

IMPEDANCE ANALYZER FOR LOCAL MEASUREMENTS OF ELECTRICAL CONDUCTIVITY IN BIOLOGICAL OBJECTS

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Abstract – The subject of the publication is a portable device for measurements of impedance magnitude in the range of $25 \Omega \div 2.5 \text{ k}\Omega$, with the phase angle of $-90 \div 0^\circ$, within the frequency band of $100 \text{ Hz} \div 100 \text{ kHz}$. Small measuring current, $10 \div 100 \mu\text{A}$, allows for the use of miniature sensors for the measurement of sample with volume as small as several mm^3 . The device was designed specifically for local measurements of electrical conductivity in biological objects.

Keywords: electrical conductivity, local measurement, properties of biological tissue

1. INTRODUCTION

Electrical conductivity of biological objects is related to their micro-structural and physico-chemical properties. Electrical conductivity measurements can be applied in determining the type and assessing the condition of tissues and organs. Biological objects are usually heterogeneous and the influence of properties of particular parts of the object on the measurement results is not identical. The results of such measurements are difficult to interpret if they reflect the properties of the object averaged in too large measurement area [1,2]. If this area is sufficiently small, then heterogeneous object can be treated as locally homogeneous. In such conditions it is called local measurement and its example is conductivity measurement of objects with very small volume. The main problem in this case is to determine the appropriate size of the object sample. This is particularly important in measurements of biological objects, where the volume of an available object sample is usually limited. Local measurements also allow to considerably reduce the invasiveness of the *in vitro* examination.

2. METROLOGICAL CHALLENGES IN LOCAL CONDUCTIVITY MEASUREMENTS

Carrying out local measurements is a difficult issue. Reduction of the sample volume in the area where the measurement is being made can be achieved through the use of specially designed electrode arrangement and/or through sensor miniaturization. Most studies on conductivity sensors used in local measurements focus on technological aspects connected with fabrication of miniature sensors, whereas metrological problems caused by miniaturisation are often ignored or omitted.

Miniaturisation of the sensor reduces electrodes surface area and the spaces between electrodes. This process leads to a considerable increase in impedance value of electrode polarization combined with reduced measured impedance of the object (measurand) and thus is raising the requirements for the measuring circuit compared to averaging measurements performed in large volumes [3,4]. The measurement must give correct results in the case when impedance value of polarization is many times higher than the measured impedance of the object itself [5]. For this reason, in local conductivity measurements a four-electrode measurement of impedance is used, which effectively limits the polarization error value. Very high values of polarization impedance, together with high density of the current near the surface of miniature electrodes pose a threat of local heating of the object, which may lead to changing its properties, or even cause damage. In order to avoid this, in local measurements the amount of energy delivered to the object should be limited, i.e. the current flowing through the object should be reduced to the value ranging from a few to about a hundred μA . With such small values of current flowing through the object the signal received by means of voltage electrodes is weak, often nearing the level of noise occurring in the measuring circuit, which is an additional complication. In measurements of biological objects it is often very important to be able to conduct impedance measurements, including the modulus and the phase angle, in a suitably wide frequency band. The measuring circuit should therefore work as a wide-band impedance analyzer.

3. IMPEDANCE ANALYSER ASSUMPTIONS

The realization of the circuit for measurements of local conductivity in biological objects is a difficult task. Majority of commercially available instruments designed for wide-band impedance measurements cannot be directly used in local conductivity measurements of biological objects [6,7]. It is usually necessary to use additional auxiliary circuits which make it possible to perform measurements with reduced current values. This can be sometimes impossible or at least unsatisfactory due to the high cost of the device and its adaptation to the new tasks, too complicated handling, or its oversize. Moreover, in the case of *in vivo* examinations most of the available instruments do not meet safety requirements and require the use of still other, additional auxiliary protective circuits. Also, in examinations of biological objects it is often necessary for the measuring set

to be portable. Considering the above-mentioned requirements the proposed aims and the problems the authors came to the conclusion that it would be best to propose an own dedicated measuring device designed especially for local conductivity measurements in biological objects. Such device, together with its software, has been designed and fabricated by the authors and described in this article.

