Development of a Low Complexity System to Measure Electrical Signals in Plants

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Abstract: Electrical signals are generated and transmitted through plants in response to stimuli caused by factors localized in the external environment. Furthermore, these signals allow the whole plant structure to be informed almost instantly. By analyzing the electrical responses of a specific plant, it is possible to describe the impact of external aspects, such as insect infestation and light quality, in the plasma membrane potential, and identify the electrical signal origin. This project presents useful digital filters and a system consisting of a signal conditioning circuit and another one for measuring environmental parameters. Besides, they can be applied to signals of various plants types. The environmental parameters acquiring circuit consists in using a microcontroller alongside five sensors, which have unique functions. These aspects have got to be measured since they may interfere with the plant's electrical response.

Resumo: Sinais elétricos são gerados e transmitidos através das plantas em resposta a estímulos causados por fatores localizados no ambiente externo. Ademais, esses sinais permitem que toda a estrutura da planta seja informada quase instantaneamente. Ao analisar as respostas elétricas de uma planta específica, é possível descrever o impacto de aspectos externos, como infestação de insetos e qualidade da luz, no potencial elétrico da membrana plasmática, e identificar a origem do sinal elétrico. Este projeto apresenta filtros digitais úteis e um sistema composto por um circuito de condicionamento de sinais e outro para medição de parâmetros ambientais, nos quais podem ser aplicados para sinais de diversos tipos de plantas. O circuito de aquisição dos parâmetros ambientais consiste no uso de um microcontrolador e cinco sensores, que possuem funções próprias. Esses aspectos precisam ser medidos visto que podem influenciar na resposta elétrica do vegetal.

Keywords: Electrical Signals; Plant Electrophysiology; Response to Stimuli; Electronic Instrumentation.

Palavras-chaves: Sinais Elétricos; Eletrofisiologia Vegetal; Resposta a Estímulos; Instrumentação Eletrônica.

1. INTRODUCTION

Plants are organisms aware of numerous aspects of the environment they are located. In general, the electrical activities of the plants are related to transient changes in the potential of the plasma membrane (Fromm and Lautner (2007)). According to de Toledo et al. (2019), a significant point is the fact that plants have four different types of electrical signals: *(i)* action potentials (APs); *(ii)* local electrical potentials (LEPs); *(iii)* variation potentials (VPs); and *(iv)* system potentials (SPs). Furthermore two methods for performing electrical potential measurements can be used: intracellular and extracellular.

Countless studies, such as Fromm and Lautner (2007); Trebacz et al. (2006), were done in the past years. These studies showed that different types of stimuli could generate specific electrical responses in living plant cells. These responses, once initiated, spread to adjacent excitable cells. Plant cells' excitability is linked to their balance with the environment, and the coordination of internal processes, as detailed in Labady Jr et al. (2002).

Studying motivation in this field is that electrical responses emitted by plants are closely related to external environmental factors. A complete and automated system for real-time monitoring of these signals allows the user to be aware of what happens in the environment where the plant is located. It is possible to detect the presence of acid rain, landslides (Aditya et al. (2013)), or even an increase in air pollution. Moreover, it is conceivable to monitor the plants' growth and know whether pests are attacking a particular plant in the plantation, the soil it is inserted is dry, or the plant receives too much light or not. Another critical point is that this work can be used to create an equipment that aims to notify the user about the previously mentioned data.

Therefore, this work's main objective is to provide crucial information about measuring electrical signals in plants and the development of a modular and open hardware system capable of measuring parameters related to the environment using sensors. Besides, a signal conditioning circuit was made and had to be used in the process of acquiring plants' electrical signals.

2. PROPOSED METHODOLOGY

Figure 1 illustrates the process of the proposed methodology. It is possible to see in this diagram two processes. The first one is related to the detection and signal conditioning of a plant's electrical response. The second refers to the environmental parameters acquisition system. In the first process, the plant electrical response is initially extracted, entering a signal conditioning circuit. The response is then measured by a data acquisition instrument (i.e., analog-to-digital converter) and transferred to a storage system. Finally, the signal is filtered applying MATLAB software to remove residual noise. In the second process, the environmental parameters are acquired using sensors connected to an Arduino Mega 2560. In order to finish, the data obtained is saved on an memory.

