

IMPEDANCE SPECTROMETER FOR APPLICATION IN BIOLOGY AND FOOD QUALITY CONTROL

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Abstract – The electrical impedance spectroscopy is widely used in material science, because this quantity and its frequency spectrum are important features of any material. This method became popular in measurement of biological and biochemical objects, including foods, especially in the specification of material ingredients and their concentration. This application requires quite different approach to the construction of impedance spectrometers, because they are usually designed for measurements in laboratory conditions [1], [2], while many biological materials are investigated in situ or are processed under rough climatic conditions (low temperature, high air humidity, etc.). Besides, portability and measurement automation are the accented features of the instrument. To reach these requirements, the new impedance spectrometer has been built. Specific impedance measurement conditions of biological objects [3], the state of the art, the instrument construction, used measurement method and achieved results are discussed.

Keywords: Impedance spectrometer, signal processing, quadrature detection

1. INTRODUCTION

Biological materials consist of cells and intracellular medium. If the electrical impedance of such material is to be measured, it is necessary to take in account the fact that a material on the boundary of the solid and liquid phase is investigated.

1.1. Electrical impedance

Electrical impedance describes the measured plant behaviour in electrical field, its capability to conduct electrical current, or dielectric or inductive properties. To express these physical quantities mathematically, it is necessary to use the complex numbers. In this case, the electrical impedance is usually written in one of the complex forms

$$Z = R + jX \text{ or}$$

$$Z = |Z|.e^{j\varphi} \text{ or } Z = |Z|. \cos\varphi + j. |Z|. \sin\varphi$$

R is the real part of the impedance, which represents the electrical resistance of the material, j is the imaginary unit and X is the imaginary part of the impedance, which is the reactive (wattles) resistance. If the impedance is placed in the complex plane $\text{Im} = f(\text{Re})$, it creates a vector which is turned to the real axis by the angle φ , called phase. $|Z|$ is the module of the impedance vector, which determines its length, and is given:

$$|Z| = \sqrt{R^2 + X^2}$$

Generally, both, the real and imaginary impedance parts change their magnitudes with frequency of the supplying AC signal. The goal of impedance measurement is to get this dependency, the frequency spectrum.

1.2. Impedance of biological objects

To explain the biological tissue impedance, a one-cell system placed in the a.c. electric field will be assumed. The cell membrane is a dielectric material and intracellular liquids are electrolytes so that a simple electrical model shown in the Fig. 1 can be used [3].

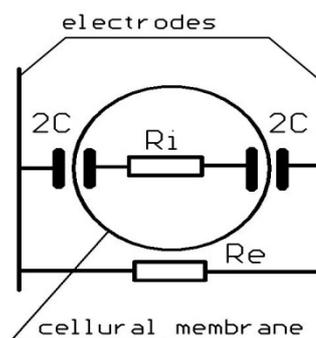


Fig. 1. Electrical model of one cell placed in the electric field.

This scheme can be simplified to the diagram shown in the Fig. 2.

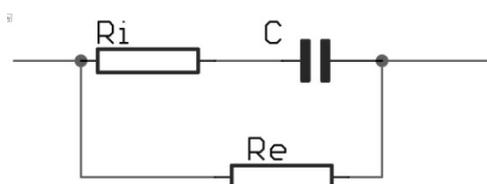


Fig. 2. Simplified scheme of a one cell system.

The frequency characteristic of this circuit in the complex plane is in the Fig. 3.

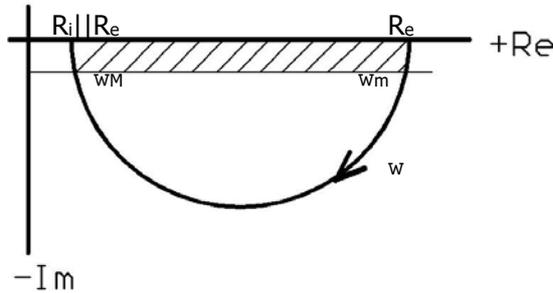


Fig. 3. Frequency characteristic of the circuit shown in the Fig. 2 in the complex plane. The model is valuable within the range of frequency from 1 kHz to 10 MHz.

