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Virtual instrumentation for biological process monitoring based on electric field perturbations

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Abstract— **We present a low-noise virtual instrument programmed in LabView platform for biological processes monitoring. Measurements are based on electric field perturbations due to temporal variations of a biophysical process over an interdigitated capacitance sensor. Blood clotting process was monitored in real time with around 5µl drop volume of whole blood sample. Blood drop was placed between 150 microns thick glass slabs over an interdigitated capacitance sensing device. Analog temporal signals were digitalized and processed with low-noise virtual instrumentation. Temporal signals of biophysical process presented a good signal to noise ratio showing that the sensitivity and resolution of the measuring system proposed is suitable for other biological processes and bioelectric signal characterization.**

Key words — **Biophysical process, electric field, interdigitated capacitor, low noise.**

I. Introducción

Electric field perturbation measurements based in electrode coplanar array capacitance sensors is an applicable technique for physical-chemical process characterization like solvent evaporation and dielectric coating [1]. Also, it has been used for biological processes like tissue characterization [2], bioelectric signal characterization, etc. However, bioelectric signals can be so small that they are usually immersed in electromagnetic noise produced by system hardware such as electronics, cables, connectors, high voltage sources etc. Thus, low noise measurements techniques must be used in order to recover just the bioelectric signal of a particular biophysical process in study. Low-noise measuring instrumentation with Lock-in amplifiers techniques have been used successfully performing these tasks [3].

Blood impedance measurement is a measuring technique to determine electrical parameters of its constituents such as white and red blood cells (WBCs, RBCs), platelets, serum and plasma. These constituents can be used to characterize the whole blood coagulation process, a very complicated dynamic physiological process. Electrical properties of blood give additional information of interest like those as physical, biological and chemical for biological studies or medical diagnosis to determine some disorder in its fundamental function inside the human body. Hematological and biochemical disorders in blood coagulation can carry on complications as hemorrhage, thrombosis and embolism in vascular system. Furthermore, important blood parameters such as the levels of glucose, urea, lactate, cholesterol and nitric oxide, have been detected with Bioimpedance Spectroscopy techniques [4,5]. However, electric field sensing devices must be functionalized and characterized to obtain its electric parameters in order to determine the best sensing performance in a measuring system [6,7].

The measuring system proposed is based in Virtual Instrumentation (VI) programmed with LabView software from National Instruments. The main reason to implement Virtual Instrumentation with LabView was its graphic language programming. Functions and virtual instruments (VI) can be integrated to others in order to get a new VI with particular characteristics required for a specific application. The connectivity with analog to digital converters (ADC) from National Instruments is practical and easy to do, as happened with the 24 bit CAD used in the measuring system depicted in this work.

The measuring principle of the system is based in detecting electric field perturbations of the sensing device produced by biophysical processes such as blood clotting. The sensing-conditioning stage of the measurement system compensates parasitic currents due to surrounding ambience noise and hardware system (electronics, connectors, cables and glass slides) to a suitable level. Digital Lock-in stage picks up digitalized sensing data at excitation frequency in a narrow bandwidth in order to obtain good signal to noise ratio SNR of biophysical process signals.

In this work whole blood clotting process was monitored in order to determine the sensitivity and resolution of the measuring system. Around 5µl drop blood sample (BS) was placed between 150 microns thick glass slabs above 1cm^2 sensitive area of the interdigitated capacitance sensing device (IDCSD). Temporal bioelectrical signals of blood clotting process were monitored in real time.

Blood sample was selected as biological sample under test (BSUT) because its characteristics of inhomogeneity and the facility to obtain a thin film of the blood sample to cover the sensing surface. This is a very important condition required to obtain reliable measurements of the biological process. Temporal signal monitoring is possible thanks to the dielectric function of the blood sample, these measurements are important to characterize the response of the capacitive sensor device with biological sample. Finally, the development and application of biosensors with the measuring system proposed in this work is of interest for our research.