The instrument was intended to be used with four-electrode planar sensors designed by the authors for local conductivity measurements - fig. 1. Sensors are made with the use of the printed circuit manufacturing method and the electrodes and their connectors are gold-coated. These sensors may be used in both invasive and non-invasive measurements. Sensitivity maps of selected electrode settings and their other properties which are important from the point of view of local measurements were presented and analysed in a separate article [8]

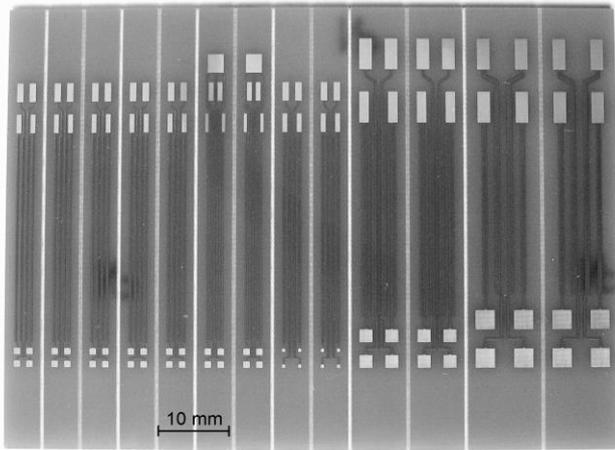


Fig. 1. Four-electrode planar sensors designed for the use in local conductivity measurements [8].

It has been assumed that the device is designed for measurements performed in two modes of operation: autonomous and controlled by an external computer via a user interface realizing a virtual instrument. In the second mode one can also define frequency spectrum of impedance in a chosen frequency range.

4. IMPEDANCE ANALYZER

The main element of the measuring system in the impedance analyzer is a 12-bit impedance converter AD5933. The structure of the analyzer is presented in fig. 2, internal parts of the converter AD5933 are marked in a dashed line. Impedance converter AD5933 is used for generating AC signals of required frequency, A/C and C/A conversions and performing operations necessary for calculating Discrete Fourier Transform (DFT). Impedance converter contains DDS (Direct Digital Synthesis) module which enables generation of sinusoidal voltages with the frequency range 10 kHz – 100 kHz. Because the project of the measuring system assumes measurements of impedance spectrum performed in the frequency band starting from

100 Hz and also in the four-electrode configuration (AD5933 was designed for 2 terminal measurements), it proved necessary to extend the basic application of AD5933.

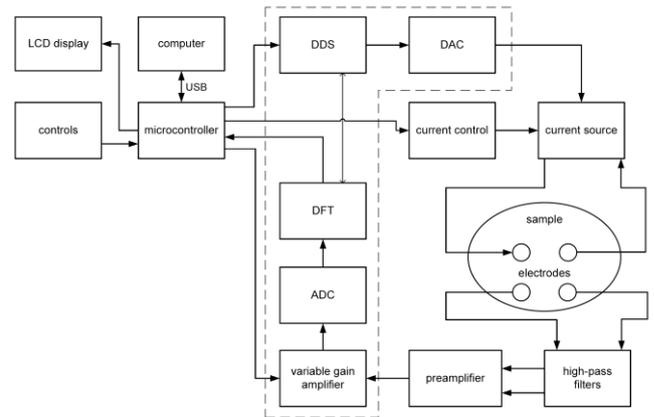


Fig. 2. Block diagram of an impedance analyzer.

External timing signal generated by programmable clock controlled by microcontroller has been applied to allow lower frequency measurements. The microcontroller used was Atmel ATmega128 from AVR family. According to project assumptions impedance measurement will be carried out using four-electrode method, with the supply current ranging from 10 μ A to 100 μ A. AD5933 converter creates sinusoidal AC voltage with constant amplitude. To meet project assumptions a voltage-current converter was used. The output of this converter is connected to current electrodes of the sensor by means of a 1 k Ω serial resistor functioning as an additional passive protection, often required in in vivo measurements.

