Development of electronic modules for their integration with impedimetric transducers applied to sarcosine detection

A Perez-Nava¹, V Vallejo-Becerra¹, S Fernández-Puig², G Oza² and J Herrera-Celis²

¹Facultad de Ingeniería, Universidad Autónoma de Querétaro, Anillo Vial Fray Junípero Serra s/n, Bolaños, 76140, Querétaro, México.
²Departamento de Ciencia, CONACYT-Centro de Investigación y Desarrollo Tecnológico en Electroquímica, Parque Tecnológico Querétaro s/n, Sanfandila, Pedro Escobedo, 76703, Querétaro, México.

Abstract. The development of fast, simple, sensitive, and minimally invasive biosensors for detecting diseases, conventionally need specialized, expensive, and highly invasive instrumentation. Furthermore, such biosensors pertinently also, need the development of optoelectronic modules that are capable of implementing specific detection techniques while interacting with the user through a friendly interface. This work highlights the development of a system whose hardware and software contributes to the detection of analytes by impedimetric sensors, especially emphasizing on the detection of sarcosine, a natural amino acid associated with prostate cancer (PCa). Dummy circuits coupled with impedimetric transducers were used to perform precise measurements using a sinusoidal signal of 20 mV in the range from 0.1 Hz to 1 MHz.

1. Introduction

Currently, prostate cancer (PCa) is the most common solid tumor in men. It is the result of uncontrolled growth of prostate cells [1]. Despite the development of diagnostic techniques over time, ranging from the practice of procedures such as digital rectal examination (DRE), introduced at the beginning of the 20th century, followed by rectal and transperineal biopsies, to the implementation of the Prostate-Specific Antigen (PSA) test in blood, nowadays, a correct and at the same time minimally invasive detection is still a necessity. Even though the PSA test is considerably less invasive than the rest, it has not yet been shown that this is a specific biomarker, as the concentration of PSA is also altered by benign prostatic hypertrophy and prostatitis [2], which leads to use invasive methods to obtain a more adequate diagnosis.

Studies realized in recent years about specific biomarkers of PCa demonstrated a significant increase in the concentration of sarcosine in patients with metastatic PCa, correlating it with cancer invasiveness [3–5]. Indeed, some works have already showed that the level of sarcosine concentration in serum and blood is related to the development of prostate cancer [6-7]. Regarding the current techniques for sarcosine detection, chromatography [8-12] and differential mobility analysis with mass spectroscopy [13] are some of the most used analytical methods. However, these techniques are expensive, depend upon a specialized instrumentation and require a qualified user to do the sample preparation and the equipment operation, which make them unfavorable for routine analysis.

These aspects have motivated research focused on the detection of sarcosine through simple, but competitively effective methods. Amongst the different transduction methods, the electrochemical
method outstand itself due to its sensitivity and easy integration. Specifically, impedimetric techniques have been tested, achieving significant detection limits, good stability over time, and reproducibility [14, 15]. Nguy et al. [14] developed an impedimetric sensor on carbon electrodes covered with a layer of gold nanoparticles and a molecular imprinted polymer (MIP), obtaining a significant detection limit below 1 nM. This sensor proved to have good reproducibility, outstanding temporal stability and selectivity over other proteins. In the formation of a molecular printing polymer, the pair functional monomer-interest compound is polymerized under appropriate conditions. Afterward, the compound is removed, which results in microspaces that are chemically and physically complementary to the compound of interest [16]. Meanwhile, Canbay et al. [17] developed an impedimetric biosensor based on sarcosine-specific reduced DNA aptamers for the determination of sarcosine in urine matrices. Its performance was evaluated by electrochemical impedance spectroscopy (EIS). This biosensor obtained a linear calibration curve at concentrations from 5 pM to 0.5 µM. In a later study, an amperometric serum sarcosine biosensor was developed. In this approach, an electrode of polycrystalline gold was functionalized with sarcosine oxidase as sensing layer. This biosensor showed a linear relationship to the concentration of the biological compound from 0.1 to 100 µM, and a detection limit of 0.01 µM [18]. Rebelo et al. [15] used screen-printed electrodes for the detection of sarcosine in biological samples, making the analyte quantification through electrochemical techniques. However, the incorporation of these developments within portable equipment continues to be a necessary step towards the massive use of minimally invasive techniques and the early detection of PCa.

