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Original article

New polypyrrole based bio-sensors for Bio-impedance measurement

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ABSTRACT

Objective: The main task of this work consists in an obtaining polarizable, dry mode, of bio-impedance sensors for non-invasive ECG monitoring, that work without any skin preparation or gel use, in two constructive models, based on conductive organic polymer polypyrrole and polypyrrole with Ag nanoparticles (NP), as sensitive materials. **Methods:** The polypyrrole was synthetized by chemical oxidative polymerization using $FeCl_3$ as oxidant agent. For sensors fabrication two technological variants have been chosen, a first variant realized by photolithographic method consisting in a substrate of Printed Circuit Board (PCB) with interdigitated copper electrode with step of 0.25 mm and over a distance of 12 mm and two pads. The polypyrrole or polypyrrole with Ag NP dissolved in ethyl alcohol was deposed on the substrate by dipping method. The second model consists in a polypyrrole powder pressed at a hydraulic press at 10 tones/cm^2 of force where one site was deposed a layer of Ag ink for conduction. **Results**: The performance of bio-impedance sensors were accessed by impedance skin-sensor interface with frequency in the range of 10 - 300 kHz measurements. The influences of technological fabrication as shape and geometry as well as the sensitive materials that used, in terms of impedance were analyzed. **Conclusions:** The introduction of Ag NP in polypyrrole, led to a better behavior, in terms of conduction and impedance response. For all tested sensors, the impedance decreases with the frequency with a good linearity.

KEYWORDS:Conductive polymers, polypyrrole based sensors, bio-impedance, bio-potential, non-polarizable electrodes, polarizable electrodes

INTRODUCTION

In modern medical applications, the desire for efficient biomedical systems along with the problem of increasing health costs has motivated the development of bio-potential electrodes [1,3], soft and hard electronics for wearable for health monitoring [4], smart textile materials electrode [5,6], flexible and stretchable electronics [7,8] and wireless wearable ECG sensor [9-12]. Among these, the bio-potential electrode, which is a transducer that converts ionic currents from the body surface into electric signals, can be identified as a critical component in the biomedical system [1]. The most commonly used bio-potential electrodes, for measuring electroencephalogram (EEG), electrocardiogram (ECG), electromyogram (EMG) or electrooculogram (EOG) signals, are standard wet silver/silver chloride (Ag/AgCl) electrodes as found in hospital based medical diagnostic and home health monitoring systems.

These electrodes typically require electrolytic conductive gel and skin preparation to reduce the skin electrode contact impedance [13]. Though the standard wet Ag/AgCl electrodes have low skin impedance with good signal stability and reproducibility, the use of a conductive gel is a significant drawback as it could dry out over time, thereby resulting in measurement errors due to increase in both contact resistance and motion artifacts. In addition, some patients could also develop skin irritation or allergic rashes [14,15]. These disadvantages have led several research groups to investigate dry ECG electrodes, as an alternative, by developing conductive materials [16,17] and using microelectromechanical systems (MEMS) based microelectrodes polymer conductor used in bio-impedance electrodes. In this work the authors present the bioimpedance electrodes sensors for ECG monitoring using two sensitive materials constituted from the conductive polymer polypirrole synthetized by chemical oxidative polymerization and polypirrole/Ag NP, electrodes type polarizable which function in dry regime. Too, frequency impedance measurements are also presented.

THE MEASUREMENT OF THE BIOELECTRICAL IMPEDANCE. A COMPARATIVE STUDY.

Bioelectrical impedance analysis (BIA) is a non-invasive method for component measuring of biological tissues and other biological samples. The BIA method is divided in principal in 2 methods in function of used electrodes number, each method presenting advantages and disadvantages. Too, in function of frequency analysis the BIA method uses a single and multiple frequency analysis. The data were presented in Table 1.