This picture shows the ideal frequency characteristic of the cell model. Differences from this line can be found at low frequencies caused by the electrode polarization [4] (under w_m – the Warburg region, Fig. 4.) and at frequencies above 10 MHz (above w_M).

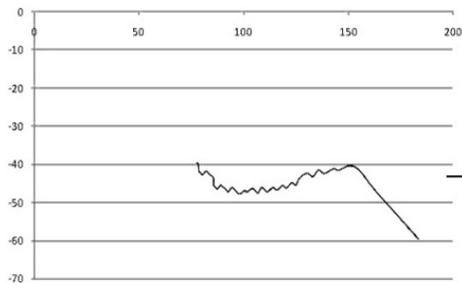


Fig. 4. The real spectrum of the pork meat in the complex plane. The sector of the circle as well as the Warburg distortion are visible. Axis scales are in ohms.

1.3. State of the Art

As mentioned, the laboratory measurement systems are available. These systems are suitable for the experimental laboratory research of biological materials impedance properties and are also frequently used in biochemistry. Systems, which offer sufficient accuracy and resolution needed for the in situ measurement and food (meat in this case) quality evaluation directly during the food production, are not in the market. This was the main reason why our institutions decided to build up a robust portable instrument capable of the electrical impedance frequency analysis in the non laboratory environment.

The main requirements involved the portability of the instrument, easy change of electrode probes (two, three or four terminal), and automated function that does not require specialized staff. Further, a good repeatability of results is necessary, because the quality evaluation takes advantage of tested material comparisons with standards or, in the case of food product ripening, the material changes observing during a time interval.

2. MEASURING METHOD

The impedance spectrometer that has been built for measurement of biological tissues applies the comparative method of the measured impedance with the standard impedance, the real resistor, which has negligible reactive components within required frequency range (Fig. 5.a and 5.b) [5].

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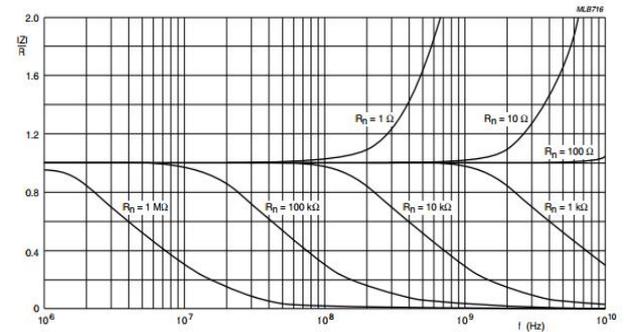


Fig. 5.a. Normalised impedance frequency characteristic of thick film resistors 0603.

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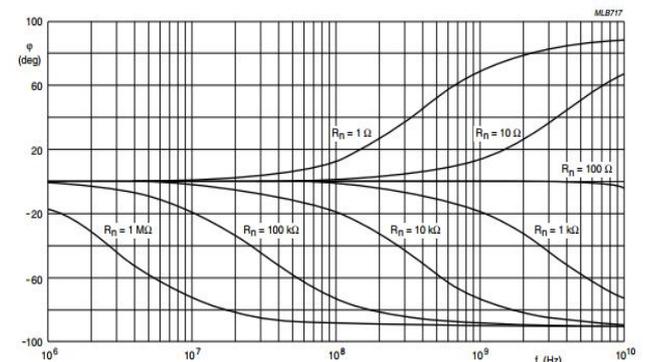


Fig. 5.b. Phase frequency characteristic of thick film resistors 0603.

As shown in both pictures, to avoid the problems with unwanted reactive components within the frequency range up to 1 MHz, the maximum resistance of the standard resistor and any resistors in measured signals circuits should be equal or less than 10 kW.