The voltage drop across the voltage electrodes of the sensor is delivered via high-pass filter to a preamplifier with constant gain value. The second stage of amplification is an internal amplifier of the impedance converter AD5933. Total amplification of the signal can be set to 20 or 100 V/V. After collecting a series of samples and completing DFT calculations the converter stores in its registers the result's components: the real (R) and the imaginary one (I), from which microcontroller computes the magnitude $|Z_p|$:

$$|Z_p| = \sqrt{R^2 + I^2} \quad (1)$$

From $|Z_p|$ the value of the magnitude $|Z|$ of the measured impedance can be calculated, if the gain coefficient G is taken into account:

$$|Z| = \frac{1}{|Z_p|G} \quad (2)$$

The value of G coefficient depends on frequency at which measurement is performed. In order to increase the accuracy of impedance measurement, calibration and G_f coefficient calculation should be done at each measurement frequency. Measurement can be performed at any frequency

between 100 Hz ÷ 100 kHz. Therefore, it is impossible to set the G value for each measurement frequency *a priori*. In such cases the application note of AD5933 converter suggests determining the G gain coefficient for a set of frequencies f_1, f_2, \dots, f_n . If this list is sufficiently long, G_f for any frequency can be calculated by means of interpolation. Calibration is done by measuring impedance of the resistor with a known resistance value. During the measurement additional procedures are carried out which average and check repeatability of the obtained measurement results.

The measuring system consists of two basic modules – fig. 3. The first one contains microcontroller and elements of the user interface, and the other – impedance converter, current source, measuring amplifier and sensor sockets.

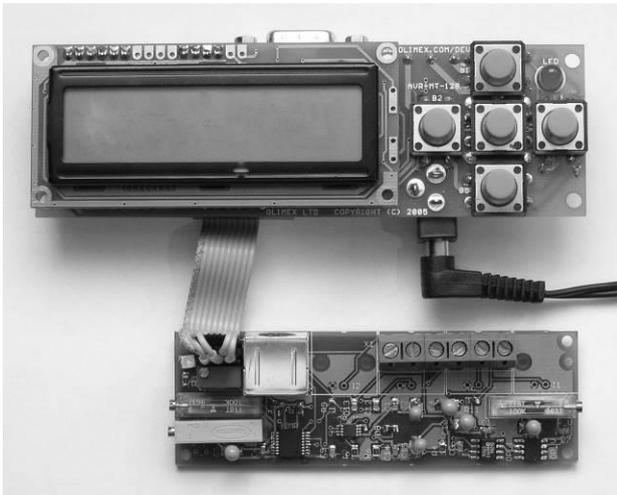


Fig. 3. The performed measuring system (micro-controller module at the top, the measuring module at the bottom).

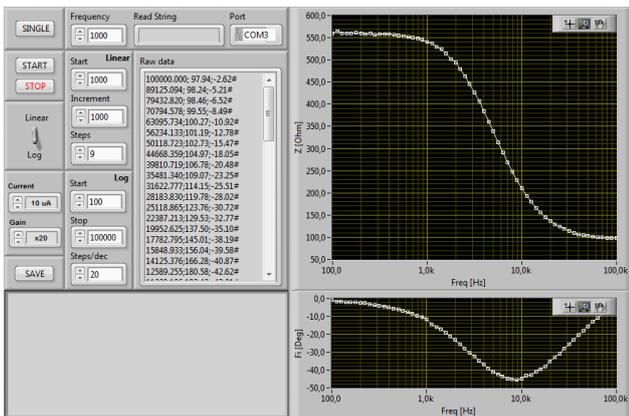


Fig. 4. Application panel managing the measurement process.

An additional small module was also fabricated and is responsible for powering and communication. Thanks to it the entire device can be powered and communicate with the computer by means of USB interface. In the case of battery powering, galvanic isolation of the data bus is used. Due to strong influence of temperature on the results of conductivity measurement, the measuring circuit was equipped with an external digital thermometer DS1820 which allows to measure temperature of the object. The

influence of temperature on measurement results may be corrected by the software of the microcontroller.

In measurements of impedance frequency spectrum the measuring system needs to be connected to a computer. Then the device communicates with the application program prepared in LabView environment, and creates a virtual impedance analyzer. Its control panel is presented in fig. 4.

