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Recent Advances on Impedimetric Electroanalysis with Non-commercial Portable Instruments: A Mini Review

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Abstract

Electrochemical impedance spectroscopy (EIS) is one of the most important methods for studying the electrochemical interface. The instrumentation for its implementation consists of an electrochemical cell and a potentiostat with a frequency analyzer. Several authors have demonstrated the feasibility of designing and constructing low-cost, in-lab portable potentiostats for EIS. This type of electrochemical instrument reduces research costs, allows in-field analysis, enables adaptation to specific experimental conditions, as well as modern capabilities such as machine learning (ML) techniques or Internet of Things (IoT) integration. The use of portable EIS devices for substance identification and quantification, i.e., electroanalysis, has been reported. Although these applications are more limited than for interface characterization, some progress has been made in recent years. This paper presents a mini-review of EIS electroanalysis performed with in-lab portable instruments. The aim is to show different options for the implementation of EIS as investigation technique when portability is needed for in-field impedimetric analysis or applications where available commercial instruments do not work.

Keywords

electrochemical impedance spectroscopy, portable, low cost, electroanalysis, machine learning, Internet of Things

1 Introduction

Electrochemical impedance spectroscopy (EIS) is one of the most useful methods for studying the electrochemical interface. It is based on the measurement of the sinusoidal response in an electrolytic cell on which an alternatig current (AC) potential or current perturbation of varying frequency is imposed [1]. The method has important applications in corrosion studies [2, 3], elucidation of electrochemical reaction mechanisms [4], battery development [5, 6], fuel cells [7], capacitors [8], among others. EIS has also been widely used as a method for the identification and quantification of both organic and inorganic species [9–11].

For the EIS method, the instrumentation consists of a three-electrode cell (although less common, a two-electrode cell can also be used [12]) and a potentiostat including a frequency analyzer. Currently, the main electrochemical methods: potentiometry [13], conductometry [14], voltammetry [15], chronoamperometry [15], and coulometry [16] have been implemented with low-cost portable instruments, resulting in a significant reduction of research and teaching costs in electrochemistry and allowing in-field measurements as well as adaptation to specific experimental requirements or implementation of machine learning (ML) and/or Internet of Things (IoT) [17, 18].

EIS electroanalysis with low-cost portable instruments has been more limited than for voltammetry, but reports have increased in recent years, especially those focused on biosensing, whose comparison with commercial instruments has demonstrated the possibility of achieving

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similar results at lower cost and with additional features such as wireless handling.

The ability to modify the features and capabilities of open-source instruments makes it possible to customize the system, which is further enhanced when it is also possible to modify the hardware. These things are usually unavailable in commercial instruments. This allows visualizing potentials for specific applications of EIS instrumentation such as point-of-care (POC) diagnostics, real-time monitoring of industrial systems, electroanalytical tools in exploratory robotic systems, remote identification of hazardous substances, and many others.

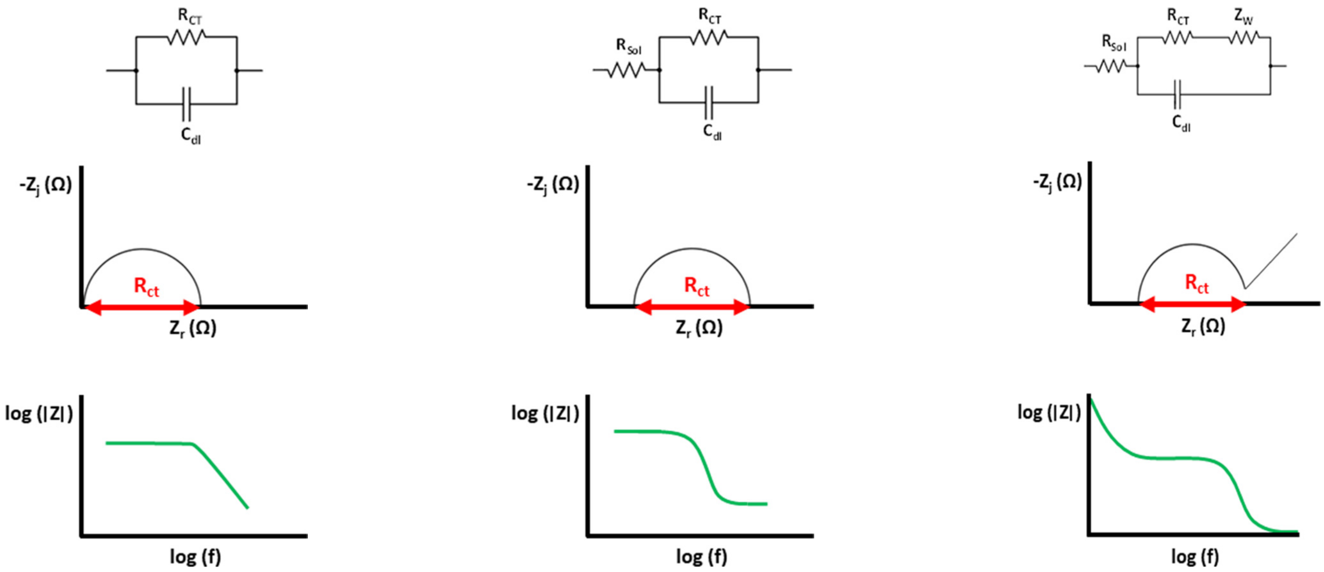
This paper presents a mini-review of EIS electroanalysis performed with in-lab constructed portable instruments. The aim is to show some of the possibilities that exist today to perform this powerful electrochemical method for analytical applications in cases where special uses or non-commercial features are required.

2 Brief fundamentals of EIS

EIS consists of applying a small-amplitude AC voltage or current signal of variable frequency to a steady-state electrochemical system and recording the sinusoidal response signal, which can be a current or voltage, although the former input-output combination is the most commonly used. The input signal is superimposed on a direct current (DC) voltage, which may be the open circuit potential (OCP) or another steady-state potential at which a particular electrochemical process occurs [1]. A data processing allows the calculation of the impedance, which is the physical quantity that measures the resistance of a material to the current flow. It is an extension of the concept of electrical resistance to AC circuits, so impedance is expressed in ohm. In DC circuits, impedance is equal to resistance, which can be considered as impedance with phase angle equal to zero, while in AC circuits, impedance has magnitude and phase angle [19]. A linear time-invariant system is one in which the output signal is linearly dependent on the input signal and the behavior does not change with time. Since the electrochemical system is linear time-invariant due to the small amplitude signal, a transfer function can model the output signal to the input signal over the frequency range [1].

Since both the perturbation and response signals are alternating, the physical-mathematical basis for EIS is more complex than for other electrochemical methods, but the important point is that the spectra derived from the experimental data allow the modeling of circuits whose electrical elements describe the phenomena at the electrochemical interface and in the bulk solution [1]. This modeling is easily accomplished using software, many of which are free.

