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D1.2 - Design, characterization and optimization of hardware and readout circuit for biosensors

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Deliverable D1.2 - Design, characterization and optimization of hardware and readout circuit for biosensors

Short summary: Deliverable D1.2 reports the work performed by NTUA in Task 1.4 for the design, integration, verification and packaging of the MOBILES electrochemical readout circuit. Development progressed from a benchtop Vertex.100mA reference setup to a portable reader based on the EmStat Pico Development Kit and controlled through PStTrace 5.13 software. The document focuses on the functional circuit architecture, WE/RE/CE electrode interfacing, USB communication and power, accessible sensor connectors, PCB mounting and the custom 3D-printed enclosure. CV and SWV measurements with the E2 aptasensor were used to verify the complete circuit-to-sensor chain.

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1. Summary

Deliverable D1.2 documents the engineering work completed in Task 1.4 to convert the electrochemical measurement requirements of the MOBILES biosensors into a portable and mechanically protected readout platform. The central output is the readout circuit and its enclosure: the electrical signal path, the three-electrode connection, the communication and power interfaces, the software-controlled measurement sequence, the mounting of the development PCB and the physical access required for repeated operation.

A two-stage development strategy was followed. A Vertex.100mA benchtop potentiostat (IVIUM Technologies, Eindhoven, the Netherlands) connected through a DRP-CAC (Metrohm DropSens, Asturias, Spain) interface was first used as the reference configuration for defining the potential windows, confirming screen-printed-electrode contact and establishing the initial CV and SWV procedures. The verified methods were then transferred to a compact platform centred on the EmStat Pico Development Kit (PalmSens BV, Houten, the Netherlands) and operated through PSTrace 5.13 software.

At circuit level, the portable platform provides the complete functional chain required by a three-electrode electrochemical sensor: generation of the programmed potential waveform, potentiostatic feedback using the reference and counter electrodes, conversion and digitization of the working-electrode current, communication with the host computer and export of the acquired voltammograms. The validated operating path uses USB for power and control and preserves access to the LEMO and screw-terminal cell connections. One electrochemical channel was used in the present work, while the underlying module retains a second channel and additional interfaces for future integration.

The circuit was integrated into a custom enclosure designed in Autodesk Fusion around the PCB geometry. The enclosure includes internal supports, dedicated connector openings, a removable lid, heat-set inserts and M3 fasteners. It protects the electronics from handling and stabilizes the relationship between the PCB, cables and disposable sensor without claiming environmental sealing or an Ingress Protection rating at this prototype stage.

Functional verification was performed with a screen-printed gold electrode (SPGE) and the 17 β -Estradiol (E2) aptasensor as a representative sensor load. The portable circuit reproduced the expected CV response of the ferri/ferrocyanide system and resolved the SWV current changes associated with aptamer functionalization and E2 binding. The analytical results are included only as evidence that the complete reader-to-electrode chain is operational; the main contribution of D1.2 remains the circuit implementation and its packaged form.

This system is now in use at NTUA, UBE and INRAE laboratories where other electrochemical biosensors are developed using the same SPGE. It will be also used to test the biosensors performances, results will be reported in a separate deliverable D1.4.





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2. MOBILES project short description

The National Technical University of Athens (NTUA) is working with another 14 partners from academia, research, and industry to develop prototypes of electronic and organism-based biosensors to monitor organic chemicals, antimicrobial-resistant (AMR) bacteria, and pathogens in water, soil, and air.

The MOBILES project is studying and developing biosensors for detecting heavy metals, antibiotics, pesticides, arsenic, microplastic and nanoplastic. It is also developing genetically modified plants and bacteria for detecting heavy metals, antibiotics, and pesticides, and the use of marine diatoms for monitoring bioplastic degradation.

MOBILES aims are to tackle chemicals, including persistent and mobile (PMCs) pollutants and contaminants of emerging concern (CECs), that degrade the environment. Another severe global health risk is associated with increasing AMR in bacteria. Foodborne pathogens, including *Listeria*, *Salmonella*, and *Campylobacter*, pose significant public health risks and are already monitored. However, current bacterial detection methods for environmental control are slow and require specialized laboratories with trained personnel. Similarly, conventional pollutant detection methods, such as chromatography and mass spectrometry, are accurate but time-consuming and require specialized equipment. State-of-the-art detection methods are unsuitable for constant on-site and real-time monitoring. The long time between sampling and detection reduces the efficiency of public health and environmental protection authorities in implementing effective countermeasures. To tackle this problem, several electrochemical biosensors will be developed within the MOBILES project.

Biosensors are devices that combine biological elements with electronic systems to detect specific pollutants. The MOBILES project enhances these sensors with advanced nanomaterials, significantly improving their sensitivity and reliability. All biosensors will have common basic electronics and functional principles (e.g., an organic ligand able to recognize target pollutants), but they will differ in the biological element employed: (i) aptasensors based on aptamers that recognize bacterial cells or spore surfaces, (ii) electronic noses for detecting and quantifying volatile organic compounds (VOCs) produced by bacteria, (iii) genosensors for detecting genes involved in antibiotic resistance, and (iv) interdigital capacitors functionalized with aptamers for estradiol, a member of CECs family.

Continual threats (such as industrial pollution and the overuse of drugs and pesticides) to sources of drinking water require real-time solutions for wide-ranging water monitoring systems to detect toxicants such as heavy metals, pesticides, and antibiotics. Conventional methods are limited in their ability to detect sub-lethal concentrations of active antibacterial compounds. The damage caused by the activity of an antibacterial agent or pesticide may stimulate different biological mechanisms of bacterial repair. Each antibiotic and/or pesticide triggers specific cellular pathways, mechanisms, and targets within the bacterial cell. This specific biological response, enabling the detection of antibiotics and pesticides using microorganisms, is being investigated in the MOBILES project through the use of genetically modified bacteria to detect toxic pollutants in water. For detecting heavy metals (cadmium, chromium, lead, mercury) in water, MOBILES is developing a flow-through





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device for continuous monitoring using biological systems (genetically modified bacteria) combined with an optical sensor and flow unit.

Highly toxic arsenic pollution can come from various sources, including industrial activities, mining, and even natural processes. Water and food contaminated by arsenic can cause serious health problems, including cancer and heart disease. For detecting arsenic pollution in soil and groundwater, the MOBILES project is developing genetically modified plants that change colour when arsenic is present in the soil or water used to grow them. The project will conduct safety evaluations to ensure that the genetically modified organisms and developed devices have minimal environmental impact. A specific work package (WP4) is dedicated to evaluate the effects of genetically modified organisms on other organisms and the environment. Safety tests and environmental impact of MOBILES organisms are performed in laboratory using EFSA guidelines.

