

# Colorimetric Fiber Optic Based Probe for Real-Time Monitoring of Dissolved CO<sub>2</sub> in Aquaculture Systems <sup>†</sup>

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**Abstract:** Dissolved carbon dioxide (dCO<sub>2</sub>) evaluation is very important in many different fields. In this work, a new, integrated, colorimetric-optical fiber-based system for dCO<sub>2</sub> monitoring in aquaculture industry was developed. The sensing chemistry is based on colorimetric changes of the used indicator—poly *p*-nitrophenol (pNPh)—in contact with CO<sub>2</sub>. Preliminary tests were done in a laboratory environment (calibration) and in a laboratory Recirculating Aquaculture System (RAS) with controlled CO<sub>2</sub> injection. The results have shown the suitability of the new sensor for assessing dCO<sub>2</sub> dynamics in RAS and its fast detection of low dCO<sub>2</sub> concentrations in an appropriate operation range.

**Keywords:** dissolved carbon dioxide; colorimetric sensor; optical fiber; aquaculture

## 1. Introduction

Dissolved carbon dioxide (dCO<sub>2</sub>) evaluation is very important in many different fields as ocean, river and lake monitoring, food industry control (e.g., aquaculture) and clinical analysis. So far, there are some different methods for CO<sub>2</sub> measurements, including gas chromatography, colorimetry, amperometry, potentiometry, UV/VIS spectrophotometry and IR spectrometry [1]. These methods are time-consuming, expensive and usually not suitable for real-time monitoring of dCO<sub>2</sub>. In the last years, several authors developed optical fiber-based technology for real time detection and quantification of dissolved carbon dioxide [2–5]. However, some complications appeared when measuring in the field due to the carbonate system complexity [2]. In this work, a new and integrated colorimetric-optical fiber-based system combined with a sensing membrane was developed for dCO<sub>2</sub> monitoring in aquaculture industry. The sensing chemistry of the membrane is based on colorimetric changes of the used indicator—*p*-nitrophenol (pNPh)—in contact with CO<sub>2</sub>. To increase the sensitivity of the sensing membrane, the indicator was polymerized (poly pNPh) and its derivatives used to make the sensing cocktail. The poly pNPh is kept deprotonated in the sensing membrane by action of a quaternary ammonium hydroxide (QA-OH). In that form, the hydroxyl group is involved in protolytic reactions by action of the hydrogen carbonate formed by the equilibrium H<sub>2</sub>O-CO<sub>2</sub>. In

the presence of carbon dioxide, the sensing layer changes its optical properties (absorption and refractive index), which changes the posterior optical response. Laboratory tests were made to ensure the suitability of the new sensor for assessing dCO<sub>2</sub> dynamics and its detection of low dCO<sub>2</sub> concentrations, important for aquaculture operations.

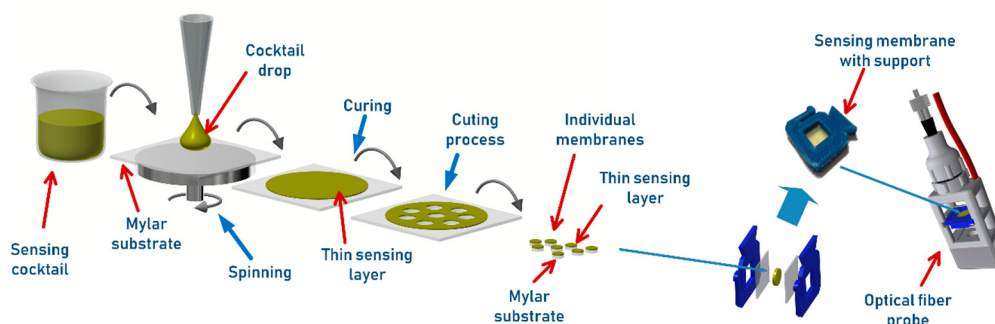
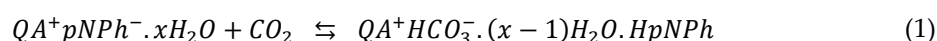
## 2. Materials and Methods

### 2.1. Chemical Reagents

The sensing membrane was developed from a cocktail with the following chemicals: p-nitrophenol (ReagentPlus®, ≥99%; Sigma-Aldrich, St. Louis, MO, USA) as a colorimetric indicator for poly pNPh synthesis; a quaternary ammonium hydroxide solution (Sigma-Aldrich, St. Louis, MO, USA) and Hydrogel D<sub>4</sub> (AdvanSource Biomaterials, Wilmington, MA, USA). To be able to use the membrane in under water conditions a thin layer of Sylgard 184 (Dow Corning, Midland, Michigan, EUA) as gas permeable silicone was produced and attached to the sensing membrane trough a plastic 3D printed support. The laboratory tests were performed with the injection of controlled concentrations of gaseous CO<sub>2</sub> under dry Nitrogen atmosphere both supplied from 50 L bottles (Linde Portugal, Lisboa, Portugal). The chemical calibration, was carried out with citric acid (Sigma-Aldrich, St. Louis, MO, USA) and sodium carbonate (≥99.5%; Sigma-Aldrich, St. Louis, MO, USA).

