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Blood components characterization for pre-analytical rapid quality controls through impedance measurements

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Abstract – **In clinical laboratories the requirements for quality control material have greatly increased in order to evaluate and monitor the reliability of performances. Pre-analytical conditions of blood samples are key factors in maintaining the high quality of the specimens and they are necessary for the reproducibility and the accuracy of measurements and procedures in clinic. Using quality control samples, day-to-day and batch variations can be limited. The quality of the serum has to be very high in order to avoid interferences due to hemolysis; for this reason, every sample should be rapidly tested with smart and portable devices. In addition, the quality of whole blood preservation should be quantified in a fast way to guarantee the complete compatibility and avoid anomalies, for example before blood transfusion. A new simple impedance-based biosensor is proposed for ensuring the reliability of patient sample results. Comparing and combining the data of impedance measurements, this technique has been applied for the fast discrimination of different blood components, the identification of hemolysis in serum samples and the rapid and automatic evaluation of blood quality, as demonstrated by the presented preliminary results.**

***Keywords*– biomedical measurements, electrical impedance, biosensors, bioengineering, blood quality controls**

I. INTRODUCTION

The use of quality controls to monitor performances of laboratory tests and blood testing devices is an essential part of any health care laboratory [1-3]. For the preparation of quality control samples, several methods are currently used in clinic [4, 5], but in practice they depend on high dimensions instrumentation and specific kits whose utilization has to be optimized. Control serum can contain minute fibrin clots or damaged red blood cells (hemolysis condition) and these situations lead to technical problems and measurement interferences [6, 7]. The quality of the serum is measured only for the samples that visibly show pale red shades, while it should be rapidly and always tested for every sample, without exclusions, in order to assure the complete absence of hemolysis. In addition, for blood collected from both healthy donors and patients, a control check has not always provided to guarantee the quality of samples or identify inadequacies related to carry or storage procedures. Anomalies involving the temperature of the containers for transporting blood components are frequent and this problem requires auxiliary investigations to preserve adequately the samples and to identify the opportune corrective actions. The quality of blood preservation should be quantified in a fast way to guarantee the complete compatibility and avoid anomalies in laboratory tests and before blood transfusion.

The relevance of impedance measurement technique in characterizing biomedical human aspects has been already proposed in literature [8, 9]. Here, we propose a new simple impedance-based sensing device. Comparing and combining the data of impedance measurements, we are able to characterize and discriminate different blood components and to perform fast, *real-time* and accurate check tests about blood samples and serum samples quality, with the possibility to confirm or reject their usability in clinic.

II. RELATED RESULTS IN THE LITERATURE

Blood is a fluid composed by a conductive liquid, plasma, and a suspension of particles (red blood cells, white blood cells and platelets). Each cell is composed of a lipidic membrane (electrically a capacitance) which

contains an intracellular fluid with dielectrical properties similar to plasma. The interaction between a biological fluid and a metal electrode forms a narrow interface, known as double layer, where the current flows as in a capacitive layer, but the phase shift of current, in magnitude, is less than – 90 degrees. For this reason the impedance of the Constant Phase Element (CPE), $\dot{Z}_{CPE}(\omega)$, was defined [10] and used in [7-9] as $\dot{Z}_{CPE}(\omega) = 1/[C_{CPE}(j\omega)^{\psi}]$, where ψ is the phase shift.

Measurements for the characterization of whole blood dynamic behavior in artificial micro-channels have been presented using electrical impedance spectroscopy, as described in [11-12, 16-17],by using an impedance meter and a low cost sensor based on printed circuit board technology. With regard to the characterization of single blood components, this is still an open challenge, not yet settled by a single multi-measurement sensor.