Microplastic and nanoplastic pollution is raising concerns about its potential impact on human health. The transfer of very small plastics through the trophic chain is a potential source of contamination at all trophic levels. Understanding the distribution, degradation, and life cycle of micro- and nanoplastics in the marine environment is limited by the intrinsic difficulties of current techniques for detecting, quantifying, and chemically identifying small particles in liquids. The MOBILES project is addressing this challenge by utilizing marine diatoms. Diatoms are known for their resilience and adaptability, making them ideal candidates for studying the biodegradation of bioplastics in marine environments. Preliminary studies have shown promising results, indicating that diatoms not only survive in environments containing bioplastics but also contribute to their biodegradation.

In addition to the development of sensors, MOBILES will undertake comprehensive metagenomic analysis, profiling the microbiota of polluted areas across Europe. This work will uncover gene clusters and reveal genetic diversity, enabling a deeper understanding of microbial functions. These insights will provide genetic markers to facilitate rapid evaluation of soil and land health. Two annual sampling rounds are planned for at least two years, and sample collection will be conducted at different locations to target microbiota related to specific pollution types: Greece for urban wastewater contamination, Poland for heavy metal pollution, Cyprus for microplastics and plastics, France for agriculture and animal farming, Italy for arsenic, and Germany for chemicals and heavy metals from former mining activities. Genomic and transcriptomic data will be analysed, visualized, and interpreted using bioinformatic tools and soil metagenomic web-based platform specifically realized by MOBILES partners. The project's data storage, located in Spain, will be connected to other well-known genomic databases in order to provide a wide range of information.

The biosensors will be rigorously tested with real-world samples from polluted sites to validate their environmental performance.

MOBILES workplan is organized in 6 WPs listed in Table 1. Management actions and collaboration with other EU funded projects are implemented within WP6 while disseminations, exploitation and communication (DEC) activities are grouped within WP5. WP1-WP2 deal with interconnected scientific and technological activities to develop electrochemical biosensors to detect specific





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pollutants, and organism-based biosensor to monitor other typology of selected pollutants. In WP3 an extensive metagenomic analysis will be performed in order to enable searches for diverse functionalities across multiple gene clusters in polluted and not polluted areas across Europe. In WP4 the safety of all genetically modified bacteria and plants will be tested using standard EU procedures (e.g., EFSA guidelines for genetically modified organisms), and a pre-industrial design of the various biosensors will be provided along with stability (shelf-life) tests.

Table 1. MOBILES WPs list

WP	Work Package Title	Lead Name	Start Month	End month
1	Electronic biosensors for environmental monitoring	INRAE	1	36
2	Detection of pollutants via biotic sensors	UR	1	36
3	Metagenomics database and fully-sequenced polluted soil microbiota	CNR-ISAFOM	1	42
4	Environmental performance and safety of developed organisms, and packaging of sensor devices	RICPA	10	42
5	Dissemination, exploitation and communication of project outcomes	GG	1	42
6	Project Management and Coordination	NTUA	1	42





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3. Introduction: readout circuit

Within Task 1.4, NTUA developed a custom portable electrochemical reader to interface the disposable biosensors of MOBILES with controlled electrical excitation and digital data acquisition. The objective was not simply to operate a commercial potentiostat, but to transform a compact electrochemical engine into an application-specific readout subsystem. The work therefore addressed the complete reader architecture: the three-electrode signal path, potential-control and current-acquisition functions, electrode and communication interfaces, software-controlled operation, printed circuit board (PCB) integration, and a purpose-designed protective enclosure. The resulting device provides a repeatable electrical and mechanical link between screen-printed electrodes (SPEs) and the digital electrochemical data required for biosensor development, comparison and validation.

At circuit level, the reader follows a three-electrode potentiostatic architecture. The sensing layer is located on the working electrode (WE), the reference electrode (RE) provides the potential reference, and the counter electrode (CE) carries the current required to regulate the electrochemical cell. A waveform-generation stage defines the applied potential profile, while a feedback-control stage drives the CE so that the programmed potential between WE and RE is maintained. The current produced at the WE is converted into a measurable voltage by a transimpedance amplifier (TIA), conditioned within an appropriate current range, digitised by an analogue-to-digital converter (ADC), and transferred to the digital control and communication layer. This common signal chain can generate and acquire the waveforms required for cyclic voltammetry (CV), differential pulse voltammetry (DPV), square-wave voltammetry (SWV) and electrochemical impedance spectroscopy (EIS). In the present work, CV and SWV were used for experimental verification, while the reader architecture preserves the capability to implement DPV and EIS for other MOBILES sensor formats.

The EmStat Pico was integrated as the compact electrochemical core of the reader rather than treated as a finished stand-alone instrument. NTUA defined the application-specific system around this core, including explicit mapping of the WE, RE and CE connections, compatibility with disposable SPEs, external access through the LEMO and screw-terminal interfaces, and retention of the USB communication path. PSTrace 5.13 software was used to configure the potential program, current range, acquisition timing and data export, thereby providing a controlled and repeatable operating layer for the hardware. Particular attention was given to connector accessibility, stable board support and short, well-defined electrode interconnections, since poor contact, cable movement and parasitic electrical effects can directly alter the low-current response of a biosensor. The reader was consequently engineered as one coordinated measurement chain rather than as a collection of disconnected commercial components.

Mechanical integration was developed in parallel with the electrical architecture. Using the manufacturer-provided STEP model of the development board, a dedicated enclosure was designed in Autodesk Fusion to reproduce the exact PCB geometry and connector positions. Internal standoffs locate and support the board, while dedicated openings maintain direct access to the screw-terminal





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block, LEMO connector and USB interface. A two-part base-and-lid construction, brass heat-set inserts and M3 fasteners allow repeatable assembly and disassembly without damaging the printed polymer. The enclosure is therefore a functional part of the reader: it protects the electronics, preserves connector alignment, reduces handling-induced movement and converts the development electronics into a stable portable device suitable for repeated laboratory use and subsequent field-oriented development.

Development and verification were performed iteratively. The Vertex.100mA benchtop potentiostat served as a reference platform for establishing electrode behaviour and defining transfer criteria, after which the operating conditions were implemented and refined on the custom portable reader. The complete circuit, interface and enclosure assembly was then verified with the E2 aptasensor using CV and SWV, demonstrating that the reader could reproduce the required electrochemical behaviour and resolve concentration-dependent signal changes. The principal outcome of Task 1.4 is therefore a reusable MOBILES readout architecture: a compact electrochemical core integrated with application-specific electrode interfacing, controlled acquisition, accessible communication and custom mechanical packaging, ready to support additional aptamer, immunosensor, genosensor and molecularly imprinted polymer platforms within WP1.

