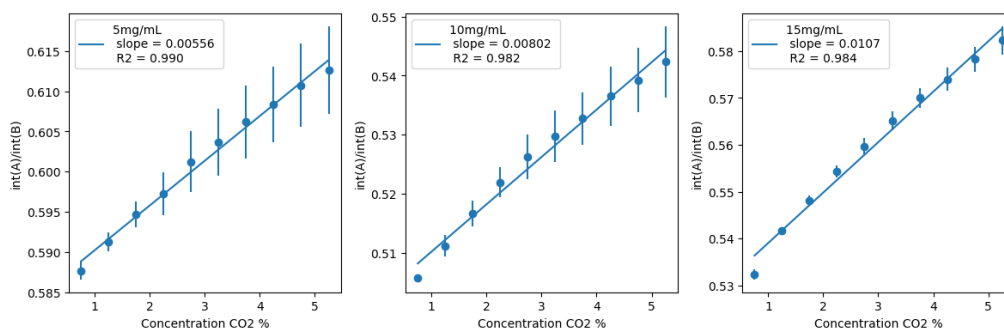


FIGURE 3.4: Linear Regression of Calibration data for the three membranes. Each calibration phase was conducted three times for each concentration of pNPh.

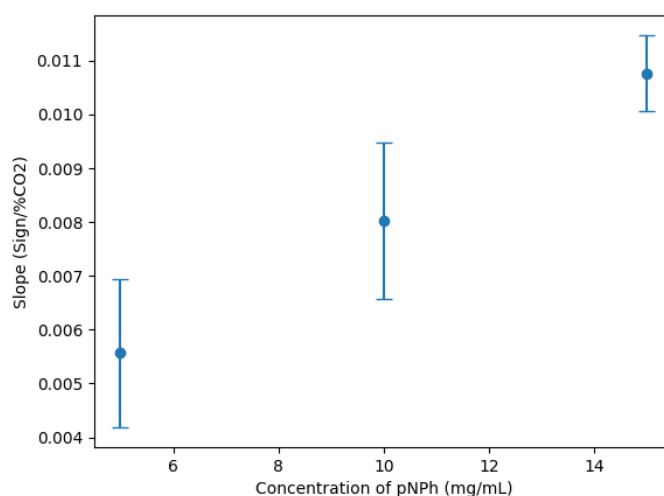


FIGURE 3.5: Membrane sensitivity as a function of pNPh concentration. Each concentration of pNPh was tested three times. The relative standard deviations for 5 mg/mL, 10 mg/mL, and 15 mg/mL are 24%, 18%, and 6%, respectively.

Table 3.2 resumes the results obtained from this experiment. There is reason to believe that higher concentrations of pNPh might reach higher sensitivities. Therefore, further experiments increasing the pNPh concentration were conducted (data not shown) to determine the limit of this sensitivity extension. However, after testing a concentration of 20 mg/mL, it became apparent that the response was similar to the one obtained with 15 mg/mL. This can be explained by the fact that there was insufficient TOA-OH to activate all the pNPh in the solution. From this point, this membrane was used for further investigations.

3.1.2 Ionic Liquids Effect

Certain ionic liquids are known to enhance CO₂ solubility, suggesting they may also increase CO₂ solubility within the membrane matrix and enhance system conductivity [47].

TABLE 3.2: Membrane's Sensitivity and pNPh Concentration

Concentration of pNPh (mg/mL)	Slope of Calibration Process
5	0.00556
10	0.00802
15	0.0108

With this in mind, two different membranes were tested: the original membrane, composed of 5mg of pNPh, 500μL of TOA-OH, 50μL of water, and 950μL of Hydrogel; and a modified version which included 50μL of BminBf4 (1-Butyl-3-methylimidazolium tetrafluoroborate).

Due to technical limitations at the time of this experiment, the experimental procedure was different: in the cycles phase the concentration was alternated from 100% CO₂ and 100% N₂ and during the calibration phase the concentrations of CO₂ applied were 5%, 10%, 15%, 20% and 25%. The signal obtained for the cycles phase is represented in Figure 3.6.

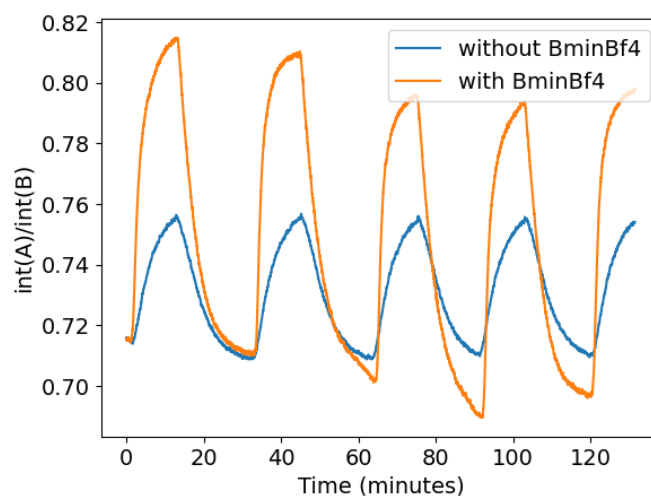


FIGURE 3.6: Signal for the cycles of CO₂ between 0% and 100% with and without BminBf4

This graph shows that for the membrane with BminBf4 the variation in response with concentration is significantly greater, with a signal amplitude approximately 2.7 times greater.

The linear regression obtained from the calibration phase is represented in Figure 3.7.

The calibration phase corroborates the results obtained on the cycles phase, thus confirming that the membrane with BminBf4 was more than two times more sensitive.

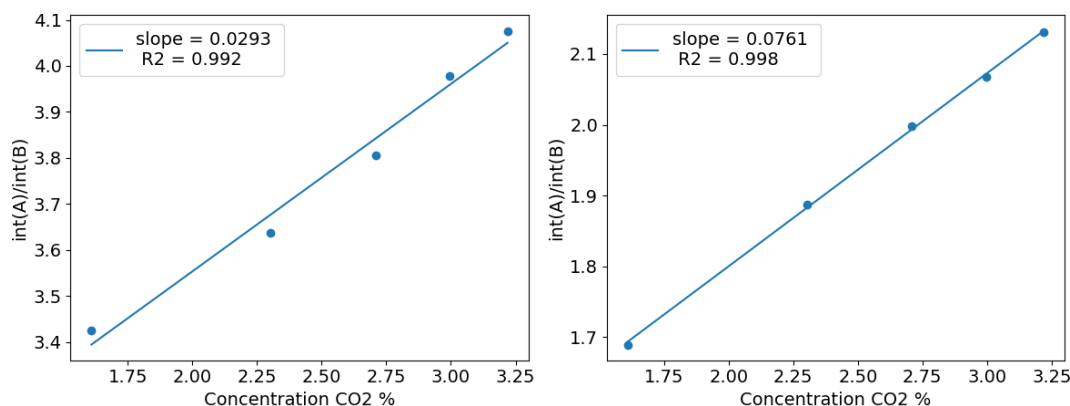


FIGURE 3.7: Linear regression of calibration data for the membranes with and without BminBf₄

It is also worth mentioning that the linear behavior was detected on the logarithm of the concentration, which contrasts what was obtained previously. The higher CO₂ concentration used on this experiment and the different range of concentrations measured do not invalidate the important conclusion of this experiment: the increased sensitivity of the membrane with BminBf₄.

