Quim. Nova, Vol. 48, No. 2, e-20250031, 1-5, 2025 http://dx.doi.org/10.21577/0100-4042.20250031

Technical Note

DEVICE FOR CALIBRATING ELECTROCHEMICAL DISSOLVED OXYGEN SENSORS

Marcus L. Sousa[a], **Isabela A. Duran**[a], **Giovana V. Teixeira**[a] **and André L. B. Formiga**[a,*]
[a]Departamento de Química Inorgânica, Instituto de Química, Universidade Estadual de Campinas, 13083-970 Campinas – SP, Brasil

Received: 01/22/2024; accepted: 04/19/2024;

Sensor calibration for dissolved gas is a critical and necessary step to ensure consistent sensor response over time and under different conditions. In this work, we describe a 3D printed device that facilitates the calibration of gas sensors. The device effectively maintained gas concentration in the solution for at least 2 h. Analytical curves were constructed in the pH range of 2 to 12, yielding determination coefficients surpassing 0.98. The chosen method proved suitable for the calibration of the sensors. Furthermore, using accurate and reliable reference sensors contributed to obtaining precise and trustworthy measurements.

Keywords: amperometric sensor; oxygen quantification; oxygen reduction reaction; microcontrollers; 3D printing.

INTRODUCTION

Dissolved oxygen (DO) is an important parameter with diverse use in chemical and biological applications. Several methods are available for determining DO in a sample, including the Winkler, electrochemical, optical, Clark, and respirometry methods. The choice of the most appropriate method will depend on the properties of the sample, the resources available, and the objectives of the analysis.

A typical method for the determination of DO is the use of electrochemical sensors. This kind of sensors can be classified based on different output signals as current, potential, or charge.[1] The Clark method is also based on electrochemical properties, but it is built as a cell that protects the electrodes with a polymeric membrane. This device significantly improved the determination of DO because it eliminates the presence of interferents. The Clark method has been used in producing commercial sensors and is highly efficient in determining DO concentration. However, requires a waiting time to perform the measurement properly. The waiting time is necessary for oxygen to diffuse across the polymeric membrane and reach the electrode surface, which can undergo electrochemical reactions and generate a sensor response.

Clark-based devices are calibrated using reference points, such as the saturation of DO or a solution with unsaturated DO. The measurement of saturated oxygen is performed by: (*i*) the exposition of the probe to air (8.28 mg L^{-1} at 1 atm)[2-4] or (*ii*) oxygen gas bubbling in a solution with an electrolyte to get a saturated solution concentration.[5,6] In case (*ii*), oxygen solubility is estimated based on the oxygen solubility in the medium with the chosen electrolyte. A solution with a DO concentration close to zero is obtained by dissolving sodium sulfite (Na_2SO_3) in an aqueous medium, which acts as a deoxygenating agent.[7]

However, for non-membrane-coated electrochemical sensors, the measurement by exposing the electrode to air is not possible due to the intrinsic physical characteristics of these sensors (the circuit will not be closed). Furthermore, bubbling oxygen in solution with electrolyte can also generate significant errors as it depends on variables like oxygen solubility under imposed conditions, ambient pressure, and solution temperature. Likewise, the saturation concentration of oxygen reported in the literature may not correspond to the ambient experimental conditions. Therefore, there is a possibility of an underestimated or overestimated measurement.

Although it is simple to calibrate the Clark-type electrode sensor, a Clark cell-based is impractical for monitoring processes within seconds (because of waiting time). To address this issue, a non-coated sensor is an alternative. To calibrate the non-coated sensors, one can compare their measurements to a reference method. We tested the determination of DO in a Britton-Robinson buffer solution using the Winkler method. However, the endpoint was not detected due to interference from the buffer components.

A better alternative is adding the uncoated and Clark-type electrode sensors to the same solution and performing the measurements simultaneously to obtain an analytic curve. Thus, the DO concentration in the solution must remain constant until the Clark-type electrode reading stabilizes and the measurement is collected from both sensors. This process uses various concentrations of DO in the solution. The available methods for performing this procedure are (*iii*) diffusion and the (*iv*) mixing of saturated and unsaturated solutions.