4. Scope and objectives of D1.2

The scope of D1.2 is the design, integration and verification of the hardware and readout circuit supporting the MOBILES electrochemical biosensors. Accordingly, the document prioritizes circuit functions, physical interfaces and enclosure engineering over a detailed discussion of recognition chemistry or analyte performance.

The main objectives are:

- to translate the electrochemical measurement needs of WP1 into explicit circuit and packaging requirements;
- to document the reference benchtop configuration and the subsequent transfer to a portable EmStat Pico-based reader;
- to describe the functional signal chain from host software and potential generation to WE-current acquisition and data export;
- to define the WE/RE/CE electrode interface, connector access and the operating path used in the prototype;
- to report the mechanical integration of the PCB, the enclosure dimensions, fabrication parameters and assembly hardware;
- to verify the complete reader through CV and SWV measurements with a representative screen-printed biosensor; and





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- to define the limits of the present prototype and the pathway toward a custom embedded reader for later project stages.

The electrochemical analogue front end inside the EmStat Pico is a tested and calibrated manufacturer module. Task 1.4 did not attempt to reproduce or redesign its proprietary transistor-level circuitry. The engineering contribution lies at the application-system level: selection of the electrochemical core, method transfer, sensor and host interfacing, connector exposure, repeatable PCB mounting, enclosure design and verification of the assembled reader. This boundary is stated explicitly so that the delivered work is technically clear and reproducible.

5. Engineering requirements for the readout circuit and enclosure

The reader requirements (Table 2) were derived from the three-electrode screen-printed sensors used in WP1, the CV and SWV methods required for method development and detection, the microampere-scale redox currents observed during validation and the need to move the electronics from an exposed development board to a portable assembly. The requirements therefore cover electrical performance, interfaces, data control and mechanical implementation.

Table 2. Engineering requirements and implemented response for the MOBILES readout circuit.

Requirement	Engineering rationale	Implemented response
Controlled potential generation	CV and SWV require repeatable potential waveforms between WE and RE.	Potentiostat core with software-defined methods and feedback through RE/CE.
Low-current acquisition	Affinity-based sensor responses are expressed as changes in microampere-scale peak current.	Selectable current ranges, transimpedance current conversion and 16-bit digitisation within the EmStat Pico core.
Three-electrode compatibility	Disposable SPEs expose separate WE, RE and CE contacts.	Explicit WE/RE/CE mapping through the LEMO/SPE path and accessible screw terminals.
Low-noise operation	Cable movement, poor contacts and noisy supply conditions can obscure small signal changes.	Short board-level paths, stable connectors, USB-powered development configuration and mechanical stabilization inside the enclosure.
Method reproducibility	Potential limits, pulse parameters and timing must not vary between sensors.	PSTrace method files, fixed operating sequence and export of raw curves with method metadata.





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Serviceable interfaces	The prototype must support sensor exchange, direct test access and host connection.	Openings for USB, LEMO and terminal access without removing the PCB.
Mechanical protection	The exposed PCB and connectors are vulnerable during repeated handling.	Custom base and lid, internal standoffs, heat-set inserts and M3 fasteners.
Expansion capability	Later sensors may require a second channel, embedded control, storage or wireless operation.	Development-board provisions are retained, while unverified options are clearly separated from the present USB workflow.

6. Readout circuit architecture

6.1. Two-stage development strategy

Circuit development (Table 3) was deliberately separated from assay development. The benchtop instrument established a reference electrochemical response before the portable circuit was introduced. This reduced the risk of attributing a chemical or electrode problem to the portable electronics and provided a clear basis for method transfer.

Table 3 Two-stage circuit-development and verification strategy followed in Task 1.4.

Stage	Configuration	Circuit objective	Output
Stage 1 - reference	Vertex.100mA + DRP-CAC + screen-printed electrode	Define potential windows, verify contact and establish reference CV/SWV traces.	Reference method and baseline response.
Stage 2 - portable	EmStat Pico Development Kit + PStace 5.13 + external SPE interface + enclosure	Transfer the method, verify signal acquisition and integrate the PCB mechanically.	Packaged portable readout verified with the E2 sensor.

6.2. Functional signal chain

Figure 1 summarizes the validated signal and data paths. PStace sends the electrochemical method to the development board through USB. The electrochemical core generates the commanded waveform, controls the cell potential by driving CE while sensing RE, measures the current entering WE and digitizes the current and potential data. The cell is connected through the accessible LEMO/screw-terminal interfaces and an SPE adapter. The resulting curves are returned to the host for visualization and export.



