Доклади на Българската академия на науките
Comptes rendus de l'Académie bulgare des Sciences

Tome 66, No 7, 2013

PHYSIQUE

Biophysique

A SIMPLE IMPEDIMETRIC DEVICE FOR IN SITU ANALYSIS OF PLANT TISSUES

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(*Submitted by Academician A. Petrov on March 5, 2013*)

Abstract

Construction and major features of a set-up suitable for impedance analysis of plant tissues are disclosed in the paper. The measuring device is based on a simple scheme operating in conjunction with the PC sound card. While many programmes running directly on sound card are capable of simulating impedance analyzer, their performance is not consistent when chemical or biological systems are explored. The main reason is a relatively low input impedance of the card itself. An addition of coordinating preamplifier between the sample and the audio input of the PC drastically improves the implementation of the set-up. On the other hand, a specially designed measuring head gives possibilities for straightforward assessment of plant tissue's electrical parameters in situ at various ambient conditions. Some examples of impedance analysis of intact leaves are given and their behaviour in regard to a preliminary imposed desiccation is briefly discussed.

Key words: impedance analysis, leaf desiccation, plant water relations

Introduction. Drought and freezing are the basic abiotic factors limiting plant growth and development. They cause various morphological, physiological and biochemical changes in plants. As far as water and ions are ubiquitous participants in these phenomena, it is clear, that electrochemical methods of investigation would be of a primary interest. Indeed, to date many such techniques are used for evaluation of the stress consequences. Conductometric measurement of electrolyte leakage from plant tissues, for example, is widely accepted for assessment of cell membrane injury [1–3]. Electrochemical impedance spectroscopy (EIS),

in particular, possesses some intrinsic advantages over other methods. The most important among them are selectivity, sensitivity and possibilities for noninvasive data collecting, not mentioning the low cost. Actually, attempts of impedance measurements of plant samples at limited frequencies have been made ever since the early seventies [4]. With the employment of larger electromagnetic spectrum of excitation, however, much more information was gained about the mechanisms of reaction of different cell compartments. This allowed the uniform plant tissue to be represented by specific models including extracellular resistance, plasma membrane capacitance, cytoplasmic resistance, tonoplast capacitance, and vacuole interior resistance [5].

The most common way to perform EIS is by applying sinusoidal AC voltage to an electrochemical cell, to monitor its reaction in the form of current passing through it. From the theory of electrical circuits, it is well known that depending on the components of the impedance, the current is also sinusoidal, exhibiting a certain phase shift with positive or negative angle θ. Most of the biological material of interest rarely shows any inductive component, so the equivalent impedance can be represented as a combination of resistances R and capacitances C, connected in a proper fashion. For the two simplest cases of "in series" or "parallel" RC connection the equivalent impedance (which is a complex quantity) is given by

$$Z = R_S + \frac{1}{i\omega C_S} = \frac{1}{\frac{1}{R_P} + i\omega C_P},$$

where resistances and capacitances are supposed to be independent of frequency $f(\omega = 2\pi f)$. It is immediately clear, that the impedance Z does not depend on the applied voltage, but is a direct function of frequency. One convenient way to represent the behaviour of Z is to use its evolution in the space $[f, \operatorname{Re} Z, -\operatorname{Im} Z]$. The respective projections of this dependence, for real and imaginary parts, are known as impedance diagrams, named as Nyquist plot in the complex plane $[\operatorname{Re} Z, -\operatorname{Im} Z]$ and Bode plots $[f, \operatorname{Re} Z]$, $[f, -\operatorname{Im} Z]$ [6].

Two main features of chemical and biological materials, however, should be stressed at the very beginning. First of all, they are prone to perturbation under application of higher voltages. Secondly, in many cases their reaction is substantially nonlinear, which means that the behaviour of the specimen could not be described solely by combination of finite number of frequency-independent RC elements (so-called Maxwell ladder). In such instances, elements with special dependence on the frequency should be introduced. For example, it is the well known Constant Phase angle Element, CPE [7], defined as: $Z_{CPE} = A(i\omega)^{-a}$, where A and a are constants. Obviously, for $a = 0$ the impedance Z_{CPE} represents ideal resistor $R = A$ and for $a = 1$ it is an ideal capacitor $C = 1/A$. Additionally, it is clear, that the Nyquist and Bode plots of CPE are straight lines in logarithmic scale.