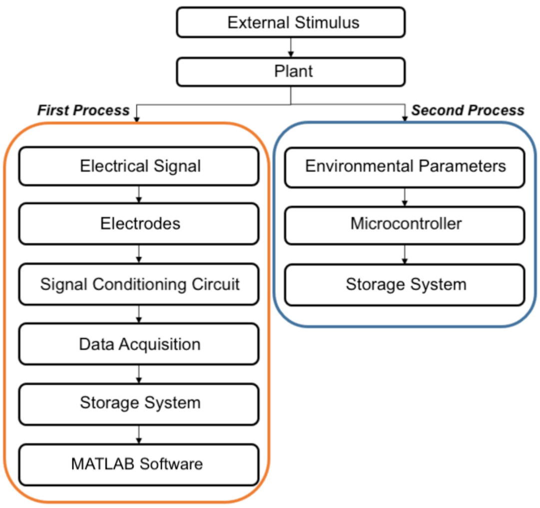

Figure 1. Flowchart of the proposed methodology.

2.1 Choosing the Electrodes

One of the electrodes recommended for extracellular measurement is the surface [1] one, made of Ag/AgCl, and used in electrocardiogram (ECG). For this electrode, authors refer to Volkov (2019). This electrode type holds an excellent cost-benefit ratio, adheres easily to the plant body, and owns an adhesive-conductive solid gel that stays in contact with the plant body and the electrode and holds the function of favoring electrochemical reactions. Moreover, an advantage of surface electrodes is the fact that it does not damage the plant body. Nevertheless, it is able to be connected to the plant only for the maximum time defined by the manufacturer, and it is necessary to remove gel residues after data recording.

The second working electrode recommended, which can also be selected as the reference one, is the needle [2]-type and was used in Aditya et al. (2013). This electrode is usually made of stainless steel. The diameter and size of the needle must be chosen based on the plant's physical characteristics that are used in the measurements. The disadvantage of the needle electrode is that it causes damage to the plant at the moment of insertion, so it is needful to wait for the plant to recover from the injury not to affect the measurements.

2.2 Signal Conditioning Circuit

Since the signals emitted by plants have got low amplitude (i.e., in the order of tens of μV to tens of mV), as explained in Tian et al. (2015), it is necessary a conditioning circuit to improve the signal-to-noise ratio (SNR) of the electrical response. It is needed to make it within the Analog to Digital Converters (ADC) dynamic range, which is the analog signal's operating amplitude range, within the converter's linear working region. It is recommended to use the entire dynamic range of the ADC because the resolution will be higher than operating only in a smaller part of the ADC dynamic range.

The input offset voltage temperature coefficient of the electrical signal pre-amplification circuit must be less than 10 $\mu V/°C$, as in Wang et al. (2009), and its input impedance must be in the order of $G\Omega$. This is because the source impedance (plant) often has a value in the order of hundreds or thousands of $k\Omega$, and the impedance value of $Ag/AgCl$ electrodes is in the order of a few $k\Omega$ (Jovanov and Volkov (2012)). So, the signal value that will appear at the input of the pre-amplification step will be practically equal to the plant's electrical response if the circuit's input impedance is as large as possible.

Furthermore, the common-mode rejection ratio must be at least $100dB$ (Wu et al. (2013)), so the power line frequency interference, which appears on the inverting and non-inverting inputs of the op-amp, can be attenuated effectively (Joachin (2000)). This parameter is fundamental since it indicates how much an undesired common-mode signal affects the measurements. The electrical signal conditioning circuit structure presented in this work is shown in Figure 2.

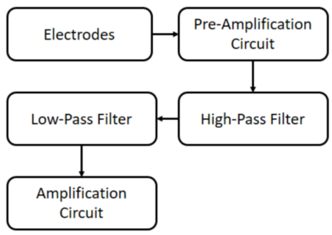

Figure 2. Signal conditioning circuit steps of the electrical signal.