II. MEASURING SYSTEM

Virtual Instrumentation Measurement system is based on a digital Lock-in stage, its function is to detect small AC signals from the sensing device reducing surrounding noise at a reference frequency. The Lock-in acts as a pass-band filter at a very narrow bandwidth at the system reference frequency. The Lock-in function is implemented principally with three modular functions or virtual instruments (VI), Phase Loop Locked, Demodulator and Low Pass Filter. The principal function of the PPL module is to measure the frequency and phase of the reference signal (f_r), this data is used to generate internal sine and cosine reference signals that are mixed with the input signal. The Demodulator module calculates some of the internally used settings for the mixer and low-pass filter as well as the actual low-pass filter time constant (τ) and setting time. Finally, the frequency component from the input signal is extracted at the frequency and phase of the reference signal (f_r) with a narrow bandwidth in order to discriminate surrounding noise.

The measuring principle is based on electric field perturbations around coplanar-electrodes array due to changing electrical properties of blood clotting process.

Analog reference signal Vac(ω) used in the system is 1.6 Vrms sinusoidal voltage @ 10KHz reference frequency (f_r) digitally generated with NI LabView software by means of a digital to analog converter NI USB-4431 (DAC) and a rms analog converter stages. Also, Vac(ω) is used as an excitation signal $Ve(\omega)$ in the interdigitated capacitive sensor device (IDCSD). Besides, $GV_{cc}(\omega\pm\varphi)$ is phase-amplitude compensated in order to obtain a similar signal of that obtained from the sensing device output $Vs(\omega)$ and get the lowest possible offset $\Delta V_d(\omega)$ at the differential output stage before begin any measurement. Differential voltage signal $\Delta V_d(\omega)$ is obtained with electrical signals $GV_{cc}(\omega\pm\varphi)$ and $Vs(\omega)$ from interdigitated capacitive compensation device (IDCCD) and IDCSD outputs respectively at the electronic differential input stage (EDIS). Output differential voltage signal $\Delta V_d(\omega)$ can be amplified by a k factor up to $1x10^6$ to obtain a suitable amplitude $k\Delta V_d(\omega)$ for 24 bit digitalization process signal at maximum sampling frequency (f_s) of 100 KS/s with the same NI USB-4431 analog to digital converter (ADC) stage. Digital Lock-in (DLI) stage picks up temporal digital data (TDD) from ADC output into 2.5Hz narrow bandwidth in order to reduce surrounding noise at 10KHz excitation frequency (f_e), same as reference frequency (f_r). Real and imaginary analog components of temporal signal $\Delta V_{LI}(t)$ and $\Delta V_{LR}(t)$ respectively are acquired in real time from a biophysical process and displayed in a personal computer, data are saved for detailed signal analysis. Block diagram of measuring system proposed is showed in figure 1.

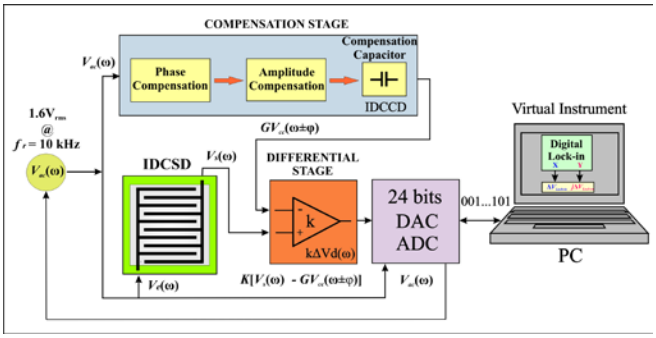

Fig. 1. Block diagram of measuring system showing sensing-conditioning, differential, DAC-ADC and digital Lock-in stages programmed in a PC.

III. MATERIALS AND METHODS

The sensitivity and resolution achievable with the measuring system proposed were determined by whole blood clotting process monitoring with around 5µl drop blood sample (BS), figure 2.

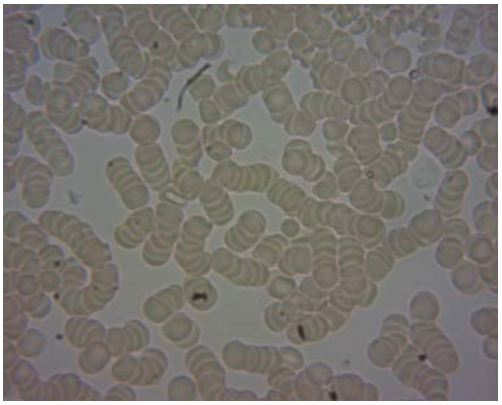

Fig. 2. View of blood sample under optical microscope with 40x/0.65 objective.