In this sense, this work proposes the design, programming, and implementation of electronic modules for their integration with impedimetric transducers such as those developed for the detection of sarcosine. The different components will be presented in the following order. First, there is exposure of the model associated to impedimetric transducers, accompanied by the measurement technique and different strategies will be used to calculate the impedance. Then, a concise description of the modules that make up the developed prototype will be given. Finally, an example for the performance of the prototype using a Dummy cell with parameters that mimics a biosensor will be developed and characterized in sarcosine detection studies.

2. Theoretical foundation

2.1. Measurement of impedimetric sensors

An electrochemical impedance meter in potentiostat mode must be capable of applying a sinusoidal signal with amplitude in the range of millivolts (usually close to 10 mV) oscillating over a DC signal (preferably adjustable), and in turn calculate the impedance of the device under test (DUT) by measuring its response signal (current passing through the DUT). The DUTs in this work are impedimetric transducers which can be modelled by means of a Randles equivalent circuit (see figure 1), a non-ideal model, with a good approximation [19]. This circuit includes the solution resistance (Rs), the charge-transfer resistance (Rct) and the double-layer capacitance (Cdl). When performing subsequent impedance measurements by varying the frequency of the AC signal, the impedance values \(Z(\omega) = Z' + jZ''\), where \(Z'\) and \(Z''\) are the real and imaginary parts of the impedance) on a Nyquist plot describe a semicircle. At low frequencies, \(Z'\) and \(Z''\) tend to \(R_s + R_{ct}\) and zero, respectively. As the frequency increases, \(Z''\) becomes more and more negative and \(Z'\) is also lowered, thus \(Z''\) achieves a maximum absolute value at \(\omega_{\text{max}}\). From that frequency, the absolute value of \(Z''\) begins to decrease. At high frequencies, \(Z'\) and \(Z''\) tend to \(R_s\) and zero, respectively. \(C_{dl}\) is given by

\[
C_{DL} = \frac{1}{R_{CT}\omega_{\text{max}}Z}
\] (1)
To guarantee that $\omega_{\text{max}}Z$ is within the measurement range and that the error in the calculation of $C_{DL}$ is low, an impedance meter using these diagrams to calculate the parameters of the DUTs must sweep over a wide frequency range and at very smaller steps.

2.2. Sampling theorem by dephasing

When the frequency of the sampled signal is known and the hardware does not allow the Nyquist theorem to be fulfilled, it is possible to reconstruct the signal using the sampling theorem by dephasing, which states that [20]:

“Let $f(t)$ be a periodic signal in time with a known period $T$, and $\tau$ the sampling period used to obtain a digital signal $g(n)$ with $N$ samples per period and a total number of samples $\mu=MN$. If the relation $\tau=T/N(1\pm 1/M)$ is fulfilled, then it is possible to reconstruct, for the discrete signal, a period with $\mu=MN$ samples regularly spaced in time.”

Figure 2 shows a 1 MHz sinusoidal signal sampled under the sampling theorem by dephasing. It can be seen here, how the 10 samples of ten different periods of the signal describe another sinusoidal signal with a period of 11 $\mu$s.

2.3. Sinusoidal regression

If there is a couple of data sets $Y$ and $X$ belonging to a signal that resembles a sinusoidal signal, it is possible to describe the signal through the following expression:

$$\bar{Y} = A_1 \cos(\omega t) + A_2 \sin(\omega t)$$

(2)

where $\bar{Y}$ is the set of values generated from the data set $X$ that describe the sinusoidal signal that most closely approximates the real signal. The values of $A_1$ and $A_2$ are given by [21]

$$A_1 = \frac{B-C}{F-G}$$

(3)

$$A_2 = \frac{D-E}{F-G}$$

(4)