EIS spectra are usually presented as Nyquist and Bode plots, some of the more common ones are shown in Fig. 1. The shape of these plots depends on the processes at the electrochemical interface and the bulk solution, which in turn define the associated equivalent electrical circuit (EEC) when modeling. The Nyquist plot is



(a) (b) (c)

Fig. 1 Nyquist and Bode-magnitude plots: (a) R-C equivalent circuit, (b) Randles equivalent circuit, (c) Randles equivalent circuit with a Warburg element. Z_r: Real component of the impedance, Z_j: Imaginary component of the impedance, R_{ct}: Charge transfer resistance, C_{dl}: Capacitance of the electrical double layer, R_{Sol}: Solution resistance, Z_W: Warburg element a representation of the opposite imaginary component (Z'') of the impedance as a function of the real component (Z'). Meanwhile, the Bode diagram is a representation of the impedance modulus ($|Z|$) or phase angle (ϕ) as a function of frequency (f) [1, 20]. Both plots allow in practice the adjustment of an EEC. From modeling it is possible to obtain the values of solution resistance (R_{Sol}), charge transfer resistance (R_{ct}), double layer capacitance (C_{dl}), or the Warburg element usually associated with diffusion of the electroactive species (Z_W, or sometimes W). Commonly, the fitting of the EEC yields constant phase elements (CPE), non-linear elements which are closer representations to reality for a capacitive or resistive component taking into account factors such as surface roughness [1]. For example, usually, for a working electrode, C_{dl} is represented by a CPE instead of an ideal capacitive element. EIS is a highly sensitive method for the study of the electrochemical interface, as it yields experimental results that allow the characterization of electrocatalytic effects [21], corrosion [22], mass and charge transfer [23], among others. The applications of EIS range from these types of physicochemical studies to the quantification of analytes. In electroanalysis, species quantification is generally based on changes in R_{ct}, which corresponds to the resistance of the semicircle on a Nyquist plot (Fig. 1) [24].

3 Impedimetric analysis based on portable instruments

Portability and wireless communication are the two main features emphasized by non-commercial EIS instruments. This is because they allow use in cases where benchtop instruments are not helpful. In addition, open source provides modification of software to tailor the instrument to specific applications. However, it is important to note that just because a portable device does not have some of these features does not mean that it does not have potential and benefits. Pruna et al. [25] present a low-cost miniaturized potentiostat for immuno-biosensor monitoring by EIS. The potentiostat was developed on an analog and a digital electronic part. The graphical user interface (GUI) as well as the post-processing algorithms were developed in MATLAB (The MathWorks, Inc.). The operation of the low-cost device is based on five stages:

1. Reference signal generation stage: to choose between a pure DC signal or DC bias plus small AC voltage.
2. Reference signal conditioning stage: to select the sign of the voltage signal excitation.
3. Sensor excitation stage: includes the connection of three conductors to the electrochemical cell.
4. Inverter sensor response acquisition stage: to transform the sensor signal into a measurable voltage.
5. Sensor response conditioning stage: is a signal adaptation stage prior to analogic-to-digital converter (ADC) acquisition.

The instrument employs two microcontrollers: a PIC24 (Microchip Technology) is responsible for the generation of the excitation waveform and the acquisition of the sensor response, as well as the subsequent transmission to a PIC32 microcontroller (Microchip Technology), which is the central control unit of the potentiostat. The instrument has miniaturized dimensions of 12 × 7 × 2 cm^3 and a mass of 70 g. The sensing system consists of a board array of eight working electrodes integrated together with the reference electrode and the counter electrode. According to the authors, the eight electrodes allow parallel measurements of different analytes or the repeatability study of a single measurement. The researchers constructed a biosensor for cytokine with EIS detection using the potentiostat, achieving analyte detection down to 266 pg/mL. There was a 98% linear relationship between the impedance at 1 Hz measured with the low-cost instrument and the same measurement, but with a commercial potentiostat.

Grossi et al. [26] present a portable system for EIS (10 × 12 × 18 cm^3) in liquid and semi-liquid media, capable of generating an electrical fingerprint of the sample that allows the identification and quantification of salts. The instrumental system was assembled in a polylactic acid structure produced by a 3D printer. The circuit was designed to perform EIS measurements on the sample contained in a disposable polypropylene container. Two cylindrical stainless-steel electrodes are used for the EIS (two-electrode cell). The sample temperature is measured using a commercially available sensor integrated into the system. The EIS analysis is performed in the potentiostatic mode, providing Bode and Nyquist plots that can be fitted to an EEC model to obtain the values of the electrical circuit elements. The electronic board of the system was designed in Kicad. The interface is provided by a Nucleo STM32 development board with an STM32F303 microcontroller (ST Microelectronics) which also includes a UART-to-USB (Universal Asynchronous Receiver-Transmitter to Universal Serial Bus) controller for data transfer to a laptop computer for storage and processing.

The EIS excitation signal is a sinusoidal voltage with an AC amplitude of 290 mV and a DC average of 346 mV, created by a programmable AD5932 generator controlled by the microcontroller *via* a serial peripheral interface (SPI). The signal is amplified with a non-inverting amplifier to obtain an AC amplitude of 1.38 V with a DC voltage of 1.65 V. Finally, another inverting amplifier generates the EIS test signal. The response current between the electrodes is converted to an output voltage by a current-to-voltage converter. The digital feedback potentiometers of the converter can be selected by two analog multiplexers to achieve measurements over a wide range of impedances. This electrochemical impedance system allows 37 different frequencies to be measured in the range of 10 Hz to 100 kHz. The pulse amplitude is selectable in 100 mV, 500 mV, and 1 V. The software was developed in LabVIEW (Texas Instruments) and allows setting the EIS test conditions, displaying the results as Bode and Nyquist plots, and storing the data.

To evaluate the on-board circuit, measurements were first made on 14 resistors and the results were compared with those obtained using a commercial instrument. The error was less than 1% for all resistors at frequencies below 10 kHz, while at higher frequencies the error increased to 3.5% up to 100 kHz. Tests were then carried out with arrays of resistors and capacitors, showing a high degree of agreement between the Bode plots obtained with the proposed instrument and those obtained with the commercial device. All these validation tests demonstrated the reliability of the board for EIS measurements in the working frequency and impedance range.

The system was applied to saline solutions: 2 M NaCl, 2 M Na_2CO_3, 2 M K_2HPO_4, and 1 M $CuSO_4$, from which dilutions were prepared and EIS experiments were performed. All EIS experiments were conducted at an amplitude of 100 mV and 23.5 °C. Because of the possible parasitic effect on measurements above 10 kHz, only frequencies between 10 Hz and 10 kHz were taken for the analysis of the Nyquist plots to model an EEC consisting of an electrical conductance (G_m) in series with a capacitive CPE. The G_m values obtained from the model fitting had a good correlation ($R^2 > 0.99$) with the concentration of each of the salts evaluated. Triplicate measurements resulted in deviations of $G_m < 40$ μS. The G_m parameter allows impedimetric quantification of salts in solution. By analyzing the spectrum over the entire frequency range, it is possible to obtain a maximum imaginary impedance value ($Im(Y)_{\max}$) at a given frequency ($f_{\max}$), which is the fingerprint of each of the salts. To make the identification, Grossi et al. [26] plotted $Im(Y)_{\max}$ as a function of $f_{\max}$ at different concentrations of each salt, dividing the graph into different regions that allowed the establishment of an impedance cluster for each electrolyte. This allowed the salts to be identified as follows: if $Im(Y)_{\max} > 0.065\,f_{\max}$, the solution is classified as Na_2CO_3; otherwise, if $Im(Y)_{\max} > 0.03\,f_{\max}$, it is K_2HPO_4; else, if $Im(Y)_{\max} > 0.0155\,f_{\max}$, it is NaCl; and else, it is $CuSO_4$. This method worked except for concentrations less than 1.25 mM $CuSO_4$, where the classification was confused with NaCl. A correlation curve between the impedimetric quantification and the prepared salt concentration showed a high analytical trueness ($R^2 = 0.9965$).

The impedimetric method proposed by Grossi et al. [26] appears to be a more complicated methodological and instrumental approach than a direct resistive method or a conductometric probe. In both cases, less expensive instrumentation is required and the results are usually highly accurate. However, from our point of view, the real advantage of this impedimetric method is the ability to identify the dissolved salts, something that is not normally achieved with conductivity measurements because it is a non-specific property that is related to the total ions in the solution. The impedimetric system for salts presented by Grossi et al. [26] is particularly interesting for industrial wastewater monitoring, pharmaceutical samples, microbiological samples, such as culture mediums, among others.