5. EXPERIMENTAL EXAMNATIONS OF IMPEDANCE ANALYZER

A series of experiments has been carried out in order to verify the correctness of the design and determine the obtained parameters of the analyzer. The stability of results in frequency domain was evaluated using thin-film metal resistors. Within the frequency range from 100 Hz to 100 kHz the measured resistance values of these resistors can be expected to remain unchanged. Examples of results are shown in figure 5, where the measurement was conducted using the resistor with the value of $(476 \pm 2) \Omega$.

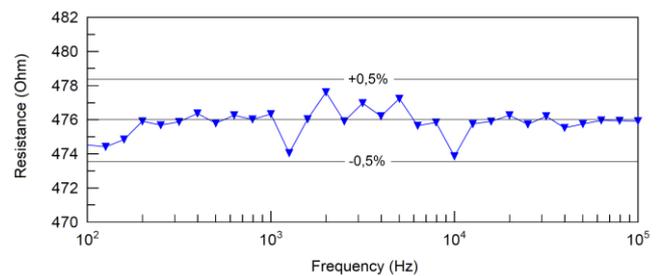


Fig. 5. Results of the measurements of the real part of impedance (resistance) for frequencies between 100 Hz – 100 kHz.

The correctness of electrolytic conductivity measurements was verified in full frequency range with the use of four-electrode planar sensor and reference KCl solutions with concentrations from 0.01 mol/l to 0.1 mol/l. The results are presented in figure 6.

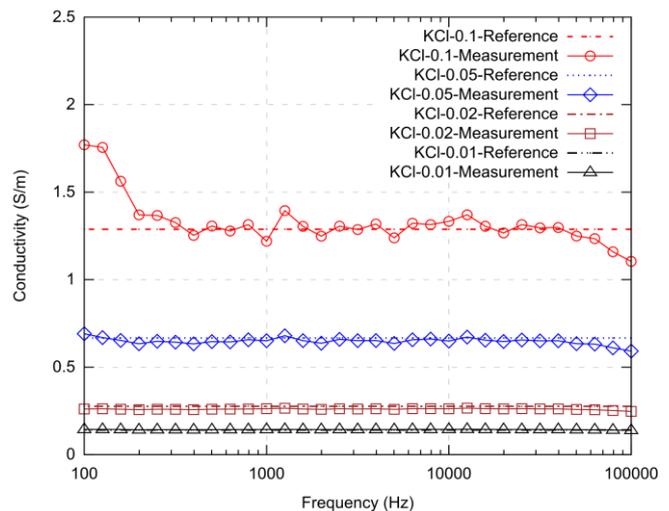


Fig. 6. Results of conductivity measurements of reference KCl solutions.

The tolerance against large values of electrode impedance was also evaluated. Figure 7 presents four-electrode measurement results of an object with the resistance of $(216 \pm 2) \Omega$. Electrode impedances were modelled by resistors of altered resistance values. The measurements were performed in three versions listed in the figure. For frequencies up to 20 kHz in all cases the results are slightly overstated (the measurement error does not exceed 2%). At highest frequencies (over 20 kHz) and larger electrode resistance values an additional error appears (can be caused by capacitances in the sensor wiring).

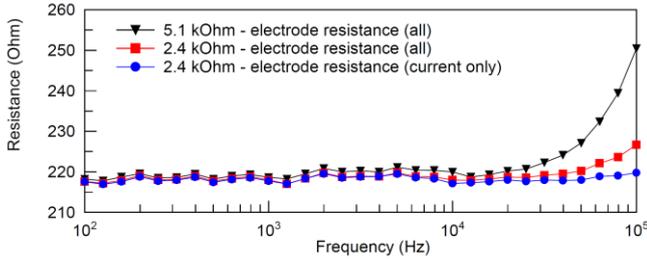


Fig. 7. Results of four-electrode measurements taking into account electrode resistances.

In order to verify the possibilities of the measurement of complex impedance values a two-terminal RC network consisting of three elements was analyzed. The diagram of this network, measurement results and fit curve of an equivalent two-terminal network are presented in figure 8.