In the pre-amplification stage, it is suggested to use an instrumentation amplifier integrated circuit, two amplifiers in the voltage follower configuration with a third op-amp, as in Wang et al. (2009), or even the classic instrumentation amplifier structure using three op amps, as shown in Joachin (2000). The differential amplifier configuration using only one op-amp can not be applied at this stage since it does not offer the required input impedance. The pre-amplification step is the most important of the entire signal conditioning circuit, since if it is appropriately built, a considerable part of the noise that interferes with the signal can be minimized. INA128, AD8221, and INA821 are

[1] https://www.medcleanprodutohospitalar.com.br/eletrodo-meditrace-adulto-200-kendall.html
[2] https://www.rhosse.com.br/eletrodo-agulha-160mmx55mm-ponta-03mm-x-15mm/p

some of the instrumentation amplifier integrated circuits that can be used in the first stage.

The second and third stages employ Sallen-Key filter topology, which is non-inverting. The second stage consists of a high-pass and the third one in a low-pass filter, both with unit gain. OP07 is an op-amp that can be used in these stages. Sallen-Key configuration was chosen for the sake of it is a low-complexity second-order filter. Besides, it is possible to apply some possible filter approximations, like Chebychev, Butterworth, and Bessel, depending on the adjustment of the quality factor (Q). These approximations determine the format of the frequency response.

Sallen-Key topology is widely employed due to the matter of the least dependent on the frequency response of the selected op-amp (Zumbahlen et al. (2011)). A bandpass filter was made by means of cascading a high-pass filter with a low-pass filter instead of using only one op-amp. The reason is the advantage of customizing the filter to have an asymmetrical response. A Sallen-Key bandpass filter with only one op-amp has cut-off frequencies equally separated from the center frequency (f_0).

Finally, the last step of the signal conditioning circuit consists of a non-inverting configuration with the gain being determined from the selected resistor values. At the end of the process, the electrical signal appears clearer at the op-amp's output, stronger and with undesired frequencies attenuated, ready for the digital filtering step. OP07 is one of the op-amps that can be applied in this second stage of amplification. The Equations related to this stage can be seen in Equations (1), (2), (3), and (4). Due to the concept of virtual ground present in op-amps:

$$V_+ = V_- = V_{in}^{'} \tag{1}$$

Resulting in:

$$\frac{V_{in'} - 0}{R_5} + \frac{V_{in'} - V_{out}}{R_6} = 0 \tag{2}$$

$$V_{in'}R_6 + V_{in'}R_5 - V_{out}R_5 = 0 \tag{3}$$

The gain is defined by:

$$A = 1 + \frac{R_6}{R_5} \tag{4}$$

Figure 3 shows the signal conditioning circuit. Sallen-Key equations for high-pass filter are given by Equations (5), (6), and (7), which represent respectively the transfer function, f_c, and Q.

$$\frac{V_o^{'}}{V_i^{'}} = \frac{s^2(R_1R_2C_1C_2)}{s^2(R_1R_2C_1C_2) + sR_1(C_1+C_2) + 1} \tag{5}$$

$$f_c = \frac{1}{2\pi\sqrt{R_1R_2C_1C_2}} \tag{6}$$

$$Q = \frac{\sqrt{R_1R_2C_1C_2}}{R_1(C_1+C_2)} = \frac{1}{2\pi f_c R_1(C_1+C_2)} \tag{7}$$