Table 1. A comparative bioelectrical impedance analysis study regarding method, advantages and disadvantages

Method	**Advantages**	**Disadvantages**
Two electrodes [18,19]	Overcome the interference that occur at interface between electrode and skin.	Two-electrode measurements method is used for single frequency applications based on detection of changes of impedance with time, e.g., impedance cardiography and respiration function monitoring. They are not in use for applications used to identify or assess tissue condition.
Four electrodes [18,20]	Measure impedance since the configuration of impedance measuring circuit is complicated in 4-electrode method. The tetrapolar arrangement eliminates the skin–electrode contact impedance and ensures that the measured impedance is essentially that of the underlying body tissues.	The four-electrode method cannot prevent the effect of stray capacitances.
Single frequency analysis Single-frequency analysis is the method of measuring the BI while applying single-frequency to the living tissues and the biological material [18,21].	Impedance can be measured in short period of time when characteristics of the living tissue and biological samples are analyzed in a particular frequency bandwidth.	BI cannot be analyzed in various frequency bandwidths.
Multi-frequency analysis is the method of measuring the impedance corresponding to a frequency range of a waveform, from low-frequency (LF) to high-frequency (HF) [18,21].	Characteristics of the living tissue and biological samples can be analyzed in various frequency bandwidth.	Measurement time is long in comparison with the single-frequency method and the measurement circuit is complicated.

TYPE OF THE ELECTRODES. A COMPARATIVE STUDY

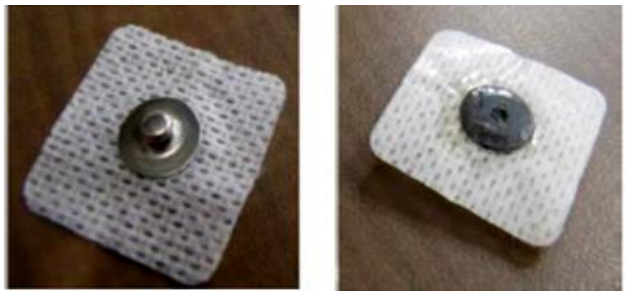
The main problem of conducting bio-signal measurement at home is a choice of an appropriate biopotential electrode that can last long time and need minimal preparation work for recording bio-signal measurements [21]. For bioimpedance analysis (BIA) the electrodes plays a great importance and the researches consist in: skin electrode impedance, shape and geometry [22], surface area, material and technological fabrication , type of gel dry or wet, the placement in body and inter electrode distance [23]. In the ECG measurements the most electrodes types used are:

Non-polarizable electrodes

Non-polarizable electrodes permit the charges to pass through the electrode-skin interface without hindrance [24]. In non-polarizable electrodes, reduction/oxidation reactions occur at the

electrode-skin interface, exchanging charge carriers from ions to electrons and vice versa. At non-polarizable electrodes reactions are electrochemically reversible.

Non-polarizable electrodes as Ag/AgCl are considered the universal electrodes in clinical measurements (e.g., ECG, EMG and EEG). They are associated with low electrode-skin impedance, low noise and low motion artifact. Ag-AgCl electrodes are now used in most bioimpedance measurements because it has a well-defined DC potential with electrolyte gel to minimize the gap impedance between skin and electrodes, Figure 1. Circular and rectangular electrode shapes with a contact area greater than 4 cm^2 are the most commonly used shape [25, 26].



(a) (b)

Figure 1. (a) Ag/AgCl electrode (electrode's snap side), (b)Ag/AgCl electrode (electrode's skin side) [25].

The electrolyte gel is used with non-polarizable electrodes to facilitate the electrochemical reactions and to reduce electrode-skin interface impedance. Another type electrode is orbital electrode Figure 2. Dry polarizable orbital electrodes are made to last longer than the common clinical wet electrodes such as Ag/AgCl. An orbital electrode's coat is made of a mixture of metals: silver/silver chloride, aluminum, gold/gold chloride, nickel and titanium. Advantages and disadvantages of these electrodes:

1. They are adhesive, thus can easily be fixed on the skin, preventing motion artifacts;
2. They show very clean and reliable ECG signals;
3. The adhesive part and the gel inside them can cause skin irritation and contact skin dermatitis if they are in use for long time;
4. They normally require skin preparation in advance, such as shaving, using alcohol to clean the contact area and even using sand paper to remove the dead layer of the skin;
5. Technically, the most significant drawback of nonpolarizable ECG electrodes is that the signal they detect will degrade when the gel inside them dries out.

These electrodes are not appropriate for long-term use and are considered disposable electrodes. It is inconvenient to replace electrodes, making them problematic for the users in home healthcare and telemedicine.