The final results can be summarized in the following table 3.3.

TABLE 3.3: Membrane's Sensitivity and BminBf₄

Conclusion Table	Normal Membrane	Membrane BminBf ₄	Ratio M2/M1
Max amplitude	0.051	0.14	2.7
Sensitivity (slope)	0.029	0.076	2.6

After this positive result was achieved, repeated attempts to replicate these results were unsuccessful, likely due to the difficulty of mixing BminBf₄ with TOA-OH. Many strategies were attempted, including changing the concentration of TOA-OH and BminBf₄, adding water, and extending the mixing time. Several other Ionic liquids were tested to find a more compatible alternative to BminBf₄.

With this in mind, several membrane recipes were tested by adding 50 µL of different ionic liquids to the previously tested 15mg/mL pNPh membrane. The various ionic liquids are identified in Table 3.4.

The calibration process was also conducted for each of the recipes. Only Figure 3.8, which compares the sensitivity (corresponding to the linear regression slope), is displayed for simplicity.

TABLE 3.4: Number Designation and Chemical Names

Number Designation	Chemical Name
IL08	1-Butyl-1-methylpyrrolidinium tris(pentafluoroethyl)trifluorophosphate
IL00	1-Ethyl-3-methylimidazolium tris(pentafluoroethyl)trifluorophosphate
IL23	1-Butyl-3-methylimidazolium tris(pentafluoroethyl)trifluorophosphate
IL09	1-Butyl-3-methylimidazolium bis(trifluoromethylsulfonyl)-imid

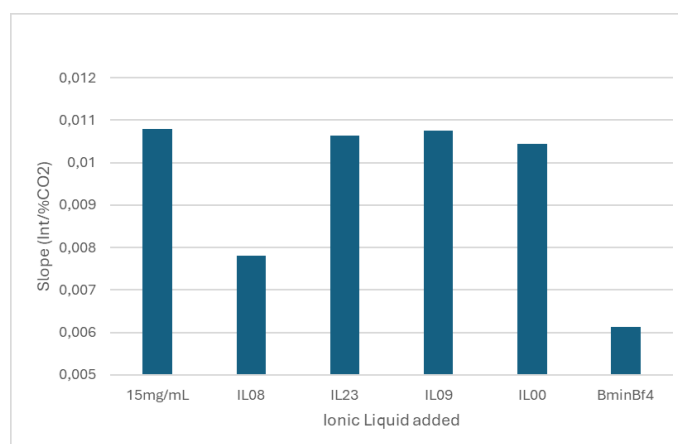


FIGURE 3.8: Comparison of the sensitivity of the calibration regression obtained for membranes with different ionic liquids. Repeated only once for each ionic liquid.

It can be concluded from these results that these ionic liquids (at least on the concentrations tested) did not improve the quality of the membrane. An exception might be raised for those that did not dissolve into the cocktail, such as the BminBf₄, as good results could have been achieved had the chemical dissolved well with this cocktail. The ionic liquid can likely be dissolved into the cocktail by adjusting the concentration of the reagents.

These results indicate that further research is needed before ionic liquids can be integrated into the membrane fabrication process. While BminBf₄ has demonstrated increased membrane sensitivity, the conditions under which this occurs remain unclear.

3.1.3 Experimenting with Low CO₂ Concentrations

This experiment used a process similar to the previously explained calibration phase, consisting of 11 steps ranging from 500ppm to 1500ppm with a constant gas flow of

1000mL/min with the default 15mg/mL of pNPh membrane. The results are shown in Figure 3.9. It can be seen that this sensor is unable to detect CO₂ concentrations below 700ppm.

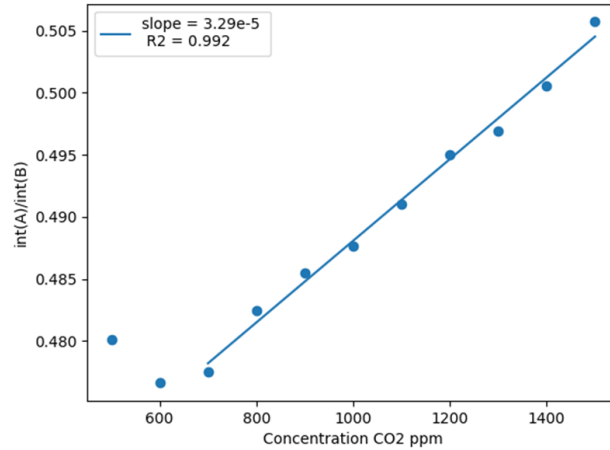


FIGURE 3.9: Linear regression analysis of calibration data for low CO₂ concentrations (500–1500 ppm)

In order to test the applicability of these results in a real scenario, a repeated experiment was conducted which consisted of injecting specific CO₂ concentrations in the range from 500ppm to 1500ppm. The same membrane was used and was stored for four days at around 5°C.

The linear equation obtained from the previous calibration was compared with the data obtained from the second experiment. An offset had to be applied so that the data would coincide with the linear equation (Figure 3.10).

As shown in Figure 3.10, starting at 900ppm, the predicted concentration seems to follow the expected very well. However, under this concentration, the sensor cannot produce reliable results.

These results allow some important conclusions to be taken. First, it is possible to reliably measure CO₂ with the same membrane from an older calibration at concentrations above 900ppm. Second, it is necessary to do at least a single-point calibration in order to account for the signal offset.

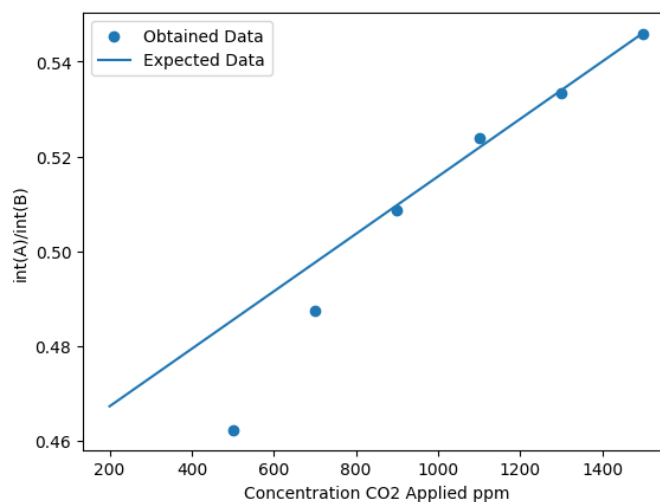


FIGURE 3.10: Predicted signal from previous calibration compared to obtained signal after applying an offset

3.2. Dissolved CO₂ Experiments with RedBlue Sensor

This section presents experiments conducted in a water environment to measure dissolved CO₂. All the experiments were conducted with the default membrane of 15mg/mL of pNPh, developed previously.

The primary objective of this section is to assess the system's reliability in detecting and measuring dissolved CO₂ while also comparing the sensor's performance in water with its performance in gas.

The same CO₂ concentrations were used to maintain consistency for air comparison and to simplify the process.

The signal obtained during the phase of the cycles is represented in Figure 3.11.

It is noticeable that the cycles take significantly longer when performed in water than in a gaseous environment due to the time that the gas takes to dissolve into the water.

On the other hand, the signal amplitude is significantly higher in water. This phenomenon is due to the higher refractive index of water, which significantly reduces the angle of light transmission when changing the medium according to Snell's law.