The calibration of sensors by natural oxygen diffusion is performed by removing oxygen from the solution (bubble nitrogen, for instance). Subsequently, the solution undergoes monitoring as atmospheric oxygen naturally diffuses into the solution.[8] This technique allows for more data to be collected to construct the analytical curve since diffusion occurs gradually. Nonetheless, this approach presents additional issues. One of these concerns is the development of a concentration gradient in the solution, which impedes the assumption that two points within the same solution have equal oxygen concentration. Additionally, obtaining a curve across a vast concentration range is time-consuming since the oxygen diffusion process is slow. The volume of solution employed also affects the concentration gradient, which lengthens the time required to construct the curve. Furthermore, data collection must be continuous, and the experiment cannot be paused at any point for data collection.

An alternative way to quickly obtain a solution with different oxygen concentrations is to blend saturated and unsaturated oxygen solutions.[9] A solution with a lower DO content is obtained through nitrogen bubbling. Conversely, a solution with higher oxygen content is prepared by oxygen bubbling. Subsequently, an aliquot of the saturated solution is added to the unsaturated solution and equilibrium is waited before measurement.[10,11] A limitation of this approach pertains to the isolation of the system. The environmental oxygen diffuses to the solution, leading to inaccurate results. Furthermore, strict control of adding solution aliquots is necessary to ensure the reliability and accuracy of the measurements.

---

*e-mail: formiga@unicamp.br

Associate Editor handled this article: Eduardo M. Richter

Thus, developing a device to isolate the solution mixture is important, which prevents both the release of oxygen to the atmosphere and the diffusion of atmospheric oxygen into the solution. This enables precise and reliable measurement obtained through the electrochemical sensor with a wide range of DO concentrations. Therefore, the present invention aims to improve the calibration of sensors not covered by a membrane by using a controlled mixing system to obtain solutions with different concentrations of DO.

EXPERIMENTAL

Device assembly

The 3D models were created using SolidWorks[12] software. The design was exported in ".STL" format (see Supplementary Material) with a fine mesh and imported into Chitubox[13] software for slicing. When required, appropriate supports were included in the print. The dental resin was used to print the part using a Creality LD-002H 3D printer (digital light process, DLP), with a print layer thickness of 0.05 mm and an exposure time of 2 s. The printing process took approximately 4 h under these conditions. Finally, the part was cured in a chamber with ultraviolet light for 5 min.

A peristaltic pump was built by modifying the model described by Behrens *et al.*[14] The design was adapted for a NEMA 17 motor with a 24 mm shaft length and 3 × 10 × 4 mm bearings (623 ZZ model). An Arduino Uno prototyping platform and an L293D shield were also utilized to control the stepper motor.

Sensors

A platinum wire was encased with a plastic tube and used as an uncoated electrochemical oxygen sensor. The electroactive area of the uncoated sensor was determined ($A = 4.59\ mm^2$) using cyclic voltammograms of 1 mM $[Ru(NH_3)_6]^{3+/2+}$ redox probe and applying Randles-Ševčík equation, according to Crapnell and Banks[15] guidelines. A septum was then added to the center of the tube to ensure a secure connection to the calibration device. All measurements were conducted in potentiostatic mode. Electrochemical analyses were performed on an Autolab PGSTAT 302N (Metrohm) using three electrodes. A pseudo-reference electrode was assembled utilizing platinum and used in all measurements.

A Hanna HI98193 probe (Hanna instruments, Woonsocket, USA) with a Clarck-type sensor was utilized as a reference to measure the DO in the device and correlated with a non-coated electrochemical sensor. The probe was calibrated at two points, one with a zero oxygen solution and the other with a supersaturated oxygen solution, as *per* the guidelines of the manufacturer. The probe automatically corrected for ambient pressure and temperature during the process.

Solutions

Britton-Robinson (BR) buffer solutions with pH ranging from 2 to 12 were prepared as described by Britton and Robinson.[16] Saturated and unsaturated oxygen buffer solutions used in the device were prepared by bubbling gaseous oxygen and nitrogen, respectively, for 15 min.