Materials and methods. Attempts to use computer facilities for the purposes of impedimetric analysis of plant tissues have been made earlier [8]. Our device utilizes the conveniences of the PC sound card, which offer easier collecting and manipulation of the data. As it is mentioned above, the major problem in this respect is the relatively low input impedance of the sound cards. This can be overcome by addition of high input buffer preamplifier with unity gain. Thus any nonlinearity of amplification with frequency is avoided.

In the implementation of the preamplifier we follow the solution chosen in [9]. The approach works as follows. One of the two output channels (e.g. right, R) of the sound card (SC) drives a voltage divider, consisting of a reference resistor R_{ref} and the sample under test Z_x, with a sine wave (Fig. 1*a*). The second output of the SC is unused. The two input channels measure the two voltages on the voltage divider, which allows the ratio between these two quantities to be measured at any instant. In principle it would be possible to assume that the voltage applied to R_{ref} is simply proportional to the voltage that has been output by the programme to the SC. R_1 references the SC output to ground. The two operational amplifiers, each with a gain of one, act as buffers with a high input

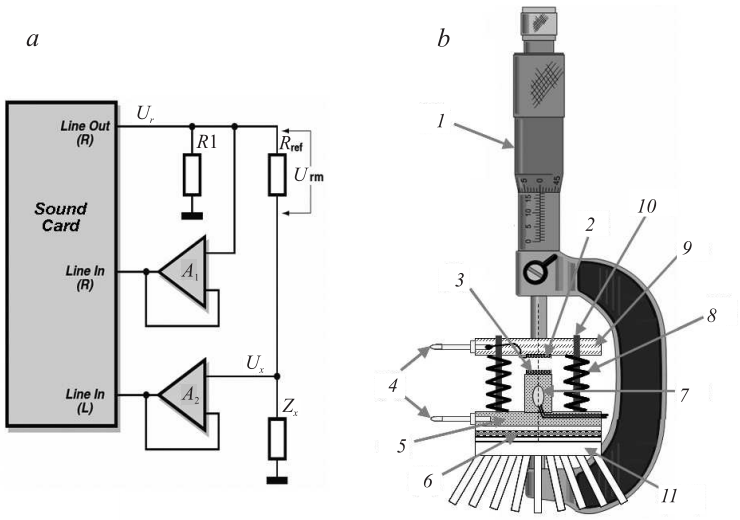

Fig. 1. (*a*) Block diagram of the measuring principal, modified from [9]. See text for details. (*b*) Schematic of the set-up measuring head: *1* – micrometer screw; *2* – upper electrode; *3* – lower electrode; *4* –gold plated pin connectors (type Amphenol Ø 1.5 mm) of the electrodes; *5* – brass holder of the lower electrode; *6* – Peltier element; *7* – embedded thermo resistor; *8* – spring; *9* – Plexiglas holder of the upper electrode; *10* – steel leading rods; *11* – radiator of the Peltier element

and a low output impedance. The sinusoidal voltage U_r (from 'line out'), which is applied to the test circuit, is measured on the right input channel. The voltage across the sample under test, Z_x, is measured using the left input channel. The operational amplifier used is a type LM358, although any similar device would do equally well. R_{ref} can be varied to change the measurement range. For best results the value of R_{ref} should be comparable to the Z_x. The ratio between the amplitudes of the voltages dropped across the R_{ref} and the Z_x, and the phase angle between these voltages, are the keys to computing the impedance of the sample. The series combination of the reference resistor and the sample is driven by the signal U_r, which is one output of the SC; a voltage is dropped across the sample whose amplitude and voltage depend on its impedance Z_x, so

$$U_x = I_{\text{ref}}.Z_x = \frac{U_r - U_x}{R_{\text{ref}}}.Z_x.$$

As a software, manipulating the above mentioned quantities, we have used the specialized programme PhysLab 5.0 developed by S. P. Palto from Shubnikov Institute of Crystallography at the Russian Academy of Sciences, Moscow [10]. This programme, working on the basis of SC, possesses several virtual instruments especially suitable when phase dependent measurements are involved. It can generate the sinusoidal voltages, necessary for the sample excitation, and is capable to lock-in the incoming signals, so giving possibility for the impedance definition. Thus, a simple and inexpensive device for impedimetric analysis, operating in the range of audio frequencies i.e. from $\sim$ 10 Hz to $\sim$ 10 kHz, is practically realized.