Blood drop was deposited between 150 microns thick glass circular slabs directly from a pricked finger with a lancing device. Blood sample (BS) under microscope view shows blood cells with no damage, figure 2. A blood film is formed over $1cm^2$ sensing surface of the IDCSD with 20 coplanar electrode array of 20 µm width and gap, as it is shown in figure 3.

Temporal electric field perturbations are presented over the sensing coplanar electrode array that is covered by the BS film placed between circular glass slabs. Electric field perturbations were sensed as temporal differential potential variations $\Delta V_L(t)$ of blood clotting process. Measurements were performed without ambience room control at 24 ºC and 60% H_R average.

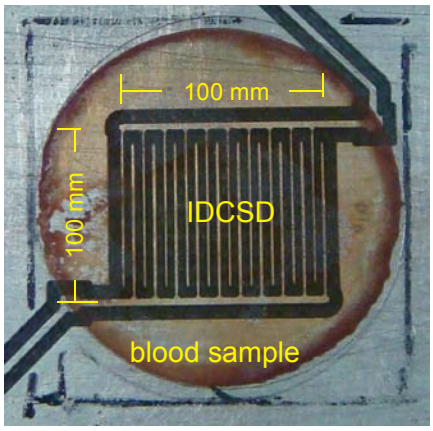

Fig. 3. Blood sample between circular glass slabs placed over interdigitated capacitive sensor device (IDCSD).

IV. RESULTS AND DISCUSSION

The sensing-conditioning stage of the proposed measurement system compensates parasitic currents due to hardware (electronics, connectors, cables, circular glass slabs). A Digital Lock-in picks up data at the excitation frequency f_e = 10KHz in a narrow bandwidth of BW = 2.5Hz with a 100ms time constant (τ).

Before performing any measurement, minimal differential reference value $k\Delta V_d(t)$ was set, compensating the double glass slabs without material under test (MUT) over the IDCCD (used as a mirror device) and the IDCSD. This way, the output noise of sensing-conditioning stage could be set as it is shown in figure 4.

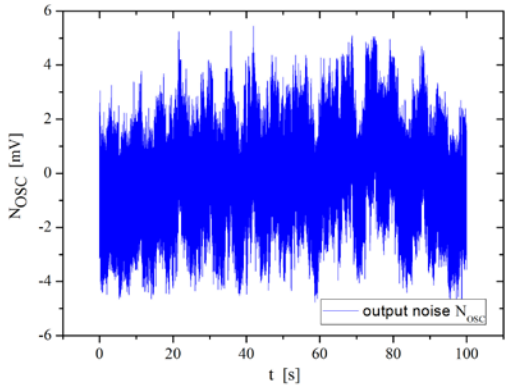

Fig. 4. Sensing-conditioning stage output noise, around 10 mV_{pp}.

As it can be seen sensing-conditioning stage maximum output noise peak to peak amplitude (N_{OSCpp}) is around 10mV_{pp}. This noise amplitude is too big for temporal signals thousands of times smaller than those presented in a real biophysical process. N_{OSCpp} signal is digitalized in an ADC stage in order to be processed by the DLI and obtain base noise peak to peak present in the IDCSD, shown in figure 5.

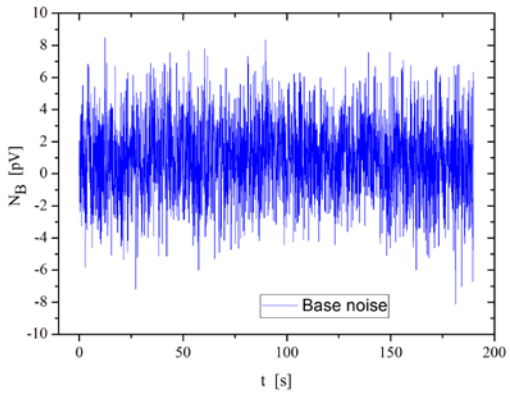

Fig. 5. IDCSD base noise, around 16 pV_{pp}.