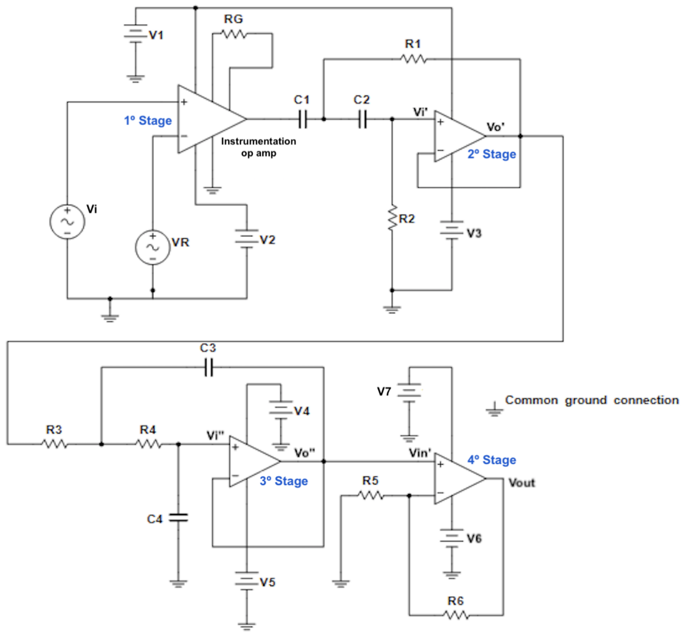

Figure 3. Representation of the developed signal conditioning circuit.

Sallen-Key equations for low-pass filter are given by Equations (8), (9), and (10), which represent respectively the transfer function, f_c, and Q.

$$\frac{V_o^{''}}{V_i^{''}} = \frac{1}{s^2(R_3R_4C_3C_4) + sC_4(R_3+R_4) + 1} \tag{8}$$

$$f_c = \frac{1}{2\pi\sqrt{R_3R_4C_3C_4}} \tag{9}$$

$$Q = \frac{\sqrt{R_3R_4C_3C_4}}{C_4(R_3+R_4)} = \frac{1}{2\pi f_c C_4(R_3+R_4)} \tag{10}$$

An important point about the gain given to the signal is that the higher the gain value, the narrower the bandwidth op-amp will work without attenuating the signal. It is crucial to take these factors into account since, according to Zhao et al. (2015), plant signals frequency range from very low frequencies to several hundreds of Hertz. So, the bandwidth the op-amp is working with a determined gain covers all frequencies of the plant's signal selected to perform measurements without suffering attenuation.

2.3 Environmental Parameters Acquiring Circuit

The proposed circuit consists of a soil moisture sensor, a real-time clock module RTC DS3231, a DHT-11 temperature and humidity sensor, a GY-302 sensor to provide information about the amount of light that strikes the place that the plant is located, an SD card module applied for storing data from other sensors, and an air quality MQ-135 sensor that is employed to measure the CO_2 levels in the environment in part per million ppm. All of them were connected to a microcontroller ATmega 2560, as shown in Figure 4.

This microcontroller ADC has 10 bits of resolution, and the measured voltage, which needs to be valued between

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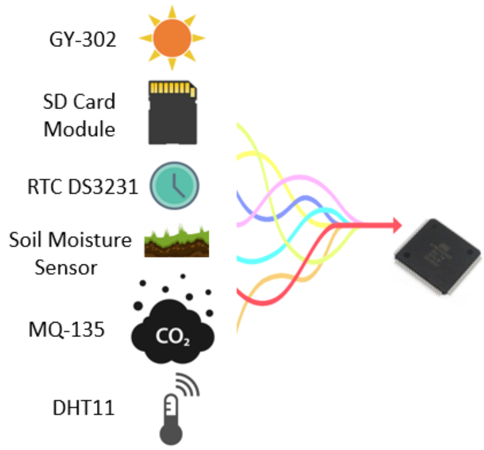

Figure 4. Sensors connections into the microcontroller ATmega 2560.

0 and V_{ref}, will be converted into values between 0 and 1023.

2.4 Digital Filtering

Even after going through the analog conditioning circuit, signals often still have interference generated by the power line's frequency and other undesirable frequency components. Two digital filters can be used to improve the acquired signal: a high-pass and a low-pass filter. It can be applied FIR filters, which have a constant group and phase delay, and a linear phase response if symmetry exists in the time domain (Equation 11). Besides, the order (N) and sampling frequency of the filters must be adjusted according to the signal and noise level.

$$h[n] = \pm h[N - n] \tag{11}$$

It is suggested to be applied just a high-pass and a low-pass FIR filter if frequency components of plant signal are lower than the power line frequency. Otherwise, it might be necessary to employ a notch filter too.

