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Abstract: An advanced, cost-effective and portable DNA biosensor capable of detecting multiple bacteria simultaneously have been developed. The biosensor consists of a fast and inexpensive potentiostat that controls the applied potential to a screen-printed electrochemical array platform functionalized with MoS_2 flakes and bacteria DNA probes, and monitors the current response obtained by the use of *à la carte* thionine functionalized carbon nanodots (Ty-CDs) as electrochemical indicator of the hybridization event. The design of the potentiostat prioritizes achieving an optimal signal-to-noise ratio and incorporates a user-friendly interface compatible with various devices such as computers, mobile phones and tablets. The device is compact, lightweight, and manufactured at a low cost. The key components of the potentiostat include a data acquisition board capable of analyzing multiple samples simultaneously and a controller board. The results obtained confirm the ability of the developed device to successfully detect specific bacteria DNA sequences, demonstrating the reliability and performance of the developed multiplexing portable biosensor with better sensibility than using a traditional, more complex and laboratory oriented potentiostat.

1. Introduction

Food poisoning is a problem that has affected most of the population for years. There are different sources of food and water poisoning, such as the presence of heavy metals, highly toxic pesticides, or foodborne pathogens. Thus, food analysis has become a very critical area from a public health and economic point of view. Foodborne pathogens can be found in various foods, and it is important to detect them to provide safe food supply and to prevent foodborne diseases. There are many identified foodborne pathogens in the world and it is estimated that viruses are the primary causes of illnesses whereas bacteria are the primary causes of hospitalizations and deaths [1]. The common foodborne pathogens which are responsible for most of the foodborne disease outbreaks includes *Listeria monocytogenes* and *Salmonella enterica*. They are two of the main pathogenic bacteria causing listeriosis and salmonellosis respectively, two food- and water-borne diseases with a high infection and mortality rate. The main symptoms experienced after ingestion are meningitis, septicaemia, gastroenteritis, fever, or nausea [2]. Bacteria detection is especially important in food products and water because these organisms might spoil or contaminate the food, causing serious risks to consumers. Currently, the most used detection methods are polymerase chain reaction (PCR) and enzyme-linked immunosorbent assays (ELISA). Despite the selective, specific, and reproducible detection of target bacteria provided by these techniques, they also involve long analysis times, sample pre-treatment and low sensitivity [3]. Therefore, the search for alternative techniques for early detection of these bacteria is an area to be explored. In this sense, electrochemical DNA biosensors emerge as a potential alternative due to their simplicity, speed, sensitivity, robustness, capacity for miniaturization, automation, and their low cost. Despite there are some studies focused on the development of DNA biosensors for the detection of bacteria [4,5] is an emerging area where

researchers are still developing novel methods with improvements in terms of multianalyte detection, better rapidity, specificity, and sensitivity. In this sense, research involving the use of nanomaterials in the development of new electrochemical biosensors with better analytical properties is a great deal of interest. Among the different types of nanomaterials, molybdenum disulphide (MoS_2) has become one of the most attractive from an electrochemical point of view and in the development of biosensors due to its ability to act as a sensing interface and its ability to interact with thiolated molecules (such as the thiolated DNA probes) [6]. The method employed for the synthesis of molybdenum disulphide used in this work (liquid phase exfoliation), allows the formation of internal and perimeter edges in the crystalline structure of the nanomaterial, which have a high molecular affinity, thus favouring the immobilization of the probe with a specific orientation that could favour the subsequent hybridization with the analyte sequence [7].

In electrochemical DNA biosensors, one of the most widely used techniques, due to its simplicity and efficiency, for the detection of the hybridization event is the indirect detection by means of a redox active molecule, known as an electrochemical indicator. These are electroactive substances that have different interactions with the double stranded DNA (ds-DNA) and single-stranded DNA (ssDNA). In addition, they must exhibit chemical stability of both the oxidized and reduced species, a reversible redox response at low potentials, low toxicity, and low cost. The signals provided by these molecules are focused on changes in the potential values, or in current intensity. Considering the characteristics of a good electrochemical indicator, in this work we propose the use of thionine-modified carbon nanodots (Ty-CDs) as a redox indicator. Carbon nanodots (CDs) are a nanomaterial of the nanocarbon family that consist of spherically shaped nanoparticles essentially composed of carbon, oxygen and hydrogen. Their easy methodology of synthesis, using bottom-up strategies, allow the selection of the appropriate precursors with the aim of determine some of their properties and prepare *à la carte* compounds. In this sense the used of phenazine and phenothiazine compounds have been employed during carbon dots synthesis, with the incentive of granting them with excellent optical [8] and electrochemical [9] properties. Despite CDs have been employed in DNA hybridization detection strategies [8,10–12] it is an emerging area to be exploited. Furthermore, the benefits of preparing *à la carte* thionine functionalized carbon nanodots (Ty-CDs) respect to the used of single thionine molecules and using as electrochemical indicators, is the ability of concentrate over the modified electrode a great amount of electrochemical active moieties, allowing higher electrochemical currents, which is traduce in better sensibility for our devices.

Existing approaches for DNA biosensor-based pathogen detection have limitations in terms of multiplex detection, portability and Point-of-care (POC) systems application. Conventional potentiostats are complex, expensive and bulky, rendering them unsuitable for POC implementation, also budget constraints pose a significant challenge in developing POC devices based on single-chip potentiostat platforms [13,14]. To address this issue, exploration in the field is ongoing, and one promising technology is the use of single-board computers (SBCs). SBCs, such as the Raspberry Pi model 3B+ [15], offer high computing power, low power consumption, ease of programming and cost-effectiveness, making them ideal for tailored solutions in POC and point-of-need applications. The controller module of the electrochemical DNA biosensor system is particularly complex and costly, and employing an SBC helps reduce expenses. Furthermore, to equip the device with the capability of analyzing multiple samples simultaneously, a high-frequency multiplexer should be incorporated into the design. Nevertheless, this addition presents the challenge of managing events such as synchronizing the chemical reaction occurring in the electrochemical cell and configuring the electronic components to deliver the desired signal from the corresponding analyte to the control and signal processing unit, to ensure signal and data consistency. To address this issue, a widely adopted engineering resource should be employed, a multiplexation technique. In the field of engineering, it refers to a fundamental technique employed to optimize the transmission and reception of multiple signals or data streams over a shared communication channel. The primary objective of multiplexation is to enhance the efficiency of the system by maximizing the utilization of available bandwidth and resources. This technique is particularly valuable in scenarios where there is a need to transmit multiple streams of information while avoiding unnecessary duplication of

communication channels, which would be both impractical and resource-intensive. For this reason, in this work, Time Division Multiplexing (TDM) is used by allocating discrete time slots to individual signals for sequential transmission.

Based on the above described, in this work, we present the develop a multianalyte electrochemical DNA biosensor based on the use of MoS_2 and *à la carte* thionine functionalized carbon nanodots for the simultaneous detection of *Listeria monocytogenes* and *Salmonella enterica* coupled with a portable potentiostat specifically designed for micro-power electrochemical sensing applications. The portable potentiostat, built around a Raspberry Pi 3B+ SBC, incorporates filters and analog-to-digital converters to minimize electrical noise in the system. It also includes a main controller board for fast and accurate sampling and can be operated from any device, such as computer, mobile or tablet using a WiFi or Ethernet connection.

2. Materials and methods

2.1. Chemicals

Sodium phosphate monobasic monohydrate ($NaH_2PO_4 \cdot H_2O$), sodium phosphate dibasic (Na_2HPO_4), sodium chloride, thionine (3,7-diaminophenothiazin-5-ium chloride), bulk MoS_2 and synthetic DNA sequences summarizes in Table 1 were supplied by Merck (https://www.merckgroup.com/). Millipore Milli-Q system 18.2 Ω was used for the experiments.

Table 1. Synthetic DNA sequences used in this work.