Jenkins et al. [27] present a completely open-source, wireless, portable potentiostat (ABE-Stat) that allows EIS in addition to voltammetry and potentiometry. The hardware consists of a modification of a previously reported potentiostat named DStat [28], which does not allow EIS, only voltammetry and potentiometric measurements. The ABE-Stat incorporates a network analyzer chip and has a control amplifier network reconfiguration that allows EIS to be performed in the frequency range of 0.1 Hz to 100 kHz at one of 4 pre-programmed AC amplitudes (100 mV, 50 mV, 20 mV, or 10 mV). In addition, the ABE-Stat has wireless communication *via* Bluetooth and Wi-Fi, as well as battery power and operability from Android (Google LLC), features that the DStat does not have. The cost of the ABE-Stat is approximately 105 USD.

The ABE-Stat showed smaller deviations from a reference instrument in the EIS experiments in ferricyanide solution when the scan was performed with a small AC amplitude of 10 mV and a bias of 200 mV, close to the redox potential of ferricyanide. When a larger AC amplitude of 100 mV was used, larger deviations were observed, which were related to the nonlinear nature of the redox processes and some limitations of the impedance estimation approach. The ABE-Stat was not used in the electroanalysis, but it is an EIS instrument for general purposes, and therefore it can be used for any EIS application within its instrumental capabilities, where impedimetric analysis fits perfectly. However, the device still presents some aspects that can be improved in terms of current noise and consistency of EIS measurements to ensure better electrochemical performance.

Salahandish et al. [29] show the development of a bioanalytical EIS method for the clinical detection of nucleocapsid proteins of SARS-CoV-2, the causative agent of COVID-19 disease. For the analysis, they use screen-printed electrode-type biosensors and a portable low-cost bi-potentiostat (40 USD). The prefix "bi" refers to the fact that sensing with two working electrodes is possible. According to Salahandish et al. [29], multi-sensing has several beneficial effects on the analysis:

- Elimination of matrix effect in complex biological fluids.
- Self-validation of the analysis by the use of different detection methods.
- Increased selectivity.

The potentiostat developed is highly accurate, with connection *via* USB to a computer, and also *via* Bluetooth to a smartphone. The researchers present the Nyquist plots, from which they determined the value of the R_{ct}, which depends linearly on the protein concentration, allowing linear calibration and quantification. The two working electrodes allowed a limit of detection (LOD) of 116 fg/mL (WE1) and 150 fg/mL (WE2), respectively. The linear range was 1 pg/mL − 10 ng/mL. The results obtained were better than those evaluated with a commercial potentiostat. The EIS method based on the portable instrument allows the detection of SARS-Cov-2 and the quantification of the viral load of patients.

Burgos-Flórez et al. [30] report a low-cost instrumentation-based EIS method for the POC detection of S100B protein, which is a calcium-binding dimeric protein that has been used as a biomarker in plasma/blood serum of traumatic brain injury (TBI). The protein was detected using a biosensor based on an antibody (Anti-S100B) functionalized thin-film gold interdigitated electrode (IDE) platform (Fig. 2 (a)). For the EIS measurements they employed a fully open-source, modular, wireless (Bluetooth module), battery-powered, smartphone-controlled, portable, low-cost potentiostat (TBISTAT) developed by the authors. The electrochemical measurement system consists of a front-end analog-digital potentiostatic electronic circuit and a Teensy LC microcontroller board (PJRC). The instrument allows to perturb the electrochemical system with AC frequencies from 1 Hz to 10 kHz. Fig. 2 (b) shows the system components and Fig. 2 (c) shows the photograph of the TBISTAT operated from the application on a smartphone.

The instrument was validated using a dummy cell, on which perturbation signals were imposed and Bode plots were obtained. These results were compared with those obtained with a commercial potentiostat Autolab/M204 equipped with an Autolab Frequency Response Analyzer (Autolab FRA32) (Metrohm AG), matching the results of both instruments (Fig. 3 (a)). The authors mentioned that the largest discrepancy is observed below 10 Hz; however, in practice, this difference is not very noticeable when observing the graphs. Bode plots were presented for 10 mM $K_3[Fe(CN)_6]$ in 0.2 mM KCl solution, in which correspondence with the results obtained using the commercial instrument is also observed, with a phase angle difference close to 2° (Fig. 3 (b)). Using the biosensor, the authors obtained Bode and Nyquist plots for 316 pg/mL of S100B spiked human plasma samples (Fig. 3 (c) and (d)), which also coincided with those obtained using the commercial instrument, although in the Nyquist plots greater variability is observed from Z' values greater than 2.5×10^4 Ω.

A statistical *t*-test was performed for the comparison of the average capacitance for five measurements obtained using the TBISTAT and the benchtop reference instrument; there were no statistically significant differences. From here, the Nyquist plot was constructed for different analyte concentrations in a linear range of 31 pg/mL to 316 pg/mL, with a LOD of 35.73 pg/mL (Fig. 4). The authors point out some limitations of the instrumentation developed, among which we can mention:

- Only frequencies from 1 Hz to 10 kHz can be used to measure impedances between 100 Ω and 35 kΩ.
- The effects on electrochemical measurements of factors such as temperature, humidity, electrical noise, and tests in real settings are not known.
- The use of an additional redox solution could represent a limit to scaling the technology to a marketable stage.

From an analytical point of view, one aspect that can be criticized in our opinion is the fact that the calibration was performed with only three concentrations. A statistically acceptable calibration curve must have at least 5 experimental points. However, despite the limitations, Burgos-Flórez et al. [30] demonstrate the possibility of constructing high-performance experimental instrumentation for