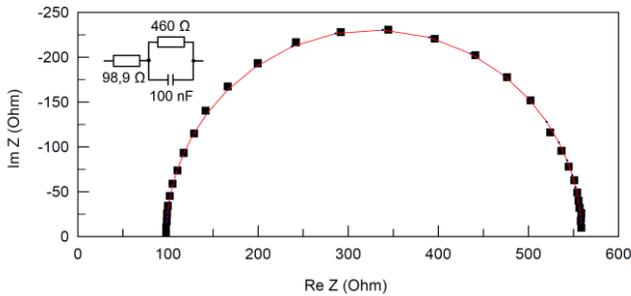


Fig. 8. Results of measurements of two-terminal impedance and the fit curve for its equivalent circuit.

The fabricated measurement circuit, its software as well as the conductivity sensors were used for performing conductivity measurements in biological objects.

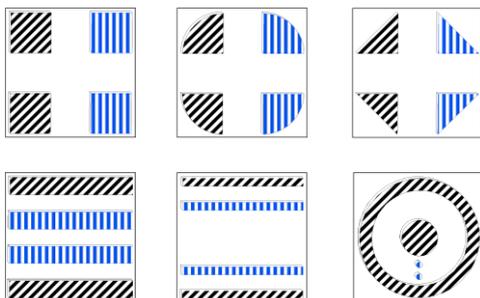


Fig. 9. Electrode setting for local conductivity measurements (current electrodes are marked in a diagonal line).

In the former stage of the project, four-electrode planar sensors dedicated to local conductivity measurements were designed and fabricated [8]. A few versions have been designed, varying in arrangement, shape and size of the electrodes. Examples of such electrode settings are shown in figure 9.

The measurements started with checking repeatability of the measurement results. The measured object was an apple cut in half. A sensor with concentric electrodes, and the external radius of the current electrode of 3mm (as in the last electrode configuration from figure 9) was placed on the cut surface of the apple. The results were presented in figure 10.

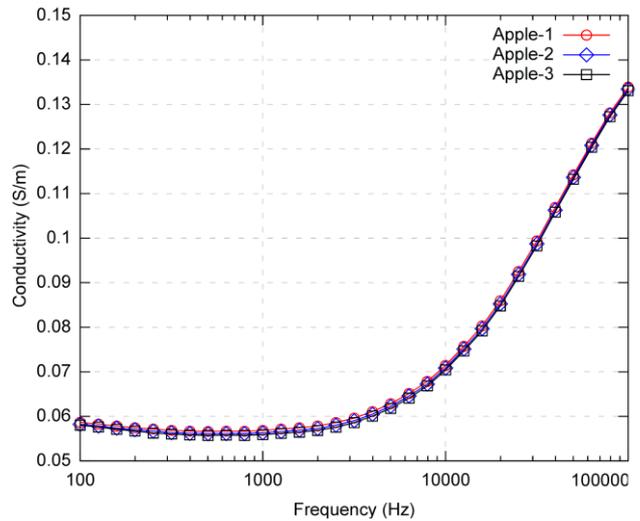


Fig. 10. Conductivity measurement of an apple - measurement repeatability.

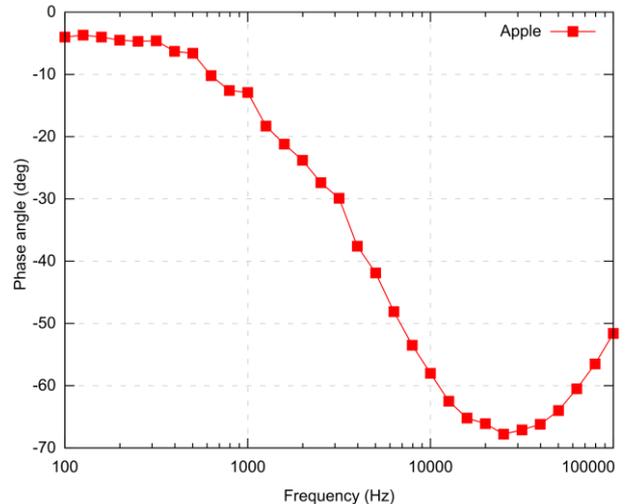


Fig. 11. Phase angle shift – an apple.