Where

$$B = (\sum_{i=1}^{n} Y_i \cos(\omega X_i))(\sum_{i=1}^{n} \sin^2(\omega X_i))$$

(5)
\[ C = \left( \sum_{i=1}^{n} Y_i \sin(\omega X_i) \right) \left( \frac{1}{2} \sum_{i=1}^{n} \sin(2\omega X_i) \right) \]  
\[ D = \left( \sum_{i=1}^{n} Y_i \sin(\omega X_i) \right) \left( \sum_{i=1}^{n} \cos^2(\omega X_i) \right) \]  
\[ E = \left( \sum_{i=1}^{n} Y_i \cos(\omega X_i) \right) \left( \frac{1}{2} \sum_{i=1}^{n} \sin(2\omega X_i) \right) \]  
\[ F = \left( \sum_{i=1}^{n} \cos^2(\omega X_i) \right) \left( \sum_{i=1}^{n} \sin^2(\omega X_i) \right) \]  
\[ G = \frac{1}{4} \left( \sum_{i=1}^{n} \sin(2\omega X_i) \right)^2 \]  

To obtain an expression of the form
\[ \bar{Y} = A \cos(\omega X + \varphi) \]  

The following equations can be applied
\[ A = \sqrt{A_1^2 + A_2^2} \]  
\[ \varphi = \tan^{-1} \left( \frac{A_2}{A_1} \right) \]  

Finally, the error of this approximation is given by
\[ e = \sum_{i=1}^{n} (Y_i - A \cos(\omega X + \varphi))^2 \]  

2.4. Circular regression

From a couple of data sets Y and X belonging to a curve that resembles a circle with center at C (h, 0), an expression that describes the circumference can be obtained in the following form
\[ \bar{Y}^2 = R^2 - (X_i - h)^2 \]  

where \( \bar{Y} \) is the set of values generated from the data set X that describe the circumference that best fits the data Y and X. The h and R values are given by [21]
\[ h = \frac{\sum_{i=1}^{n} X_i^2 + \sum_{i=1}^{n} X_i Y_i^2 - \left( \frac{1}{n} \sum_{i=1}^{n} X_i \right) \left( \frac{1}{n} \sum_{i=1}^{n} X_i^2 + \frac{1}{n} \sum_{i=1}^{n} Y_i^2 \right)}{2 \left( \sum_{i=1}^{n} X_i^2 - \frac{1}{n} \sum_{i=1}^{n} X_i \right)^2} \]  
\[ R = \left[ h^2 - \left( \frac{2}{n} \sum_{i=1}^{n} X_i \right) + \frac{1}{n} \left( \sum_{i=1}^{n} X_i + \sum_{i=1}^{n} Y_i^2 \right) \right]^{1/2} \]  

The error of this approximation is given by
\[ e = \sum_{i=1}^{n} (\bar{Y}^2 - Y_i^2)^2 \]  

3. Description of electronic modules

The block diagram with the different electronic modules of the proposed impedance meter is shown in figure 3. There are five main parts: signal processing and device control, sine wave generator, analog control system, voltage and current sensing, and signal processing. Each of these parts will be addressed below.

3.1. Signal processing and device control

The microcontroller selected to this function was the MC56F8367 manufactured by NXP Semiconductor. This microcontroller is a digital signal controller (DSC) of 16 bits that operates at 60 MHz and has enough program memory for this application, as well as a flexible set of peripherals. This device performs two types of functions: control of other devices by means of its peripheral modules, as well as signal processing and calculation of the impedance. Some of the configured peripherals are the serial peripheral interfaces (SPIs), pulse width modulators (PWMs), timer modules, and general-purpose...
input/output (GPIO) modules among others. This DSC was programmed through the Codewarrior® software. The code charged to the DSC includes the control tasks, the execution of the sampling theorem by dephasing and the mathematical implementation of the sinusoidal and circular regression needed for impedance calculation.

Figure 3. General block diagram of the impedance meter.

3.2. Sine wave generator
This stage is formed by a programmable waveform generator (AD9833) and a signal attenuator (LMP7312), both programmed via SPI modules. The signal generator delivers a sine wave signal with an amplitude of 0.6 Vpp in a frequency range from 0.1 Hz to 1 MHz. The operational amplifier associated to the attenuator reduces the amplitude of the sine signal to 20 mV. The output signal of the attenuator corresponds to the voltage applied to the working electrode.

3.3. Analog control system
A set of operational amplifiers conforms the analog control system with positive feedback loop. The idea behind this system is to guarantee the voltage at the working electrode, following the set point defined by the attenuator (described above), and at the same time, to provide the power required by the DUT (impedimetric transducer). With this purpose, operational amplifiers with offset level less than 100 µV, maximum voltage output swing from supply rails of 30 mV, input bias current less than 1 pA, minimum output current of 1 A for the power stage, and dual supply of ±2.5 V among others were selected. Additional to the AC level, this system applies to the DUT a voltage with a DC level in the range of -2 to 2 V.