Polarizable electrodes

In contrast to non-polarizable electrodes, in polarizable electrodes no actual electrical current flows. Instead, a displacement current occurs as a result of a change in ionic concentration in the electrode–skin interface. These electrodes work based on the capacitive coupling between a conductive material and skin. They do not need any kind of gel; instead, they operate by moisture and sweat on the skin. Dry electrodes vary from simple stainless steel plates to new flexible textiles capable of conducting electrical potential. Dry electrodes can be categorized as contact or noncontact [24,25].

Another type of polarizable electrode is orbital electrode. Dry polarizable orbital electrodes are made to last longer than the common clinical wet electrodes such as Ag/AgCl. An orbital electrode's consists in a mixture of metals: silver/silver chloride, aluminum, gold/gold chloride nickel and titanium. Also, stainless steel electrodes represent polarizable electrodes.

(a) Orbital electrode - electrode's skin side. (b) Orbital electrode - electrode's snap side.

(c) Stainless steel electrode - electrode's skin side. (d) Stainless steel electrode - electrode's snap side.

Figure 2. Polarizable electrodes; (a,b) orbital electrode types and (c,d) stainless steel electrode types [25].

They are the most common polarizable electrodes used in modern wireless sensor technologies for monitoring biological signals (e.g., chairs, shirts). In Figure 2 are presented a structures for these type of electrodes [25].

TYPE OF THE ELECTRODES THAT WAS MADE BY THE MICROELECTROMECHANICAL SYSTEMS (MEMS) TECHNIQUE. WET ELECTRODES-GEL THE WET ELECTRODES REQUIRE SKIN PREPARATION. DRY ELECTRODES.

The most dry electrodes were made by the microelectromechanical systems (MEMS) technology [24]. Figure 3 shows a conductive polymer electrodes containing ~50% of carbon and having 2 mm pin length and 10 mm base size were used for ECG recordings. To connect the polymer dry electrode to the recording system and to place the dry electrode on chest, a conventional wet gel ECG electrode type (Meditrace 200 Series ECG Electrodes, Kendall) was taken, and the gel was removed leaving only the snap and the skin adhesive part as attachment for the dry electrode. The backside of the dry electrode was attached to the snap using copper double sided tape as shown in Figure 3 (a). In order to correctly compare the signal of a dry electrode with that of a wet electrode, two conventional wet electrodes were placed next to two dry electrodes to obtain two very similar ECG signal, see Figure 3 (b). Two such pairs of wet-dry electrodes were placed on the subject's chest and combined with one dry electrode as bias signal at the chest locations shown in Figure 3 (c) [27].

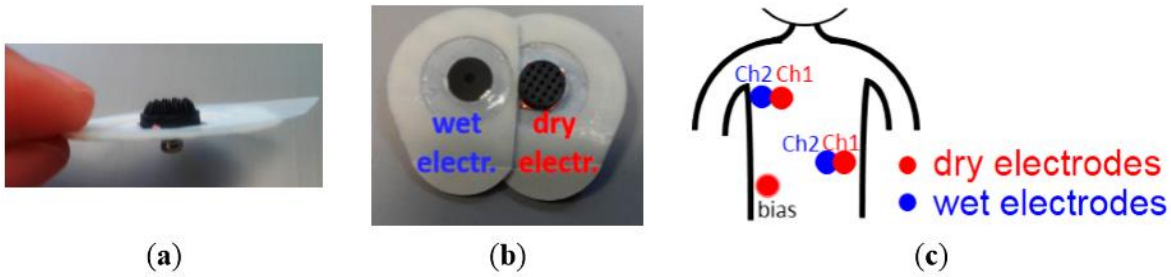
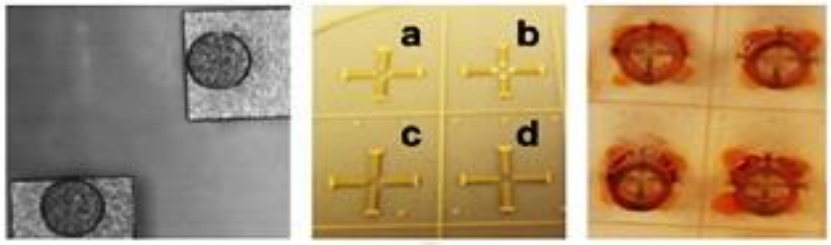


Figure 3. (a) Conductive polymer dry electrode connects with gel removed wet electrode; (b) a pair of wet and dry electrode; (c) electrode locations for ECG measurements [27].