The measurement marked “1” had been performed beforehand, and the subsequent measurements were performed at 2 minutes’ intervals. It is worth noting that the later the measurement was carried out, the lower conductivity value obtained. The difference in conductivity values between subsequent measurements is about 1% at

lower frequencies and about 0.5% at higher frequencies. The probable reason for the reduction in conductivity values over time is drying out of the object's surface. The duration of a single measurement was about 60s. At frequencies higher than 1 kHz conductivity is increasing. This is characteristic of biological objects and is connected with current permeating through cell membranes [3, 9]. Changes in conductivity in this respect correspond to changes in the phase angle shift, which is illustrated in figure 11.

In order to prove that the method allows to perform local measurements in heterogeneous objects, conductivity measurements were carried out on carrot. Carrot contains two types of tissues – the central cortex and the outer stele. The radius of the cortex of the carrot under examination was 1 cm, while the outer radius was 1.5 cm. This means that the stele was only 5 mm thick. Due to the size of the stele concentrically arranged electrodes were used (as in the apple measurement). The choice of geometrical arrangement of the sensor electrodes results from the simulation of measurements of heterogeneous objects described in previous publications of the authors [8]. This publication proves that the chosen electrode configuration allows for measurements of 5 mm objects without the need for corrections resulting from object's geometry and electrical properties of its environment. Conductivity measurements of the carrot were conducted in two areas – the cortex and the central stele. Measurement series were carried out at 5 minute intervals. The second series of results enable to assess the change in conductivity values taking place between the measurements and is caused by the drying of the damaged (cut) tissue. The obtained results are presented in figure 12.

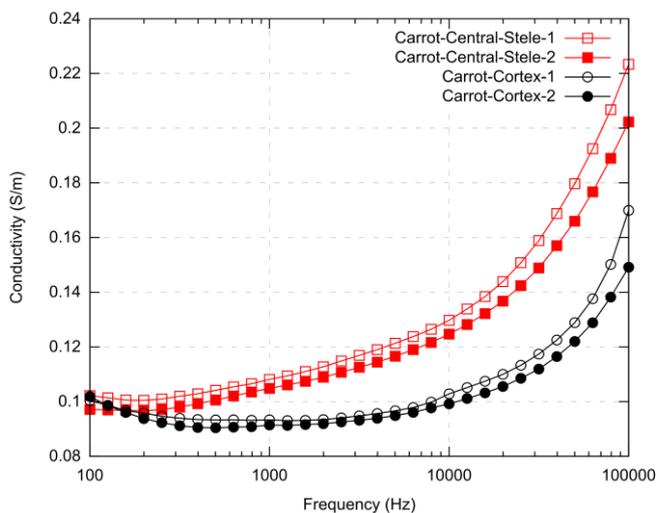


Fig. 12. Results of a carrot conductivity measurements with the use of four-electrode method.

6. FUTURE IMPROVEMENTS

The measuring module presented in figure 3 (the lower board) can be manufactured in considerably smaller sizes. At present, nearly $\frac{3}{4}$ of the surface of the printed board is occupied by connectors and potentiometers. The connectors can be removed from the board altogether, and

potentiometers, used for setting parameters of the current source and amplification in the final realization of the module may be replaced by resistors. The measuring module can therefore be reduced to the size of about 1,5 x 6 cm, which would allow to manufacture the measuring circuit in a new form. Instead of placing the microcontroller and the measuring circuit in one bigger casing, as it was done in this project, one can place the measuring module together with exchangeable probe in a small separate casing in the shape of a pen. This solution would definitely prove to be more convenient and would reduce the undesirable influence of capacitance of the wires connecting the sensor with the measuring circuit.

7. SUMMARY

A measuring system providing the functions of impedance analyzer designed to be compatible with miniature conductivity sensors is presented in the article. The system is powered by a battery, or a USB. It is really portable – small enough to be held in one hand (e.g. it may be a handle of a sensor). For biomedical applications a wider range of measuring frequency available in the impedance analyser would be useful, however, application of impedance converter AD5933 adapted for four-electrode impedance measurement limits upper measuring frequency to 100 kHz. Thanks to application of a commercially available chip the device is very small and also inexpensive.

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