3.4. Voltage and current sensing
The voltage and current sensing are carried out by two different strategies. To obtain the voltage in the terminals of the DUT, one programmable-gain operational amplifier is connected to the feedback loop, right at the output of an amplifier connected in buffer mode to the reference electrode. To sense the current passing through the DUT, one transimpedance amplifier is connected between one terminal of the DUT (no polarization terminal) and the analog ground. The gains of these amplifiers are not enough to set the signal levels according to the voltage input range (2 Vpp) of the dual analog to digital converter (ADC), which has 14 bits of resolution, a sampling rate of 40 MSPS, 71.6 dB of SNR and 80.5 dBC of SFDR. For that reason, one dual extra amplifier with a -3 dB bandwidth of 175 MHz, a slew rate of 220 V/µS and a rail-to-rail output is used to increase the amplitude of both signals. These signals enter in a conditioning stage, which gives the DC level required by the ADC. The DC levels of the voltage and
current applied to the DUT are suppressed by capacitors connected in series to the input of the dual extra amplifier. Finally, the signals are converted to 14-bit digital data by one ADC operating at a sampling rate controlled by one PWM module of the DSC. The ADC and the conditioning amplifier are supplied at 3.3 V, whereas the other devices are supplied at ±2.5 V.

4. Operation test

The prototype was proved with a Dummy cell, which is frequently used for calibration of potentiostats and impedance meters, and whose equivalent electric circuit is similar to Randles (see figure 1). The selected values were 10 Ω, 100 Ω and 1 μF for $R_S$, $R_{CT}$ and $C_{DL}$, respectively. The Table 1 resumes the main parameters and results. The values were calculated using the data processing described in section 2. The figures 4 and 5 show the Bode and Nyquist plots corresponding to the measured values. As mentioned above, because of the approximation in the frequency value at which the maximum $Z''$ occurs, an error greater than 1% in $C_{DL}$ is obtained.

| Parameter of the Dummy cell | Nominal value | Real value | Measured value | Error % |
|-----------------------------|---------------|------------|----------------|---------|
| $R_S$ (Ω)                  | 10 ± 1%       | 10.000     | 10             | 0.00    |
| $R_{CT}$ (Ω)               | 100 ± 1%      | 100.000    | 101            | 1.00    |
| $C_{DL}$ (μF)              | 1.0 ± 5%      | 0.989      | 0.948          | 4.15    |

Table 1. Parameters of the Dummy cell and results (measured value and error) obtained with the prototype.

Figure 4. Bode plot of the impedance of the Dummy cell according to the real and measured values. a) Magnitude and b) Angle of the impedance.
Figure 5. Nyquist plot of the impedance of the Dummy cell according to the measured values.

5. Conclusions
An electronic prototype for impedimetric biosensing was developed. The different constitutive blocks were designed according to the requirements of the application. The prototype includes a keyboard and a screen through which the users give instructions and receive the results of the measurement. The proposed system is compact, portable, and displayed good performance in the first approximation towards a deeper evaluation of its real potential for the application.

These results prove the potential of the system for the application. Additionally, it offers a route to early diagnosis of prostate cancer as well as other pathologies using less invasive techniques. Future work will be focused on a deeper evaluating of the prototype using impedimetric biosensors and quantification of analyte concentration.

Acknowledgments
This work was supported by the Center for Research and Technological Development in Electrochemistry (CIDETEQ), National Laboratory for Micro and Nanofluidic and National Council for Science and Technology (CONACYT) through the Cátedras CONACYT project No. 746. The authors thank CIDETEQ for the thesis grant given to Alejandra Perez-Nava. S. Fernandez-Puig, V. Vallejo-Becerra, and G. Oza are grateful to the UT system and CONACYT for the CONTEX-076B award.