In recent years the microelectrodes fabricated by microelectromechanical MEMS technology are very used and by great interest. Figure 4 shows the 4 microelectrodes realized by Abdur Rub Abdur Rahman et. al. [22] by photolithographic technology using a glass substrate by deposing first a chromium layer by 250 Å and a gold layer in 1400 Å thickness. The sensor diameters are: 500 µm, 250 µm, 100 µm and 50 µm. After device fabrication, a cloning cylinder of 3.4 mm internal diameter was attached to microelectrode device [22].



(a) (b) (c)

Figure 4. Optic image of a microelectrode device with 2 lithographically defined electrode sensors; (a,b) photograph of four gold plated microelectrode devices on a glass wafer; (c) photograph of electrodes with cloning cylinders attached using photoresist as adhesive [22].

E-skin electrode are realized by photolithographic method using a Si wafer where was deposited conductive layers composed from multiwall carbon nanotube MWCNT and conductive polymer polydimethylsiloxane PDMS. This sensor detects the most receptors of human skin as temperature, force, pressure and visualizes the distribution of sensations. The role of MWCNTs is to form a conductive network, and the change of resistivity of the network was used to sense various stimuli. Figure 5 shows the process fabrication composed by the 5 stages, referred to below A-E:

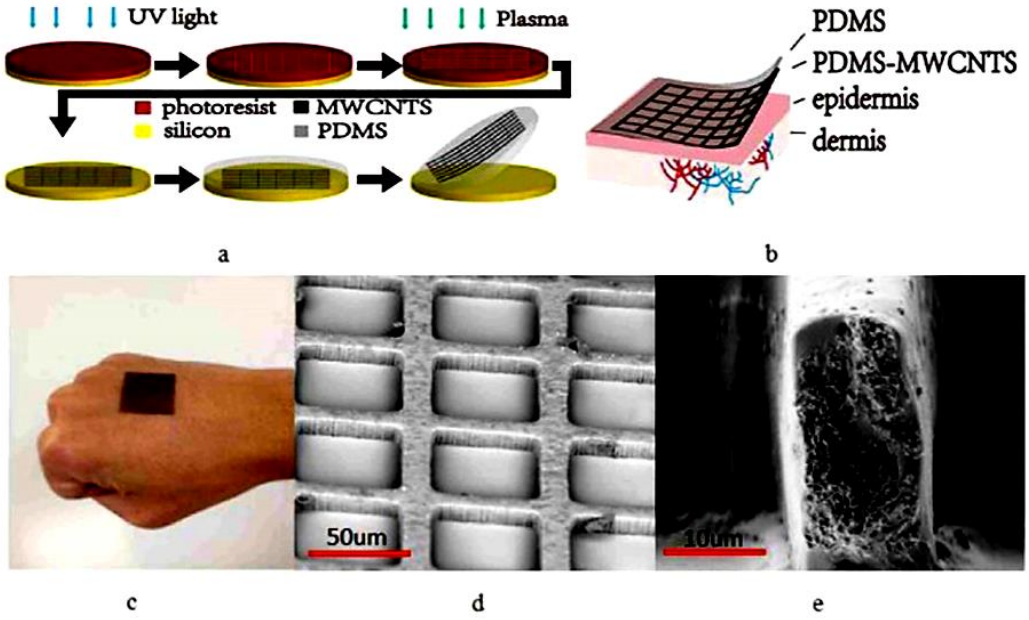
A. Crisscross network channels patterned by photolithographic process using positive photoresist on a 4-inch silicon substrate with the size of 4 × 4 cm^2. It was then etched by deep reactive ion etch to form the crisscross network channels of 50 μm deep and 10 μm wide. The Si wafer was used as the mould to fabricate the sensor;

B. The network channels of the silicon mould were filled with MWCNTs powder with shake, and then the excessive CNTs above the surface of the Si wafer were removed. The weight of the MWCNTs was ca. 8.5 mg (± 10%) after the excessive nanotubes were removed;

C. The silicon mould with MWCNTs deposed in the channels was filled with the degassed prepolymer PDMS (in ratio of 10:1 for the base to the crosslinker by mass);

D. The PDMS composite on the Si mold was solidified in oven at 90 °C for 30 min;

E. The PDMS E-skin was peeled off the silicon wafer, ready for use. The cross section of the PDMS/MWCNTs composite lines is 50 × 10 μm^2, while the thickness of the top PDMS support layer is 250 μm. Figure 5 (b,c) represents the microplates sensor. Figure 5 (d), shows the SEM images, showing conductive crisscross network and cross section of conductive line. The sensor is characterized by a robust structure, is flexible and biocompatible with human body [28].