It is also important to note that the signal is quite unstable, showing significant variation even when the same concentration is applied, which is undesirable. The cause of this instability can be found when representing both red and blue signals independently (Figure 3.12).

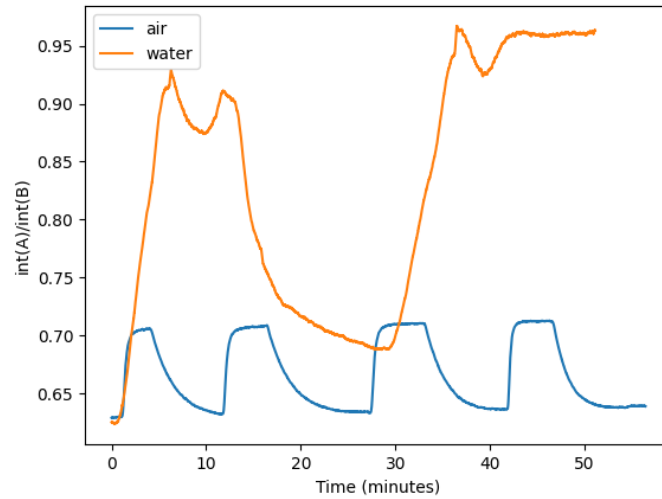


FIGURE 3.11: Comparison of sensor's signal in gas and in water (cycles phase)

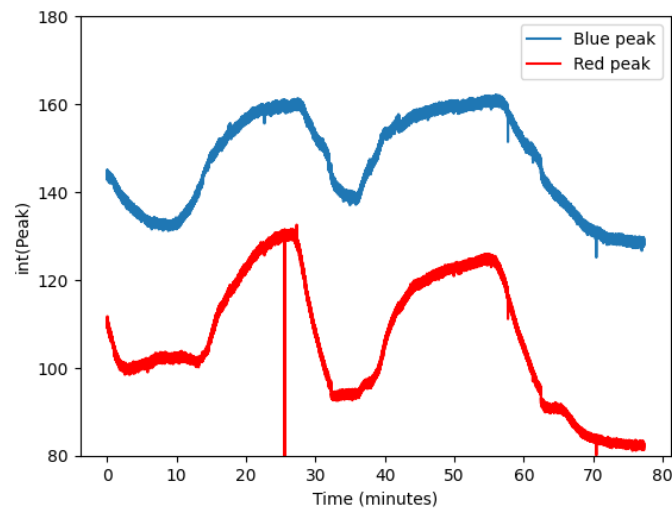


FIGURE 3.12: Graph of the unprocessed signal data for both blue and red in water (cycles phase).

In Figure 3.12, it is clear that the red signal is responding to CO₂ concentration, unlike what is happening in Figure 3.13, which contrasts with what should happen in theory. Since pNPh does not absorb red wavelength, it should not be able to detect CO₂. Multiple membranes were developed and experimented, displaying the same behavior.

Despite the unpredictable results obtained in the phase of the cycles, the calibration phase was still conducted in water (Figure 3.14).

Observing Figure 3.14, it can be concluded that calibration in water was still possible despite the difficulties. Even though the sensitivity is higher, the data obtained seems more unstable in water than gas. This is expected considering the results obtained during

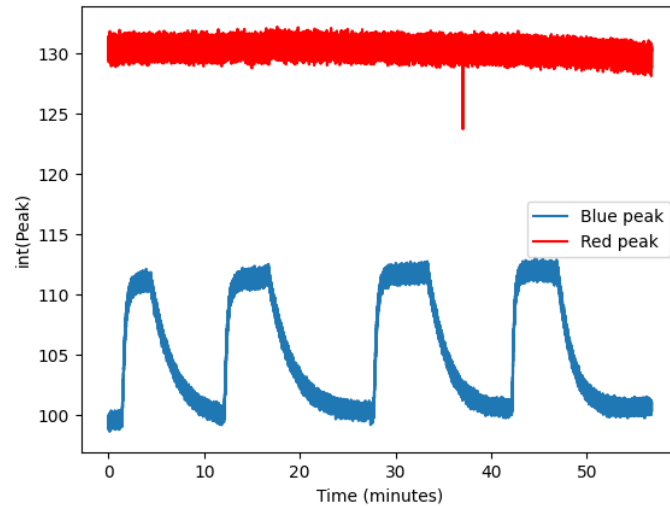


FIGURE 3.13: Graph of the unprocessed signal data for both blue and red in gas (cycles phase).

the phase of the cycles.

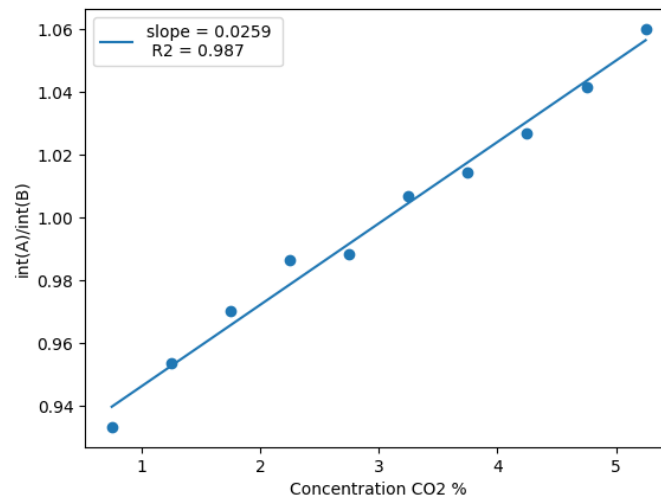


FIGURE 3.14: Linear Regression obtained from calibration data in gas.

3.3. Gaseous CO₂ Experiments with White Sensor

Since the RedBlue sensor presented significant limitations in the dissolved CO₂ experiments, a new sensor was developed to overcome the problems associated with the previous sensor.

Before testing the sensor in water, this sensor was first experimented in gas. In some of these trials, both sensors were placed inside the chamber simultaneously, allowing for a direct comparison in performance between the two. This also served to understand

whether the different behavior of the White sensor was related to the different experimental setup or if it was a characteristic of the sensor itself.

With this objective, the cycle phase was performed with both sensors conducted for both sensors simultaneously. Each sensor was equipped with a sensitive membrane produced from the same cocktail and spin-coating process. Both sensors were thus inserted into the same chamber, where gas with the desired concentration was injected, following the standard procedure.

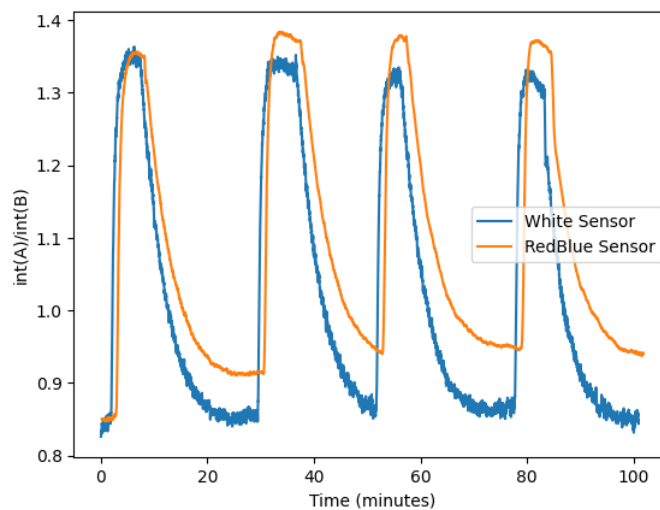


FIGURE 3.15: Comparison of the signal obtained from both sensors in gas (cycles phase). Both sensors were operated simultaneously within the same chamber.