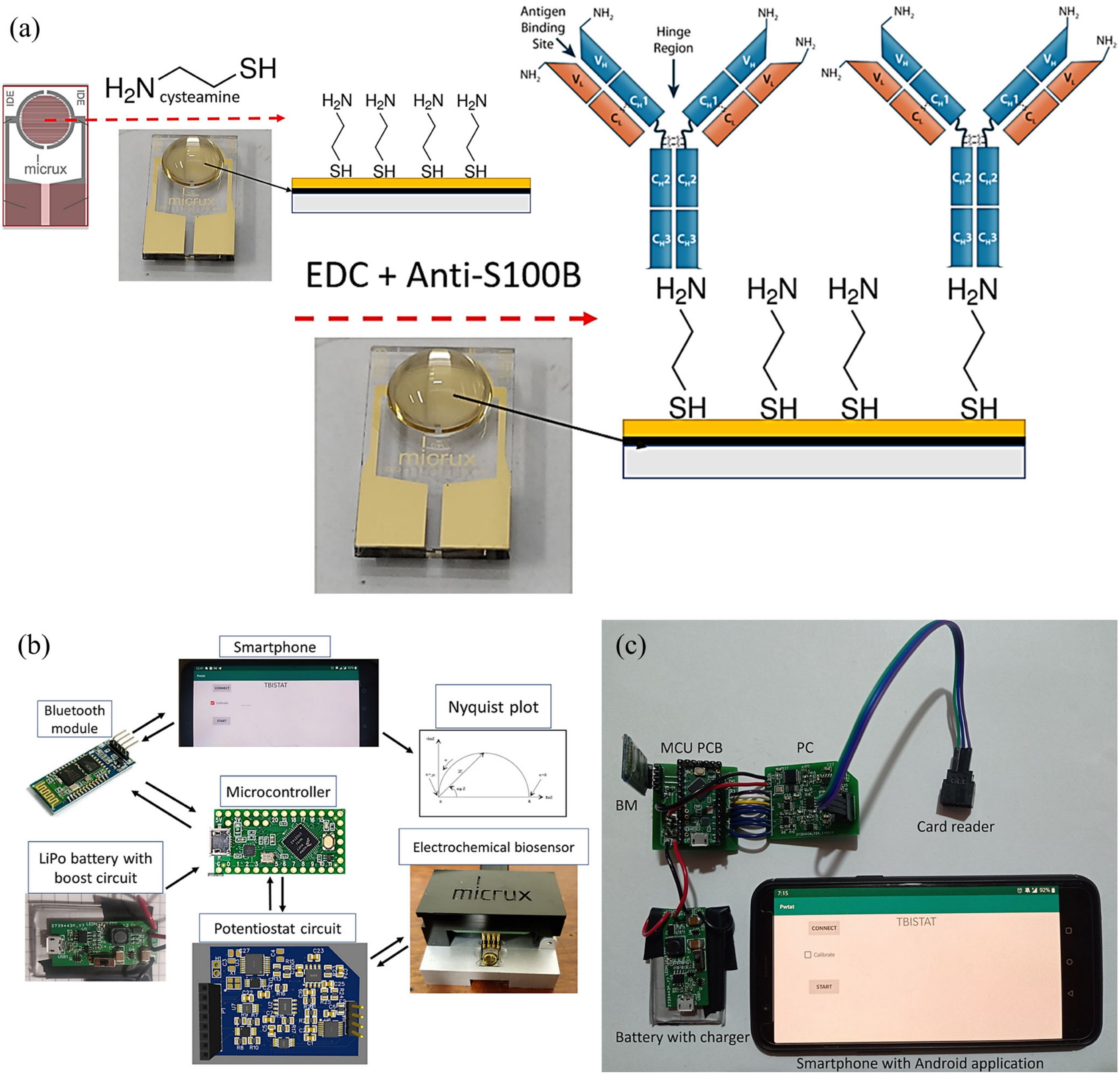


Fig. 2 Low-cost EIS instrumentation reported by Burgos-Flórez et al. [30] for the POC detection of S100B protein; (a) Scheme of the biosensor fabrication, (b) Scheme of the system components, (c) Photograph of the TBISTAT. All figures were taken from Burgos-Flórez et al. [30] under CC BY 4.0 license [31]

bioanalytical EIS. In addition, throughout the paper, they offer proposals for improving the instrumentation. It is important to note that the modular design of the system allows modification of some of the instrumental modules to improve the performance of the device. The TBISTAT has an approximate manufacturing cost of 80 USD.

Buscaglia et al. [32] present a low-cost portable impedance analyzer (Simple-Z) that allows the imposition of the excitation signal and recording of the response signal to obtain electrochemical impedance spectra. The portable instrument costs about 100 USD and its fabrication could be targeted for POC diagnostic systems and for electrochemistry education. The Simple-Z is based on an AD5933 integrated circuit and peripheral circuits for regulation, control, calibration, and external communication. The implementation of software solutions allowed to improve the performance of the Simple-Z. In addition, a GUI was developed for the intuitive use of the instrument. The results obtained with the Simple-Z in EECs mimicking electrochemical sensor systems and in

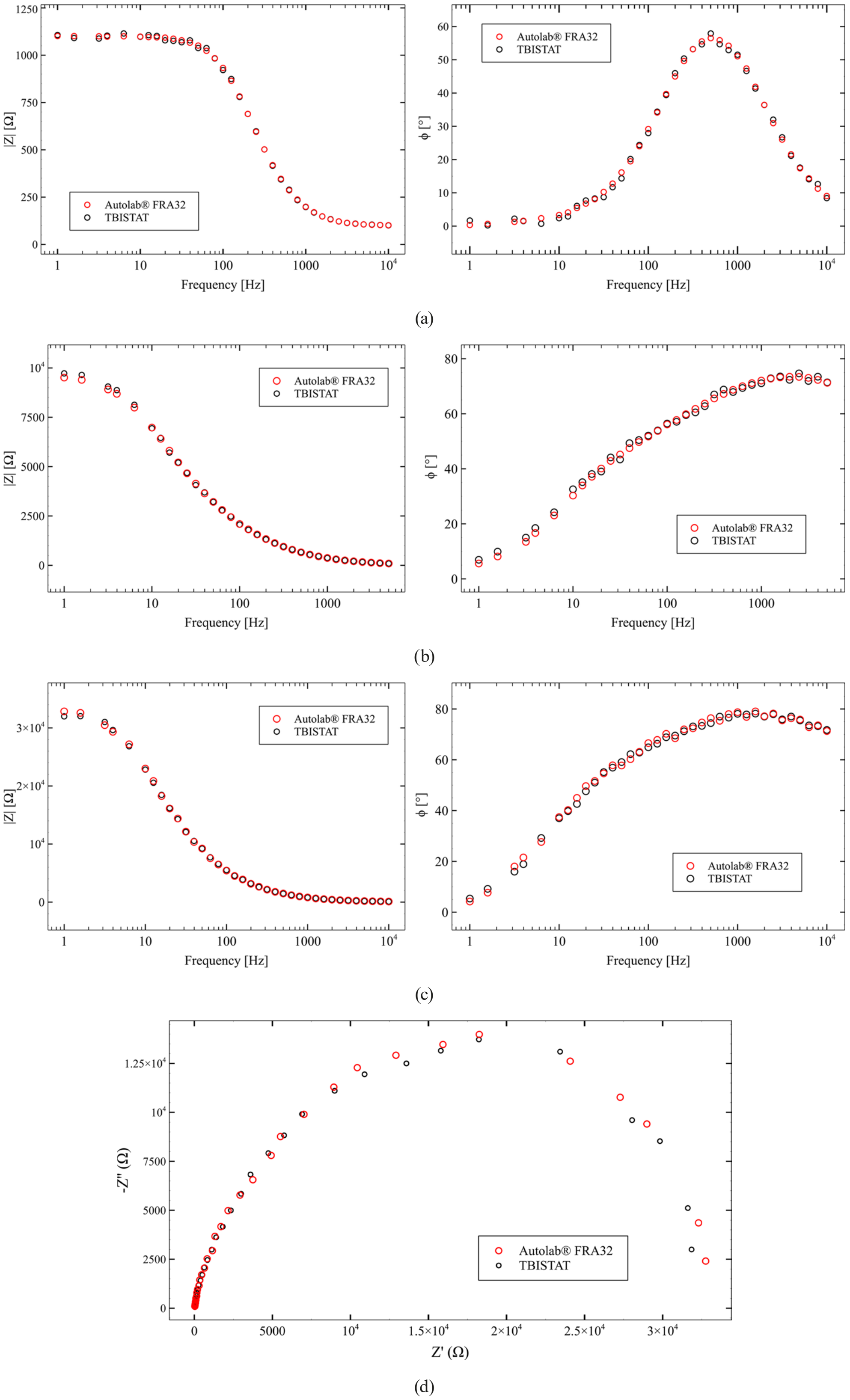


Fig. 3 EIS experimental results using the low-cost instrumentation reported by Burgos-Flórez et al. [30] for the POC detection of S100B protein; (a) Bode plots for measurements in a dummy cell using the TBISTAT and the commercial instrument Autolab FRA32, (b) Bode plots for measurements in 10 mM $K_3[Fe(CN)_6]$ in 0.2 M KCl solution using the TBISTAT and the Autolab FRA32, (c) Bode plots for measurements in 316 pg/mL S100B spiked human plasma samples with the biosensor using the TBISTAT and the Autolab FRA32, (d) Nyquist plots for measurements in 316 pg/mL S100B spiked human plasma samples with the biosensor using the TBISTAT and the Autolab FRA32. All figures were taken from Burgos-Flórez et al. [30] under CC BY 4.0 license [31]