Synthetic DNA sequences		
Listeria probe	5´-[SH-C_6H_{12}]-CCTAGCAGGTCTAACCGCACTCACT	List-SH
Salmonella probe	5´-[SH-C_6H_{12}]-GCCGCGCGCGAACGGCGAAGCGTAC	Salm-SH
Listeria target	AGTGAGTGCGGTTAGACCTGCTAGG	List-C
Salmonella target	GTACGCTTCGCCGTTCGCGCGCGGC	Salm-C
Probe-FAM	5'-[SH- C_6H_{12}]-CCATAACCTTTCCATTTTTTTTTTACATTCCTAAGTCTGAAACATTACAG CTTGCTACACGAGAAGAGCCGCCATAGTA3'-[6-FAM]	SH-Probe-FAM

2.2. Apparatus

Electrochemical experiments were performed on an Autolab multiplex-potentiostat from EcoChemie with GPES 4.9 software. An eight-channel connector (DRP-CAC8X) and eight screen-printed electrode plates (DRP-8X220AT) were used as interface and transducers respectively. Both were supplied by Metrohm DropSens.

For hardware development, the ALTIUM DESIGNER v21.0.9 PCB design environment has been used, as well as the RIGOL DS1104Z oscilloscope, the RIGOL DP832 power supply and the JBC NASE-2C soldering station; regarding the software development, the IDEs PyCharm and IntelliJ, from JetBrains, have been used.

2.3. Procedures

2.3.1. Molybdenum disulphide (MoS_2) flakes synthesis

In a 250 mL round-flask, 200 mg of the bulk MoS2 were dispersed in 200 mL of N-methyl-2-pyrrolidone (NMP) [16]. Then it was cooled down at 2 °C and sonicated (ultrasonic probe Vibracell 75115, Bioblock Scientific, 500 W) for 1 hour with a 37% of amplitude. A black suspension was formed after sonication. This suspension was separated in six 50 mL conical centrifuge tubes, and it was centrifugated for 30 minutes at 5000 rpm (Allegra X-15R Beckman Coulter centrifuge, FX6100 rotor, 20 °C). The olive-color supernatant was separated from the black sediment (non-exfoliated material) by decanting and filtering under vacuum (Omnipore 0.45 μm PTFE membrane filters, 45 mm in diameter). The retained exfoliated MoS2 was cleaned by redispersion and filtration steps. This

cleaning process was performed three times in acetonitrile (60 mL) and three times in isopropanol (60 mL). The collected solid was dissolved in water and used for further experiments.

2.3.2. Thionine functionalized Carbon nanodots (Ty-CDs) synthesis

Ty-CDs were synthetized irradiating the mixture of 72 mg Thionin acetate salt, 87 mg L-arginine, 86 µL 3,3'-diamino-N-methyldipropylamine and 100 µL Milli-Q water into a microwave system at temperature and pressure of 235 °C and 20 bar respectively for 180 seconds. The blue solid obtained was then dissolved with 10 mL of ultrapure water and filtered with 0.1 µm porous filter. Then, the suspension was dialyzed for 1 week in a 0.1-0.5 kDa dialysis membrane. Finally, a 2.83 mg/mL Ty-CDs solution was obtained and stored for it use at 4 °C.

2.3.3. DNA solutions preparation

Stock 10 µM probe solutions were prepared using 10mM phosphate buffer pH 7.0 solution as solvent, after a previous treatment with dithiothreitol (DTT) and purification through a NAP-10 column of Sephadex G-25. Stock and 100pM Listeria and Salmonella target solutions were prepared in 10mM phosphate buffer pH 7.0 with 0.4 NaCl. All solutions were stored at -20°C.

2.3.4. Biosensing platform development

Nanostructuration of the array of screen-printed electrodes (SPE)

Screen-printed electrochemical array formed by eight electrodes (8xSPE) was nanostructured with MoS_2 flakes by drop-casting to improve the analytical properties of the biosensor. For this purpose, 5 µL of a colloidal dispersion of MoS_2 in water was deposited on the surface of the gold working electrode by drop-casting over a hot plate to evaporate the organic solvent. The resulting platform is denoted as 8xSPE/MoS_2.

DNA capture probe immobilization

8xSPE/MoS_2 platform was then modified with 5 µL of 10 µM thiolated DNA Listeria and Salmonella capture probe by drop-casting and was kept for 24 hours at room temperature (SPE/MoS_2/List-SH or SPE/MoS_2/Salm-SH). The platform was then washed with Milli-Q water.

Hybridization reaction and electrochemical detection

The platform with the immobilized probe was then hybridized with 5 µL of 100 pM of the target sequences of Listeria or Salmonella, for 1 hour at 40°C. To detect the hybridization evet carbon nanodots modified with thionine (Ty-CDs) were used as redox indicator. For this purpose, 5 µL of 2.83 mg/mL Ty-CDs solution were incubated for 1 hour at room temperature over the working electrode. Finally, the electrodes were rinsed with Milli-Q water and the differential pulse voltammograms (DPV) were recorded in each case using PB 0.1M pH 7.0 as the supporting electrolyte. Measurements were carried out on the designed portable multiplex-potentiostat (Single-pulse potential measurement) and on the commercial Autolab multiplex-potentiostat to compare the results.

Study of the selectivity of the biosensor

To study the selectivity of the biosensor, 8xSPE/MoS_2/List-SH and 8xSPE/MoS_2/Salm-SH platforms were incubated in the same conditions of time and temperature (1hour, 40°C) with Salmonella and Listeria target sequences respectively, acting then as non-complementary sequences. Then, in the same way as in the previous step, they were incubated for 1 hour with Ty-CDs, the electrodes were washed with sterile water and the DPVs were recorded in 0.1M PB pH 7.0.

### 2.3.5. Multiplex-potentiostat design and development

The developed multiplex-potentiostat presents a comprehensive device for conducting end-to-end measurements. The design has been meticulously executed at both the hardware and software levels.

At the hardware level, the design led to the creation of two distinct modules: the Main Controller Board and the Data Acquisition Board. The first one acts as a repository for all necessary software and takes charge of overseeing the entire system's functioning, running all the necessary algorithms and commanding the second module, which, in turn, can be seen as a group of other three modules, this is, the Data Multiplexing Module, the Signal Conversion Module and the Signal Acquisition Module. The first one is the core part that allows to perform multiple measures, by routing properly the signals between all chemical cells and the Main Controller Module. The second one incorporates all the essential electronic components required to process the signal, including analog-to-digital and digital-to-analog converters and a set of filters that allows to achieve a low electrical noise level in the system. At last, the third one is composed of all the essential electronic components and signal conditioners required to acquire the raw signal from the electrochemical cell. A scheme of the hardware design can be seen in the Scheme 1.