MOBILES portable electrochemical readout: functional circuit architecture

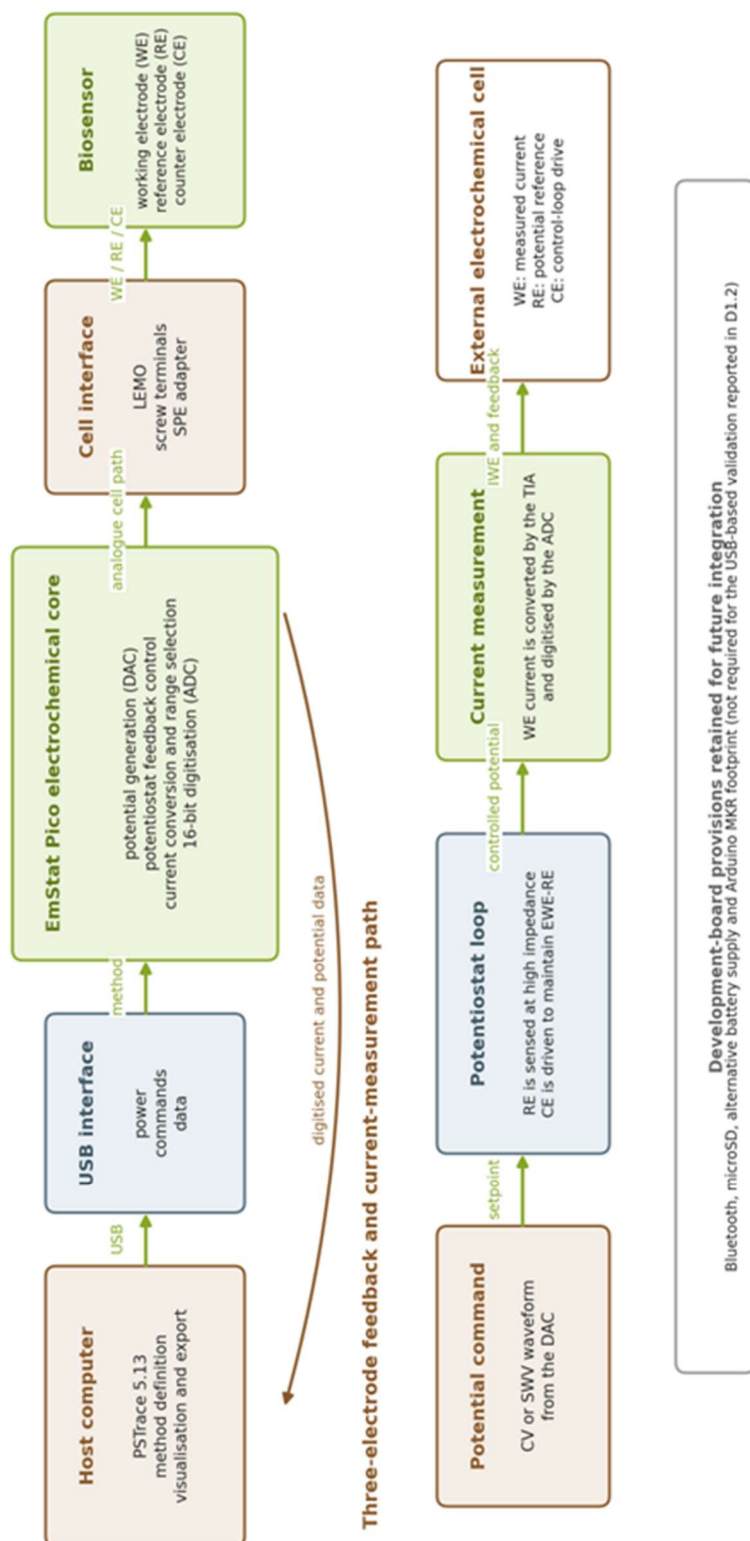


Figure 1. Functional architecture and signal flow of the portable electrochemical readout circuit. The solid path identifies the USB-controlled configuration verified in Task 1.4; expansion resources of the development board are shown separately. Prepared from the implemented configuration and manufacturer documentation.



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6.3. Functional circuit blocks

Table 4 illustrates the functional circuit blocks.

Table 4. Functional blocks of the portable readout and their responsibilities.

Block	Function in the circuit	Task 1.4 implementation
Host and method layer	Defines potential limits, scan or pulse parameters, timing and data export.	PSTrace 5.13 on a Windows host.
Communication and power	Transfers commands and data and supplies the development board.	Micro-USB operating path used throughout the reported validation.
Potential-generation block	Converts the programmed method into the analogue excitation applied to the electrochemical cell.	DAC resources integrated in the EmStat Pico module.
Potentiostat feedback loop	Maintains the commanded WE-RE potential by sensing RE and driving CE.	One of the EmStat Pico potentiostat channels used for the three-electrode SPGE.
Current-measurement block	Converts WE current to a measurable voltage, selects the current range and digitises the response.	TIA/current-range resources and 16-bit ADC integrated in the module.
Cell interface	Maps the analogue channel to WE, RE and CE and provides exchangeable sensor access.	LEMO and screw-terminal connections with SPE adapter.
Mechanical subsystem	Supports the PCB, avoids cable-induced movement and exposes the required interfaces.	Custom FDM-printed base and lid with inserts and fasteners.

6.4. Electrochemical core and board-level interfaces

The EmStat Pico (Figure 2) module integrates (Table 5) two potentiostat channels, high-impedance RE inputs, current-measurement channels, DAC resources and a 16-bit ADC. Manufacturer documentation specifies a maximum channel current of ± 3 mA, current ranges extending down to the 100 nA range, and impedance-measurement capability from 0.016 Hz to 200 kHz. The D1.2 validation used one channel and voltammetric operation; the second channel and impedance capability remain available for later sensor variants subject to the installed module version and method requirements.



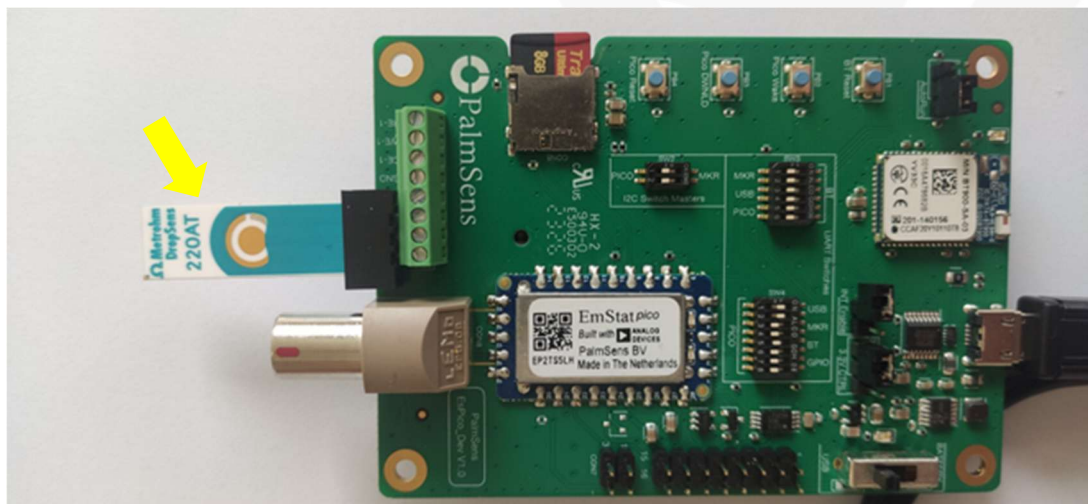


Figure 2. EmStat Pico board used by MOBILES biosensors connected to a SPGE (yellow arrow)

Table 5 Physical and logical interfaces of the development platform

Interface	Role	Status in the D1.2 prototype
Micro-USB	Board power, host connection, method transfer and return of measurement data.	Used and exposed through the enclosure.
LEMO cell connector	Robust external connection to the electrochemical sensor cable/SPE adapter.	Used for the packaged reader and exposed through a circular cutout.
Screw-terminal block	Direct access to cell connections during development, troubleshooting or alternative fixtures.	Retained and accessible through the enclosure.
SPE adapter/connector	Provides repeatable contact between the reader cable and disposable screen-printed electrodes.	Used for the validation electrodes.
Bluetooth, microSD, alternative battery input and Arduino MKR footprint	Development resources for embedded or wireless operation.	Board capabilities retained but not required or validated in the reported USB-based workflow.

6.5. Design boundary and system-level customization

The portable reader is not presented as a new discrete potentiostat schematic. The calibrated analogue core is a commercial module, while the MOBILES engineering work defines how that core is used as a complete biosensor reader. The implemented customization includes the reference-to-portable method transfer, selection of the validated communication and power path, explicit cell-



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interface mapping, accessible test and sensor connectors, software-controlled execution, raw-data handling and the mechanical conversion of the exposed development PCB into an assembled instrument. This is the appropriate integration level for the current project maturity and creates a clear route toward a later custom carrier PCB using the same electrochemical module.