As seen in Figure 3.15, it can be concluded that the White sensor can detect gaseous CO₂, albeit with slightly more noise. Although the White sensor is considerably noisier, it proved to be more stable than the RedBlue sensor as the signal did not deviate from its original level. Most likely, this is related to the RedBlue sensor's optical system, which relies on a multimode optical fiber, on which slight changes in the curvature and position of this fiber can cause signal deviation. Since all optical components of the white sensor are fixed in place, this deviation never occurs, even if this sensor is moved around.

The calibration phase was conducted with only the White sensor as there would not be any significant benefit in conducting it with both sensors at the same time.

Figure 3.16 shows the calibration data obtained from the cycles phase with the White sensor. Similarly to the RedBlue sensor, the trend observed is linear. However, this sensor shows significantly more signal instability, which is probably related to the higher noise characteristic of this sensor.

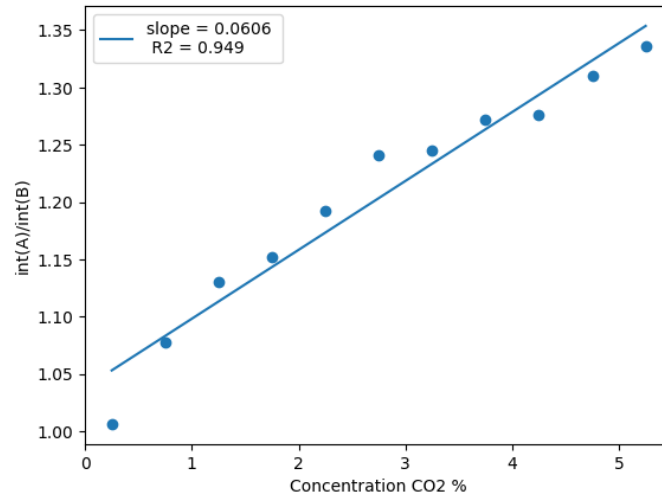


FIGURE 3.16: Linear regression obtained from calibration phase with White sensor in gas

It is hard to know the source of the increased noise observed in this sensor since the source cannot be the membrane as both sensors were equipped with similar membranes. It is most likely related to optical sources. It could be that the white LED implemented on the White sensor is slightly noisier than the RGB LED implemented in the RedBlue sensor. However, the most probable cause is the photodetector. Since the RedBlue sensor uses a bulky and expensive spectrometer composed of multiple photodiodes, it should have less noise than the White sensor, which employs only two (one for each color channel).

After this calibration process, the next logical step was to assess the capability of this sensor to detect low concentrations of CO₂. This way, a calibration phase with concentrations of hundreds of ppm was conducted.

As shown in Figure 3.17, the sensor does not provide a clear response below 1500 ppm. The initial rise observed below 1500 ppm was most likely due to cross-sensitivity to humidity. Starting from 1500ppm, the sensor gives a linear response with increasing concentrations of CO₂.

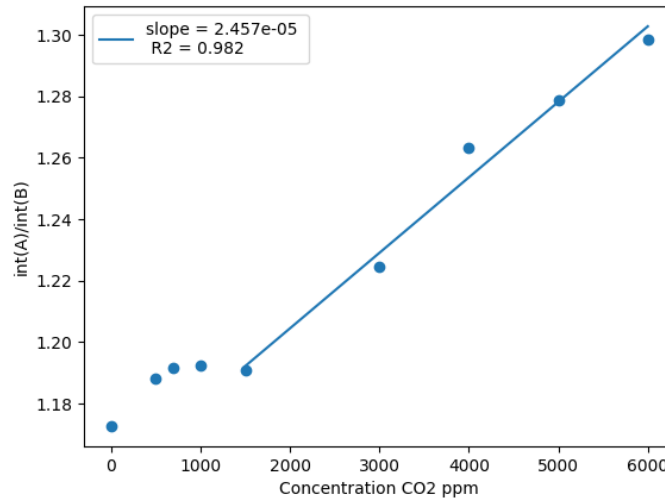


FIGURE 3.17: Linear regression obtained from calibration phase with White sensor for low CO₂ concentrations in gas

3.4. Dissolved CO₂ experiments with White Sensor

Experiments with White sensor in water followed a similar protocol. However, operating both sensors simultaneously in the same chamber was impossible. The RedBlue sensor's LED emission interfered with the signal detected by the White sensor. This was most likely due to internal reflections in the water where both sensors were submerged.

Figure 3.18 represents the signal detected by the White sensor in water during the cycles phase. In contrast with the detected signal by the RedBlue sensor in Figure 3.11, this signal shows great stability and accuracy.

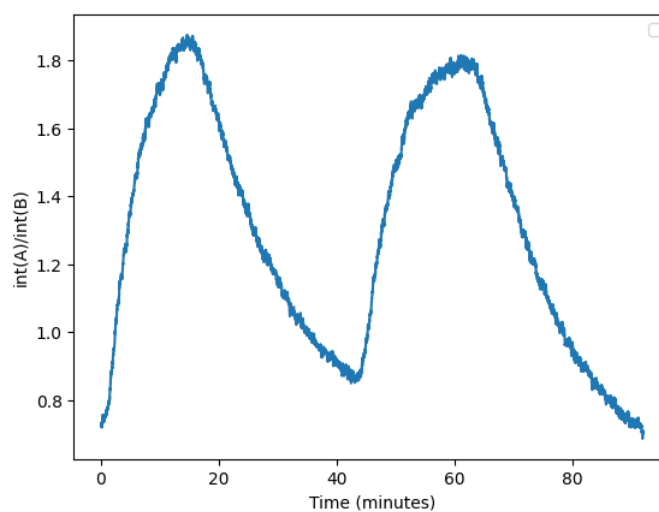


FIGURE 3.18: Signal obtained from cycles phase with White sensor in water

As shown in Figure 3.19, it can be concluded that this sensor does not possess the same

limitations as the RedBlue sensor. As was seen previously, the RedBlue sensor shows CO₂ detection on the red signal, which is supposed to work as a reference. This confirms the hypothesis that this behavior arises from the RedBlue sensor's optical system, likely due to coupling effects between the fiber and its environment.

After confirming the success of the cycles phase, the calibration phase was conducted following standard procedure.

Unlike previous experiments, Figure 3.20 shows that signal response does not follow a linear fit. However, by taking the logarithm of the concentration of CO₂ an excellent linear approximation can be achieved with an R² = 0.994 as seen in Figure 3.21.

Note that the concentrations on the x-axis refer to the concentration of dissolved CO₂ in water calculated with Henry's law and not the injected gas concentration. Still, the injected concentrations were the same as the ones applied during calibration in gas.