As it can be seen in figure 5, maximum IDCSD base noise peak to peak (N_{Bpp}) is around 16 pV_{pp} so, comparing N_{OSCpp} with respect to N_{Bpp} we obtain a noise reduction factor (NRF=N_{OSCpp}/N_{Bpp}) of around 6x10^6. The rms system base noise (N_{Brms}) was calculated in a determined time lapse with minimal differential $\Delta V_L(\omega)$ value setting in a bandwidth BW = 2.5 Hz at a reference frequency f_r = 10KHz. Thus, N_{Brms} = 2.6 pV√Hz, calculated with one of the statistical functions that perform the virtual instrument (VI).

The electric field temporal perturbations due to blood clotting process were sensed by the IDCSD in real time as temporal differential-voltage signals $\Delta V_{LR}(t)$ and $\Delta V_{LI}(t)$, real and imaginary components of the signal $\Delta V_L(\omega)$ are shown in figure 6 and 7, respectively.

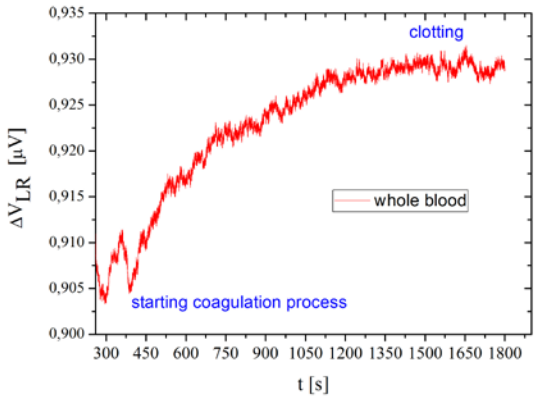

Fig. 6. Real component $\Delta V_{LR}(t)$.

It can be observed in figures 6 and 7 that $\Delta V_{LR}(t)$ and $\Delta V_{LI}(t)$ are over an offset of around 0.9μV and -1 μV respectively because whole blood film is sensed as a biological material film with a predominant composite dielectric function across

the electric field presented in the sensitive surface of the IDCSD. Opposite polarity presented in the graphs are due to DLI stage phase compensation module. Temporal differential voltage signals $\Delta V_L(t)$ of whole blood clotting process could be monitored by the measuring system just over the offset presented in both components, starting from around 400 seconds to 1150 seconds, a 750 seconds period approximately. Blood clotting starts after 1150 seconds approximately, where minimal signal variations are monitored.

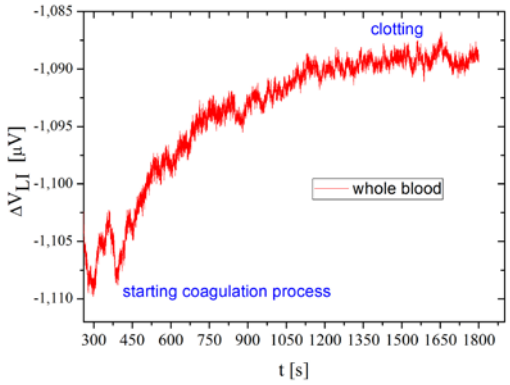

Fig. 7. Imaginary component $\Delta V_{LI}(t)$.

This behavior is present in both graphs $\Delta V_{LR}(t)$ and $\Delta V_{LI}(t)$, figures 6 and 7. However, maximum amplitude of temporal signal of blood clotting process is around 25nV for $\Delta V_{LR}(t)$ and around -20nV for $\Delta V_{LI}(t)$. Due to blood clotting process, the signal to noise ratio SNR was estimated from maximum amplitude of temporal signal variation with respect the rms system base noise, ($\Delta V_L(t)$ / N_{Brms}). Thus, SNR was $9.6x10^3$ and $7.7x10^3$ for $\Delta V_{LR}(t)$ and $\Delta V_{LI}(t)$ respectively. Temporal signal monitoring before 200 seconds time process are not shown in figures 6 and 7.