Conversely, the software-level design yielded five modules: the Communications Module, which is responsible for managing the data transfer among all system components; the Control Module, that governs the behaviour of each individual component within the system; the Digital Filter Module is dedicated to digitally processing the acquired signal using the Savitzky-Golay algorithm; the Data Acquisition Module obtains and processes the signal from the biosensor; and, lastly, the User Web Interface Module, which purpose is serving as the user interface for the operator, allowing for seamless interaction with the multiplex-potentiostat system. A scheme of the software design can be seen in the Scheme 2.
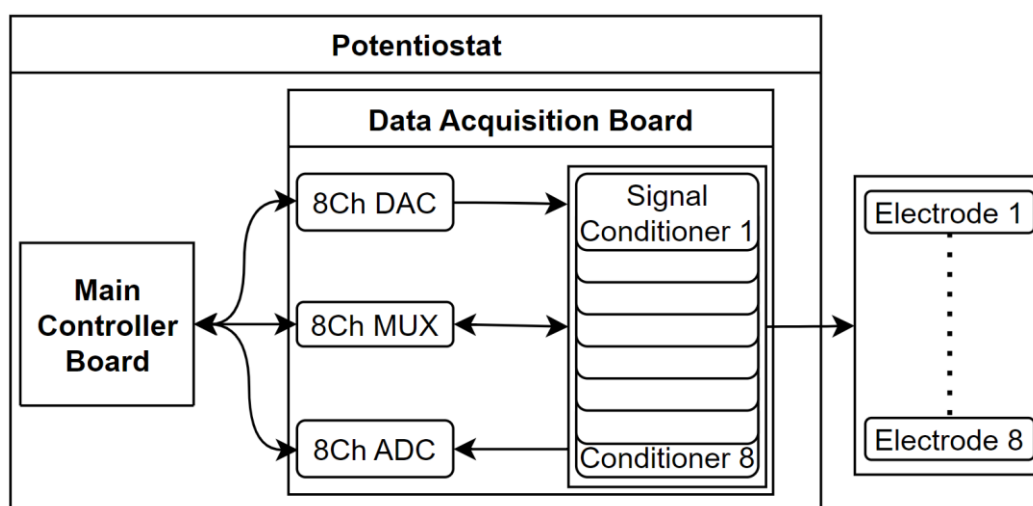
Scheme 1. Hardware design scheme.
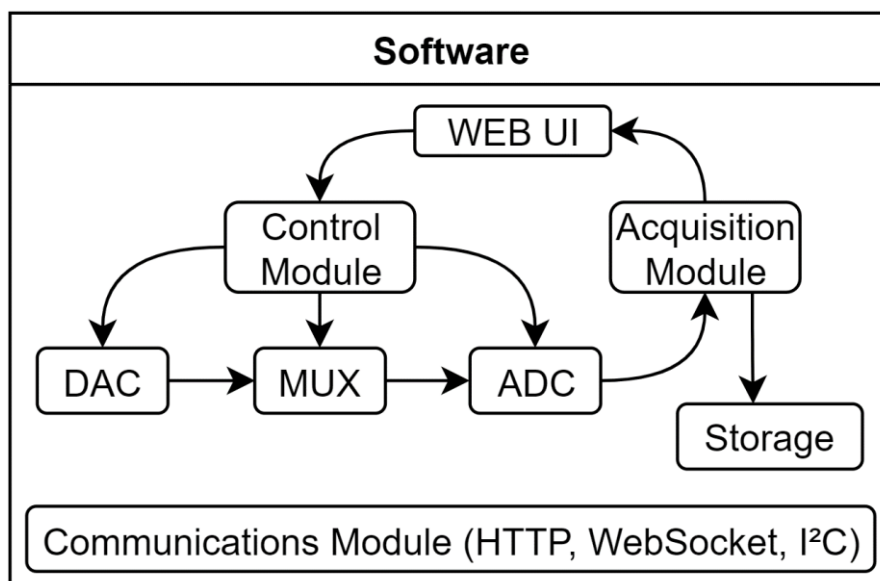
Scheme 2. Software design scheme.

2.3.5.1. Main Controller Board

The primary objective of the Main Controller Board is to offer an affordable and portable device. To achieve this goal, the design emphasizes modularity, aiming to utilize commercially available modules as much as possible. This approach reduces the need for custom electronic boards, thus lowering manufacturing costs. Among the various modules within the system, the controller module is particularly complex and expensive. In the prototyping phase, a complete computer was initially used. However, to reduce costs, a commercial development board was chosen as the controller.

Several development boards, including *Arduino YUN*, *BeagleBone Black*, and *Raspberry Pi model 3B+* [17–19], were evaluated to determine the most suitable option for the project. Considerations such as communication and processing capabilities, as well as flexibility in software development and maintenance, were considered during the selection process. Based on the analysis, the *Raspberry Pi 3B+* development board emerged as the most suitable choice due to its high core speed, communication capabilities, and cost-effectiveness. Although it lacks analog ports, this issue can be easily resolved by incorporating an Analog-to-Digital converter, which will be discussed later.

An additional advantage of the selected development board is that it features HDMI and USB ports, enabling standalone usage by simply connecting a screen, mouse, and keyboard to it. To utilize the *Raspberry Pi 3B+* board, a few additional elements are required, including a μSD card with a minimum capacity of 4 GB and a 5V μUSB power adapter with a current output of 2.5A is also necessary. Additionally, an Ethernet cable can be used to connect to the board through SSH protocol, or alternatively, an HDMI screen with a USB mouse and keyboard can be employed.

2.3.5.2. Data Acquisition Board

The Data Acquisition Board serves as an integration point for essential electronic components involved in the sensing and physical treatment of the signal generated during the chemical reaction. These components are configured and controlled by the Controller Board, and the captured signal is subsequently returned to it for further processing. The primary modules housed on the Data Acquisition Board includes the Data Multiplexing Module, which enables the routing of the signal from the electrochemical cell to the main controller board using the TDM technique; the Signal Acquisition module, that consists on several signal conditioners; The Signal Conversion module, which includes analog-to-digital converters (ADC) and digital-to-analog converters (DAC); and a set of non-configurable components that contributes to maintaining a low noise level within the system.

To facilitate the required functionalities and interactions with the Controller Board, the Data Acquisition Board incorporates I²C communication bus [20] through the SCL (Serial Clock) and SDA

(Serial Data) ports. This communication enables the programming of the components and the retrieval of measurement data from the sensor.

The multiplexing module is the core circuit that allows performing multiple measures using the same device. It is based on a key component that is the *Texas Instruments PCA9548 8-channel I²C-bus multiplexer*. This chip plays a crucial role as it provides up to eight channel communications, which enables having up to eight electrochemical cells and, thus, perform up to eight measures on distinct analytes at the same execution. This enables the system to analyze multiple samples simultaneously, understanding the simultaneity not as parallel execution but as obtaining all results at the end of an operational window, with a maximum capacity of 8 samples. The multiplexer works using a TDM technique, by selectively exposing one of the connected signal conditioners, connected to a chemical cell associated with it, routing the I²C bus signals of the desired channel to the Controller Board. This functionality allows for the parallel processing and analysis of multiple samples, providing greater efficiency and throughput in the system. By leveraging the capabilities of the PCA9548 multiplexer, the system gains the flexibility to handle a higher number of samples and perform several measurements on them, enhancing the overall performance and productivity of the electrochemical sensing setup. One additional reason to use the multiplexing technique is that the signal acquisition module, LMP91000, has a non-configurable I²C address, which means that without a multiplexing technique, the Controller Board would be able to work only with one electrochemical cell.

The signal acquisition module is based on a *Texas Instruments LMP91000 configurable AFE Potentiostat*, specially designed for low-power chemical sensing applications, was chosen as the signal conditioner. This component plays a critical role in conditioning and preparing the raw signal for further processing. It may involve amplification, filtering and any necessary adjustments to ensure the signal is suitable for subsequent conversion and analysis. Furthermore, the signal conditioner requires a reference voltage, which is supplied through the V_{ref} port. This reference voltage is essential for performing a potential sweep on the chemical sample, enabling precise control and measurement of the electrochemical reaction. To ensure the integrity and quality of the acquired signal, the signal conditioner incorporates a capacitor connected to ports *C1* and *C2*. This capacitor plays a vital role in analog filtering, effectively attenuating electrical noise and disturbances from the signal. The result is an output signal, available at V_{out} port, that is as clean and noise-free as possible, enhancing the accuracy and reliability of the measurements.