RESULTS AND DISCUSSION

Device design

The device shown in Figure 1 has a total volume of 110 mL. The part has been split in half to simplify printing, machining, and cleaning. In addition, a clamp and plastic film (parafilm®) was used to connect the two halves. The device was designed specifically to prevent the formation of gas bubbles during both the introduction of the unsaturated oxygen solution and the data collection process for constructing the analytical curve. Performing these procedures using standard laboratory flasks, such as three-nozzle flasks, for example, would be problematic due to their curved shapes, which can hinder the removal of gas bubbles during the initial solution addition and make their elimination a challenge.

Figure 1. *Calibration cell used to perform the solution mixtures*

The first characteristic of the device is that it keeps the internal space free of bubbles due to the design of the electrode connections. These connections are shallow to prevent bubble formation. The shape of the electrode connections is inclined relative to the device body. The oval shape was chosen to allow the bubbles to be directed towards the top of the device. The liquid inlet and outlet facilitate solution insertion when the device is full, making it user-friendly.

An o-ring has been incorporated on the inside of the cap to facilitate the sealing. Additionally, the cap includes a bump cylinder that causes the liquid to overflow when the device is filled with the unsaturated oxygen solution (similar to the pycnometer mechanism). The device has three smaller connections for attaching the electrochemical sensors that will undergo calibration and a larger one for attaching a Clark-type reference sensor. Rubber seals were employed to seal the sensors that are connected to the device. These rubber connectors were selected due to their ability to accommodate sensors of varying sizes, which can differ depending on the sensor developer's specifications.

The device has a liquid inlet and outlet on the bottom and top, respectively. These inlets were designed for easy connection to a medical scalp due to their accessibility and low cost. During testing, an Arduino prototyping platform was used to control a peristaltic pump inspired by Behrens *et al.*[14] work. A detailed account of the operation of the device can be found in the study. Before use, the pump's liquid injection quantity was calibrated and exhibited a good linear correlation with time and motor speed (Figure 2).

Device usage

Figure 3 illustrates a depiction of the typical use of the device. Before initiating the analysis, two solutions are prepared as follows: (*i*) an unsaturated oxygen solution obtained by bubbling nitrogen gas (Figure 3a); (*ii*) a saturated oxygen solution produced by bubbling oxygen gas (depicted in Figure 3b). Both solutions should be bubbled

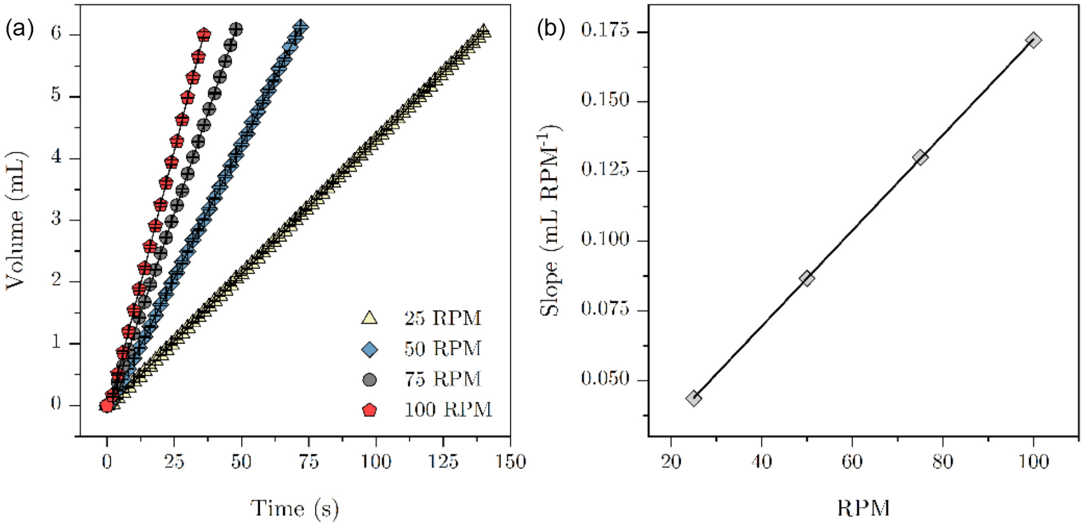

Figure 2. *(a) Peristaltic pump calibration based on the motor speed (rpm, revolutions per minute). (b) The slope of the fitting curves of (a) as a function of the motor speed. All data were collected in three repetitions*

for a minimum of 15 min. Subsequently, the dissolved oxygen concentration in both solutions is measured using a commercial oxygen sensor, based on a Clark-type electrode.