### 2.2. Sensing Chemistry and Sensing Layer Preparation.

The sensing chemistry is based on the acid-basic equilibrium of p-nitrophenol (pNPh) derivatives, a well-known colorimetric indicator for titrations with a pK<sub>a</sub> of around 7.2, that is converted into the anionic form by addition of a quaternary ammonium hydroxide. The sensing layers were attained by dissolution of an amount of poly pNPh in MeOH:H<sub>2</sub>O mixture and mixed with the QA-OH. After total mixing it was added the Hydrogel D<sub>4</sub> solution (10% in Ethanol, 96%) and stirred till obtaining a homogeneous solution. The resulting cocktail was spread on a Mylar foil by spin coating and allowed to dry. After that, the sensing layer was protected with a thin layer of a gas permeable silicone. Furthermore, the total isolation of the sensing layer avoids the direct contact of the sensing film with the tested media making it impossible that the sensor responds to a media pH variation, allowing just the gas passage through it till the detection layer. In its turn, the CO<sub>2</sub> will react chemically with the ion pair formed by the polymer with the QA-OH (QA<sup>+</sup>pNPh<sup>-</sup>·xH<sub>2</sub>O) as shown in equation 1. That reaction will decrease the internal pH of the membrane become it more colorless. The resulting membrane was attached to an optical fiber probe in transmission mode illuminated with integrated dual wavelength LED combined with collimating lenses to improve the light signal. The signal is received by a micro detector and analyzed by a computer software specially developed for this purpose. Figure 1 shows the preparation scheme of the sensing membrane and the optical fiber probe configuration.

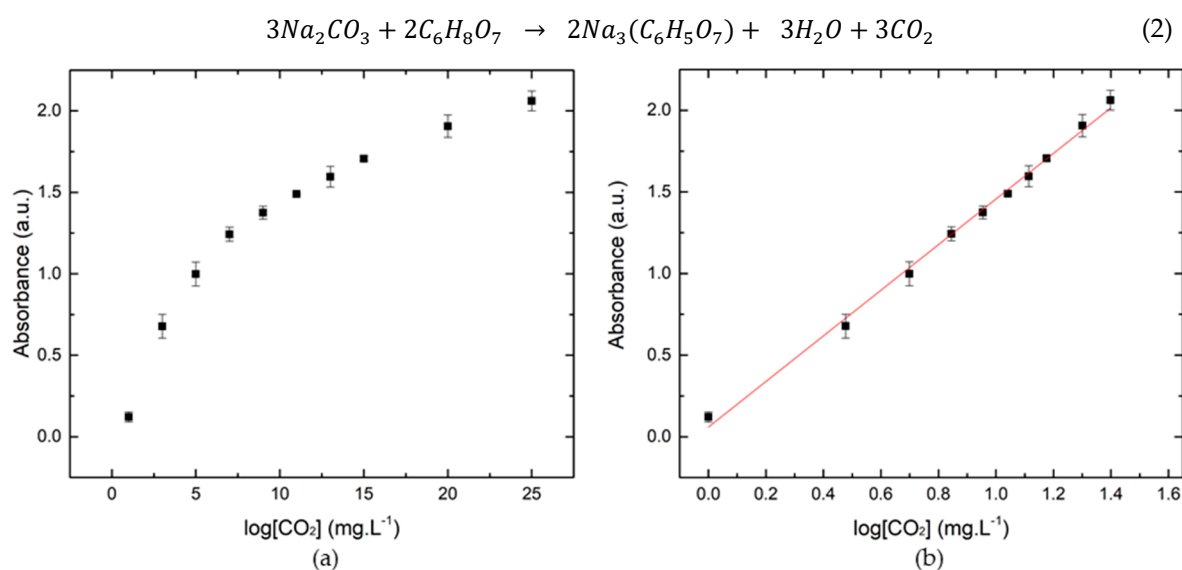


**Figure 1.** Preparation scheme of the sensing membrane and the optical fiber probe configuration.

### 3. Results and Discussion

#### 3.1. Sensor Calibration

For a more precise dCO<sub>2</sub> evaluation it was made a chemical calibration using standard solutions with known concentrations of CO<sub>2</sub>. The formation of carbon dioxide results from the reaction of sodium carbonate in a citric acid solution as shown in Equation (2). The sensor calibration was made in a range between 1 and 25 mg·L<sup>-1</sup> (Figure 2a). Furthermore, for a linear correlation, the graph in Figure 2b is presented in the form of logarithmic of the CO<sub>2</sub> concentration (log[CO<sub>2</sub>]) versus the registered absorbance at each CO<sub>2</sub> concentration. The calibration values obtained from data calibration are presented in Table 1.



**Figure 2.** (a) Nonlinear calibration curve for freshwater obtained by acid calibration and (b) linear curve for the same calibration using the logarithm of the CO<sub>2</sub> concentration.