The *Raspberry Pi 3B+* development board chosen for the system lacks analog GPIOs, which means it can only handle digital signals. To overcome this limitation, the signal conversion module is designed using analog-to-digital converters (ADC) and digital-to-analog converters (DAC). The first one, *Texas Instruments ADS1115*, is responsible for converting the analog signal received from the biosensor, V_{out} port of the signal conditioner, into a digital format that can be processed by the Controller Board. This component stands out for its 16-bit resolution, which translates to greater precision in working with the signals, and sigma-delta conversion procedure, which offers several advantages, including inherent linearity, monotonicity, and minimal requirements for external anti-aliasing filters. The digital nature of this conversion method ensures that the performance remains stable over time and temperature. However, it requires complex digital circuitry and operates at an oversampled rate significantly higher than the maximum signal bandwidth. Conversely, the second one, *Microchip MCP4728*, is utilized to convert digital signals from the Controller Board into analog signals, V_{ref} signal, used by the signal conditioners. These integrated circuits are specifically designed to operate with high precision and low noise, which are crucial factors for handling weak signals and minimizing electrical noise susceptibility. This component incorporates a high-precision output amplifier, enabling precise and stable output voltage generation.

Additionally, the Data Acquisition Board incorporates various non-configurable components that contribute to maintaining a low noise level in the overall system. These components work in tandem to reduce or eliminate unwanted electrical noise, interference, or disturbances that could adversely affect the quality and accuracy of the acquired signal. Capacitors are involved in the power line to ensure a smooth and clean power signal throughout the circuit, also, decoupling capacitors are placed at the power entry of each integrated circuit on the board to clean the power signal for

each chip. Moreover, *Texas Instruments OPA388 Precision Operational Amplifiers* (OPAs), are placed in buffer configuration. These OPAs are strategically positioned between the signal conditioners and the ADC/DAC integrated circuits. This component is designed to meet high precision and ultra-low noise requirements, and it integrates filters to mitigate electromagnetic and radiofrequency interference.

Together, these components as a whole serve as a vital intermediary between the physical sensing of the chemical reaction and the digital processing performed by the Controller Board. They enable efficient signal capture, conditioning and conversion, ensuring that the acquired signal is of sufficient quality and fidelity for subsequent analysis and interpretation by the system.

2.3.5.4. Software design

The software implementation of the device can be divided into two main blocks: the backend and the frontend. The backend comprises all the software necessary for managing the system but is not directly accessed by the operator. This includes the communication protocols between the various components of the device, internal controls of each chip, digital filtering algorithms, and data acquisition algorithms. Thus, it ensures that the commands are correctly interpreted and executed. On the other hand, the frontend encompasses the software that allows the operator to interact with and manage the system. This includes a user web-based interface where the operator can visualize the results and control various aspects of the POC device. The frontend module is designed to be user-friendly and accessible, enabling operators to work from any device, such as computer, tablet, or mobile phone.

Together, these software modules collectively contribute to the efficient functioning and usability of the system, enabling seamless communication, precise signal treatment, accurate data acquisition, and an intuitive user experience.

2.3.5.4.1. Communications Module

The Communications Module plays a crucial role in facilitating communication between different components of the POC device and enabling interaction with the operator through the user interface. This module utilizes various protocols to ensure effective and reliable data transfer.

The I²C protocol is utilized for communication between the Controller Board and the Data Acquisition Board. It enables the Controller Board to configure and control the multiplexer, signal conditioner, ADC, and DAC on the Data Acquisition Board. Through I²C, the Controller Board can send commands and receive data from these components, facilitating the overall operation of the POC device.

TCP (Transmission Control Protocol) is employed for data transfer between the operator's user interface (UI) and the device. HTTP (Hypertext Transfer Protocol) is specifically used within TCP to enable the operator to interact with the POC device through a web-based UI. The HTTP protocol allows the operator to send commands, configure device settings, and retrieve information from the device in a user-friendly manner.

In addition to HTTP, the Communications Module utilizes WebSocket, which is built on top of TCP, to achieve real-time data display on the UI plot part. WebSocket enables a persistent, full-duplex communication channel between the UI and the device. This allows the acquired data to be streamed and displayed on the UI plot in real time, providing the operator with immediate feedback and visualization of the measurements.

By leveraging these protocols, the Communications Module ensures seamless communication between the different components of the POC device and facilitates user interaction through a web-based UI. This enhances the usability and functionality of the device, enabling efficient configuration, control, and real-time data visualization for the operator.

2.3.5.4.2. Control Module

The Control Module of the software is responsible for handling the commands received from the operator through the web-based UI and configuring the internal registers of the components on the Data Acquisition Board accordingly. Here's an overview of the configuration requirements:

- Multiplexer (*PCA9548*): The Control Module configures the multiplexer to select the desired channel corresponding to a specific signal conditioner on the Data Acquisition Board. This allows the system to choose which signal conditioner to expose for measurement, allowing analyzing up to 8 samples simultaneously, remember that simultaneously means obtaining all results at the end of the same operational window.
- Signal Conditioner (*LMP91000*): The Control Module configures internal registers of the signal conditioner based on the operator's commands. This configuration includes:
    - Transimpedance Control Register (*TIACN*): This register sets the amplifier gain (*RTIA*) and the load resistance (*RLOAD*).
    - Reference Control Register (*REFCN*): This register determines the voltage reference (*V_{ref}*) source (internal or external), sets the internal zero value as a percentage of *V_{ref}*, and configures the bias sign and value as a percentage of *V_{ref}*.
    - Mode Control Register (*MODECN*): This register enables or disables the *FET* feature and selects the operation mode of the sensor.

The Control Module ensures that the signal conditioner is properly configured to provide the desired amplification, reference voltage, biasing, and operating mode for the electrochemical cell.

- DAC (*Digital-to-Analog Converter*): The DAC integrated circuit on the Data Acquisition Board has a single register that needs to be configured by the Control Module. This register sets the desired output voltage, which will be used as the *V_{ref}* (reference voltage) for the signal conditioner. Having a variable voltage reference makes the device very flexible as it can be configured to analyze a large diversity of samples.
- ADC (Analog-to-Digital Converter): The ADC integrated circuit on the Data Acquisition Board also requires configuration, but these settings are handled automatically and are not accessible for operator modification. The Control Module ensures that the ADC is properly calibrated and set up during system startup, allowing it to accurately convert the analog signal obtained from the signal conditioner.

By properly configuring the registers of each component, the Control Module ensures that the Data Acquisition Board is correctly set up for signal conditioning, conversion and measurement of the electrochemical cell's output.

2.3.5.4.3. Digital Filter Module

The Digital Filter Module in the software implementation is embedded in the Data Acquisition Module, but it is relevant enough to dedicate this paragraph to it. This module utilizes a *Savitzky-Golay* [21–25] filter to enhance the precision of the data obtained from the ADC. The *Savitzky-Golay* filter is a type of digital *Finite Impulse Response* (FIR) filter that aims to improve the accuracy of the data without significantly distorting the signal trend.

The filter operates through a process known as convolution, where successive subsets of adjacent data points are fitted with a low-degree polynomial using the method of linear least squares. By finding a set of "convolution coefficients," which can be determined analytically when the data points are equally spaced, the filter estimates the smoothed signal at the central point of each subset.

The original work by Savitzky and Golay demonstrated that the smoothed output value obtained by sampling the fitted polynomial is equivalent to a fixed linear combination of the local set of input samples. This means that the output samples can be computed through discrete convolution.

The *Savitzky-Golay* filter has two key parameters: the window length, which determines the number of samples used for the filtering process, and the polynomial order, which specifies the degree of the fitting polynomial. These parameters are calibrated and set up in the Control Module of the software, ensuring optimal filtering performance. However, they are not accessible for modification by the operator.

By applying the *Savitzky-Golay* filter, the Digital Filter Module enhances the representation of the signal, providing a smoother and more precise visualization of the data for the operator.

2.3.5.4.4. Data Acquisition Module

The Data Acquisition Module is responsible for sampling the V_{out} output signal from the signal conditioner and processing it for various purposes.

Firstly, the module is responsible for presenting the acquired data on the user interface (UI), allowing the operator to monitor the measurements and observe any changes or trends in the data.