7. Hardware implementation and enclosure integration

7.1. Benchtop reference configuration

Initial measurements were performed with a Vertex.100mA benchtop potentiostat (IVIUM Technologies, Eindhoven, The Netherlands). A DRP-CAC connector (Metrohm DropSens, Asturias, Spain) provided stable contact to the screen-printed electrode. This setup was used to verify the electrode/redox-probe response, define the first CV and SWV parameter sets and identify workflow variables that would later need to be fixed in the portable implementation.

7.2. Portable circuit assembly

The portable implementation uses the EmStat Pico Development Kit as the carrier for the electrochemical module and its supporting communication, power and connector circuitry. The PCB is mounted horizontally on internal supports so that the terminal block, LEMO connector and USB interface align with the enclosure openings. The sensor remains external to the enclosure, separating the wet electrochemical area from the protected electronics and allowing disposable electrodes to be exchanged without opening the reader.

Table 6. Main hardware, software and fabrication resources used for the readout circuit.

Element	Specification/source	Function
Reference potentiostat	Vertex.100mA, IVIUM Technologies	Reference method definition and comparison.
Reference SPE connector	DRP-CAC, Metrohm DropSens	Stable electrical contact during benchtop development.
Portable electrochemical core	EmStat Pico Development Kit, PalmSens	Potential control, current acquisition, digitisation and communication.
Disposable transducer	DRP-250AT-U75 screen-printed gold electrode	Three-electrode sensor used for verification.
Control software	PSTrace 5.13	Method definition, execution, visualisation and data export.
Mechanical CAD	Autodesk Fusion	Enclosure design around the PCB and connectors.
Fabrication materials	PLA, brass heat-set inserts and M3 fasteners	PCB protection and repeatable assembly.





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7.3. Electrode and connector integration

The readout circuit is connected to the sensor through the conventional three-electrode mapping: WE is the current-measurement input, RE is the high-impedance potential-sensing input and CE is the control-loop output. Maintaining this mapping is essential because a swapped or intermittent contact can generate apparently valid but physically incorrect voltammograms. The packaged implementation therefore preserves a robust external connector for routine use and direct terminal access for verification or alternative electrode fixtures.

The SPE connector also provides an important mechanical function. It defines the insertion position of the disposable sensor and prevents the user from applying load directly to the PCB. During measurement, the sensor and cable should remain stationary to minimize contact variation and triboelectric or movement-related noise.

7.4. Enclosure CAD and assembly

A custom enclosure was developed in Autodesk Fusion using the manufacturer-provided three-dimensional model of the development board. The base supports the PCB on internal standoffs, while the lid closes the assembly without contacting components. Dedicated openings maintain access to the cell connector, terminal block and USB interface. The exploded assembly and fabricated prototype are shown in Figure 3.

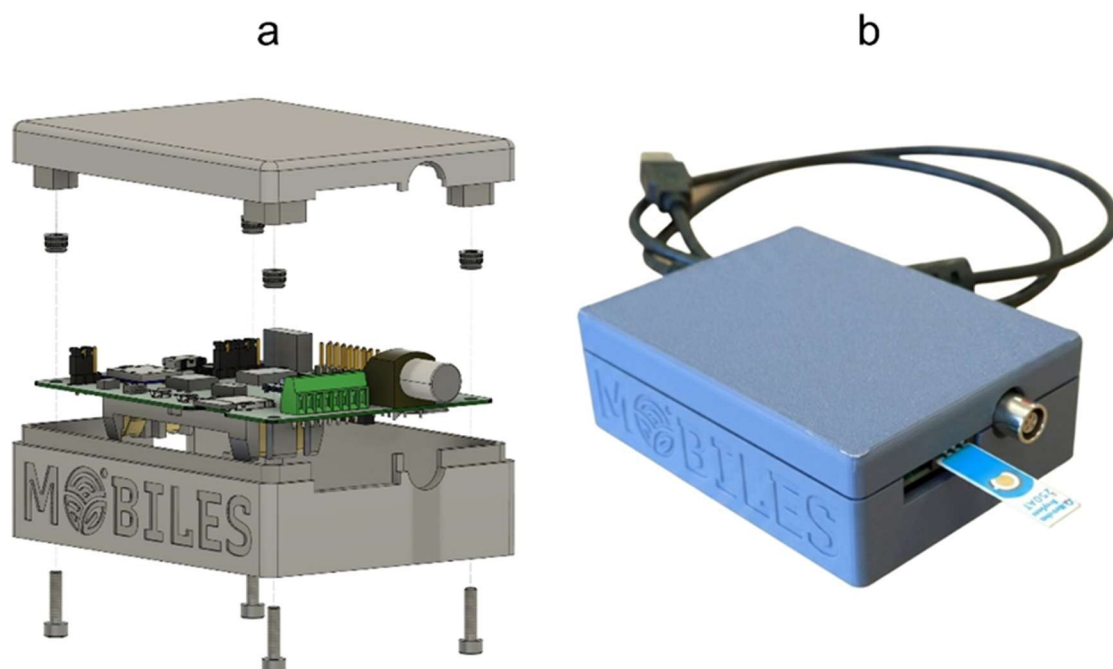


Figure 3. Portable enclosure for the electrochemical readout circuit. (a) Exploded CAD assembly showing the base, development PCB, lid, heat-set inserts and fasteners; (b) fabricated reader connected to a commercial screen-printed gold electrode.



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The upper and lower parts are secured with four M3 x 12 mm ISO 4762 socket-head screws. Tapered brass M3 x 0.5 mm heat-set inserts, 3.8 mm long, are embedded in the printed part so that the enclosure can be opened repeatedly without degrading the polymer threads. This choice is especially relevant during development, when the PCB may need to be inspected or reconfigured several times.

7.5. Enclosure dimensions and fabrication

Figure 4 presents the technical drawing of the EmStat Pico PCB board, whereas Table 7 lists its mechanical and fabrication parameters.