Windowing Method: A design method of the FIR filter that can be used is windowing. The window must be chosen based on the frequency response characteristics of the electrical response measured. If the magnitude values of the frequency components are rather different, then the background noise can hide the components with the lowest amplitude values. However, if magnitude values of frequency components have similar values, the background noise is not a problem. In situations where the signal's frequency components are close to each other, it is necessary to choose a window with better frequency resolution. If frequency components of the signal are more spaced apart, the frequency resolution is not an issue.

FIR filter development by windowing method arises by means of multiplying the impulse response of an ideal filter by the selected window function. Nonetheless, one of the hassles provoked by truncation of an ideal filter by a window is that this action results in the Gibbs phenomenon, which is defined by an oscillatory phenomenon in the frequency domain that takes place in transition band proximity. In the case of aiming to reduce the Gibbs phenomenon, it is required to select a window with a smoother band transition. The greater the intensity of the side lobes in the frequency response of the window, the higher will be the intensity of ripples in the stop and passbands. Considering the main lobe, the narrower it is, the shorter the transition band will be.

2.5 General Guidelines

The sampling frequency (f_s) of the equipment that will receive the plant signals is extremely important since it converts the waveform of the signal from the device's analog input into digital data. According to the Nyquist theorem, to sample the original signal correctly, without occurring aliasing, the sampling frequency must be at least twice the highest frequency component of the signal being acquired. The plant studied, growth stage, the stimulus applied, and the measured tissue influence electrical signal frequency, according to Zhao et al. (2015). Thus, depending on the aspects mentioned above, the user will have to use an instrument with a sampling frequency that is appropriate to the signal to be measured.

ADC with f_s between $40Hz$ and $100Hz$ are generally used in plants (Macedo et al. (2021)). It is suggested that when starting electrical signal measurements, a device with a relatively high sampling rate, something between $1kHz$ and $10kHz$, can be employed, and as the user is analyzing the signal frequency, it is possible to decrease the f_s up to a value that does not occur aliasing. It is recommended to decrease the f_s, since the higher the f_s, the larger the created files size (Mousavi et al. (2014)). Besides that, a higher f_s will acquire, alongside the electrical response, a huge amount of noise. Consequently, the signal of interest will occupy a narrow fragment of the spectrum, making it worse to examine the desired signal frequency range. The f_s choice and, consequently, the ADC sampling frequency is ultimately determined by the plant characteristic monitored and thus by the circuit application.

Data acquisition (DAQ) device input impedance (Z_{in}) must be much higher than the output impedance of the signal conditioning circuit (Z_{out}), by reason of the greater the impedance of the former, the closer the input signal value of DAQ instrument will be to the output voltage of the conditioning signal circuit, as shown in Equations (12) (13) (14).

In case of the output voltage of the signal conditioning circuit (V_{out}) is equal to $1V$, Z_{in} is equal to $6k\Omega$ and Z_{out} is equal to 60Ω. The voltage at the input of the data acquisition device (V_{in}) will be:

$$V_{in} = V_{out} \times \frac{Z_{in}}{Z_{in} + Z_{out}} \tag{12}$$

$$V_{in} = (1) \times \frac{6000}{6000 + 60} \approx 0.99V \tag{13}$$

When Z_{in} is 100x greater than Z_{out}, V_{in} has a value equivalent to 99% of V_{out}.

In case of V_{out} being equal to $1V$, Z_{in} being equal to 600Ω and Z_{out} being equal to 60Ω. V_{in} will be:

Table 1. Components values of each signal conditioning circuit stage.