Additionally, the Data Acquisition Module facilitates data storage for future reference and analysis. The acquired data is saved in a CSV (Comma-Separated Values) file format, which is a common format that can be easily accessed and processed by various third-party applications such as spreadsheet software or data analysis tools. Storing the data in a CSV file enables the operator to retrieve and utilize the data outside of the POC device, enhancing its usability and compatibility with other applications or analysis workflows.

2.3.5.4.5. User Web Interface Module

The User Web Interface Module provides a user-friendly and intuitive control panel to the operator, enabling them to configure various parameters of the system with ease. The interface offers a simple and interactive interface where the operator can make selections or click buttons to adjust settings or choose from pre-set configurations.

The User Web Interface Module also facilitates the visualization of the analysis results on a graph, allowing the operator to easily interpret and analyze the data obtained from the biosensor. The graph provides a visual representation of the measurements, trends, and changes over time, aiding in the understanding of the analyzed samples.

One of the key advantages of the User Web Interface Module is its accessibility. Being a web-based interface, it can be accessed from any device that is connected to the same local network as the POC device. This eliminates the dependency on a specific computer or the need for installing third-party drivers, making it highly convenient to use. The operator can access and control the system from their computer, laptop, tablet, or even a mobile phone, providing flexibility and ease of use.

The User Web Interface Module enhances the overall user experience by offering a user-friendly interface, real-time visualization of results, and accessibility from various devices within the local network. It simplifies the control and monitoring of the system, making it more convenient and efficient for the operator.

3. Results

In this work we propose the develop of a multiplex portable device capable of detecting bacteria with high sensitivity, quickly and easily applicable at the point of care for *Listeria monocytogenes* and *Salmonella enterica* detection. To this end, the work has focused on the design and development of a portable, simple, and fast multiplex potentiostat coupled to an electrochemical DNA sensing platform. Scheme 3 shows the main components (electrochemical DNA biosensor and portable multiplex potentiostat) and the principal steps for the multiplex portable device development.

To develop of a portable multiplex potentiostat with the ability to work with multiple analytes, both at hardware and software levels, should be take into account. This portable concept of the device requires a miniaturization of the electronics in small area as it is described in detail in experimental section. The main obstacle in this work has been providing the system with the ability to process the

signal from several electrochemical cells at the same time. For this, the time division multiplexing [26] technique has been used, obtaining a device capable of analysing up to eight samples at the same time.

The electrochemical DNA biosensor preparation (see Scheme 3) is based on the use of two different nanomaterials, MoS_2 for nanostructuring SPE surface and *à la carte* thionine functionalized carbon nanodots (Ty-CDs) for hybridization detection. As it is portrayed in Scheme 3 the first step in the biosensor development is the nanostructuration with molybdenum disulfide (MoS_2) of the array of screen-printed electrodes (SPE). This nanomaterial allows to improve the analytical properties of the biosensor generating a stable platform (MoS_2/SPE) for the immobilization of the specific bacteria DNA probe [12]. The second stage is the immobilization of the thiolated DNA probes (List-SH or Salm-SH) on the SPE/MoS_2 due to the ability of the thiol group to eliminate the sulfur vacancies of the MoS_2 layer on the electrode surface, leaving the probe to be available for subsequent hybridization with its complementary sequence [6]. The next step is the hybridization of the platform with the analyte (complementary sequence (List-C or Salm-C) or with a non-complementary one. Finally, the detection of the hybridization event, was carried out using thionine-modified carbon nanodots (Ty-CDs) as redox indicators. These nanomaterials have been reported by us as efficient redox indicators [12]. Some characterizations of the Ty-CDs have been included in the Supporting Information (see Figure S1). In order to choose the best procedure to incorporate the redox indicator in the dsDNA immobilized layer, two different strategies have been studied. Figure S2 shows biosensor signal in 0.1 M PB pH 7.0 buffer after incubation with the complementary sequence when Ty-CDs are incorporated by cycling the potential from -0.70 to -0.15 V (black curve) or via accumulation at open potential (red curve). As can be observed both strategies studied to incorporate the redox indicator in the immobilized DNA layer make possible to accumulate the redox indicator. However, the peak current ascribed to the oxidation of the Ty-CDs by incubating at open potential is higher than that obtained by successive scans of potentials suggesting that more Ty-CDs are accumulated. Taking into account these results, the accumulation of the redox indicator was carried out by incubating at open potential was chosen as efficient and simpler methodology for POC system development.
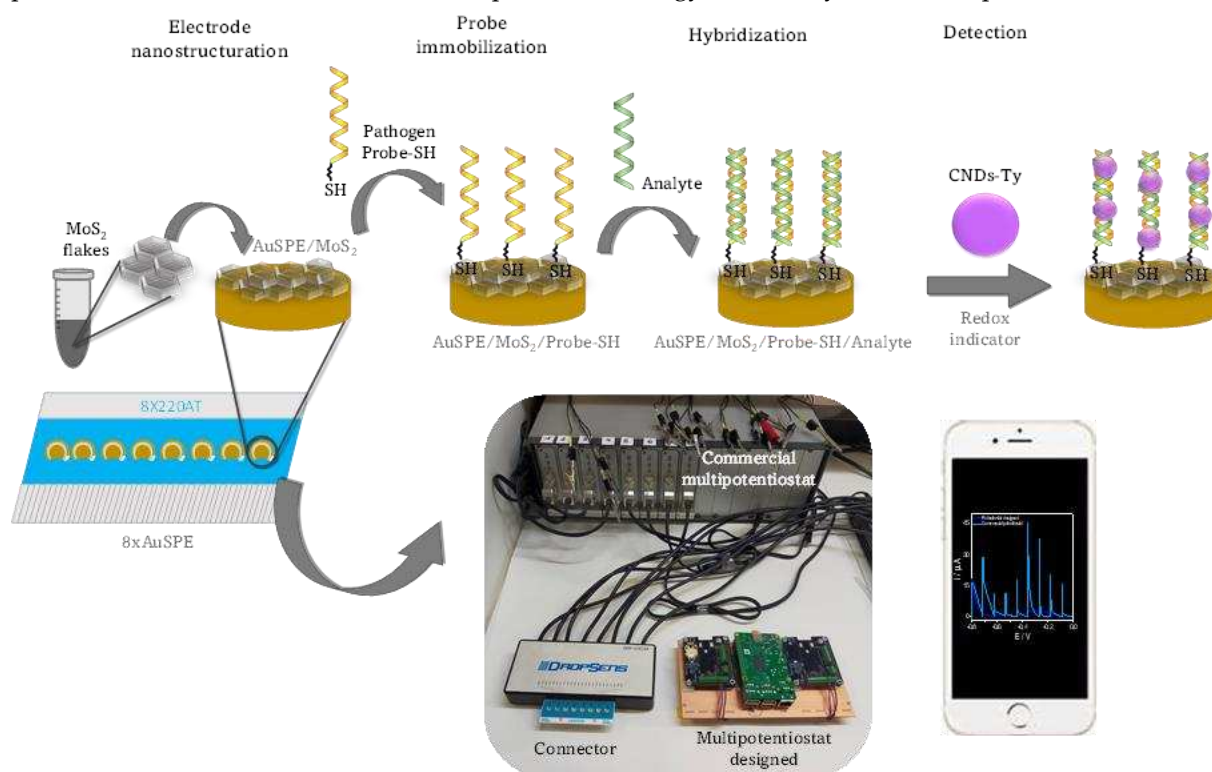
Scheme 3. Scheme followed for the development of the multiplex portable DNA sensing device.