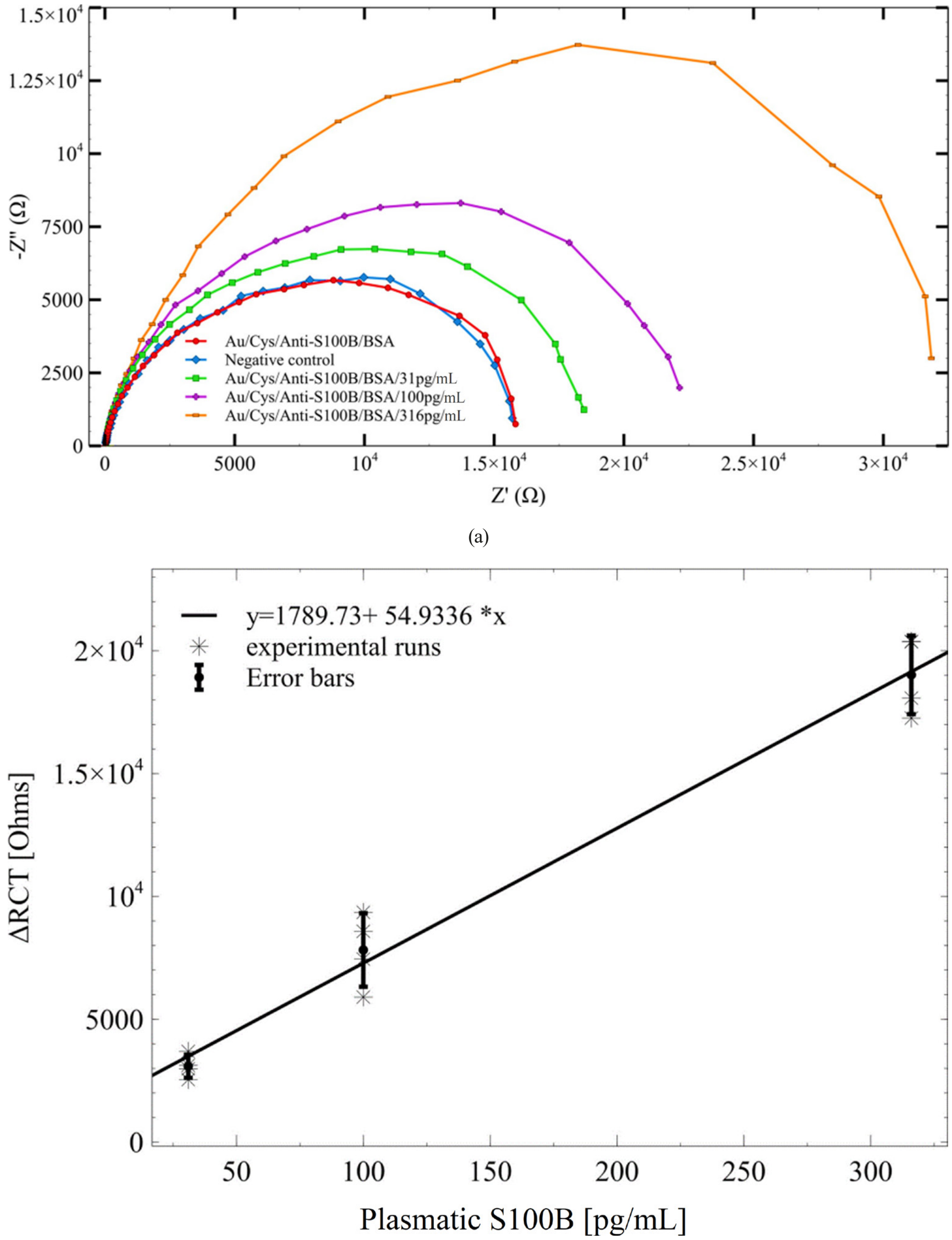


Fig. 4 Calibration of the EIS method reported by Burgos-Flórez et al. [30] for the POC detection of S100B protein; (a) Nyquist plots for different S100B plasmatic concentrations using the TBISTAT, (b) Calibration plot from the Nyquist plots. All figures were taken from Burgos-Flórez et al. [30] under CC BY 4.0 license [31]

solutions of different Na_2SO_4 concentrations were consistent with those obtained with a commercial benchtop instrument. The analytical use of portable impedimetric instruments is mainly focused on POC tests, as opposed to voltammetric methods performed with this type of device, which have more diverse applications such as quantification of analytes in natural waters, control of industrial wastewater, and also, POC diagnosis [18, 33, 34].

Ye et al. [35] present a low-cost (95 USD), portable and high-performance device for biomarker detection using an 8-well IDE chip. The electrodes consist of a Cr/Au bilayer with a thickness of 10 nm and 60 nm, respectively. The system for EIS consists of a field-programmable gate array (FPGA) module for digital input and output signal control, computation, and communication. An analog front-end circuit generates the excitation signal and collects the feedback signals from the IDE. The IDE chip forms a device under test with biological fluid samples whose impedance changes with content, being the principle of biomarker detection. The circuit was implemented on a printed circuit board (PCB). It is known that in an EIS experiment, the impedance measurement in the low-frequency range can be delayed because the system needs to acquire one or more complete sine waves. Therefore, the developed system uses the synthesis of multi-sinusoidal signals with low crest factor for frequencies below 1 kHz and single-tone signals for frequencies above 1 kHz. The multi-sinusoidal signal is the sum of several sine waves at different frequencies. This increases the speed of impedance measurements while maintaining accuracy. The excitation signals are generated by an AD9742 digital-to-analog converter chip (Analog Devices) controlled by the FPGA module. As in the work presented by Jenkins et al. [27], a low excitation amplitude of 10 mV is used to maintain impedance linearity while protecting the biological samples. The current signal is converted to a voltage signal by a transimpedance amplifier (TIA, a current-to-voltage converter). This signal is then adjusted to the correct magnitude using a programmable amplifier. Finally, the signal is digitized using an ADC. The system exhibited a low mean magnitude accuracy error of only 0.30% and performed measurements in only 0.46 s while scanning 28 frequencies from 10 Hz to 1 MHz. The bioanalytical EIS system was used to detect anti-SARS-CoV-2 IgG in serum. There was a high statistical significance with a *p*-value of 7×10^{-6}. The relative difference in impedance of the positive group was significantly higher than that of the negative group (27.2% ± 13.6% vs. 4.1% ± 1.7%), which allowed the two groups to be discriminated with high accuracy. This type of molecular detection approach is very fruitful for building artificial intelligence (AI) models to automatically identify positive cases with higher accuracy. In addition, IoT technologies have been implemented in voltammetric detection [17], which can be a way to improve the performance of EIS-based POC testing. For example, the user can receive the result of the diagnostic test on the mobile device or PC through data transmission *via* the cloud.