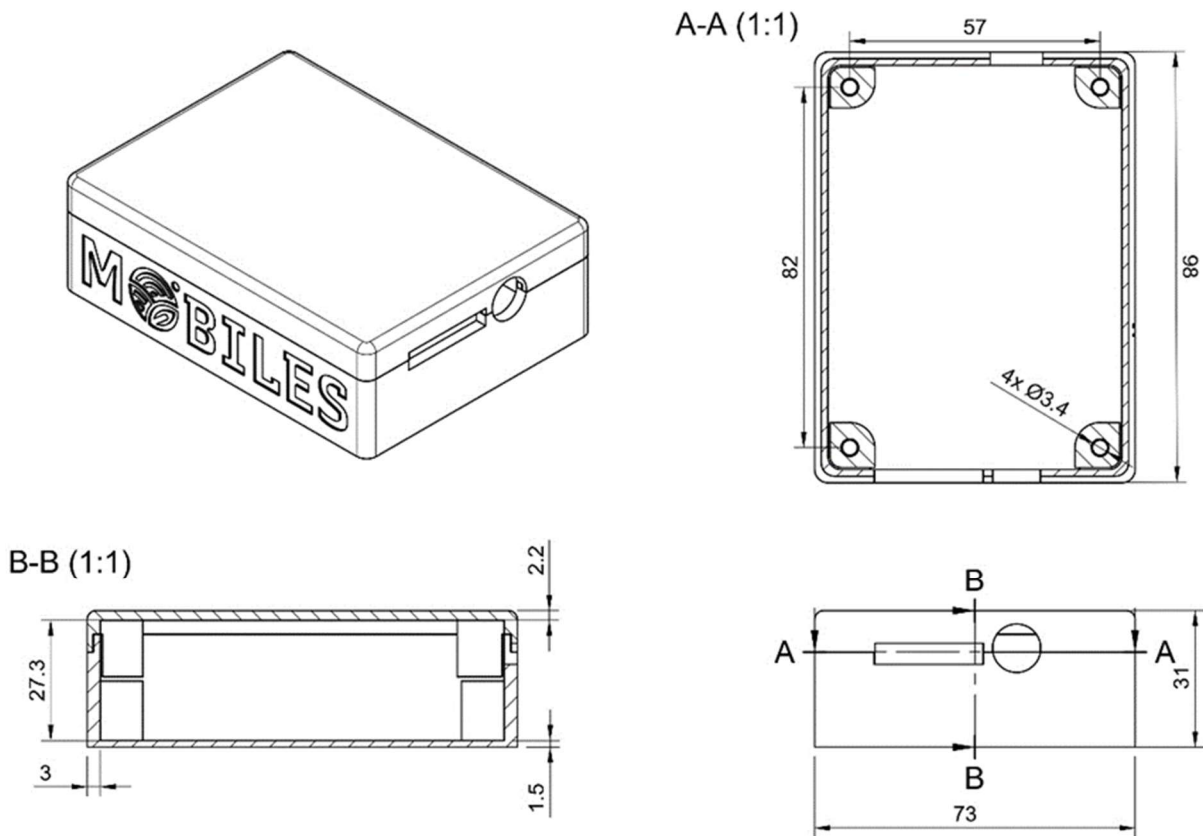


Figure 4. Engineering drawing of the enclosure showing the nominal external envelope, wall sections, connector openings and four mounting features used for replication.



Table 7. Mechanical and fabrication parameters of the enclosure prototype

Parameter	Implemented value
Nominal external envelope	73 mm x 86 mm x 31 mm, as indicated in the engineering drawing.
Principal internal span shown in section A-A	57 mm x 82 mm.
Wall and floor features	Approximately 3 mm side wall, 2.2 mm lid section and 1.5 mm base floor.
Mounting features	Four nominal diameter 3.4 mm corner features with internal standoffs.
Fasteners	Four M3 x 12 mm ISO 4762 socket-head screws.
Thread reinforcement	Tapered M3 x 0.5 brass heat-set inserts, 3.8 mm long.
Printing process	Fused deposition modelling on a Bambu Lab X1 Carbon CoreXY printer.
Material and settings	1.75 mm PLA filament, 0.2 mm layer height and 20% infill.

7.6. Mechanical assessment and present limitations

The enclosure meets the immediate Task 1.4 needs: the PCB is supported, the most frequently used interfaces are accessible, the sensor remains external, and the lid can be removed repeatedly. The design also reduces the risk of electrostatic or mechanical contact with the exposed board during routine handling.

The current prototype should not be interpreted as a final field enclosure. It has no documented gasket, ingress-protection rating, conformal coating, electromagnetic shielding or cable strain-relief test. Its purpose is to provide a stable portable research reader and a mechanically defined reference for a subsequent iteration. A field-ready version should add sealed connectors, controlled venting where required, battery retention, impact testing and environmental qualification.

8. Circuit operation, software control and verification

8.1. Software and communication workflow

PSTrace 5.13 software was used to define the electrochemical methods, transmit them to the reader, display the acquired curves and export the raw data. For the reported prototype, USB provides the most controlled development path because it combines communication and power and avoids introducing additional variables from wireless links or battery state. The development platform can later reproduce methods through MethodSCRIPT or custom software, but that embedded path was not required for the present verification.



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The operating sequence used for circuit verification was:

- connect the reader to the host by USB and confirm communication in PStace;
- load the approved CV or SWV method file and verify the electrode-channel configuration;
- connect the SPE through the defined WE/RE/CE interface and keep the cable mechanically stable;
- apply the redox-probe solution and allow the specified equilibration period;
- execute the defined scan without changing method parameters during the series; and
- export the raw voltammogram together with the method identifier and sample metadata.

8.2. Electrical and electrochemical functional verification

A bare SPGE in ferri/ferrocyanide solution was used as a practical end-to-end circuit test. The CV response contained well-defined anodic and cathodic peaks of approximately $\pm 37 \mu\text{A}$, showing that the reader could generate the potential scan, maintain the three-electrode feedback loop and acquire both current polarities. SWV then resolved the progressive current reduction produced by aptamer immobilization and E2 binding, demonstrating sensitivity to interfacial changes rather than only gross cell continuity (Figure 5).