The method takes advantage of the circuit that consists of a serial connection of the measured impedance and the standard resistor with low parasitic impedances. The circuit is supplied from a generator of needed a.c. signal. The current vector is the basic vector of the circuit vector diagram (Fig. 6).

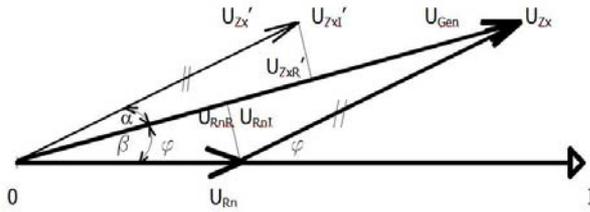


Fig. 6. Vector diagram of impedance components at the comparative method application. The signal components which are obtained by the quadrature detection are also shown.

In this picture, the basic electric current vector is signed as I . The standard resistor vector U_{Rn} is in phase with the current vector, while the measured impedance vector U_{Zx} is phase shifted. If the standard resistor magnitude is well known, it is possible to evaluate the real and imaginary parts of the impedance. To be able to measure both orthogonal impedance components, it is necessary to use a proper method of the signal detection, the quadrature detection of a.c. voltages on circuit components.

3. SYSTEM DESIGN

The system design has been determined by requirements of food producing companies, where material is processed at low temperatures and high relative air humidity. Further, the system should be portable and its handling easy. All previous instrument solutions assumed the construction in a form of an impedance data logger equipped with its own keyboard, LCD and an internal high volume memory for data archiving. The measurement consisted of two phases, the data logging in the process, and, after the data transport, the off-line processing in a host computer. This way brought problems with measurement identification and a loss of information.

The need of off-line processing led us to the innovative approach - to apply available technique, the tablet, which makes possible to handle the process easily, without any need of handling elements or memory medium for data transport, and offers available results in the real time of the experiment and helps to recognize eventual measurement errors immediately and gives a chance for the experiment repeating. The tablet graphic makes possible to create the curves of frequency characteristics

in required format and to program the experiment parameters.

The system design can be divided to several parts:

3.1. Analogue signal generation and processing

The basic concept of the analogue signal trace comes from the requirement of the complex quantity measurement, in which the orthogonal real and imaginary parts are to be determined. For this purpose, the technique of the quadrature detection of the signals is used. The principle of the method is based on the multiplication of the measured a.c. signal with the reference signals of the same frequency, but phase shifted. The phase shifts of the reference signals are switched between two values, 0 and 90 degrees against the signal exciting the measured circuit. If the $|Z|$ and f are the characteristic quantities that determine the measured signal position in the complex plane, the measured signal (the voltage drop on the Zx) can be expressed as:

$$U_{Zx} = A_1 \cdot \sin(\omega t + \varphi)$$

And the reference signals

$$U_{ref1} = A_2 \cdot \sin \omega t$$

$$U_{ref2} = A_2 \cdot \cos \omega t$$

If the quadrature multiplication is applied, and the reference signals with the frequency ω and amplitude $A_2 = 1$ are used, two signals are obtained, the parameters of which can be calculated as:

$$U_{ZxR} = 0.5 \cdot A_1 (\sin \varphi - \sin 2\omega t)$$

$$U_{ZxI} = 0.5 \cdot A_1 (\cos \varphi - \cos 2\omega t)$$

As the reference signal is used the signal which is phase coherent with the signal generated by the a.c. current generator U_{Gen} , so that it is necessary to detect the U_{Rn} signal the same way.

These signals contain two components, the d.c. and a.c. ones. Filtering out the a.c. components from signals causes that the final signals are only the d.c. ones, and their magnitudes are complying with the trigonometric expression of imaginary numbers.