Components	Values
Resistor	R_G=2.7$k\Omega$
	R_1=39$k\Omega$
	R_2=82$k\Omega$
	R_3=10$k\Omega$
	R_4=10kΩ
	R_5=1$k\Omega$
	R_6=100$k\Omega$
Capacitor	C_1=5.6μF
	C_2=5.6μF
	C_3=560nF
	C_4=270nF

$$V_{in} = (1) \times \frac{600}{600+60} \approx 0.91V \qquad (14)$$

When Z_{in} is 10x greater than Z_{out}, V_{in} has a value equivalent to 91% of V_{out}.

It is extremely indispensable that the plant, alongside the unshielded components of the measuring system, must be placed inside a Faraday cage, which can be custom-make without complications, for the purpose of improving SNR of the measured electrical response. The connections must be made as shown in Figure 5, as also showed in Tian et al. (2015).

All cables employed for grounding must be connected to a single point, and then, this point has to be attached to the power ground provided by a wall socket (Mousavi et al. (2014)). This procedure needs to be performed in order to avoid ground loop, which occurs when two or more points of an electrical system are connected to each other, and they are connected to the ground separately, creating a potential difference between the points, which gives rise to the circulation of a current in a low impedance loop.

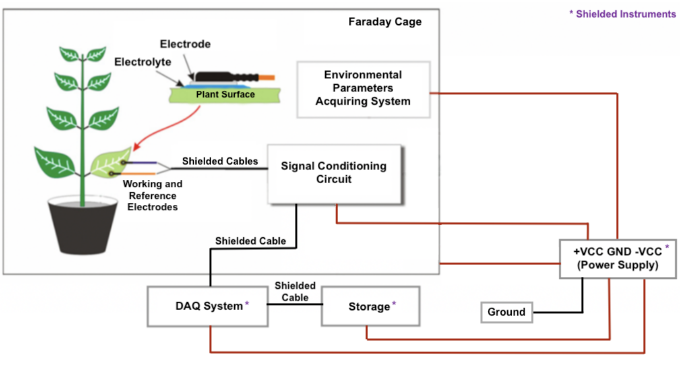

Figure 5. Connections applied in the process of measuring plants electrical signals. Adapted from Cabral et al. (2011).

3. RESULTS AND DISCUSSION

The signal conditioning circuit, shown in Figure 3, was simulated in Multisim 14.2 Student Edition software to testify the functionality for which it was proposed. The values chosen for the components are shown in Table 1 and represent commercial values. The tolerance of the resistors used in the simulation is 1%.

3.1 Simulation Results

In the pre-amplification stage, a gain of 19.93x was set, for that, according to the datasheet of the selected instrumentation amplifier, a resistor (R_G) with a value of 2.61$k\Omega$ should be chosen, however, as long as it was employed commercial values components, it was used a 2.7$k\Omega$ resistor. As shown in Table 2, it is possible to see that the gain value given to the differential signal at the input (V_+-V_-) is very close to what was expected. Additionally, when input signals are pure sinusoidal waves having frequencies which are within passband of the filters, electrical response is not attenuated throughout the stages. Voltage values applied in the circuit are in the range from the tens of µV to the tens of mV, as stated in Tian et al. (2015) for plants electrical signals.

Table 2. Voltage value showed in voltmeter after passing through each circuit stage.

Source Configuration	Stage	Voltmeter
V_i=700μV$_{rms}$; V_R=100μV$_{rms}$; f=0.05Hz	First	16.477mV
	Second	163.601μV
	Third	165.174μV
	Forth	16.662mV
V_i=700μV$_{rms}$; V_R=100μV$_{rms}$; f=0.5Hz	First	16.379mV
	Second	11.848mV
	Third	11.865mV
	Forth	1.199V
V_i=700μV$_{rms}$;V_R=100μV$_{rms}$; f=20Hz	First	16.021mV
	Second	16.500mV
	Third	16.140mV
	Forth	1.615V
V_i=700μV$_{rms}$;V_R=100μV$_{rms}$; f=40Hz	First	16.407mV
	Second	16.410mV
	Third	12.030mV
	Forth	1.213V
V_i=700μV$_{rms}$;V_R=100μV$_{rms}$; f=60Hz	First	16.414mV
	Second	16.450mV
	Third	7.041mV
	Forth	710.500mV