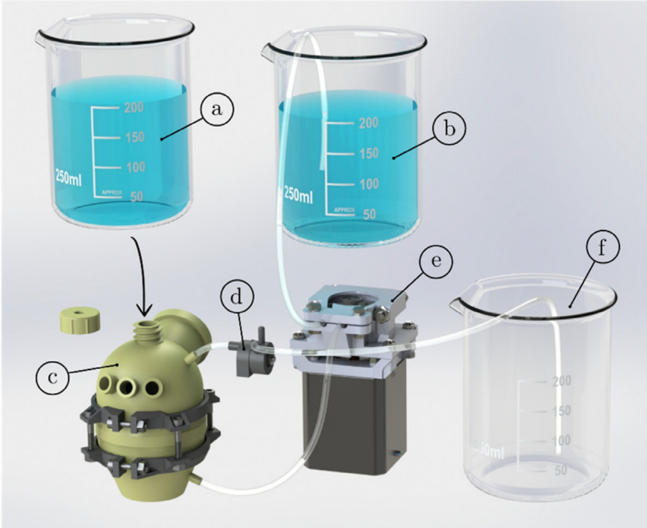

Figure 3. *Example of assembling the device to use. (a) Oxygen unsaturated solution; (b) oxygen saturated solution; (c) device to mix solutions and measurement; (d) valve (can be replaced by a binder clip); (e) peristaltic pump; (f) outlet reservoir*

Next, the Clark-type electrode (commercial reference) is connected to the device at the larger connection, and in the three smaller connections, the electrode to be calibrated (standard electrochemical cell: working electrode, reference electrode, and counter-electrode). The peristaltic pump (shown in Figure 3e) is briefly triggered to fill the hose connected to the device, aiming to prevent gas bubbles from being injected into the system. Next, nitrogen gas is purged into the device to remove the oxygen from the local atmosphere. Following this step, the unsaturated solution (Figure 3a) is inserted into the device until it overflows. Then, the cap is placed on top, causing the solution inside to overflow again, ensuring the device is gas-free.

The analytical curve is obtained by successively adding saturated oxygen solution to obtain different mixing ratios. To ensure proper mixing, a magnetic bar must be added to the device before the liquid injections, and the magnetic stirrer should be activated in between each injection. The Equation 1 was used to calculate the oxygen concentration:

$$C_{device} = \frac{(C_i \times V_{device}) - (C_i \times V_{add}) + (C_{sat} \times V_{add})}{V_{device}} \qquad (1)$$

where: C_i is the initial oxygen concentration inside the device; C_{sat} is the concentration of the oxygen saturated solution (Figure 3b), C_{device} is the oxygen concentration in the device, V_{device} is the volume of the device = 110 mL and V_{Add} is the aliquot of saturated solution added.

The analytical curve shown in Figure 4a was obtained from the calibration device using the Clark-type electrode. In this instance, a solution with an initial dissolved oxygen concentration of 0.56 mg L^{-1} was submitted to successive additions of 5 mL of a 20.7 mg L^{-1} oxygen solution. The curve exhibits a good determination coefficient, indicating the effectiveness of the device. Additionally, a stability curve (Figure 4b) was generated to demonstrate the capability of the device to maintain a constant gas concentration.

Device application

Optimization of the buffer concentration

To enhance coupling in microdevices, we employed a miniaturized reference electrode (RE) that acts as a pseudo-reference electrode, which needed adjusting the electrolyte concentration to achieve a suitable ionic strength for electrochemical analyses. Our study investigated three different concentrations (0.033, 0.04, and 0.1 mol L^{-1}) of the buffer, revealing that a concentration of 0.1 mol L^{-1} for each buffer component has minimal impact on the potential of the oxygen sensor (Figure 5). Furthermore, the 0.1 mol L^{-1} curve exhibits only a slight potential variation compared to the other concentrations. It should be noted that this slight variation is unavoidable due to the influence of pH, which agrees with the Nernst equation.