In this context, it is important to note that AI is related to IoT technology. ML is a branch of AI very useful in instrumental analytical systems such as spectroscopy and electrochemistry [18, 36–38]. ML aims to develop learning techniques that can solve tasks based on training experience from large amounts of data [39]. The transmission of data to and from the cloud, the availability in real time, as well as the amount of electrochemical and analytical information offered by EIS are a perfect mix to build ML models for automatic identification based on fingerprint data, also for analytical autocalibration, real-time reporting, and many others. Fotia et al. [40] show the use of EIS data for water quality in ML models that can be loaded into low-power microcontrollers for portable applications. The real and imaginary impedance components for each frequency within the range of 0.18 to 1 Hz were used as features (input) of the ML models. Five different lead concentrations were utilized as output. Since the difference in percentage between the results of the measurements in the dataset was less than 2%, a synthetic dataset increased in magnitude (number of observations) was generated using a mathematical equation that introduces a randomized factor. The lead concentration data was increased by the same proportion. The dataset consisted of 330 features and 930 observations, with 186 observations for each lead concentration. The models were trained on 16 possible scenarios to reduce the number of features while maintaining model accuracy and decreasing training time. This translates into efficiency. These models have approaches towards classification (Decision Trees, Discriminant Analysis, Naive Bayes, Support Vector Machine, Nearest Neighbors, and Ensemble), which allows identification of chemical species, and regression (Linear Regression, Tree, Support Vector Machine, Ensemble, and Gaussian Process Regression), what allows quantification. Classification accuracy ranged from 97.1% to 100% depending on the model, while regression models achieved coefficients of determination of 0.99 and 1. These types of developments appear to be less explored for analytical EIS than for other electrochemical methods. The control of metal ions in wastewater and natural waters is a topic of particular importance due to their toxicity. The application of advanced analytical systems, such as the one described by Fotia et al. [40] is of great interest for industries that handle high levels of metals, such as mining or metallurgy, given the concentrations of these contaminants released in wastewater. In addition, the control of metal species is essential in the quality control of hydrometallurgical processes.

Akhter et al. [41] report an IoT-enabled portable impedimetric sensing system for nitrate in water for smart agriculture. Sensing is achieved using a multi-walled carbon nanotube/polydimethylsiloxane sensor. The device has dimensions of $110 \times 78 \times 86$ mm^3, and in addition to nitrate concentration, it also measures the dissolution temperature, which is intended to ensure applicability in real scenarios where this parameter can vary significantly and modify the measured resistance. It is composed of an AD5933 impedance analyzer, the sensor, an LCD screen to display the nitrate quantification results, an Arduino Uno microcontroller for data processing, a LoRa shield to send the data wirelessly to a server in the cloud, a battery charging unit, and a battery for powering the circuit. The AD5933 allows highly accurate determination of real and imaginary impedance using a discrete Fourier transform algorithm. The sensor's selectivity for nitrate in the presence of other ions was achieved through an ML algorithm called K-nearest neighbor (KNN). This allows the microcontroller to be trained to determine the probable concentration of the analyte individually in the presence of phosphate and calcium. The training matrix was constructed at three concentration levels of the three analytes, at three pH, three temperatures, and the impedance and resistance response of the sensor for each mixture. The sensor developed allowed the detection of nitrate in a concentration range of 0.01 to 30 ppm at a frequency of 1.3 kHz. The electroanalytical response was repeatable, stable for up to 90 days, and reproducible for different sensors. Nitrate determinations in six real samples: river water, stream water, canal water, creek water, fisheries water, and tap water, were acceptably consistent with the spectrophotometric method, showing more than 95% accuracy. In a similar work, Akhter et al. [42] also used the KNN model to quantify phosphate in water in the range of 0.01 to 40 ppm. Determination with repeatability, stability, and reproducibility was achieved. Likewise, the EIS electroanalysis in real samples showed an accuracy greater than 95% compared to the UV-visible spectrophotometric method.

The two previous works [41, 42] are very interesting, demonstrating the potential of ML and IoT to significantly minimize the interfering effect by training microprocessor-controlled portable electroanalytical systems. This allows the device to automatically quantify substances and establish real-time monitoring processes. This is generally not possible with traditional benchtop analytical systems. In addition, these works show how the temperature measurement allows corrections to be made to the resistance, enabling the device to operate in real-world scenarios. Details like these should be considered in other prototypes to increase their potential for real applications.

Park et al. [43] present an all-in-one electroanalytical device (AED) for POC testing. The miniaturized portable instrument (48 × 37 mm^2) allows EIS as well as amperometry, voltammetry, conductometry, and potentiometry. In addition, it enables the connection of up to eight screen-printed electrochemical sensors simultaneously. Communication is possible both *via* a wired connection using a USB port and wirelessly *via* low-energy Bluetooth. A GUI was designed for the operation of the electrochemical instrument and the sending of the results. Fig. 5 (a) shows a block diagram of the device proposed by these authors. The AED consists of a connector for screen-printed electrodes, an analog multiplexer, a microcontroller unit, a USB, and a Bluetooth unit. The microcontroller unit includes components such as an analog-to-digital converter and vice versa, op-amp, and TIA, which, as mentioned above, are essential in electrochemical devices. A 3.7 V rechargeable lithium-ion polymer battery or a USB connection can be used to power the AED. The device allows electrochemical experiments in the range of ±2 V voltage

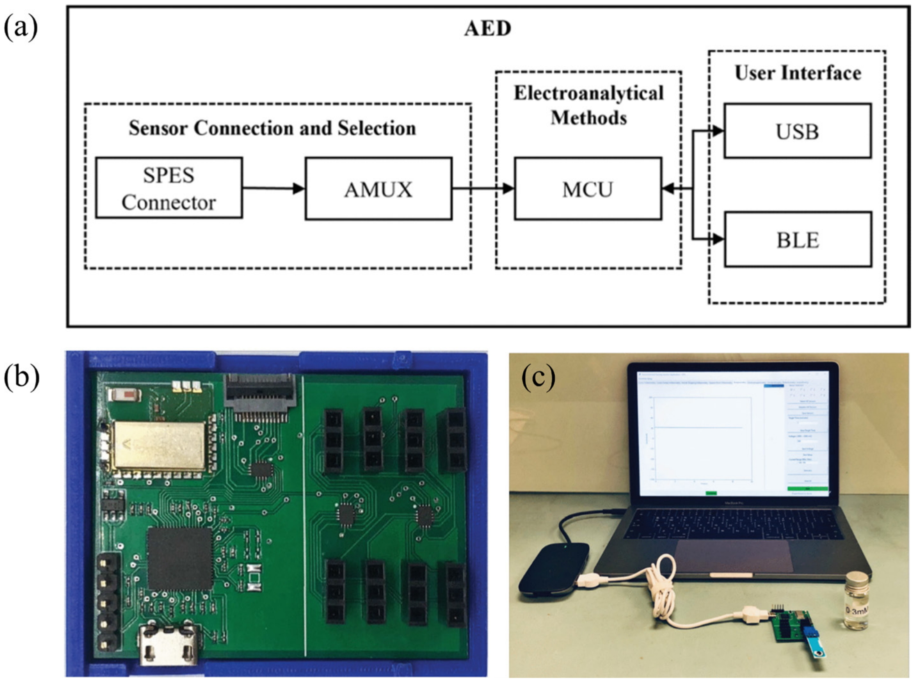


Fig. 5 All-in-one electroanalytical device for POC testing proposed by Park et al [43]; (a) Block diagram of the electrochemical device for POC testing, (b) Photograph of the device, (c) Photograph of the device during a test with a screen-printed electrode. All figures were taken from Park et al. [43] under CC BY 4.0 license [31]

and ±100 μA current. The AED is shown in Fig. 5 (b), while in Fig. 5 (c) it is shown connected to a computer and a sensor during an electrochemical measurement. A test was performed on a Randles circuit to validate the instrument's capability for EIS, replacing a sensor. Impedance and phase versus frequency plots showed the characteristics of this type of equivalent circuit. This work has the disadvantage that no application tests were performed on analytes using impedimetric analysis, contrary to what was done for amperometry and voltammetry, which were applied for glucose and uric acid testing, respectively.