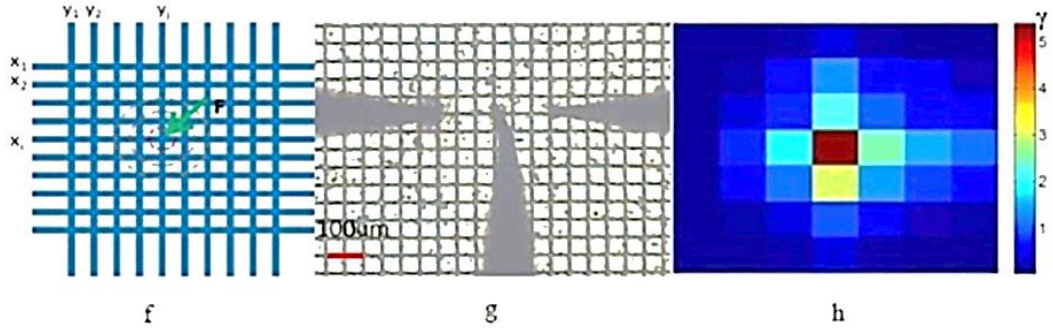


Figure 5. (a) fabrication process for the E-skin; (b) a schematic diagram of the skin-like sensor; (c) a photo of the fabricated sensor on a hand; (d,e) SEM images of the PDMS/MWCNT's networks and the cross section of a conductive line; (f) a measurement scheme used to map the variation of resistance; (g) a photo of the sensor under test using three probes. The mid probe was used to press the conductive line, while the other two were used to measure the resistance change and (h) is the reproduced color mapping of resistance change, hence the deformation induced by the probe tip. The color of the unit is shown in the scale bar on the right of the color map, red color presents the larger change of the resistance, the resistance change in the blue area is smaller [28].

MATERIALS AND METHODS

The sensitive materials for new polypyrrole based bio-sensors fabrication

For sensitive materials were used: pyrrole monomer was purchased from Merck with 97% purity and $FeCl_3x6H_2O$ was purchased from Alpha Aesar with purity grade, pa, ethyl alcohol purity pa was purchased from CHIMOPAR ROMANIA. The synthesis of polypyrrole was made using the chemical oxidative polymerization using $FeCl_3$ as oxidizing agent with a molar ratio of 1/3. The synthesis was carried out at room temperature in an aqueous solution. The black precipitate formed was spun and dried at 50 °C for 3 days.The synthesis of pyrrole matrix composite and Ag nanoparticles was carried out under the same conditions as the polypyrrole synthesis described above, with the modification as in the second synthesis in the initial solution silver nanoparticles were introduced so that it was 10% of the mass of pyrrole. The formed precipitate was spun and dried at 50 °C for 3 days. The Ag nanoparticles was obtained from a 10 mM silver nitrate solution that was precipitated with a hydrazine solution having a molar ratio of nitrate/hydrazine of 1/3 to a temperature of 80 °C.

The new polypyrrole based bio-sensors fabrication

Three bio-sensors S1, S2 and S3 with capabilities to measure the bio-impedance, which will be referred to below such as the bio-impedance sensors, were manufactured in the following way:

S1. On a PCB, an interdigital grid is formed consisting of eight pairs of Cu electrodes with a constant interdigital step of 0.25 mm over a distance of 12 mm and two pads. Then, a layer of polypyrrole/Ag organic polymer-sensitive solution of ethyl alcohol was deposited on the substrate of the interdigital grid followed by drying in the oven at 60 °C for 24 hours. After drying, the thickness of the polymeric sensitive layer is 400 nm.

S2. On the PCB (printed circuit board) substrate it is realizing an interdigitated array electrode from copper by photolitographic method consisting of eight pairs of Cu electrodes with a constant interdigital step of 0.25 mm over a distance of 12 mm and two pads. Then, a layer of organic polymer sensitive polypyrrole material, a solution in ethyl alcohol, was deposed on the substrate with electrode by diping method, followed by drying in the oven at 60 °C for 24 hours. After drying, the thickness of the polymeric sensitive layer is 400 nm.