Analytical curves at different pH values

A comprehensive investigation was conducted to assess the behavior of analytical curves across a broad pH range of 2 to 12, aiming for widespread applicability. The results obtained from analyzing eleven different curves are presented in Table 1. Figure 6 shows the curve obtained at pH = 7. To evaluate the correlation, coefficients of determination (R^2) were calculated for all the analytical

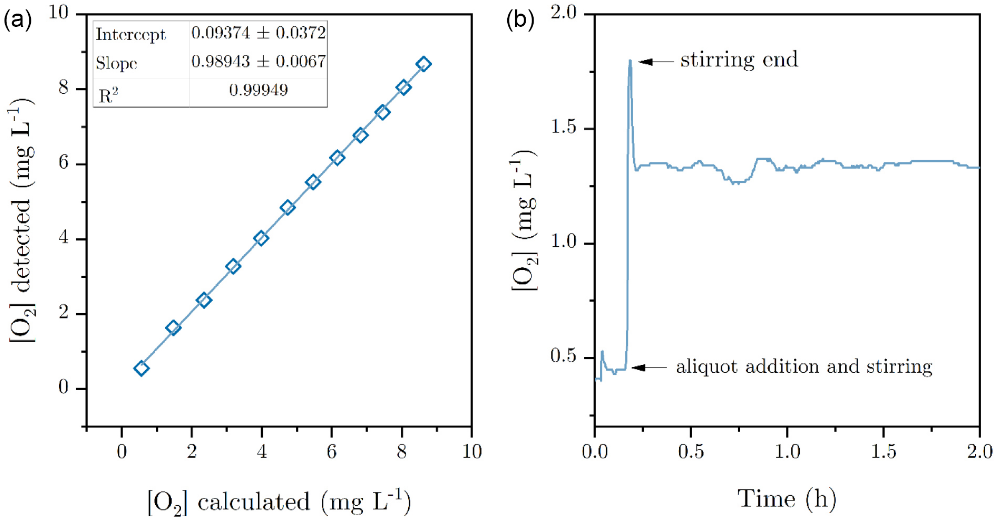

Figure 4. *(a) Analytical curve of oxygen concentration generated using the commercial Clark-type electrode sensor. (b) Gas concentration stability inside the device as a function of time. Conditions: sensor = commercial, pH = 7.008*

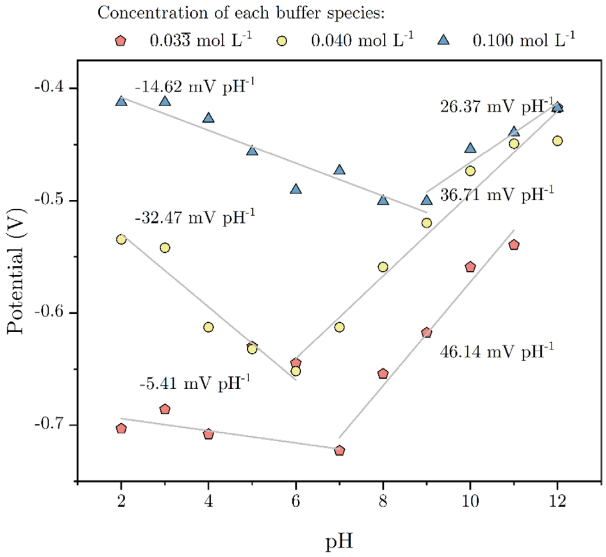

Figure 5. *Plot of the peak potential as a function of the pH for different BR buffer concentrations*

Table 1. Parameters extracted from the analytical curves at different pH conditions

pH	Slope / (μA mg⁻¹ L)	Intercept / μA	Number of points	R²
2	–0.695	–0.303	15	0.9954
3	–0.771	–0.229	15	0.9965
4	–0.603	–0.812	12	0.9838
5	–0.625	–0.502	9	0.9962
6	–0.554	–0.284	16	0.9957
7	–0.517	–0.356	11	0.9984
8	–0.582	–0.302	14	0.9954
9	–0.676	–0.209	12	0.9994
10	–0.731	–0.576	15	0.9950
11	–0.936	–0.016	14	0.9954
12	–1.007	–0.096	14	0.9972

R²: coefficient of determination.