In the second stage, values of resistors and capacitors of the high-pass filter were chosen so that it could have a f_c=0.5Hz and Q=0.707. In the third stage, which is a low-pass filter, values of the resistors and capacitors were selected so that it could have a f_c=40Hz and Q=0.707. Both f_c were chosen, taking into consideration a plant electrical signal with frequency components between 5Hz and 25Hz (Wu et al. (2013); Cabral et al. (2011)). Butterworth filters were selected to be used due to their maximally flat magnitude response in the passband. In the final stage, in which the electrical signal is amplified, values of the resistors were chosen using Equation (4), so that the gain setting could be 101x. It is possible to verify in Table 2 that the gain given in this stage corresponds to the expected by theory.

It is important to point up that the components tolerance may affect the gain given in the first and last stages. Furthermore, this parameter can influence fc and Q of the filters, since they are dependent of components values.

Figure 6 presents the graph of each circuit stage, which is related to the input signals with a frequency set to $20Hz$. The other graph showed in Figure 7 gives the results of each stage when the input signals have a frequency equal to $60Hz$. It is needful to highlight that in Figure 6 the first stage graph is behind the second stage one since they both have almost the same amplitude. Also, in Figure 7, the first and second stages graphs are overlaid, for the same reason stated in the previous figure.

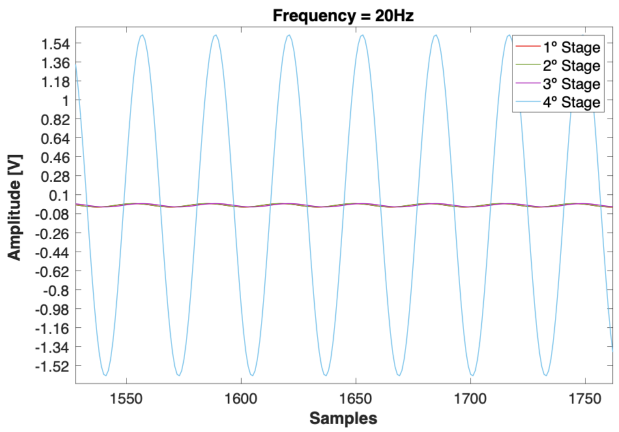

Figure 6. Graphs of each conditioning circuit stage when f=$20Hz$.

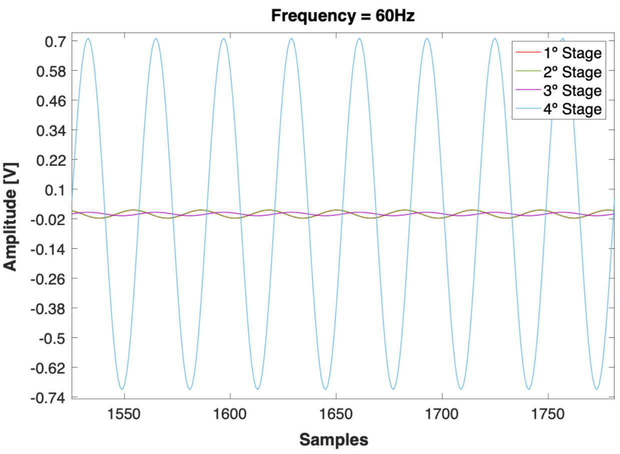

Figure 7. Graphs of each conditioning circuit stage when f=$60Hz$.

4. CONCLUSIONS AND FUTURE WORKS

This work has presented functional digital filters, and a low complexity system for measuring plant's electrical signals was explained in details. The signal conditioning and the environmental parameters acquiring circuits can be used to create a piece of low-cost equipment that includes the measurement process in a single device. Results indicated that the proposed analog filters work well and can be employed as a basis to accomplish higher order ones.

Future works include developing a more robust signal conditioning circuit, which has a high complexity, capable of making plant electrical response as clean as possible before the digital filtering stage. Another crucial topic that must be addressed is carrying out experiments using a plant, applying the project developed.

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