S3. For the third sensor, the polypyrrole powder was pressed to a hydraulic press at 10 tones/cm^2 of force in discs form with dimensions:

- Disc 1: 12.2 mm diameter, 2.1 mm thick;
- Disc 2: Diameter 12.3 mm, 1.8 thick.

On one face of the discs was deposited the silver ink as an electrode. For each of the three sensors, copper wire microwires was attached for bindings, so: for sensors S1 and S2 the pads were joined, thus creating a common potential area and gluing a copper microwires onto one of the forests; for the S3 sensor, a copper microfiber insert is placed on the face of the disc onto which the silver ink was applied. The images of the bio-impedance sensors S1, S2, S3 is showed in Figures 6,7,8, together with their frequency response.

RESULTS

The bio-impedance measurements were effected by two points method and performed using Agilent 4980 A (USA) at room temperature 25 ºC in following experimental conditions: tension: 2V, distance between sensors are 6 cm, placement of the bio-impedance sensors on the left arm, on a human subject a woman aged 60 years old. The characteristics of the dry polarizable S1, S2 and S3 ECG bio-impedance sensors have been investigated by measuring skin-sensor interface bio-impedance with frequency, without the use of gel and no skin preparation. The bio-impedance measurements were effected by attaching of the sensors on left arm with a medical adhesive tape for a constant pressure contact, in the frequency range of 10 - 300 kHz and signal stabilization time 1 minute.

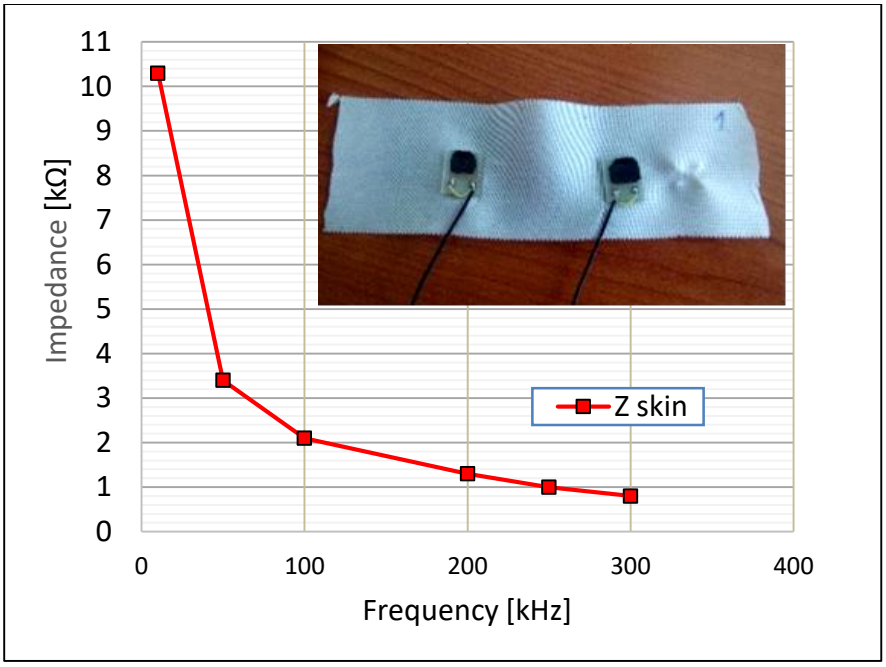


Figure 6. Skin interface bio-impedance response with frequency, for dry polarizable bio-impedance sensors S1 (PCB substrate with polypyrrole/Ag NP).