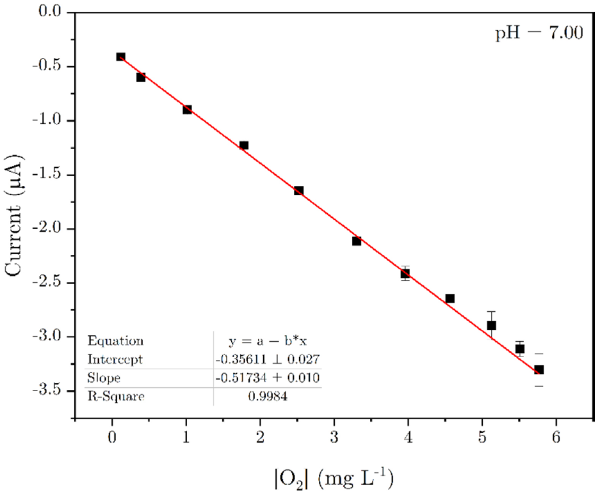

Figure 6. *Analytical curve of oxygen concentration generated using the developed platinum sensor*

curves, exceeding 0.98. This high coefficient indicates a robust correlation between the data acquired using the Hanna probe and the developed sensor, highlighting its reliability.

A pattern was observed during the analysis of curves at various pH values: the current decreased within the pH range of 3 to 7 and increased between pH 7 and 12. This correlation is according to expectations, as variations in the pH of the medium can influence the analytical current of the specific chemical species under investigation. However, the curve corresponding to pH 2 displayed an outlier behavior decreased slope.

Interferences

3D printer resin

We conducted tests on two commercially available resins commonly used for 3D printing. When the common resin printed device came into contact with the solutions, we observed an intense current at the platinum electrode in the oxygen detection region (Figure 7). To eliminate the interference, we washed the parts in ethanol for ten days, with the solution changed daily, to remove the interfering compounds. We also monitored the release of these compounds in the 200-300 nm range using UV-Vis spectroscopy (data not shown). However, despite our efforts, the compounds were not eliminated and the part was damaged.

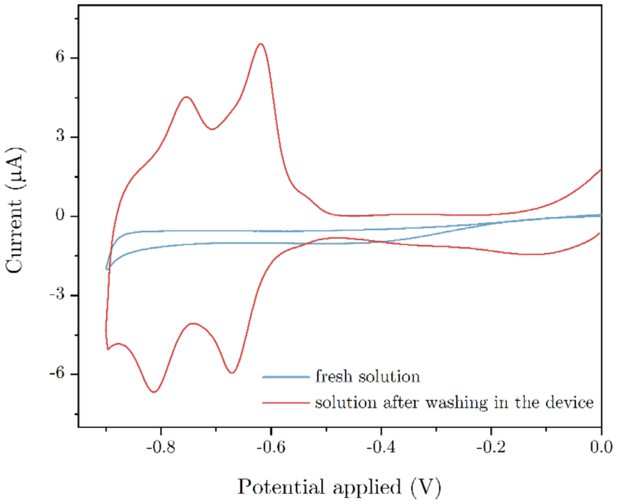

Figure 7. *Comparison of voltammograms between fresh and washed solution in the common resin printed device. The voltammogram of the solution washed in the resin-printed device for dental application overlaps with that of the fresh solution and has, therefore, been omitted*

To address this issue, we switched to printing the parts using a dental application resin. With this material, we did not detect any electrochemical or spectroscopic signals in the analyzed regions.