Demirhan et al. [44] present a miniaturized EIS device for early diagnosis of gastrointestinal bleeding by detecting hemoglobin (Hb) using an immunosensor. An optimized hardware and software strategy was used to construct a low-power consumption EIS system. A Data Distribution Service (DDS) was used to generate waveforms with a high sampling rate without using complicated hardware and software. A DDS middleware is used for data transmission in real-time systems operating in a distributed environment. The conversion of AC to DC signals was not done with an analog-to-digital converter, but through analog switches, which causes less power consumption. An ADC was then used to digitize the signal. The microcontroller unit was an EFM8LB12 (Silicon Labs). The instrument constructed is more energy efficient than other hardware alternatives such as the FPGA used by Ye et al. [35], this facilitates miniaturization since a smaller battery size is required. As we have seen throughout this review, the current from the electrochemical sensors is converted into a voltage for the electrochemical signal transduction. In this case, this was achieved by using a TIA (OPA4354 operational amplifier from Texas Instruments) whose gain was controlled by a digital potentiometer (MAX5394 from Maxim Integrated) instead of an analog switch network. Inaccuracies due to TIA gain and op-amp were calibrated with dummy cells. The goal was to achieve high-accuracy measurements at frequencies up to 100 kHz. The hardware was further optimized, e.g., by eliminating the USB, until a very miniaturized device was achieved, which was assembled in a curious pill-shaped capsule. The capsule contained two 1.55 V button batteries and two PCBs. In addition, it contained a screen-printed electrode that was specially cut to fit inside the capsule. Calibration of the device proved to be the critical factor in achieving maximum efficiency using small and inexpensive components. The approximate cost of the device for EIS is 38 USD and its miniaturization in pill form aims to show a prototype for diagnosing gastrointestinal bleeding by an endoscopic method through swallowing the pill. In the future, wireless communication protocols can be implemented to enable data transmission from the pill inside the body and outside. Fig. 6 (a) shows the schematic of the device for EIS, while Fig. 6 (b) shows the circuit board. Fig. 6 (c) shows the electronics with a flow cell whose schematic is shown in Fig. 6 (d). Fig. 6 (e) and (f) show the fully miniaturized centimeter-scale device in pill form and its comparison with the PillCam, a device for endoscopic visualization of the intestine.

For the electrochemical experiments, the device in Fig. 6 (b) was used. The sensor was able to detect Hb in redox solution (5 mM $K_4Fe(CN)_6$/$K_3Fe(CN)_6$ in phosphate-buffered saline (PBS) at pH 7.4) in a linear range of Hb concentration ([Hb]) between 5 μg/mL and 60 μg/mL, with a detection limit of 13.3 μg/mL. Fig. 7 shows the calibration of the analytical method of EIS in redox solution by Nyquist plots (taking the value of R_{ct}) and comparison with the commercial instrument Palmsens 4 (Palmsens B.V.). Similarities between the calibration curves equations is observed: $\Delta R_{ct}(\%) = 1.48 \times [\text{Hb}] + 19.61$ ($R^2 = 0.99$) and $\Delta R_{ct}(\%) = 1.61 \times [\text{Hb}] + 16.47$ ($R^2 = 0.98$) for the proposed device and the Palmsens 4, respectively. However, the constructed instrument showed a slightly greater dispersion in the calibration curve compared with the commercial device. The authors attributed this to the fact that the measurement was performed first with the Palmsens 4 and then with the proposed prototype, which caused polarization effects in the electrodes leading to larger analytical errors. In our opinion, this could be further investigated in a repeatability and reproducibility test of the EIS method for Hb with the proposed instrument, which was similarly mentioned by the authors. It was also possible to detect Hb in simulated intestinal fluid without the need for a redox solution in the range of 10 μg/mL to 10 mg/mL, with a detection limit of 2.31 mg/mL.

The work of Demirhan et al. [44] is very interesting and is a clear example of the potential of current electronics and computer science to develop miniaturized high-performance EIS instruments. Unfortunately, there does not seem to be much progress in recent years in such high miniaturization work for EIS in other areas, such as environmental diagnostics, especially for contaminants of emerging concern like pharmaceuticals, pesticides, fertilizers, cyanotoxins, industrial chemicals, and many others. Furthermore, imagine a very low-cost miniaturized device that could be introduced into industrial systems, eliminating sampling steps in tanks or chemical reactors, and allowing real-time

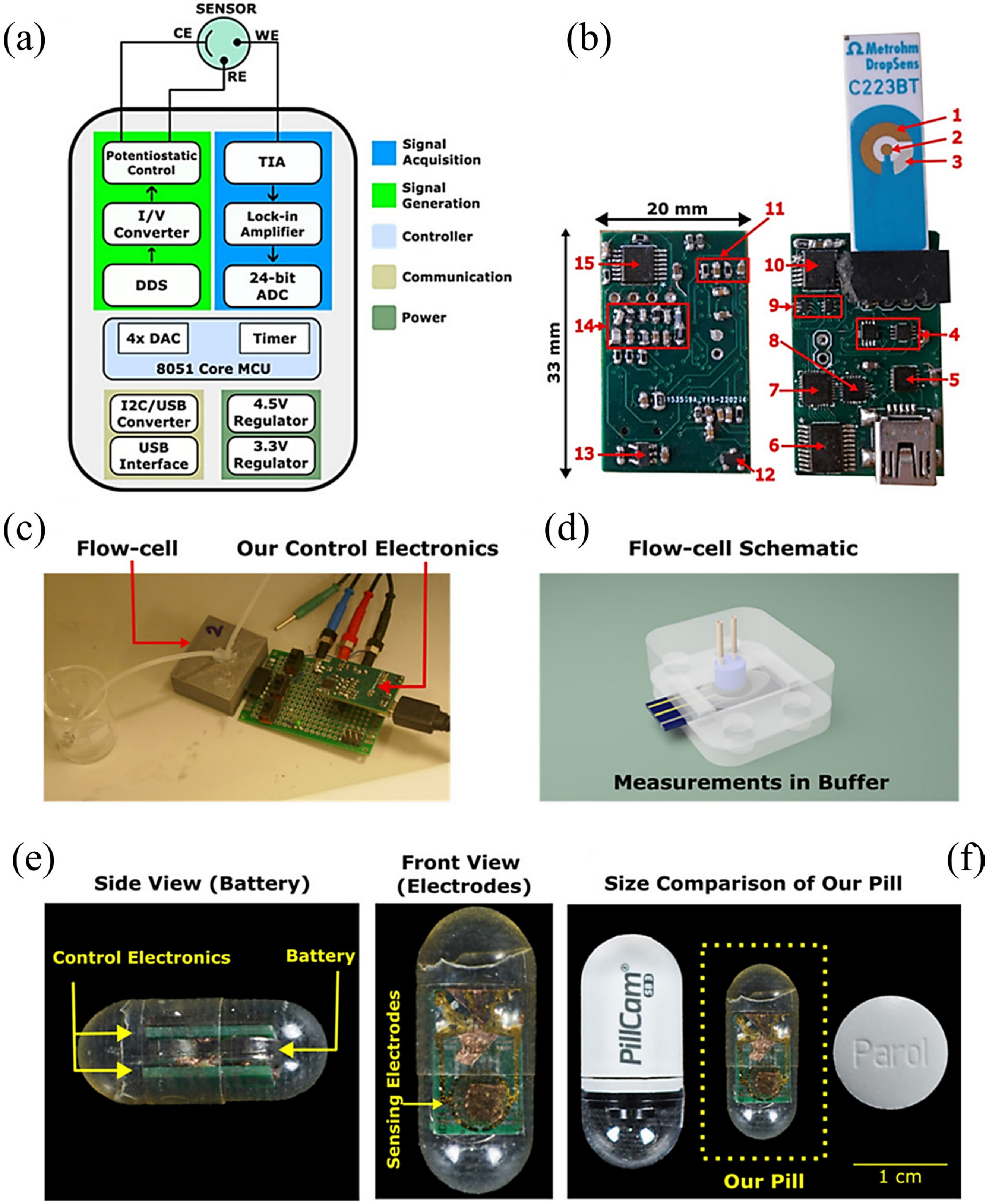


Fig. 6 Miniaturized EIS device reported by Demirhan et al. [44] for early diagnosis of gastrointestinal bleeding by detecting hemoglobin using an immunosensor; (a) Schematic of the EIS device, (b) Circuit board of the device before development into a pill, (c) Device with a flow cell, (d) Schematic of the flow cell, (e) Photographs of the miniaturized EIS device in a pill including the electrodes, (f) Size comparison with the PillCam for visualization of the intestine. All figures were taken from Demirhan et al. [44] under CC BY 3.0 license [45]