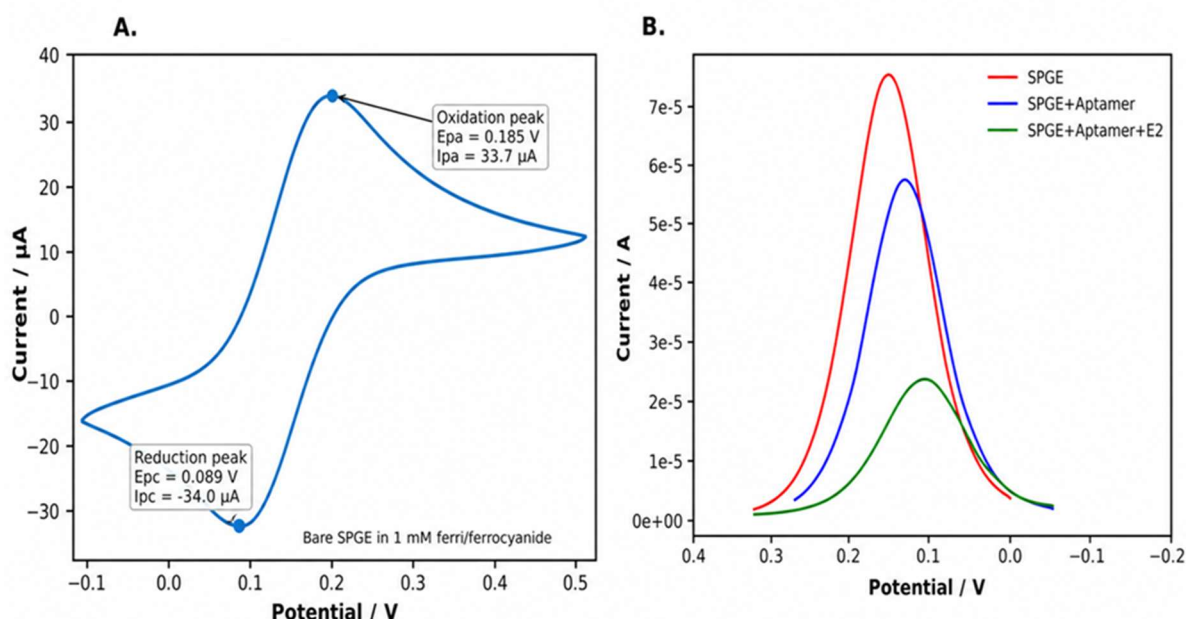


Figure 5. Functional verification of the circuit-to-electrode chain. (A) CV response of the bare SPGE in ferri/ferrocyanide; (B) SWV response after sequential interface modification. The figure confirms potential control, bidirectional current acquisition and resolution of sensor-interface changes.

8.3. Method transfer and final operating parameters

The transition from the benchtop instrument to the portable reader included a reduction of the redox-probe concentration from 5 mM to 1 mM, a defined 60 s equilibration step and refinement of the



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SWV step and amplitude. These changes reduced the peak magnitude to a more manageable level and improved consistency within the portable workflow. Table 8 separates the original reference settings from the final portable settings so that future experiments can reproduce the circuit operating conditions.

Table 8. Electrochemical methods used for reference definition and portable-circuit verification.

Stage	Technique	Main parameters	Circuit-level purpose
Reference benchtop	CV	-0.2 to 0.8 V; 50 mV/s; 5 mM ferri/ferrocyanide in 0.1 M KCl	Verify electrode contact and establish the reference redox response.
Reference benchtop	SWV	0.1 to 0.8 V; amplitude 20 mV; 10 Hz; step 8 mV	Establish the initial analytical waveform.
Portable reader	CV	1 mM ferri/ferrocyanide; anodic/cathodic peaks approximately $\pm 37 \mu\text{A}$	Confirm portable potential control and signed-current acquisition.
Portable reader	SWV	1 mM ferri/ferrocyanide; 60 s equilibration; amplitude 25 mV; 10 Hz; step 2 mV	Final readout used for sensor-response verification.

8.4. Verification matrix

Table 9 reports the verification evidence collected for the readout EmStat Pico module.

Table 9. Verification status of the circuit, interfaces and enclosure.

Item	Verification evidence	Status
Potential waveform generation	Reproducible CV and SWV traces with the defined potential programmes.	Verified
Three-electrode feedback	Stable redox peaks on the screen-printed gold electrode.	Verified
Positive and negative current acquisition	Anodic and cathodic CV peaks acquired within the same scan.	Verified
Resolution of interface changes	Distinct SWV amplitudes for bare, aptamer-modified and E2-bound surfaces.	Verified
USB communication and data return	Methods executed through PSTrace and raw curves exported.	Verified
LEMO/SPE connection	Disposable electrodes connected and exchanged during the validation series.	Verified
Enclosure fit and interface access	PCB mounted; lid assembled; USB, cell and terminal access retained.	Verified





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Wireless and autonomous battery operation	Available at development-board level but not required for the reported workflow.	Not verified in D1.2
Environmental sealing and IP performance	No gasket or ingress test included in the present prototype.	Not verified in D1.2

8.5. Operating controls for reproducible readout

The verification work showed that circuit performance and assay handling cannot be separated completely. A stable readout requires both an electrically defined method and a controlled physical sequence. The following controls were therefore adopted for subsequent measurements.

- use one approved method file for each experiment series and archive its parameters;
- confirm the WE/RE/CE mapping and full insertion of the disposable electrode before adding the sample;
- keep USB and sensor cables stationary during acquisition and avoid opening the enclosure while powered;
- use a fixed redox-probe concentration and equilibration period;
- apply one defined analytical SWV scan per electrode to limit scan-history effects; and
- export the unprocessed voltammogram together with sample, electrode and method identifiers.

9. Validation with the E2 aptasensor

The label-free E2 aptasensor was used as a representative electrochemical load for the packaged reader. A thiolated E2-specific aptamer (anti-E2 aptamer probe described in D1.1) was immobilised on a screen-printed gold working electrode, followed by E2 incubation (different concentrations), rinsing and SWV measurement in ferri/ferrocyanide. Target binding reduces the accessibility of the redox probe to the gold surface and therefore suppresses the SWV peak current.

Across 0.001-1000 nM E2 (Figure 6), the data showed an overall increase in current suppression with concentration. The logarithmic calibration reported an R^2 value of 0.883 and a calculated limit of detection of 1.51×10^{-12} M. These figures describe the current state of the biosensor assay rather than an intrinsic specification of the reader. For D1.2, their significance is that the circuit and enclosure did not prevent acquisition of a concentration-dependent electrochemical response.





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Detailed optimization of selectivity, matrix effects and inter-electrode variability remains an assay-level activity. The readout contribution is to provide a stable, method-controlled and traceable platform on which those studies can be repeated.