In our case, the reference signal was not the harmonic one, but the square wave signal was applied. This caused the imaging of the reference signal components which cause the signal distortion. In this case, the signal shape looks like two way rectified signal (if the full-wave quadrature detector is used). But these signal components do not affect the detected d.c. signal magnitude and can be rejected by the low-pass filter connected at the output of the quadrature detector.

The principle block diagram of the analogue signal trace is in the picture Fig.7.

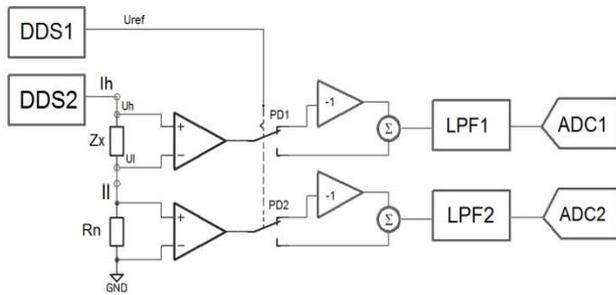


Fig. 7. Simplified block diagram of the analog signal trace of the four electrodes measuring system.

DDSx – direct digital synthesizer, PDx – quadrature phase detector, Σ – sumator, LPFx – low pass filter, ADCx – 24bit AD converter, Z_x – measured impedance, R_n – standard resistor, I_h , I_i – current terminals, U_h , U_1 – potential terminals.

The analogue trace can be divided in two basic parts. The first one generates a.c. signals for the measured circuit supply and the reference signal for quadrature detection of measured signals. It consists of two direct digital synthesizers, which are programmed by an embedded microcontroller. The synthesizers are able to generate signals and their initial phase in required range of frequencies 10 Hz to 1 MHz. Two Analog Devices synthesizers AD9837, which operate up to the clock frequency of 16 MHz, have been used. The synthesizer DDS1 generates the exciting sinusoidal signal supplying the test circuit, and the second synthesizer, the DDS2, generates square wave reference signals that are time shared. This prolongs time of measurement on one side, but the use of a single trace for the imaginary and real parts determination avoids the necessity of the phase alternation to reject two signal traces asymmetry on the other.

The second part consists of the electrode system with standard resistors, two signal traces involve the full wave quadrature detectors, low-pass analogue filters and ADCs digitizing detected signals. The electrode system makes possible to carry out two, three or four electrodes measurements. The electrodes are connected with the system via four miniature coaxial connectors. The standard resistors are automatically switched during the measurement to set the optimal range of the measurement. The detectors consist of two matched wide band lock-in amplifiers of our own construction that detect both polarities of measured signals. The low pass filters are the second order active filters. Their frequency characteristics are automatically switched with measured frequency. The SD 24bit AD converters digitize measured signals and send their output data to the microcontroller. For the digitization, the Analog Devices

AD7791 converters have been used.

3.2 Digital signal processing

The instrument conception has come from an idea to integrate the tablet to the instrument and use its batteries for the whole system supply taking advantage of the micro USB interface. This interface can be also used for the data transport. To save the batteries, an original power supply control has been used, which switches the analogue part off, if no measurement is required. The instrument is controlled by a low power microcontroller. The TI controller MSP430F235 has been selected for its low power consumption and computing power to handle the measuring circuitry. Normally, it operates in a low power mode, from which can be awoken, if the measurement is required. The microcontroller accepts the commands and experiment parameters from the host computer (the tablet) and controls the measuring trace to ensure regular experiment operation, e.g. sets the synthesizers, impedance range (automatically), low-pass filter frequency, collects the ADC data, communicates with the host etc. The instrument arrangement is in the picture Fig. 8.



Fig. 8. The system mechanical performance..

3.3. System software

The system operation is programmable from the embedded tablet and the measurement process is fully automated. The software takes advantage of four window pages. The first one is the welcome window, where the system and the institution are introduced. The second window serves to the experiment type selection (the method and the single frequency or spectrum measurement). The third window makes possible to test the connection between the units, organizes the transport of parameters, controls the experiment processing (start, stop or abortion) and stores and displays experimental data coming from the instrument. All post-processing

calculations, graphical output, result processing and store are provided in the fourth window. The main menu of the host makes possible to select either individual single frequency experiment parameter setting or the use of predefined measurement of the spectrum. An example of the software window is shown in the Fig. 9.