The biosensing platform development was characterized by SEM-EDAX, AFM and Fluorescence Microscopy. Figure S3 shows the AFM (A, B) and SEM (C) images and an EDAX (D) spectra of a bare gold substrate: a surface composed of Au nanocrystals is observed. After MoS2 modification (Figure 1 A, B), a homogeneous distribution of the MoS2 onto the Au surface can be observed on the image (1 A). Moreover, from the EDAX spectrum the peaks corresponding to the energies of Mo, S and Au are identified, confirming the MoS2 modification. After probe immobilization (Figure 1C, D, E, F), the peak corresponding to the energy of P from the DNA probe is detected. Since the peaks energy of P and Au are very close, these peaks overlap on the probe immobilization gold platform spectrum (Figure 1D). In order to avoid this overlapping, the gold substrate was substituted by carbon (Figure 1E, F). The P peak can thus be easily differentiated (Figure 1F), confirming the presence of DNA on the MoS2 modified platform.
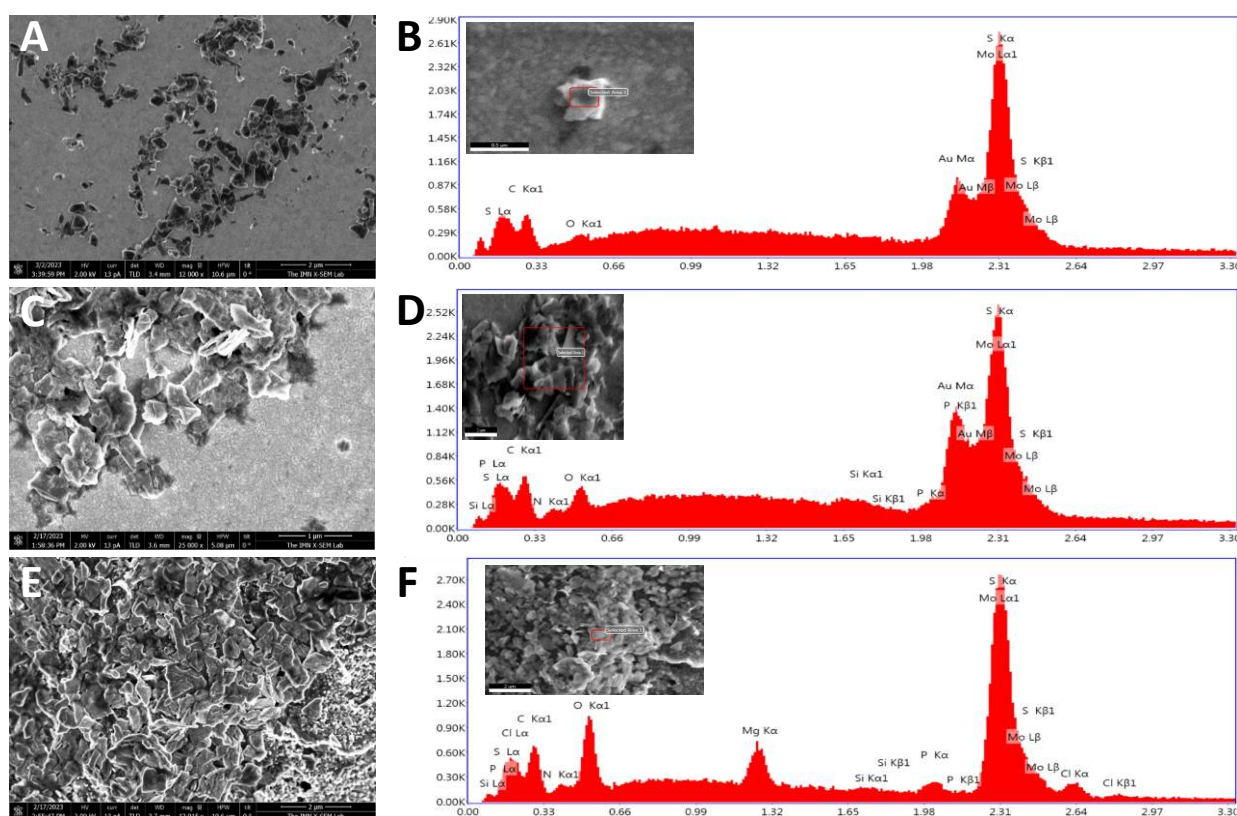
Figure 1. SEM (A,C,E) and EDAX (B,D,F) of a MoS2 modified gold substrate (1A, B), probe immobilization on the a MoS2 modified gold substrate (1C, D) and probe immobilization on the a MoS2 modified carbon substrate (1E, F). The images show the triangular structures of MoS2 flakes on the substrates. The presence of DNA can be identified by the presence of the element P in the EDAX spectra. The peaks with the energies corresponding to P are easily distinguished when the substrate is not gold (F) than when it is gold (D) since the EDAX energy peaks for Au and P are very similar.

Figure 2 shows the AFM images [27], its profile and 3D representation of the topography of MoS2 on gold (A, B and C) and of MoS2/ProbeFAM-SH on gold (D, E and F). Flakes composed of different number of stacked layers can be observed. The gold topography image and profile are shown in Figure S3.
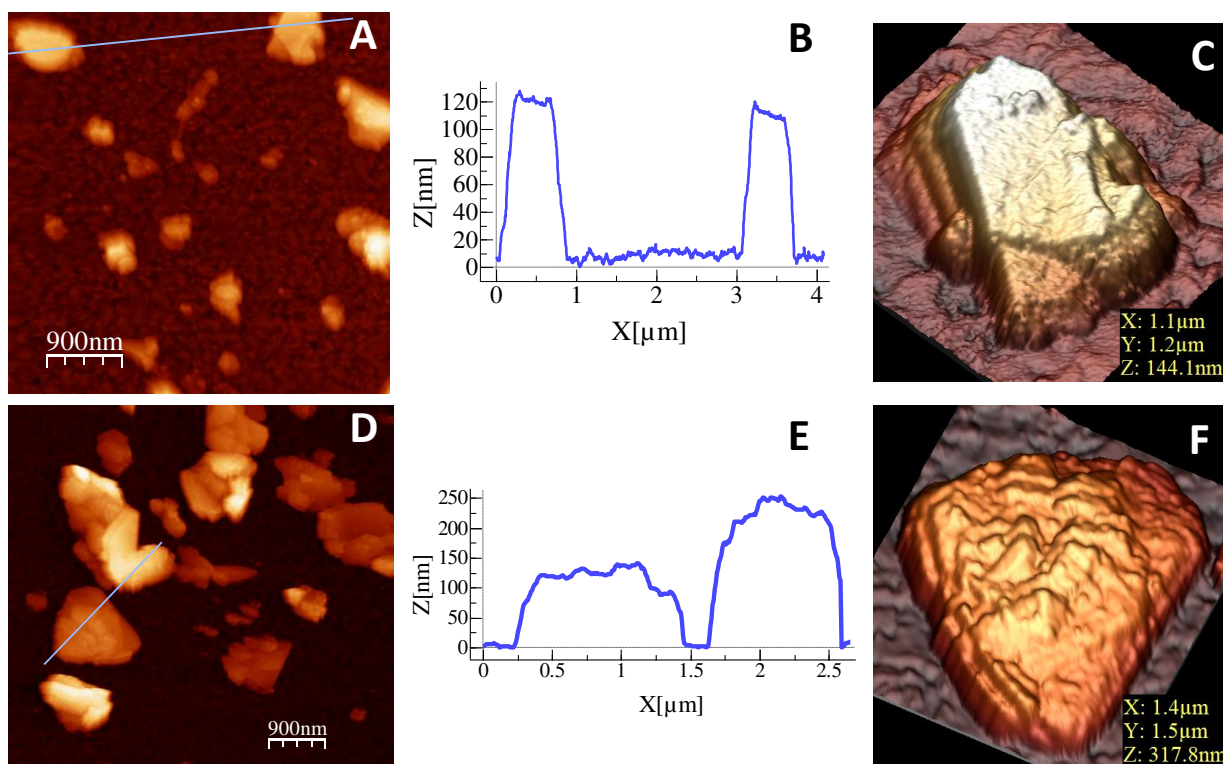
Figure 2. AFM images of (A) Au/MoS2 and (B) Au/MoS$_2$/Probe$_{FAM}$-SH, their respective profiles (B, E) and their respective three-dimentional AFM images (C, F).