CONCLUSIONS

This work presents a device for obtaining solutions with varying oxygen gas concentrations and for sensor calibration. The device is designed to be compatible with 3D printers and easily assembled using readily available accessories. With this system, it becomes possible to construct analytical curves for dissolved gases in a controlled and stable manner, enabling the calibration of sensors. The construction of the curve can be paused, allowing for the analysis of solution mixing ratios in multiple replicates without significant loss of gas concentration. The operator has excellent control over stoichiometric ratios and analysis time. The system is made from inexpensive materials, ensuring cost-effectiveness, with the possibility of broad applicability to different electrochemical sensors. The device has proven to be a reliable and effective method for obtaining accurate dissolved oxygen measurements, addressing the challenges associated with such measurements.

SUPPLEMENTARY MATERIAL

Complementary material for this work is available at http://quimicanova.sbq.org.br/, as ".STL" file, with free access.

ACKNOWLEDGMENTS

The authors thank the Brazilian funding agencies CAPES (process 001) and CNPq for financial support. Prof. A. L. B. Formiga thanks Prof. J. A. Bonacin (IQ/Unicamp) for bringing to his attention that a 3D printed device could be useful to solve the problem during a PhD defense. The authors also thank FAEPEX (proc. No. 519292) for financial support.

REFERENCES

1. Wei, Y.; Jiao, Y.; An, D.; Li, D.; Li, W.; Wei, Q.; *Sensors* **2019**, *19*, 3995. [Crossref]
2. Sun, Y.; Li, M.; Zhou, H.; Shan, G.; Cheng, Q.; Jungbluth, K.; Buescher, W.; Maack, C.; Lipski, A.; Wang, Z.; Fan, Y.; *Computers and Electronics in Agriculture* **2017**, *137*, 1. [Crossref]
3. Borodin, V. B.; *Russ. J. Plant Physiol.* **2021**, *68*, 774. [Crossref]
4. Shan, G.; Sun, Y.; Li, M.; Jungbluth, K.; Maack, C.; Buescher, W.; Schutt, K. B.; Boeker, P.; Lammers, P. S.; Zhou, H.; Cheng, Q.; Ma, D.; *Sensors* **2016**, *16*, 91. [Crossref]
5. Martin, C. S.; Dadamos, T. R.; Teixeira, M. F.; *Sens. Actuators, B* **2012**, *175*, 111. [Crossref]
6. Liebisch, F.; Weltin, A.; Marzioch, J.; Urban, G. A.; Kieninger, J.; *Sens. Actuators, B* **2020**, *322*, 128652. [Crossref]
7. Valimaki, H.; Verho, J.; Kreutzer, J.; Rajan, D. K.; Ryynanen, T.; Pekkanen-Mattila, M.; Ahola, A.; Tappura, K.; Kallio, P.; Lekkala, J.; *Sens. Actuators, B* **2017**, *249*, 738. [Crossref]
8. van Rossem, F.; Bomer, J. G.; de Boer, H. L.; Abbas, Y.; de Weerd, E.; van den Berg, A.; Gac, S. L.; *Sens. Actuators, B* **2017**, *238*, 1008. [Crossref]
9. Sosna, M.; Denuault, G.; Pascal, R. W.; Prien, R. D.; Mowlem, M.; *Sens. Actuators, B* **2007**, *123*, 344. [Crossref]
10. Lee, H. J.; Kim, H. M.; Park, J. H.; Lee, S. K.; *Sens. Actuators, B* **2017**, *241*, 923. [Crossref]
11. Maegaard, K.; Garcia-Robledo, E.; Revsbech, N. P.; *Sens. Actuators, B* **2018**, *259*, 560. [Crossref]
12. *SolidWorks*®, version 2020; Dassault Systèmes, Vélizy-Villacoublay, France, 2020.
13. *CHITUBOX*®, version 1.8.1; CBD-Tech, Guangdong, China, 2023.
14. Behrens, M. R.; Fuller, H. C.; Swist, E. R.; Wu, J.; Islam, M. M.; Long, Z.; Ruder, W. C.; Steward, R.; *Sci. Rep.* **2020**, *10*, 1543. [Crossref]
15. Crapnell, R. D.; Banks, C. E.; *Talanta Open* **2021**, *4*, 100065. [Crossref]
16. Britton, H. T. S.; Robinson, R. A.; *J. Chem. Soc.* **1931**, 1456. [Crossref]