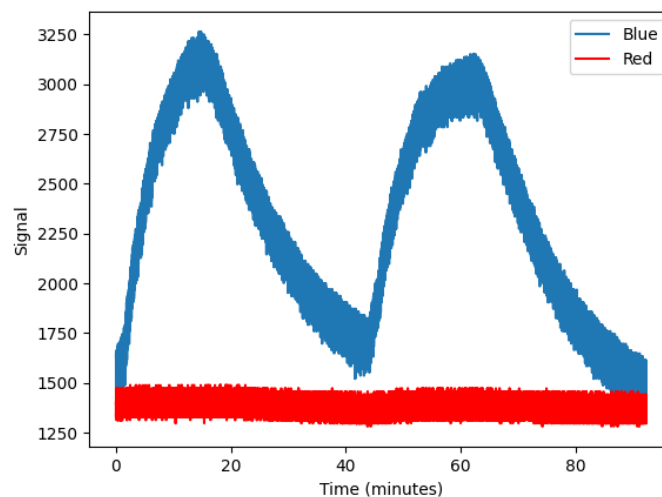


FIGURE 3.19: Graph of the unprocessed signal data for both blue and red with White sensor in water (cycles phase).

In addition, the calibration phase in water is significantly more stable and accurate than in gas, confirming this sensor's increased capabilities for dissolved CO₂ measurements.

A calibration phase with low CO₂ concentration was conducted to test the limits of the White sensor, followed by a procedure similar to the one conducted in gas.

Figure 3.22 gives insight into the limit of detection of this sensor. As can be seen, the sensor cannot detect CO₂ under the concentration of 1mg/L, even if the accuracy is lower.

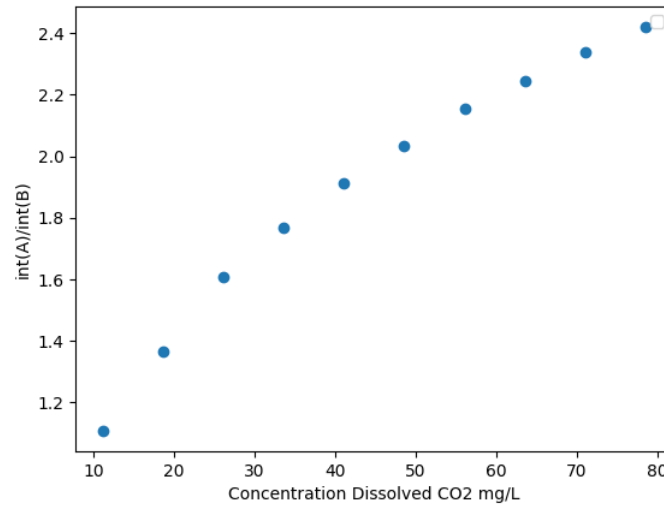


FIGURE 3.20: Data obtained from the calibration phase in water with the White sensor

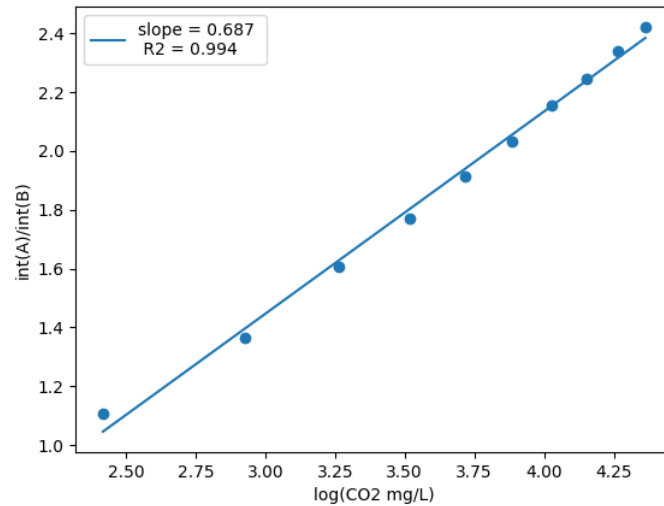


FIGURE 3.21: Linear regression applied to calibration data after calculating the logarithm of CO₂ concentration

The source of this threshold is unclear. It could be due to the chemistry of the membrane or that the CO₂ gas cannot dissolve in the water at concentrations under 1mg/L.

As shown in Figure 3.23, a good linear regression was possible by taking the logarithm of the concentration.

To properly evaluate the performance of this sensor, the degasification time needs to be measured.

With this in mind, an experiment was conducted, which consisted of submerging the sensor in water with a CO₂ concentration of 75mg/L. Afterward, the sensor was quickly removed from the chamber and submerged in another container with water at ambient CO₂

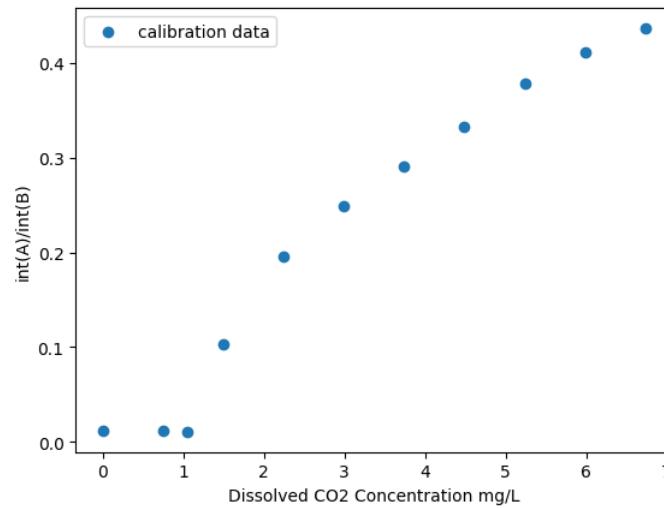


FIGURE 3.22: Data obtained during the calibration phase with White sensor for low concentrations of CO₂

concentration.

Figure 3.24 shows the signal response of the White sensor when it is moved from a concentration of 75mg/L to 0.74mg/L. With this information it is possible to conclude that the degasification time of this sensor is around six minutes for a concentration change of 75mg/L to 0.74mg/L. Note that this value includes the extra time due to the moving average calculation of 20s.

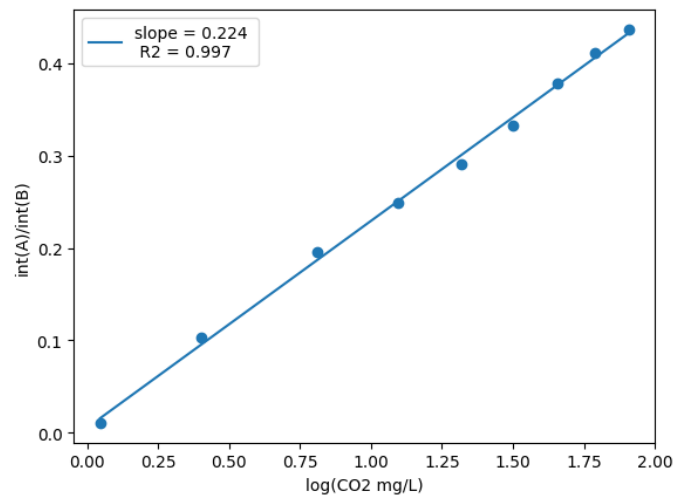


FIGURE 3.23: Linear regression obtained from calibration phase with White sensor for low CO₂ concentrations in water

The gap in data observed in Figure 3.24, is due to the removal of the sensor from the water environment which causes the signal to drop below the threshold set by the digital potentiometers.