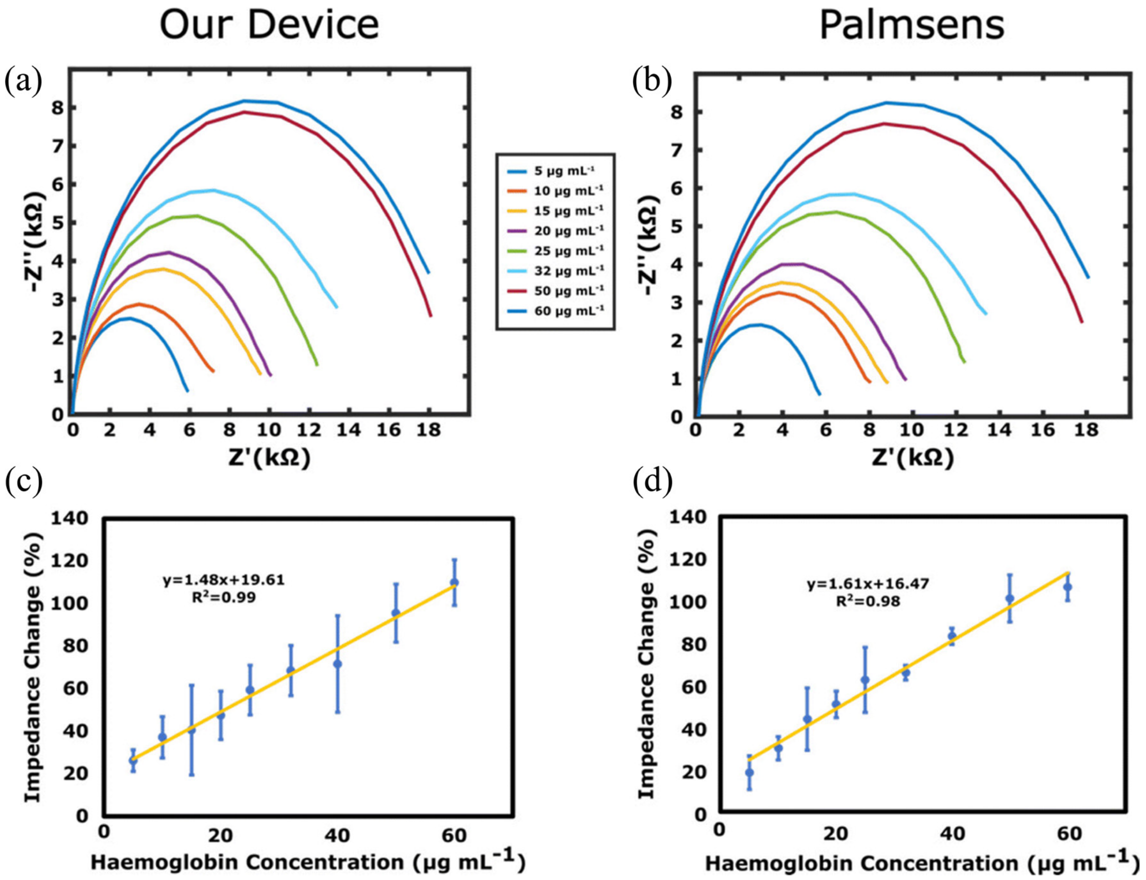


Fig. 7 Calibration of the EIS method for early diagnosis of gastrointestinal bleeding by detecting hemoglobin using an immunosensor; (a) Nyquist plots for analytical calibration for hemoglobin in redox solution using the device proposed by Demirhan et al. [44] and (b) for a commercial instrument, (c) EIS calibration curve for hemoglobin using the device proposed by Demirhan et al. [44] and (d) for a commercial instrument. All figures were taken from Demirhan et al. [44] under CC BY 3.0 license [45]

monitoring. In fact, although the technology shown by Demirhan et al. [44] is a prototype that still needs further development, it can be considered impressive, and should point the way to the electrochemical analysis of the future, in this case for quantitative EIS analysis.

Table 1 provides a summary of the portable EIS instruments presented in this mini-review. Some important features from an electrochemical point of view are shown, as well as instrumental capabilities such as wireless, IoT, and AI.

Table 1 Portable instruments for EIS electroanalysis presented in this mini-review

Work	Dimensions	Frequency (Hz)	Impedance (Ω)	Wireless	IoT	AI	Applications	Cost (USD)
Pruna et al. [25]	12 × 7 × 2 cm^3	0.1–10k	*	No	No	No	Biosensing	300
Grossi et al. [26]	10 × 12 × 18 cm^3	10–100k	80–120k	No	No	No	Characterization of saline solutions	100
Jenkins et al. [27]	74 × 89 mm^2	0.1–100k	100–100k	Yes	No	No	General purpose	105
Salahandish et al. [29]	18 × 15 × 9 cm^3	1–1400	*	Yes	Yes	No	COVID-19 diagnosis	40
Burgos-Flórez et al. [30]	216 cm^3	1–10k	100–35k	Yes	No	No	TBI diagnosis	80
Buscaglia et al. [32]	*	1–100k	100–1M	No	No	No	Biosensing	100
Ye et al. [35]	163 × 84 × 30 mm^3	10–2M	100–200k	No	No	No	COVID-19 diagnosis	95
Akhter et al. [41]	110 × 78 × 86 mm^3	10–100k	*	Yes	Yes	Yes	Water analysis	*
Park et al. [43]	48 × 37 mm^2	512–80k	*	Yes	No	No	POC testing	*
Demirhan et al. [44]	33 × 20 mm^2 (reducible to pill size)	1–100k	*	No	No	No	Gastrointestinal bleeding diagnosis	38

* No data

4 Conclusions

Identification and quantification of substances by the EIS method is possible using in-lab constructed portable instruments. This type of electrochemical device results in a cost reduction compared to commercial benchtop instruments. The possibility of open source and in-lab construction allows maneuverability with software and hardware to achieve high-performance analysis even when using very inexpensive components and allows the development of devices adaptable to specific experimental situations. Miniaturization can be such that it is possible to make instruments that could be inserted into cavities such as the gastrointestinal tract. ML and the IoT have been implemented in this type of EIS instrumentation, but the advances do not appear to be as significant as in other electroanalytical methods, such as voltammetry. The greatest advances in electroanalysis with EIS using portable instruments have been made in clinical analysis for POC diagnosis, with little progress in areas like environmental or industrial analysis.

In addition to those mentioned above, the main applications can be visualized in the remote identification and quantification of hazardous substances, industrial process control, and in-situ determination of contaminants of emerging concern such as antibiotics, anti-inflammatories, pesticides, fertilizers, and others. These are just a few of the broad applications in which these devices can be involved.

From our point of view, the main limitations are some electronic aspects that limit the frequency range in some prototypes. Also, EIS measurements are more time-consuming than other electrochemical methods. Although some prototypes are working, efforts should be made to generalize them to other devices. All of this may affect the operability of this type of analysis for field applications. In addition, work is needed to show the performance of portable EIS instrumentation in real scenarios, such as industrial systems or clinical laboratories. However, these issues are expected to be addressed as research on the topic progresses.

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