III. DESCRIPTION OF THE METHOD

For each experiment, 3 ml of venous blood were collected from healthy blood donors intocitrate as anticoagulant (hematocrit value of 38± 3 %). Informed written consent have been obtained from healthy blood donors.Afterwards, each vial of whole blood was divided in three parts, with the aim of obtaining the materials of Fig. 1. One part of whole blood was left untreated (whole blood in Fig. 1D). Another part of whole blood (1 ml) was centrifuged to obtain, as shown in Fig. 1A, plasma (liquid substance with all the coagulation proteins, but without cells) and whole concentrated blood (red cells, white cells and platelets, with an hematocrit value of 60 ±3 %). Finally, in the last part (1 ml), 250 µl of $CaCl_2$ (5mM final concentration) were added to stimulate the coagulation process and to obtain serum (liquid substance without cells and without coagulation proteins) and the red clot (red cells, white cells, platelets and fibrin).The serum thus extracted can appear yellow (serum in Fig. 1D) or it can show pale red shades (serum with low or high hemolysis in Fig. 1B, 1C, 1D), a visible indication of the presence of hemolysis, due to red blood cells damaged. In addition, serum can sporadically contain fibrin clots, here named white clots. If the serum expresses hemolysis, the white clot can contain fragments of red blood cells damaged and it looks light red (white clot with hemolysis in Fig. 1D). These anomalous situations, not always identifiable by eye, can lead to technical problems and measurement interferences if not rapidly recognized.

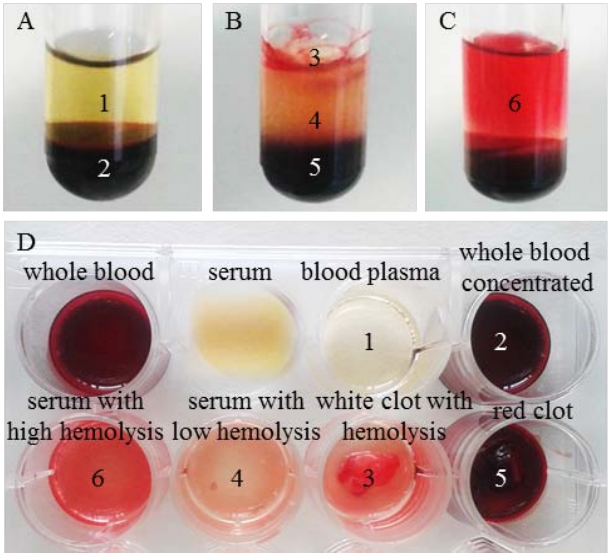

Fig. 1. Blood components characterized with impedance measurements. Whole blood centrifuged (A). Blood coagulated and centrifuged (B, C). Components disposed in plastic wells (D).

Each blood component was homogeneously disposed in a single well (300µl of substance, well diameter: 1.6 cm) and their electrical impedance was measured through a low cost, small and reproducible sensing module with a simple geometry (Fig. 2A). The sensing module appears as a capacitor with parallel planar surfaces made of copper (width: 7 mm, distance between surfaces: 4 mm). A polycarbonate cap supports the accurate positioning of the capacitor in the blood substance and, consequently, maintains the stability of measurements. The device was connected to a high precision LCR meter [18], in order to perform impedance measurement in the frequency range [100 Hz, 2 MHz], with twenty logarithmic spaced steps. A drive voltage of 50 mV was chosen, taking into consideration the electrochemical properties of materials and the open-short calibration was applied with the purpose of reducing the parasitic effects.

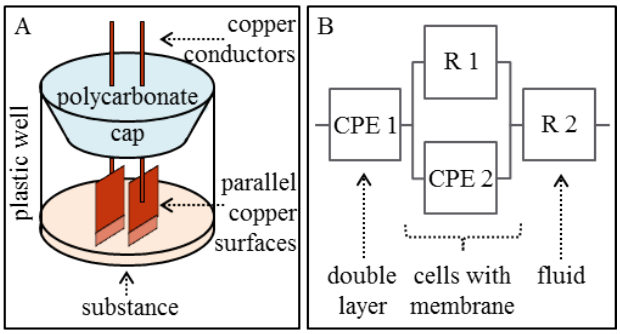

Fig. 2. Electrical impedance measurements. A polycarbonate cap is placed on each well and the parallel copper conductive surfaces are immersed in the fluid substance (A). Equivalent circuit (B).

The equivalent lumped circuit is presented in Fig. 2B. The double layer is modeled as the CPE1, in series with the parallel of R1 and CPE2 (cells and particles) and with R2 (the fluid in which cells and particles are suspended).

The blood of 10 different donors was treated, as just described, and analyzed obtaining tens of impedance signals varying with frequency and stable in time.