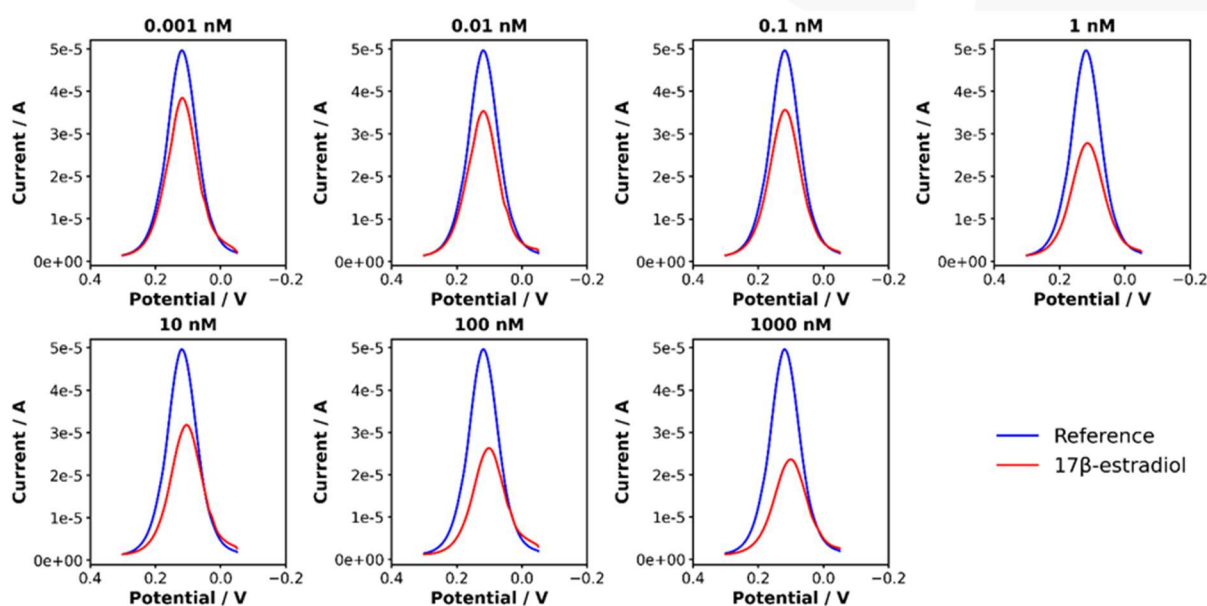


Figure 6. Representative SWV traces acquired across the E2 concentration series. The portable reader resolves the decrease in peak current relative to the reference response over the tested range.

10. Integration with MOBILES biosensors and Task 1.5

10.1. Transfer to other MOBILES electrochemical sensors

The circuit architecture is not tied to the E2 recognition layer. Its reusable elements are the software-defined potential programme, the WE/RE/CE interface, current acquisition, raw-data export and the mechanically protected connector arrangement. Other WP1 sensors (Table 10) can therefore use the same reader provided that their potential, current and frequency requirements remain within the capability of the installed module and that the appropriate method is validated.

Table 10. Compatibility of the reader developed in Task 1.4 with other MOBILES sensor families.

Sensor family	Likely readout need	Use of the D1.2 architecture
E2 label-free aptasensor	CV and SWV signal-off detection.	Fully demonstrated.
Pathogen aptasensors and immunosensors	Voltammetry or EIS with stable three-electrode contact.	Same interface; method-specific verification required.



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ARG genosensors	Pulse methods or EIS with strict metadata.	Software control and raw-data export are transferable.
Pesticide sensors	Pulse, catalytic or stripping methods.	Compatible after range and waveform verification.
MIP and hybrid sensors	Direct oxidation, DPV or EIS.	Architecture compatible; module-version dependent.

10.2. Relationship with Task 1.5 and future engineering steps

The reader is closely linked to the material and surface characterization performed in Task 1.5. The circuit defines how the interface is excited and measured, while XPS, Auger spectroscopy, AFM, CLSM and high-resolution SEM can explain the physical and chemical changes associated with the measured CV, SWV or impedance response. Combining electrical and surface information is particularly important when a signal change may originate from recognition, incomplete functionalization, fouling or electrode-to-electrode variability.

11. Conclusions

This document described how Task 1.4 lead by NTUA has delivered a complete portable readout implementation for the MOBILES electrochemical biosensors. The work progressed from a controlled benchtop reference configuration to an EmStat Pico-based circuit with defined host communication, three-electrode interfacing, software-controlled methods and raw-data export. The functional signal chain from potential generation to current digitization and returned voltammogram has been documented explicitly.

The development PCB was converted into a repeatable instrument assembly through a custom 3D-printed enclosure. The base, lid, internal supports, connector openings, heat-set inserts and M3 fasteners protect the electronics and maintain access to the interfaces required during operation. The enclosure is suitable for protected portable research use; environmental sealing and autonomous field operation remain future engineering steps.

CV and SWV measurements with the screen-printed gold E2 aptasensor verified potential control, signed-current acquisition, resolution of interface changes and concentration-dependent signal acquisition. The analytical demonstration therefore validates the integrated circuit-to-sensor chain without shifting the focus of D1.2 away from the hardware. The resulting architecture provides a technically clear basis for extension to other WP1 biosensors and for a later custom embedded reader.





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12. List of Figures

Figure 1. Functional architecture and signal flow of the portable electrochemical readout circuit. The solid path identifies the USB-controlled configuration verified in Task 1.4; expansion resources of the development board are shown separately. Prepared from the implemented configuration and manufacturer documentation.

Figure 2. EmStat Pico board used by MOBILES biosensors connected to a SPGE (yellow arrow)

Figure 3. Portable enclosure for the electrochemical readout circuit. . (a) Exploded CAD assembly showing the base, development PCB, lid, heat-set inserts and fasteners; (b) fabricated reader connected to a commercial screen-printed gold electrode.

Figure 4. Engineering drawing of the enclosure showing the nominal external envelope, wall sections, connector openings and four mounting features used for replication.

Figure 5. Functional verification of the circuit-to-electrode chain. (A) CV response of the bare SPGE in ferri/ferrocyanide; (B) SWV response after sequential interface modification. The figure confirms potential control, bidirectional current acquisition and resolution of sensor-interface changes.

Figure 6. Representative SWV traces acquired across the E2 concentration series. The portable reader resolves the decrease in peak current relative to the reference response over the tested range.

13. List of Tables

Table 1. MOBILES WPs list

Table 2. Engineering requirements and implemented response for the MOBILES readout circuit.

Table 3. Two-stage circuit-development and verification strategy followed in Task 1.4.

Table 4. Functional blocks of the portable readout and their responsibilities.

Table 5. Physical and logical interfaces of the development platform.

Table 6. Main hardware, software and fabrication resources used for the readout circuit.

Table 7. Mechanical and fabrication parameters of the enclosure prototype.

Table 8. Electrochemical methods used for reference definition and portable-circuit verification.

Table 9. Verification status of the circuit, interfaces and enclosure.

Table 10. Compatibility of the reader developed in Task 1.4 with other MOBILES sensor families.





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14. List of Abbreviations

Abbreviation	Abbreviation for
ADC	Analogue-to-digital converter
AFM	Atomic force microscopy
ARG	Antibiotic-resistance gene
Au-S	Gold-sulfur linkage
BB	Binding buffer
CAD	Computer-aided design
CE	Counter electrode
CLSM	Confocal laser scanning microscopy
CV	Cyclic voltammetry
DAC	Digital-to-analogue converter
DPV	Differential pulse voltammetry
E2	17 β -Estradiol
EIS	Electrochemical impedance spectroscopy
FDM	Fused deposition modelling
LOD	Limit of Detection
MIP	Molecularly imprinted polymer
PCB	Printed circuit board
PLA	Polylactic acid (polylactide)
PoC	Point of care
RE	Reference electrode
SEM	Scanning electron microscopy
SPE	Screen-printed electrode
SPGE	Screen-printed gold electrode
SWV	Square-wave voltammetry
TIA	Transimpedance amplifier
USB	Universal Serial Bus





WE	Working electrode
WP1	Work Package 1
XPS	X-ray photoelectron spectroscopy





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