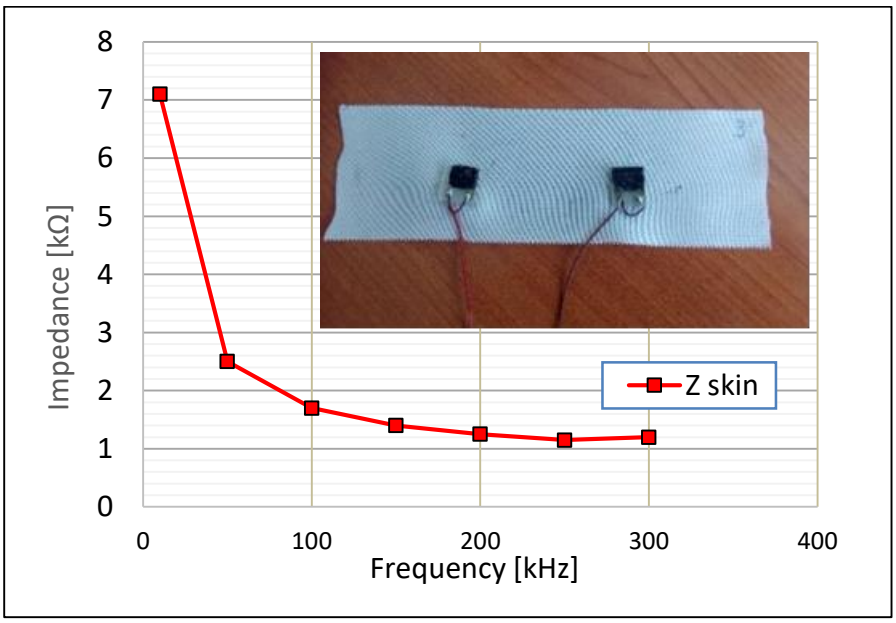


Figure 7. Skin interface bio-impedance response with frequency, for dry polarizable bio-impedance sensors S2 (PCB substrate with polypyrrole).

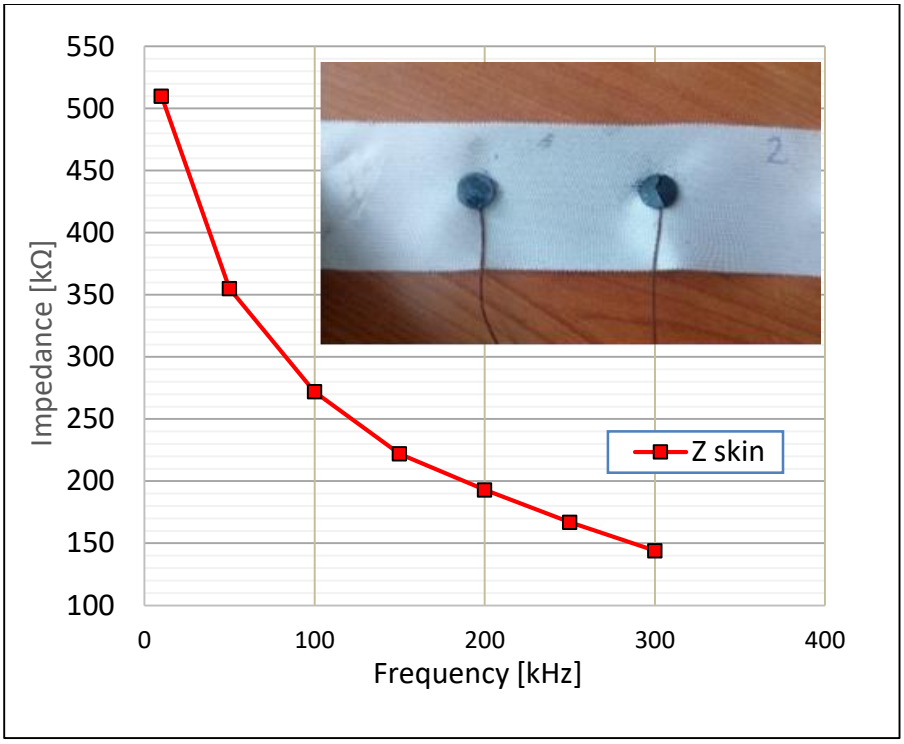


Figure 8. Skin interface bio-impedance response with frequency, for dry polarizable bio-impedance sensors S3 (disc with polypyrrole).