IV. RESULTS AND DISCUSSIONS

Impedance signals related to the representative materials of Fig. 1 are displayed in Fig. 3as magnitude and phase. For each material, one representative curve is presented. With this new prototype, it is possible to discriminate with evidence different blood components on the basis of their impedance magnitude and *phase that* are representative of their resistance to the current flow and depend on the amount of the capacitive contribution due to the lipidic membrane, mostly of the red blood cells.

In addition to the visibly obvious capability of characterizing different blood components with electrical impedance measurements (Fig. 3), the most promising results are represented by the curves of serum (with and without hemolysis) and those of the blood, in particular the combination that comprehends whole blood, red clot and whole concentrated blood. These preliminary results pave the way for comparative analysis of serum samples in order to associate pre-analytical thresholds values and quantitative hemoglobin content values to the signals obtained with our prototype, in order to program and propose a fast quality check for all serum samples with a low cost and low dimension device. Similar comparative analyses could be done with the blood collected, i.e. for transfusion, to control the transport and storage quality and identify density variations or aggregative state, situations not compatible with transfusion.

An interesting aspect that deserves attention and further experiments is represented by the white clot curves. In Fig. 3 the white clot without hemolysis seems to be the most conductive material among those analyzed and this is due to the fiber net conductive structure of fibrin, combined with the absence of resistive cellular components inside. With this approach, fibrin was electrically characterized and its formation could be identified during the coagulation whole process.

The discrimination of blood components through the impedance signals is confirmed and enriched by the inverse analysis performed with a Complex Non-linear Least Square (CNLS) fitting software.

Thanks to this, it was possible to quantify the lumped elements parameters (C_{CPE1}, ψ1, R1, C_{CPE2}, ψ2, R2) with an uncertainty in the order of 2%.In particular, the parameters related to the double layer appear almost equal for all the blood components analyzed. In fact, the double layer is common to all the blood components because it is associated to the interaction at the interface of the material with the metal. For this reason, CPE1 can be compensated with the aim of evidencing the specific electrical properties of each specific biological material. Interpolations of frequency-points are shown in Fig. 4, for some representative blood components, in a Real-Imaginary plot (without double layer compensation, on the left and with compensation, on the right).

In addition, the numerical results, shown in Table 1, confirm that the CPE2 is directly related to the presence or the absence of cells (mostly red blood cells) and indicates the possible breaking of cells membrane. In particular, CPE2 and R1 exhibit a significant difference if there are cells (the higher the number or the concentration, the higher the value), if there are cells with damaged membrane (intermediate condition, hemolysis) or if cells are absent. The phase shift of CPE2 measures exactly 1 (CPE2 is an ideal capacitor, with a very low capacitance) if cells are absent or their membrane are damaged (blood plasma and serum). Finally, imagining the natural extension of the curves in Fig. 4 (right panel) towards infinite frequencies, it is evident that they tend to blood plasma, in agreement with the decreasing of capacitive impedance to disappearance.

In diagnostic laboratories, the majority of critical issues occur in the pre-analytical phase.