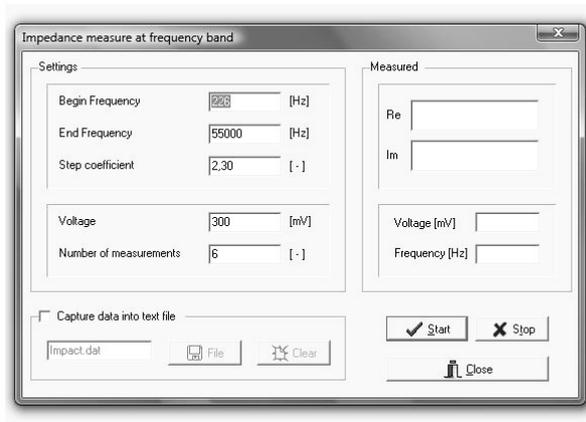


Fig. 9. The window for the spectrum measurement selection.

4. SYSTEM ERRORS AND THEIR REJECTION

The first tests offered good results at lower frequencies of the exciting signal (under 50 kHz), but the results at higher frequencies were not acceptable (Fig. 10).

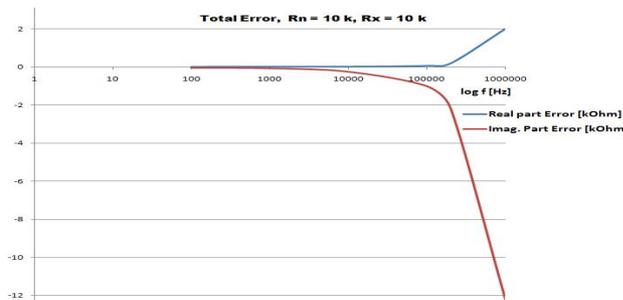


Fig. 10. Frequency characteristic of the absolute errors of the real and imaginary parts of the measured impedance.

The errors reached the magnitude in order of 10 %. To improve this status, it was necessary to carry out a careful analysis of errors, mostly coming out of imperfections of used circuit components.

There were found four main sources of these errors:

The time delay between the reference and exciting signals - the reference signal is derived from the MSB of the DDS phase accumulator, and the exciting signal takes advantage of the DAC connected to the phase accumulator output. The 198 ns delay of the DAC output has been compensated in the service routine of the exciting signal DDS initiation, where a needed phase shift

of the exciting signal is calculated and programmed.

Parasitic capacitances of the PCB, range switch and input buffer, which are connected parallel to the standard resistor, cause a decrease of the reference resistor impedance. The sum of these impedances has been evaluated and its parasitic phase shift and module are involved in the impedance calculation in the tablet program.

Offsets of signal traces and their frequency characteristic have been measured and stored in a table. Their magnitudes are subtracted from measured detected signals in the tablet software. The frequency dependence of offsets is caused by parasitic signals of the reference signal transition to measured signals via the gate capacitors of the quadrature detector switches (Fig. 11).

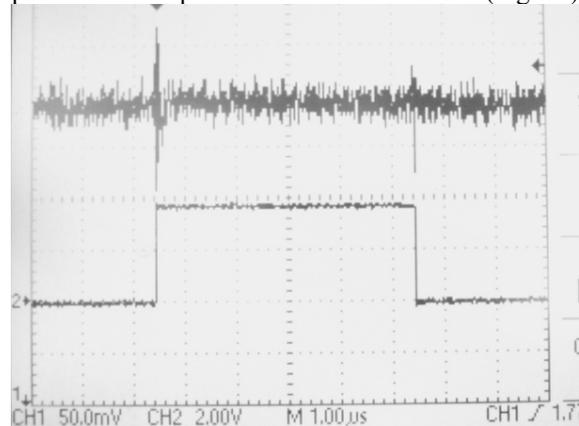


Fig. 11. Ripples caused by the reference signal transition to the detected signal contribute to the signal trace offset.