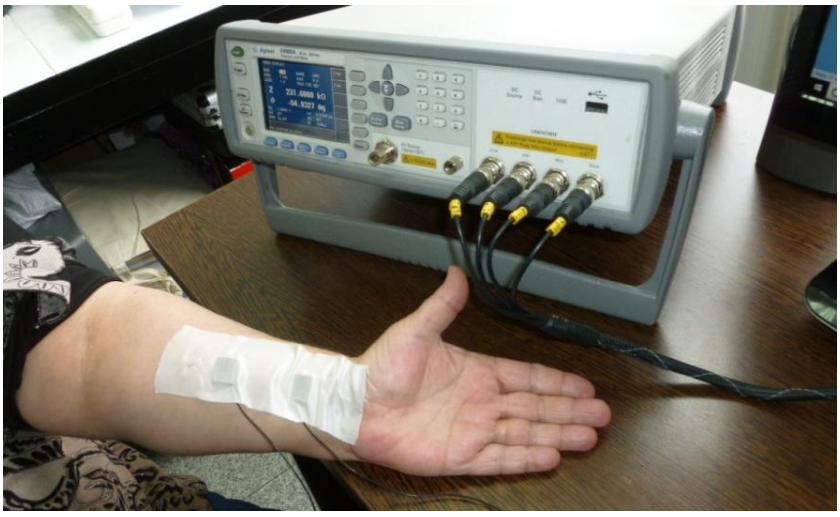


Figure 9. Test bench for measurements of the bio-impedance.

Figure 9 shows the image of the equipment that used for bio-impedance measurements.

DISCUSSION

For all tested dry polarizable bio-impedance sensors, the bio-impedance decreases with frequency with a good linearity in case of bio-impedance sensors S1 and S2 made on PCB substrate; the steep decreases of the bio-impedance being in the range of 10 - 300 kHz with values from 10.4 kΩ for 10 kHz to 858 Ω for 300 kHz for bio-impedance sensors S1, from 7.2 kΩ for 10 kHz to 1.12 kΩ for 300 kHz for bio-impedance sensors S2 and from 510 kΩ for 10 kHz to 144 kΩ for 300 kHz for bio-impedance sensors S3. It is noted that the introduction of Ag NP in polypyrrole, led to a better conduction and bio-impedance response and the bio-sensors made on PCB substrate show a better bio-impedance response against disc form bio-sensor. Also, the fabrication technology, the sensitive materials, the shape and geometry are having a real influence about the function characteristics of bio-impedance sensors.

CONCLUSION

Three of bio-impedance sensors polarizable with dry mode function S1, S2, S3 for ECG analysis were realized: S1 and S2 were realized on a PCB substrate with an interdigital grid consisting of eight pairs of Cu electrodes with a constant interdigital step of 0.25 mm over a distance of 12 mm and

two pads. The sensitive materials consist in a polypyrrole and polypyrrole with Ag nanoparticles. The polypyrrole was syntetized by chemical oxidative polymerization method using $FeCl_3$ oxidant agent in aqueous medium in molar ratio 1/3 at room temperature, the black precipitate formed was spun and dried at 50 °C for 3 days. For the polypyrrole with Ag NP, in the initial solution for polypyrrole synthesis, silver nanoparticles were introduced so that it was 10% of the mass of pyrrole. The formed precipitate was spun and dried at 50 °C for 3 days. The Ag nanoparticles was obtained from silver from a 10 mM silver nitrate solution that was precipitated with a hydrazine solution having a molar ratio of nitrate/hydrazine of 1/3 to a temperature of 80 °C. The bio-impedance sensors S1 has sensitive material polypyrrole with Ag NP, bio-impedance sensors S2 has sensitive material polypyrrole both sensitive material in 400 nm thickness.

The bio-impedance sensors S3 was realized from polypyrrole powder pressed at a hydraulic press at 10 tones/cm^2 of force where one site was deposed Ag ink for contact. The dimensions for S1 are: disc 1 diameter 12.2 mm and 2.1 mm thick also disc 2 diameter 12.3 mm and 1.8 thick. At each bio-sensor was atatched the metal wire for electric contact.

The bio-impedance measurements of sensors were effected by 2 points method.

For all tested bio-sensors, the impedance decreses with frequency with a good linearity in case of bio-impedance sensors S1 and S2 the steep decreases of the bio-impedance being in the range of 10 - 300 kHz with values from 10.4 kΩ for 10 kHz to 858 Ω for 300 kHz for bio-sensor S1, from 7.2 kΩ for 10 kHz to 1.12 kΩ for 300 kHz for bio-sensor S2 and from 510 kΩ for 10 kHz to 144 kΩ for 300 kHz for bio-sensor S3. The introduction of Ag NP in polypyrrole, led to a better conduction and bio-impedance response and the bio-sensors made on PCB substrate show a better bio-impedance response against disc form bio-sensor.

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Competing interest: The authors declare that they have no competing interests.

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