Furthermore, Fluorescence microscopy images were also taken in order to follow the probe immobilization onto Au/MoS$_2$, a thiolated probe modified also with a fluorophore (FAM) was employed. As can be observed (Figure 3), white light optical microscope image before (Figure 3B) and after (Figure 3C) probe immobilization are quite similar. However, fluorescence images (Figures 3D, E, F) are very different since only the Au/MoS$_2$/Probe$_{FAM}$-SH (Figure 3F) gives a fluorescence image with contrast, confirming that the DNA probe is bound to Au/MoS$_2$ (Figure 3F). As can be observed, neither bare Au (figure 3D), nor Au modified with Au/MoS$_2$ (Figure E) show any fluorescence contrast.
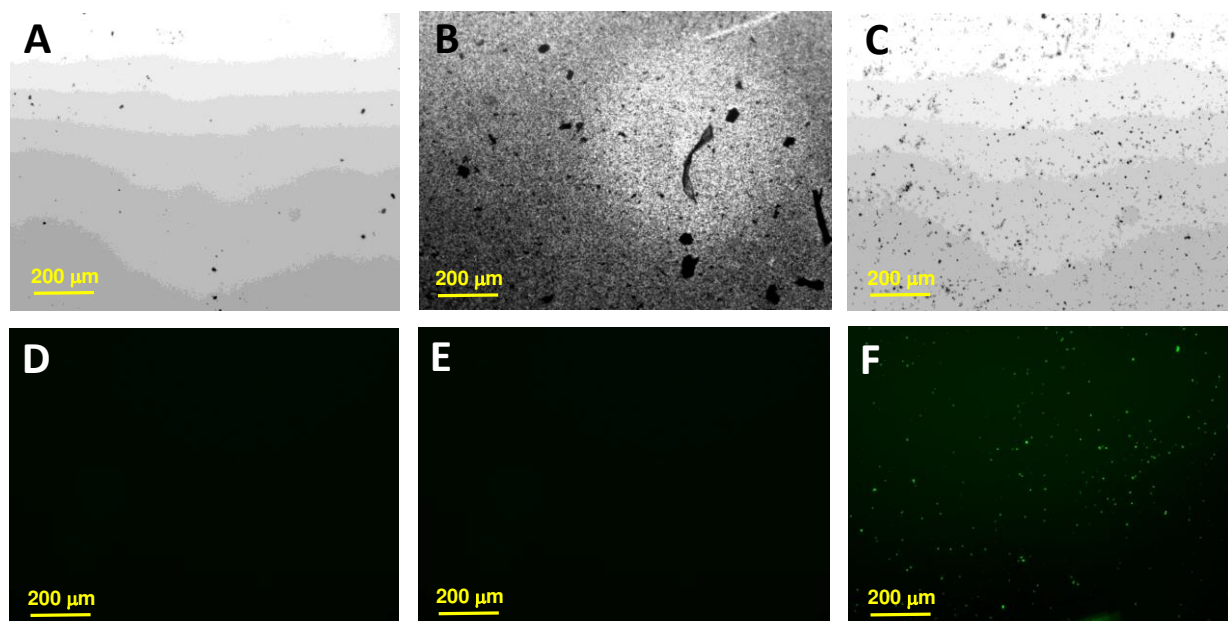
Figure 3. Bright field (A, B, C) and Fluorescence (D, E, F) optical images of bare gold substrate (A, D), Au/MoS2 (B, E) and Au/MoS2/ProbeFAM-SH. (C.F). Only the Au/MoS2/ProbeFAM-SH shows contrast on the fluorencence image (F): bright dots that correspond to the black dots (MoS2 flake aggregations) of image C.

Once we confirm DNA biosensor development, we decided to validate the use of the designed multiplex potentiostat. For this reason, we compare the biosensor response obtained by the commercial potentiostat and the designed portable multiplex potentiostat before and after hybridization with the complementary sequence. Figure 4 shows the intensity registered applying different potentials with the portable multiplex potentiostat (A) and the commercial one (B).-As can be observed the maximum current is recorded, in both cases, at a potential of approximately -0.4V, which is ascribed to the oxidation of the redox indicator (Ty-CDs). On the other hand, the intensity at this potential is higher in the case of the portable multiplex potentiostat (95.8 μA) compared to the register with the commercial one (5 μA). This points out that the designed multiplex potentiostat, besides being more accessible and portable, has a higher sensitivity than the commercial ones. This is another advantage when it comes to applying them as POC devices.
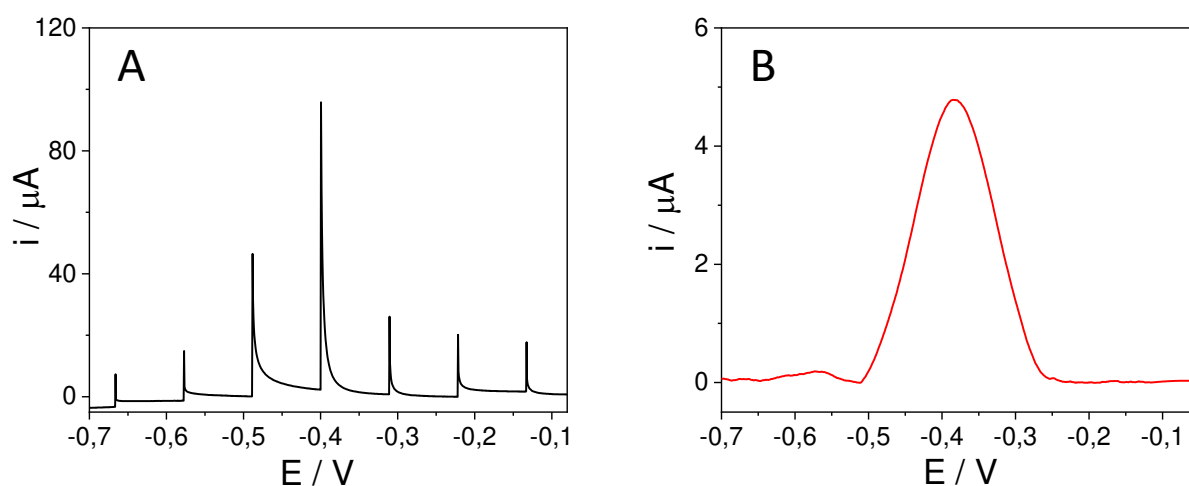

Figure 4. Signal registered for the platform SPE/MoS2/Salm-SH after incubating with 10 μL of 100 pM Salmonella complementary sequence with the designed portable multiplex-potentiostat (**A**) and the commercial multiplex-potentiostat (**B**).