References
[1] American Cancer Society 2021 Key Statistics for Prostate Cancer American Cancer Society. Atlanta, Ga
[2] American Cancer Society 2019 Screening Tests for Prostate Cancer American Cancer Society. Atlanta, Ga
[3] Goo Y and Goodlett D 2010 Advances in proteomic prostate cancer biomarker discovery. Journal of proteomics. 73 pp 1839–50.
[4] Stephan C, Ralla B and Jung K 2014 Prostate-specific antigen and other serum and urine markers in prostate cancer. Biochim Biophys Acta. 1846 pp 99-112.
[5] Stephan C, et al. 2009 New markers and multivariate models for prostate cancer detection. Anticancer RES. 29 pp 2589-2600.
[6] Khan A, Rajendiran T, Ateeq B, et al. The role of sarcosine metabolism in prostate cancer progression. Neoplasia. 15 pp 491-501.
[7] Sreekumar A, et al. 2009 Metabolomic profiles delineate potential role for sarcosine in prostate
cancer progression. *Nature*. **457** pp 910–4.

[8] Meyer T, Fox S, Issaq H, Xu X, Chu L, Veenstra T and Hsing A 2011 A reproducible and high-throughput HPLC/MS method to separate sarcosine from a- and b-alanine and to quantify sarcosine in human serum and urine. *Anal. Chem.* **83** pp 5735–40.

[9] Burton C, Gamagedara S and Ma Y 2013 Partial enzymatic elimination and quantification of sarcosine from alanine using liquid chromatography–tandem mass spectrometry. *Anal. Bioanal. Chem.* **405** pp 3153–58.

[10] Jiang Y, Cheng X, Wang C and Ma Y 2010 Quantitative determination of sarcosine and related compounds in urinary samples by liquid chromatography with tandem mass spectrometry, *Anal. Chem.* **82** pp 9022–9027.

[11] Bianchi F, et al. 2011 Fully automated solid-phase microextraction-fast gas chromatography-mass spectrometry method using a new ionic liquid column for high-throughput analysis of sarcosine and N-ethylglycine in human urine and urinary sediments *Anal. Chim. Acta*. **707** pp 197–203.

[12] Wu H, Liu T, Ma C, Xue R, Deng C, Zeng H and Shen X 2011 GC/MS-based metabolomic approach to validate the role of urinary sarcosine and target biomarkers for human prostate cancer by microwave-assisted derivatization. *Anal. Bioanal. Chem.* **401** pp 635–646.

[13] Martínez-Lozano P and Rus J 2010 Separation of isomers L-alanine and sarcosine in urine by electrospray ionization and tandem differential mobility analysis mass spectrometry. *J. Am. Soc. Mass Spectrom.* **21** pp 1129–32.

[14] Nguy T, Phi T, Tram D, Eersels K, Wagner P and Lien T 2017 Development of an impedimetric sensor for the label-free detection of the amino acid sarcosine with molecularly imprinted polymer receptors. *SNSR ACTR Elsevier*. **247** pp 461-470.

[15] Rebelo T, Pereira C, Sales M, Noronha J, Costa-Rodrigue J, Silva F and Fernandes M 2014 Sarcosine oxidase composite screen-printed electrode for sarcosine determination in biological samples. *Anal. Chem. Acta*. **850** pp 26-32

[16] Özkütük E, Diltemiz S, Avci Ş, Uğurağ D, Aykanat R, Ersöz A and Say R 2016 Potentiometric sensor fabrication having 2D sarcosine memories and analytical features Materials Science and Engineering. **69** pp 231-235

[17] Canbay Z, Ozyurt C, Mengulluoglu U, Dinçkayal E and Evran S 2018 Optimization of aptamer-based impedimetric biosensor for detection of sarcosine. *1* pp 26–8.

[18] Kumar P, Narwal V, Jaiwal R and Pundir C 2018 Construction and application of amperometric sarcosine biosensor based on SOxNPs/AuE for determination of prostate cancer. *Biosens. Bioelectron.* **122** pp 140-6.

[19] Banica, F 2012 Chemical sensors and biosensors: fundamentals and applications ed John.Wiley & Sons (United Kingdom).

[20] Miranda D and Barrero J 2007 Teoría, método, análisis de Fourier y error del muestreo por desfase. *Revista UIS Ingenierías*, **6** pp 25-33.

[21] Amaris J and López J 2004 Elaboración del software para la caracterización de una celda electroquímica usando DSP familia 56800 de Motorola [tesis de pregrado]. Bucaramanga, Colombia: Universidad Industrial de Santander, Escuela de Ingenierías Eléctrica, Electrónica y Telecomunicaciones.