The second basic part of the set-up is a measuring head containing two electrodes forming the electrochemical cell (Fig. 1*b*). They are made of gold coated MOS (Si/SiO_2) structures placed on holders. The lower holder is machined from brass and contacts a Peltier element, regulating its temperature. The upper holder is moving up and down and its position is controlled by a micrometer, so the distance between the electrodes can be precisely defined.

Results and discussion. Thus far, EIS has been widely used for investigation of different plant tissues and plenty of models have been proposed for description of their impedance behaviour under various conditions [11, 12]. However, no one of them could be regarded as ultimate and universal. On the one hand, the large number of equivalent circuits used to mimic the samples' impedance stems from the wide diversity of plant material tested in any particular case. On the other hand, the enormous complexity of the processes on cellular and subcellular level, contributing to the plant electrical features, hamper to a great extent the correct identification of impedance elements. Nevertheless, as an illustration of the performance of our device, we present here the results of impedance measurements of a leaf from crane's bill, *Geranium sp.* under two quite different conditions. In Figure 2*a* the Nyquist plot of a freshly cut leaf

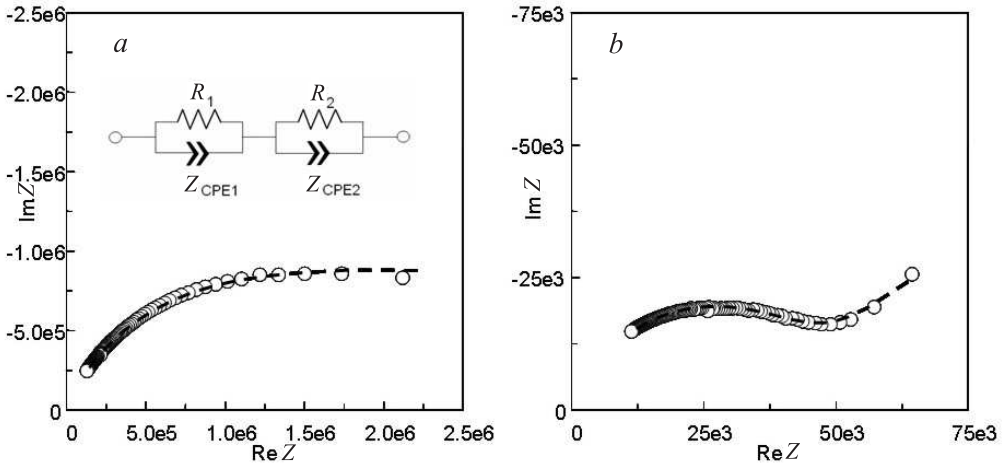

Fig. 2. Nyquist plots of the impedance of a leaf from crane's bill, *Geranium sp.* (a) before and (b) after a treatment with UHF in a microwave oven for few seconds. Hollow circles are experimental data and the dashed line is the fit with the equivalent circuit shown. The respective parameters of the equivalent circuit for the two cases are: (a) $1/A1 = 3.001 \times 10^{-8}$; $a1 = 0.77253$; $R_1 = 1.856$ MΩ; $1/A2 = 5.046 \times 10^{-9}$; $a2 = 0.76383$; $R_2 = 1.646$ MΩ; and (b) $1/A1 = 4.002 \times 10^{-6}$; $a1 = 0.472$; $R_1 = 1$ MΩ; $1/A2 = 3.132 \times 10^{-8}$; $a2 = 0.858$; $R_2 = 38$ kΩ. Notice the great differences in the scales

is shown, whereas Fig. 2b demonstrates the drastic changes of the impedance after a treatment of the leaf with ultrahigh frequencies (UHF) for few seconds. Obviously, the two plots are markedly differing. As far as mainly the water is responsible for the formation of the impedance, our model includes two basic interfaces in leaves tissue – the cell wall and the cell membrane, which impedances are represented by the so-called ARC elements Z_{ARC}, comprising ideal resistor R and Z_{CPE} in parallel. Such a model is used earlier in [12]. It is tempting to interpret the obtained results in terms of changes namely of these peculiar plant compartments. Water significantly absorbs electromagnetic waves in the UHF region and, it is reasonable to expect that due to elevated local temperatures, it causes strong structural distortions in the wall as well as in the membrane. Indeed, according to the data fit, the second set of equivalent circuit parameters shows clear tendency of resistance lowering for both parts of the scheme. Such behaviour is characteristic for injured walls and membranes, which become more conductive [5]. The model presented here, of course, has no pretensions for completeness, but rather is aimed only to demonstrate the abilities of the device under consideration. So, its more thorough verification is a matter of future research with variety of plant species and tissues.

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