The blood clotting process signals response can be explained with the equivalent electrical model of whole blood tissue which is represented for red blood cell interior resistance R_{ci} in series with red blood cell membrane capacitance C_{cm} and both in parallel with plasma resistance R_{pl} [4], as it is shown in figure 8.

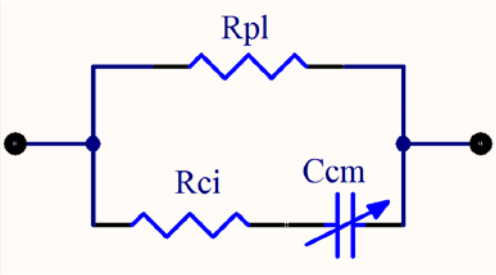

Fig. 8. Equivalent electric circuit of whole blood.

Assuming the system is monitoring just the differential potential variations of whole blood clotting process, we can notice that real and imaginary components of blood impedance both have similar behavior at f_e = 10KHz with an absolute offset value of around 0.1μV between them. The absolute variation difference of 5nV of maximum blood clotting process for $\Delta V_{LR}(t)$ and $\Delta V_{LI}(t)$ could be due to the temporal impedance value of C_{cm} at frequency f_e in $\Delta V_{LI}(t)$ signal [5].

Dielectric function variations of whole blood sample are detected as electric field perturbations in the sensitive surface of the sensing device. An impedance analysis of the measuring system was done in order to obtain an approximation of the whole blood sample capacitance $C_S(\Delta V_{LI}(t))$. Sensitivity was estimated as the ratio of the variation of the amplitude of the signal $\Delta V_{LI}(t)$, imaginary component of the signal, to the variation of the dielectric function of the blood sample as the capacitance C_S. So sensitivity of the measuring system for blood clotting process was estimated as $\Delta V_{LI} / \Delta C_S = 10$ [nV / aF].

The resolution of the measuring system was estimated with respect the base noise peak to peak (N_{Bpp}), showed in figure 5. The resolution in the measurements are based in experimental results as $3N_{Bpp}$ = 48pV. The estimation of the resolution allows define a minimal change in amplitude of the temporal signal respect to $3N_{Bpp}$ in experimental measurements.

The main advantage of the VI was the integration of the principal modules necessary to add portability to the measuring system. It is possible improve the algorithm in case two more digital channels of the ADC need to be used. This way, two more sensor devices could be added to the system to perform a total of three measurements at the same time. The modules used to perform the Lock-in stage are protected for LabView and cannot be modified. However, the results obtained showed that is possible to develop low-noise measurement instruments digitally. Sampling frequency (f_S) limits the reference frequency (f_r) of the measuring system, it is recommended to use a sampling frequency of eight times the reference frequency, $f_s=8f_r$, for a reliable signal digitalization and data processing. Improvement sensor device fabrication is desirable to improve measuring system performance.

V. CONCLUSIONS

The measuring system proposed reached the resolution and sensitivity necessary to monitor 5μl of whole blood sample during its clotting process by means of differential potential variations due to electric field perturbations of IDCSD. Differential signals $\Delta V_{LR}(t)$ and $\Delta V_{LI}(t)$ presented a N_{Brms} of around 2.6 pV / $\sqrt{H_z}$ and total blood clotting process of around 25nV and 20nV absolute potential variation, respectively. Thus, signal to noise ratio SNR obtained was

$9.6X10^3$ and $7.7X10^3$ for $\Delta V_{LR}(t)$ and $\Delta V_{LI}(t)$, respectively. The biophysical process was monitored just to probe the measuring system response with the conditions mentioned before. However, particular blood characterization is possible by functionalizing the IDCSD with a suitable bio-receptor. The results obtained in this work show that sensor fabrication improvement is necessary to obtain better response of the measuring system. Portability feature of the measuring system was possible thanks to the virtual instrumentation implemented in the measuring system. This feature will be important to perform and validate experiments with diverse biophysical, biochemical or pharmaceutical processes in specialized research centers labs.

Virtual Instrumentation code can be modified in order to improve the usage of all input channels of the CAD. This way a total of three sensors could be used at the same time.

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