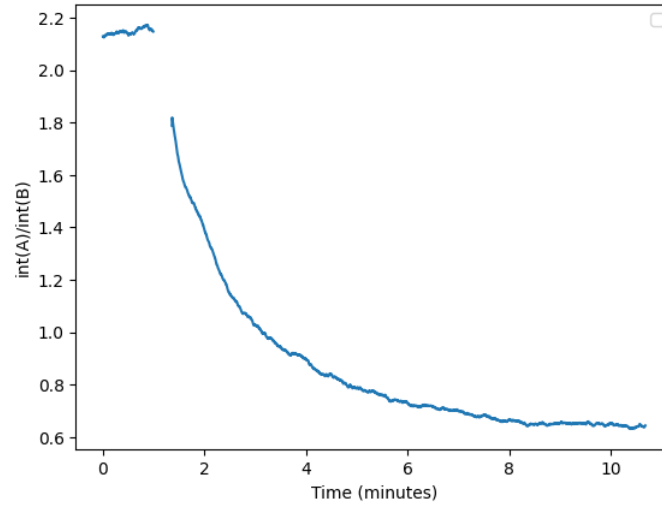


FIGURE 3.24: Sensor's signal response when CO₂ concentration is quickly switched with an applied moving average of 100 points or 20s

3.5. Overall Discussion

3.5.1 Calibration Curve

The signal response detected by both sensors is not linear with CO₂ concentration. However, within a small concentration range, a simple linear regression is an excellent approximation of the actual ideal calibration. When a linear calibration does not fit the data well, a linear regression with the logarithm of CO₂ concentration is found to be adequate. As such, similar sensors found in the literature confirm this hypothesis [48–50]. However, the linear regression does not fit wider measurement ranges. This phenomenon is evident when comparing the slope obtained in Figure 3.20 with the slope in Figure 3.23. In order to achieve the best results and allow the sensor to function at a wide concentration range, a proper calibration curve is required.

The proper calibration curve formula can be obtained theoretically [50]:

$$a \times C_{CO_2} + b = \frac{Abs(C_{CO_2} = 0) - Abs(C_{CO_2})}{Abs(C_{CO_2}) - Abs(C_{CO_2} = 100\%)} \quad (3.1)$$

Equation 3.1 shows how the concentration of CO₂ (C_{CO_2}) is related to the absorbance of the membrane. Since $Abs(C_{CO_2} = 0)$ and $Abs(C_{CO_2} = 100\%)$ are constants, this equation can be reorganized as follows 3.2:

$$Abs(C_{CO_2}) = \frac{a + b \times C_{CO_2}}{c \times C_{CO_2} + d} \quad (3.2)$$

$$Abs(C_{CO_2}) = \frac{aC_{CO_2} + b}{C_{CO_2} + c} \quad (3.3)$$

This way, equation 3.3 gives a curve that relates membrane absorbance with CO₂ concentration. However, the signal measured by the sensor is proportional to transmittance and not absorbance. Conversion can be applied by considering $Abs = -\log_{10}(Tra)$. Thus, the final calibration curve should be given by the following equation:

$$Signal = 10^{\frac{aC_{CO_2} + b}{C_{CO_2} + c}} \quad (3.4)$$

where a, b, and c are the calibration curve parameters.

Figure 3.25 represents the fit of the data obtained with the White sensor in water following equation 3.4.

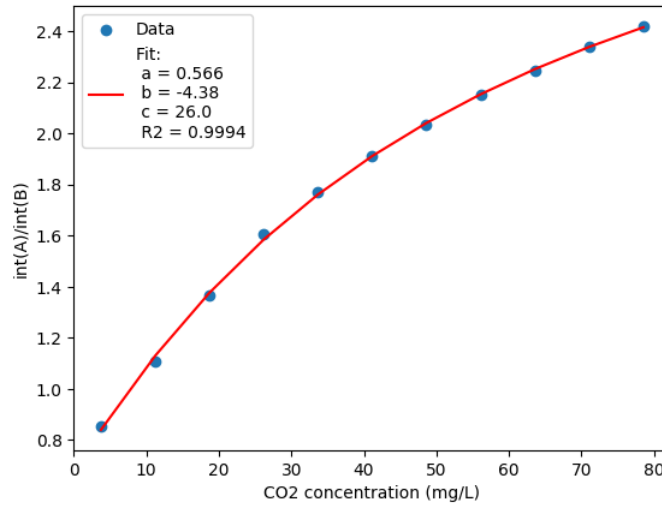


FIGURE 3.25: Curve fitting obtained from calibration phase with White sensor in water

The high R^2 score indicates that this is the correct equation that fits the data, thus confirming the theoretical deduction.

By applying this formula to the calibration phase conducted with low CO₂ concentration, figure 3.26 is obtained.

Once again, this fit gives a high R^2 score and could fit the data for concentrations as low as 1.5mg/L.

Resorting to this calibration curve instead of a linear regression allows the sensor to work

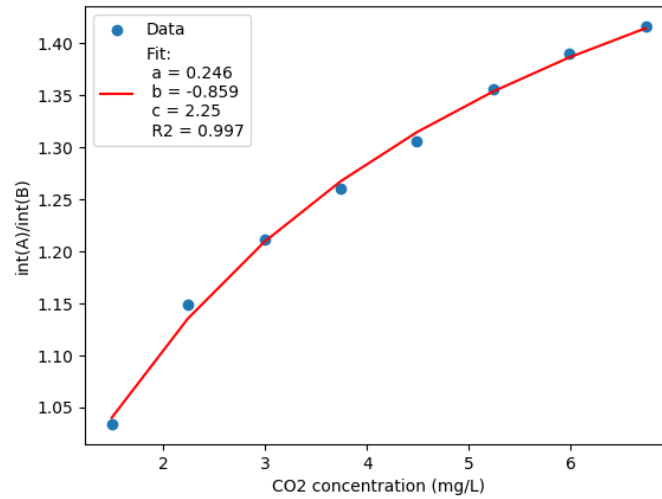


FIGURE 3.26: Curve fitting obtained from calibration phase with White sensor in water for low CO₂ concentrations

at a higher range of concentrations with high accuracy, especially for low CO₂ concentrations. However, this comes with the disadvantage of requiring at least three points for calibration.

3.5.2 Henry's Law Evaluation

To evaluate the reliability of the results obtained with the Henry's Law, another calibration of dissolved CO₂ was conducted with sodium carbonate (Figure 3.27).

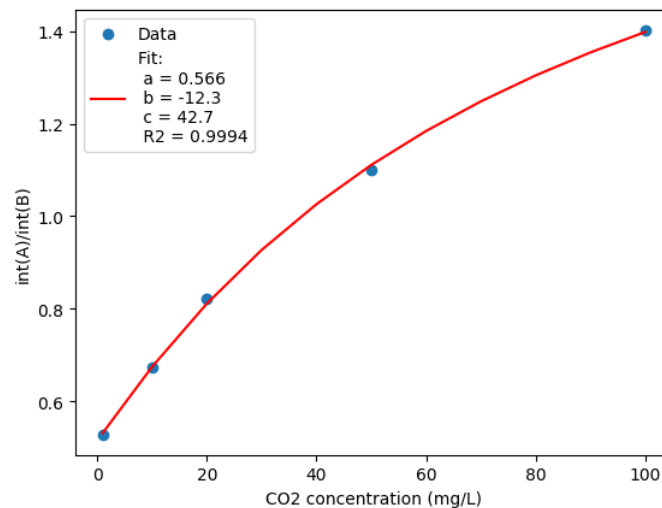


FIGURE 3.27: Curve fitting of calibration data obtained with sodium carbonate experiment with the White sensor

As expected, the data follows the same equation curve that was observed in the previous experiments conducted with gas injection.