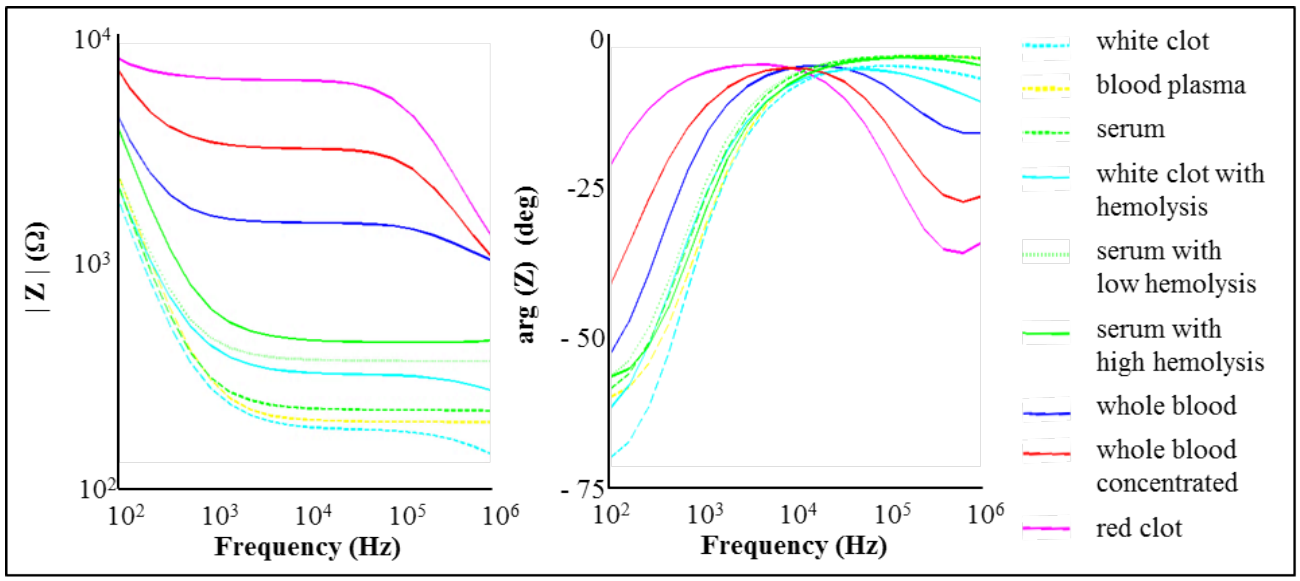

Fig. 3. Electrical impedance representative signals displayed as magnitude and phase.

	C_{CPE1}[F]	ψ1	R1 [Ω]	C_{CPE2} [F]	ψ2	R2 [Ω]
Plasma	3.6e-7	0.86	660	2.6e-12	1	50
Serum low hemolysis	7.2e-7	0.85	690	4.7e-12	1	180
Serum high hemolysis	7.7e-7	0.85	850	4.1e-12	1	180
Whole blood	7.4e-7	0.82	1060	1.1e-10	0.82	1100
Concentrated	7.3e-7	0.83	3200	8.6e-11	0.85	1200
Red clot	6.1e-7	0.84	4050	5.9e-11	0.83	1100

Table 1. Extracted lumped elements according to the equivalent model. The biologic sample discrimination can be achieved mainly by observing the values of R1, C_{CPE2}, ψ2 and R2.

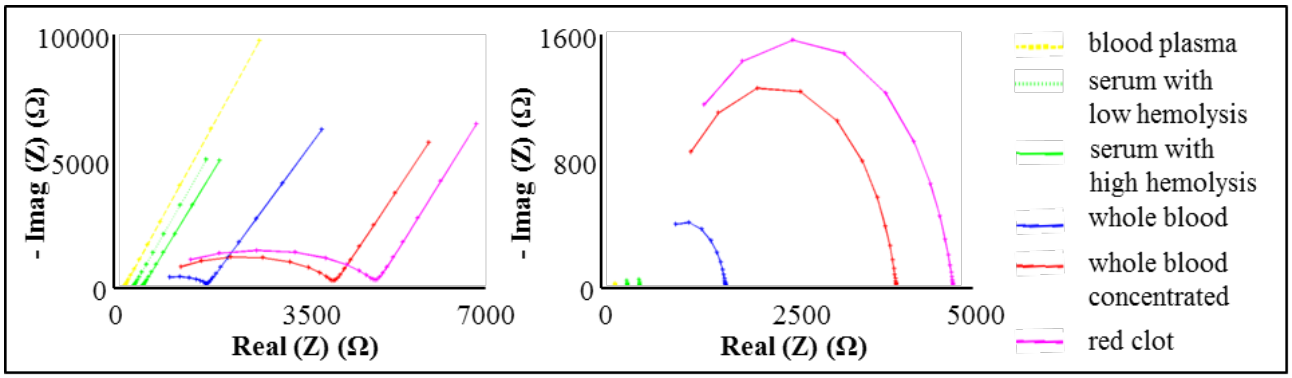

Fig. 4. Electrical impedance representative signals displayed as real – imaginary plot, with and without the compensation of the double layer effect.

There are different means and techniques today available, but the way towards a complete and effective rationalization of these laboratories is still long. Thanks to the impedance measurements and the electrical characterization of different components of blood samples, a further optimization of costs, resources and means in performing quality controls, especially on serum and blood in the pre-analytic phase, is proven to be feasible. Through static electrical impedance measurements and analysis, it is possible to carry out very fast and automated controls for every sample, thus reducing the risk of errors in laboratory processes and limiting the propagation of these errors from the laboratory to the clinic.

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