**Table 1.** Calibration values obtained from data represented in Figure 2b.

| Equation            | Intercept<br>(a)    | Slope<br>(b)        | Residual Sum of Squares | Pearson's | R-Square | Adj.<br>R-Square |
|---------------------|---------------------|---------------------|-------------------------|-----------|----------|------------------|
| $y = a + b \cdot x$ | $0.0589 \pm 0.0315$ | $1.3974 \pm 0.0311$ | 0.0122                  | 0.9980    | 0.9961   | 0.9956           |

#### 3.2. Sensor Validation

Tests were made in a laboratory aquaculture system ( $V = 1 \text{ m}^3$  of dechlorinated freshwater), with a culture tank and water filtering systems (Figure 3a). This system recreates the environment of a Recirculating Aquaculture System (RAS) existent in several aquaculture facilities.

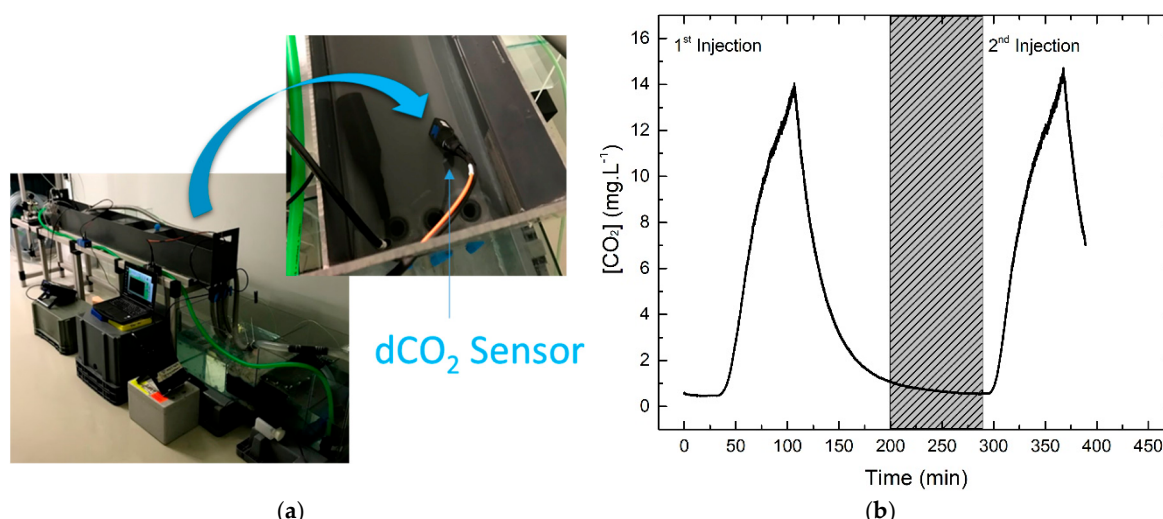
Gaseous carbon dioxide was injected in the tank to simulate the presence of a certain biomass of fish, controlled by a flow controller ( $7.5 \text{ mL} \cdot \text{min}^{-1}$ ) and the experience was repeated twice in the same day. Each CO<sub>2</sub> injection lasted 1 h and the registered sensor data is plotted in Figure 3b.

The performance of the new dCO<sub>2</sub> sensor is almost the same after two injections, showing up to approximately  $14.5 \text{ mg} \cdot \text{L}^{-1}$  (see Figure 3b). Furthermore, the carbonate hardness (KH, Aquapex kit test) and the pH of the water (after full injection) were also accessed and from these parameters the dCO<sub>2</sub> was calculated to be in the range between  $14.3$  and  $18.0 \text{ mg} \cdot \text{L}^{-1}$  (Table 2).

**Table 2.** Water parameters used to estimate the amount of dissolved CO<sub>2</sub> in water.

| KH (Degrees, German Units) | T (°C) | pH      | [dCO <sub>2</sub> ] (mg·L <sup>-1</sup> ) |
|----------------------------|--------|---------|-------------------------------------------|
| 5–6                        | 15     | 7.0–7.1 | 14.3–18.0 *                               |

\* [http://www.aquahobby.com/articles/b\\_phxkh.php](http://www.aquahobby.com/articles/b_phxkh.php).



**Figure 3.** (a) Laboratory set-up of a Recirculating Aquaculture System using freshwater and (b) registered data obtained from 1 h of CO<sub>2</sub> injection (7.5 mL·min<sup>-1</sup>).

#### 4. Conclusions

In conclusion it is possible to say that the new colorimetric fiber optic based probe is appropriate to work in low CO<sub>2</sub> levels situations. These tests have shown the suitability of the sensor and its fast and precise detection in close to real applications. The dissolved carbon dioxide showed by the colorimetric sensor is in the range of concentrations estimated by the pH, carbonate hardness and temperature. It is secure to say that the sensor needs a complementary work on the sensing scheme but also in the sensor body to optimize its functionality and avoid the effect of the external interferences present in the real environment.

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**Conflicts of Interest:** The authors declare no conflict of interest.

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