In order to properly compare the obtained calibration curves, both of them are represented in Figure 3.28.

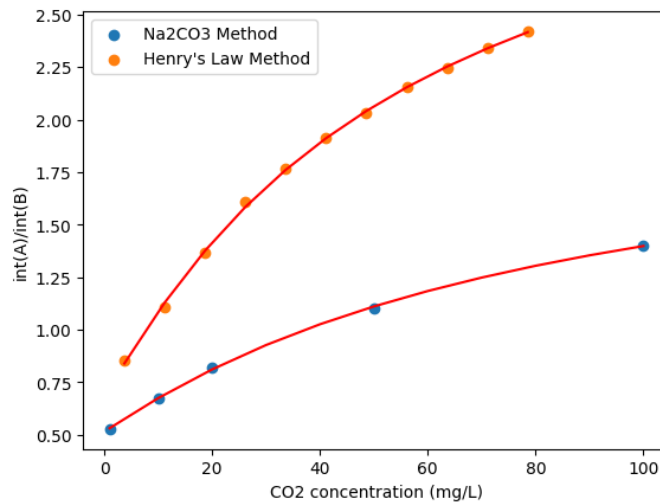


FIGURE 3.28: Comparison of the calibration curve obtained with Henry's Law method with Na₂CO₃ method after adjusting the signal offset.

Since there is a high degree of confidence with the data obtained from the sodium carbonate solution, it can be concluded that Henry's law calculation is unreliable. It is unclear why Henry's constant does not correspond to the one found in literature. A reasonable explanation might be related to the special conditions in which the experiment is conducted. For example, gas is injected into the water in this experimental setup. However, this law generally applies to natural diffusion on the water-gas interface. Besides, this law is not very established in the scientific community.

3.5.3 Algae Tank Measurement

Considering the previously presented results, it is now possible to obtain dissolved CO₂ measurements from an algae tank that is present in the lab.

A small 300mL sample was removed from the tank and filtered. Afterward, the sensor was submerged in the sample and the signal was measured after signal stabilization. Subsequently, N₂ gas was injected into the water sample, thus removing the CO₂ dissolved in the water and allowing the measurement of a reference signal in order to account for external factors such as salinity and turbidity which induce an offset at the sensor's signal.

With the calibration curve obtained in Figure 3.27, the CO₂ concentration was calculated at 8.4mg/L following Equation 3.5:

$$C_{CO_2} = \frac{b - \log_{10}(S)c}{\log_{10}(S) - a} \quad (3.5)$$

where S is the signal detected and C_{CO_2} is the calculated concentration of CO₂.

The concentration was measured previously with a commercial sensor (OxyGuard[®] CO₂ Portable), displaying 8mg/L. This result provides experimental evidence of the sensor's effectiveness in real-world applications, as it accurately measured CO₂ concentration despite external factors like temperature, turbidity, and salinity, commonly encountered outside the lab. In theory, it should be possible to account for these variables without requiring a reference signal. However, further research is needed for a better understanding.

3.5.4 Limit of Detection

The standard deviation associated with the signal response is stable throughout all concentration levels. With this in mind, the signal's standard deviation was calculated with data from 20 minutes in blank state (0% CO₂ concentration) after applying a 100 point (or 20 seconds) moving average. The value obtained is $\sigma_S = 0.00802$.

In order to estimate the accuracy of this sensor, the uncertainty of the response signal is propagated to the uncertainty of CO₂ concentration [51]. However, the signal's response is not linear with CO₂ concentration which means the accuracy of this sensor is dependent to the concentration. In other words, the sensor is more accurate for lower concentrations and less accurate for higher concentrations.

Since the performance of this sensor is more important in lower concentrations of CO₂, the slope of the graph represented in Figure 3.27 is taken at 10mg/L.

The derivative of the calibration function is given by 3.6.

$$\frac{\partial}{\partial x} \text{Signal} = - \frac{(ac - b) \log 10 \times 10^{(ax+b)/(c+x)}}{(c + x)^2} \quad (3.6)$$

Considering the fit obtained for CO₂ concentrations with sodium carbonate and the point $x = 10\text{mg/L}$, the slope of the calibration curve at this point is $\text{slope} = 0.0148$.

Since the error associated with the slope is insignificant when compared with the variance of the data, the relationship between the uncertainty of the signal and that of the concentration is written in equation 3.7.

$$u(\text{CO}_2) = \frac{u(\text{Signal})}{\text{slope}} \quad (3.7)$$

where $u(\text{Signal}) = \sigma_{st}$ and considering a confidence interval of 95%, t is equal to 3.2. Thus, the accuracy of the White sensor at a 95% confidence interval is given by 1.7 mg/L.

The response time of the sensor was determined using the sodium carbonate experiment. This was calculated by measuring the time it took for the signal to reach 90% of the gap between CO₂ concentrations of 10 mg/L and 20 mg/L, resulting in a response time of 70 seconds.

3.5.5 Performance in Aquaculture Applications

After analyzing the results and characterizing this sensor, it is evident its capabilities for aquaculture applications. This sensor has a fast response time and high accuracy.

In fish ponds, it is desirable and ideal that the concentration of dissolved CO₂ is maintained under 10mg/L. Moreover, concentrations above 100mg/L can be deadly to fish. Since this sensor has a detection limit of at least 1.7 mg/L, it is clear that it can be employed to detect CO₂ concentrations that might be undesirable for fish growth. Besides, algae health is also vital in a multitrophic system. Algae show significantly higher growth when in the presence of high CO₂ concentration (above 100+ mg/L). However, this sensor cannot measure the minimum concentration of CO₂ required for photosynthesis as it cannot measure concentrations below 1 mg/L [23, 52].

Even though this sensor is not capable of detecting concentrations of CO₂ below 1.7 mg/L, its range of operation is ideal since it can detect CO₂ concentrations well below the maximum desirable for fish growth.

TABLE 3.5: White Sensor Characteristics

Characteristic	Value
LoD	1.7 mg/L
Response Time (10-20 mg/L)	70 sec

4. Future Work

While the results achieved with the White sensor were satisfying, there is still much room for improvement and work before this sensor can be applied to aquaculture applications.

4.1. Further Optimization

4.1.1 Membrane Fabrication

Experiments with membrane fabrication produced positive results, and it was possible to increase the sensitivity of the membrane two fold simply by adjusting the concentration of pNPh. However, there is a high chance that higher concentrations of pNPh could increase sensitivity even more.

Further experiments with other cocktail's reagents should be carried out to further increase the sensitivity of the membrane. Most notably, ionic liquids (ILs) have proven to increase membrane sensitivity significantly, but more research is needed.

Moreover, the relationship between membrane's sensitivity and thickness (controlled by spin coating speed) has not been studied and favorable results can probably be expected.

Another important variable is the membrane's lifespan in use and storage. This variable has not been studied rigorously, but it seems to stay in good quality for at least two weeks.

Regarding the encapsulation process, while repeated trial and error allowed for the development of a fabrication process with a success rate of around 80%, this process is still relatively slow as the Sylgard needs to be cured three times. Moreover, encapsulation occasionally fails during experiments due to water pressure and turbulence.