This transition causes ripples, the influence of which on the signal trace offset rapidly increases with the reference signal frequency.

The gain difference of the standard and measured impedances traces caused the error in order of 0.1 %, in spite of careful components match. To reject this effect, it is necessary to evaluate this difference at low frequencies measuring the resistor the resistance of which is well known.

After correction of these influences, the frequency spectrum of the real impedance represented by the 1206 resistor, the actual resistance of which is 9.9231 kW is shown in the picture Fig. 12.

It can be seen the relative errors of the real and imaginary do not exceed 2 % of measured value (0.2 kW) in the entire frequency range up to 1 MHz.

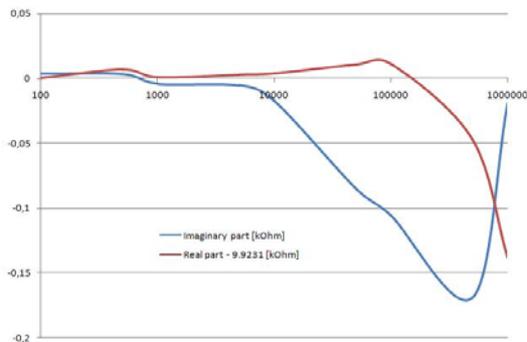


Fig. 12. Errors of the instrument after compensation.

5. CONCLUSIONS

At present, the system is completely finished and tested. It operates within the range of impedances 10 W to 1 MW and with operating frequencies from 10 Hz to 1 MHz. Its properties have been tested on real resistors, complex reference impedances and on biological objects. Its accuracy of impedance module, phase and repeatability are on sufficient degree, needed for the system application.

The practical results are shown in the Fig. 13.

The method of impedance spectroscopy has been tested in the slaughterhouse to assess the meat quality – the fat content. For the experiment, meat from one pig breed (Přeštice Black Pied) has been chosen. Their fattening time to get the slaughter weight (100 - 130 kg) varied from 140 to 200 days. The electrode probe was placed in the left half of the *Musculus longissimus lumborum et thoracis*. The picture shows the graph of the impedance frequency characteristic displayed in the complex (Gaussian) plain, $X = f(R)$ with the parameter of f . Individual curves belong to the typical samples of animals, the typical strength of their back fat is featured at the appropriate curve. It can be seen the direct correlation between the impedance and the fat layer thickness at lower frequencies.

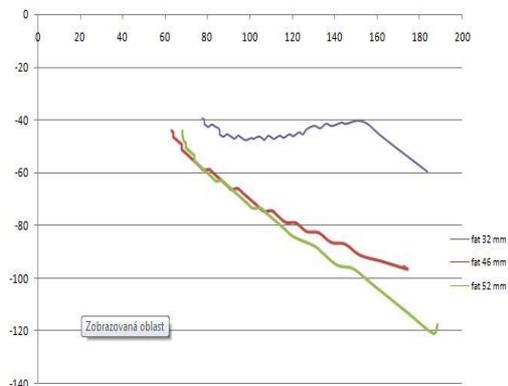


Fig. 13. The frequency spectrum of meat samples with different fat layer thickness.

The system is prepared for the application in the food industry. Good results of test measurements and tests in rough conditions of the food industry are very promising. At present, the new series of control measurements are prepared. These new tests give the finite answer dealing with the impedance spectroscopy method usefulness. Another questions, how to interpret the spectra to get the maximum information, are waiting for the applicants of the method.

As to the future of the electronics, the new electronic circuitry and its PCB revision is prepared to decrease the parasitic capacitances influence on the results and the and the wireless communication between the impedance spectrometer and the tablet is planned.

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