In addition, to confirm multianalyte detection we study the biosensor response (at -0.4 V) using both potentiostats, before and after hybridization with the specific sequence of each bacterium (List-C or Salm-C) and with a non-complementary sequence. Figure 5 shows the biosensor response for each case measuring at the potential characteristic of the redox indicator. As can be shown, for both analytes, a clear difference can be observed in the intensity of the current recorded when the platform is hybridized with the complementary sequence with respect to the unhybridized control. In contrast, when the platform is hybridized with a non-complementary sequence, the current intensity is similar to that of the control. These results confirm the applicability of the developed device to multiplex detection of specific sequence of the bacteria of interest using Ty-CDs as electrochemical indicators and MoS2 as immobilization platform. Furthermore, as can be observed in Figure 5 the signals obtained in the case of using the portable multiplex potentiostat are higher (at least 3 time more) than those obtained with the commercial potentiostat suggesting a better signal/ noise ratio in the developed potentiostat.
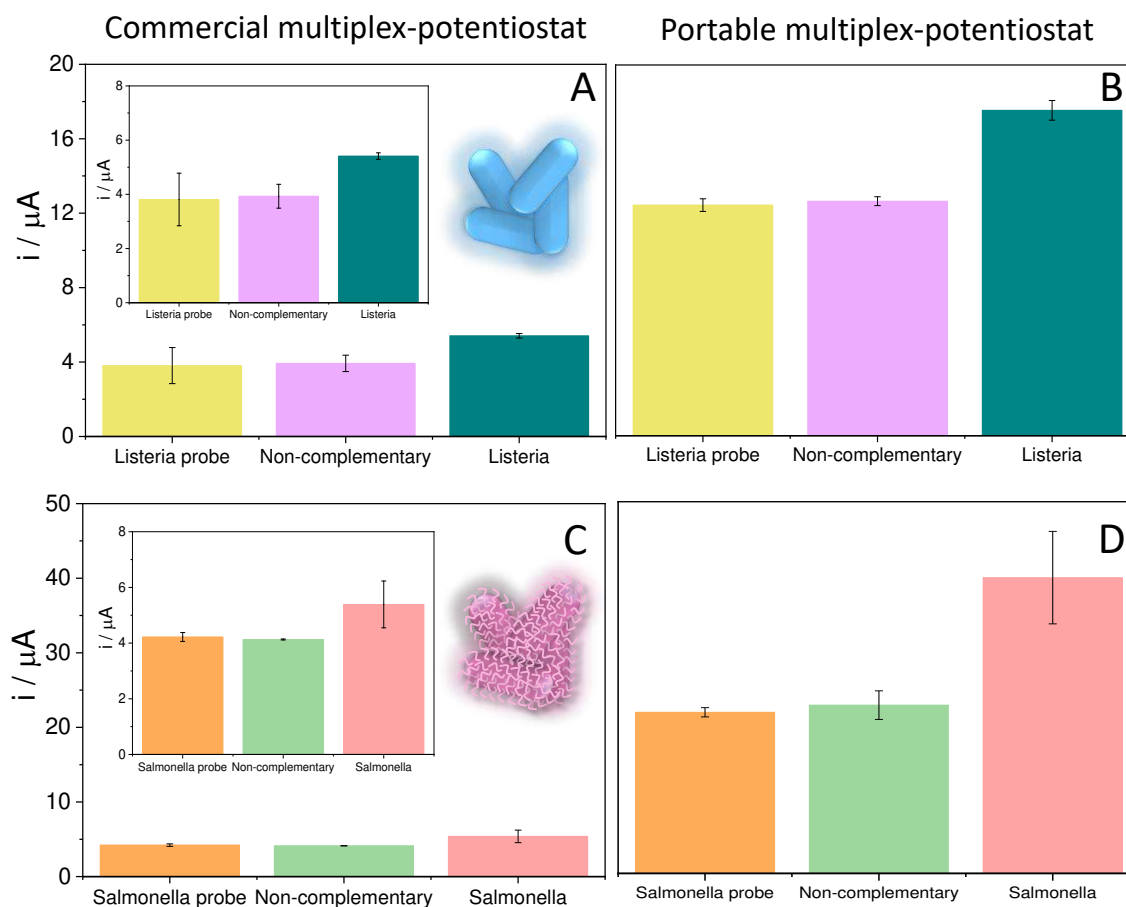
Figure 5. Bar diagrams for the signal obtained with the commercial multiplex-potentiostat (**A** and **C**) and the portable multiplex-potentiostat (**B** and **D**) for the $SPE/MoS_2/List-SH$ (lime), $SPE/MoS_2/List-SH/NC$ (purple), $SPE/MoS_2/List-SH/List-C$ (blue), $CSPE/MoS_2/Salm-SH$ (orange), $SPE/MoS_2/Salm-SH/NC$ (green), $SPE/MoS_2/Salm-SH/Salm-C$ (pink) platforms.

In order to obtain a better comparison of the results obtained using both potentiostats, we represented the percentage of the signal increase after incubating the $SPE/MoS_2/List-SH$ and the $SPE/MoS_2/Salm-SH$ platforms with a non-complementary and the complementary sequences using the commercial (Figure 6A) and the developed multiplex potentiostat (Figure 6B). As can be observed in Figure 6, the % signal increase registered with the portable multiplex potentiostat designed after hybridization event is higher than the obtained with the commercial one, 3 time higher in the case of salmonella detection. Thus, confirming again the advantages in sensitivity of designed biosensor present in terms of multiplex bacteria biorecognition.
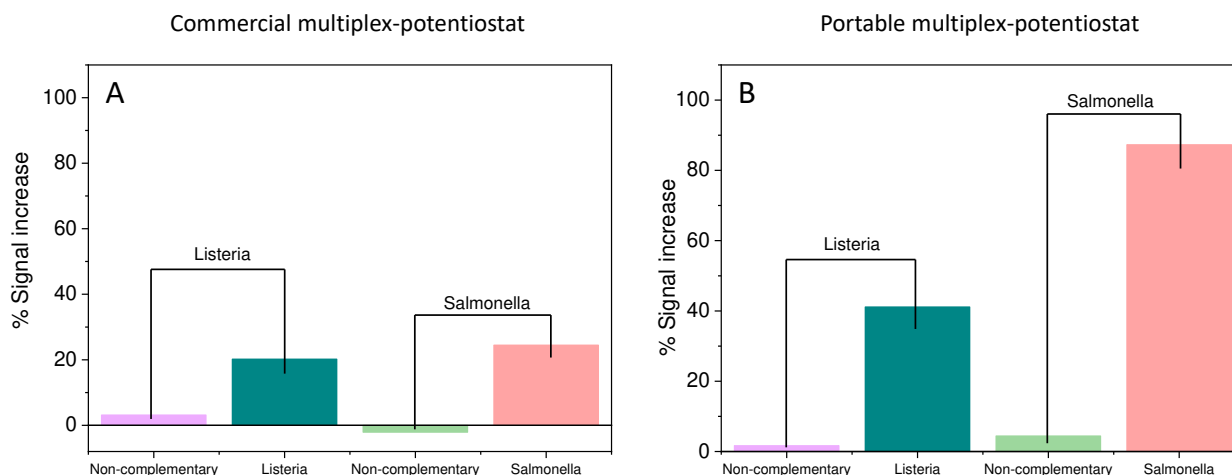
Figure 6. Bar diagrams comparing the % signal increase obtained with del commercial multiplex-potentiostat (**A**) and the portable multiplex-potentiostat (**B**) after incubating the SPE/MoS$_2$/List-SH and the SPE/MoS$_2$/Salm-SH platforms with a non-complementary sequence (red and grey), or with the listeria and salmonella complementary sequences respectively (blue and green).

4. Conclusions

In this study, a portable DNA biosensor for the multiplex detection of bacteria (*listeria and salmonella)* is presented, serving as a proof-of-concept for a point-of-care (POC) DNA sensing device. The biosensor combines a disposable DNA sensing electrochemical platform based on the use of MoS$_2$ and *à la carte* thionine functionalized carbon nanodots (Ty-CDs), with a portable multiplex potentiostat for signal acquisition. The design, construction, and validation of each component of the prototype are described in detail. The system is built around the following main blocks: a main controller board responsible for overall equipment management, a data acquisition board housing the necessary components for fast and low-noise data acquisition.

The primary objectives of this work were to develop a portable, cost-effective, and user-friendly DNA biosensing device for the specific detection of *listeria and salmonella* DNA sequences. These objectives were achieved through compact dimensions, affordability, and the ability to interface with a computer, smartphone, or tablet via a local network connection or the internet. The device offers a user-friendly interface for operation and control.

The portable multiplex potentiostat was validated by successfully detecting the specific DNA sequences of two bacteria. The performance of the developed device was comparable to that of a commercial potentiostat, indicating the advantages in sensibility and potential application of the prototype in POC testing scenarios. Overall, this work presents a promising development in the field of multiplex DNA biosensing.

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