By optimizing these variables, the sensor's accuracy is expected to be increased by at least two or more, especially considering the potential results of incorporating ionic liquids.

4.1.2 Optical System

While membrane optimization will increase the strength of the signal and thus increase accuracy and signal-to-noise ratio, optical considerations can be taken to reduce noise and environmental effects.

The most significant cause of noise is related to optical effects as noise amplitude is way larger than the ADC's minimum resolution (although this is adjustable with the potentiometers). Thus, there are two main noise sources: Noise caused by LED emission and noise due to the photodetector.

For example, the noise could be reduced by changing the light source with a Laser as they usually have higher SNR. However, this comes with all kinds of problems such as higher price, size and energy supply and thus might not be worth it.

Another possible improvement to this build would be changing the reference signal to another parallel blue light that passes through another membrane that does not contain the sensitive cocktail. This would eliminate errors related to the unequal wavelength of both signals.

4.1.3 Electronic System

The electronics of this sensor are well optimized; however, some adjustments could be implemented that would surely increase this sensor's capabilities.

First off, the values of the capacitors could be adjusted so that lower frequency signals are eliminated.

Experimenting with the sensor shows that the allowed light intensity offset and sensitivity range are not ideal. For example, the sensitivity option for the red channel is at the minimum, while the sensitivity option for the blue light is relatively high. This occurs because the light received by the photodiode is not uniform in wavelength, and the different photodiodes for the different color channels have different intrinsic sensitivities. A possible solution to this problem would be to use 50k Ω potentiometers or adjust the values of other resistors so that the maximum range of allowed light intensities is increased without affecting precision too much.

4.1.4 Data Processing

The data processing is generally easier to modify and optimize compared to other systems as it does not require any physical modifications to the sensor's components. However, some room for improvement was not taken into consideration.

As explained previously, the signal goes through a moving average calculation to obtain the desired signal and reduce noise amplitude. However, this moving average is applied

to the signal that is received by the computer at five points per second. However, this is limited by the serial communication speed and not by the number of points the ADC can measure. By taking multiple points provided by the ADC, calculating their average, and only sending them to serial communication, it might be possible to substantially decrease the signal's noise.

4.2. Adjusting the Sensor for Practical Applications

While the White sensor works well in laboratory conditions, more factors must be considered in real-world conditions.

4.2.1 Accounting for Additional Variables

All the experiments were conducted while keeping external variables such as temperature, external light, and water quality as constant as possible. However, in practical, real-world conditions, some variables change and can affect the signal detected by the sensor.

Due to the reasons mentioned above, experiments regarding the effect of each of these variables in signal response must be considered so that data points are adjusted for these external conditions. The conditions likely affecting the sensor's signal include temperature, external light (such as day and night cycle) and turbidity.

4.2.2 Automatic Calibration

The current calibration process takes a long time and requires manual participation. It should be possible to completely adjust the values of the digital potentiometers automatically through trial and error as the program can repeatedly change the potentiometers' value and check the sensor's response.

Moreover, the calibration curve can be determined accurately with three appropriate points of measurement. this process can also be done automatically through software, although inserting the sensor in an environment with a known CO₂ concentration is required.

5. Conclusion

This project aimed to develop a colorimetric sensor for monitoring dissolved CO₂ in aquaculture applications in the framework of the European project INNOAQUA. Experiments were taken with two prototypes, the RedBlue sensor and another developed for this thesis called White sensor. While both sensors make use of the same colorimetric membrane, they differ in their optical and electronic systems. The White sensor is more affordable, compact, and better suited for measuring dissolved CO₂.

By adjusting the concentration of the membrane's components, the sensor's sensitivity and accuracy were significantly improved. Initial experiments also indicate that incorporating ionic liquids may further enhance membrane sensitivity, but more research is required.

The White sensor showed promising performance as it is accurate and fast, proving to be capable of being implemented in aquaculture applications. Moreover, this sensor is substantially more reliable than the RedBlue sensor.

Compared to existing commercial sensors such as Oxyguard[®] CO₂ analyzer, this sensor is substantially faster, operates over a wider measurement range and maintains similar accuracy. The White sensor could measure dissolved CO₂ continuously, although this has not been fully implemented yet.

Further research is still necessary before this sensor can be deployed in real-world applications, particularly to assess the impact of external variables on sensor performance.

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Appendix

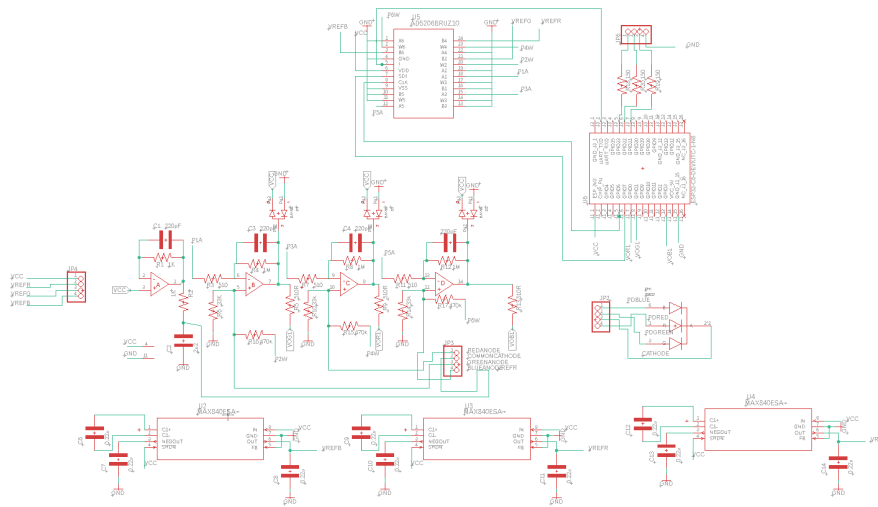


FIGURE 1: Scheme of the electronic circuit designed for the White Sensor

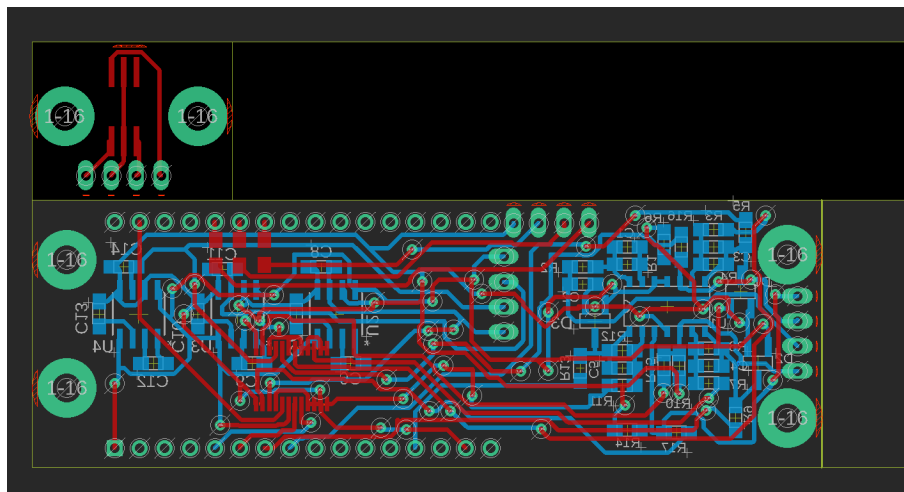


FIGURE 2: PCB layout and electronic circuit of the White sensor