

Electrical Modeling and Impedance Analysis of Biological Cells

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Abstract- *It was proved that the external electric field intensity has significant effects on the biological systems. The applied electric field intensity changes the electrical behavior of the cell systems. The impact of electric field intensity on the cell systems should be studied properly to optimize the electric field treatments of biological systems. Based on the cell dimensions and its dielectric properties, an electrical equivalent circuit for an endosperm cell in rice was developed and its total impedance and capacitance were verified with measurement results. The variations of impedance and conductance with respect to applied impulse voltage at different frequencies were plotted. This impedance analysis method can be used to determine the optimum voltage level for electric field treatment and also to determine the cell rupture due to electric field applications.*

Keywords- Electric field intensity, biological cells, cell dimensions, endosperm cell, equivalent circuit.

INTRODUCTION

It was proved that the treatment of seeds in higher electric field intensities improve the seed germination rate [4][5]. The application of an external electric field can induce a critical electrical potential across the cell membrane, which leads to rapid electrical breakdown and local structural changes of the cell membrane. This first field effect results in a drastic increase in permeability due to the appearance of pores in the membranes. Based on this phenomenon, many practical applications of high electric fields for the reversible or irreversible permeabilization of various biological systems have been studied [3].

The process development must be based upon the cell-specific critical transmembrane voltage induced, (for many different types of cell and artificial membranes, it is found to be approx. 1 V) or of the critical external electric field strength E_c . In soft coat seeds, the irreversible breakdown leads to complete rupture of the seeds. But in hard coat seeds, the irreversible breakdown improves the water imbibitions of seeds and thus improves the germination rate [1]. So the magnitude of electric field intensity for seed treatment should be limited based on the type of seeds to avoid the irreversible membrane breakdown due to higher transmembrane potential. Very little information is available regarding membrane permeabilization kinetics, and on reversible, irreversible structure changes of cells during and after the application of high electric field intensity. A fundamental

understanding of these phenomena is essential for optimal process design and for the characterization of critical process parameters in seed processing.

During the processing of seeds, attention needs to be given to the maximum electric field applied, type of seeds, exposure time and number of pulses. Due to inhomogeneous structure of biological cells, the development of electro physical model of intact and processed cells can be a valuable tool for studying the processing effects on the biological cell systems. This paper summarizes some of our activities on modeling of equivalent circuit of a plant cell and impedance approach for detecting the effect of electric field intensities on the cells.

METHODS AND MATERIALS

Generally, the compartments of a cell are separated from the surrounding medium by a cell membrane, which consists mainly of highly structured, electrically insulating phospholipids. The behaviour of biological tissue or suspended cells exposed to an alternating electrical field can be described using Maxwell-Wagner theory of heterogeneous dielectrics. Modifications of this basic concept were applied to biological, biochemical, or biomedical problems. In the field of plant physiology, electrical impedance analysis is used to detect the effect of electric field intensity on the plant cell [2].

THEORETICAL CONSIDERATIONS

The presence of intact membranes with very low electrical conductance in a cellular sample (with conductive inner and outer phases) produces alternating current (AC)-frequency-dependent changes of the macroscopically detectable electrical conductivity. The β -dispersion is the result of the repeated charging process of the membranes in the altering electrical field. For biological systems, it is more pronounced in a frequency range between 1 kHz and 100 MHz. The β -dispersion may be regarded as a special case of the Maxwell-Wagner polarization effect, which generally explains the frequency behaviour of the impedance due to the presence of nonconductive interfaces separating two conductive aqueous

phases, such as a dielectric in a parallel plate capacitor. Therefore, in an equivalent circuit, the electrical behaviour of the cell membrane can be assumed as a capacitor connected with one resistor in parallel. The liquid phases on both sides of a membrane can be introduced to this circuit as two additional series resistors. At higher frequencies (greater than approximately 0.1-0.2 GHz), dipole rotation of molecules in biological solution will further influence the complex conductivity. An approximation of an elementary cell within a tissue consisting of extra-cellular compartments, cytoplasm membrane, cytoplasm, tonoplast and vacuole yields a more complex equivalent electrical circuit. Similar circuits were frequently used in plant physiology for the purpose of impedance analysis. The complete rupture of the cytoplasm membrane and the tonoplast of plant cells reduce the equivalent electrical circuit to a parallel connection of three ohmic resistors, formed by the electrolytes of the cytoplasm, the vacuole, and the extracellular compartments, respectively. The tissue in homogeneities (such as gas vacuoles or oil droplets in raw or processed material) are to be added to the equivalent circuit as ohmic resistors (as far as charge polarization can be excluded) [2].

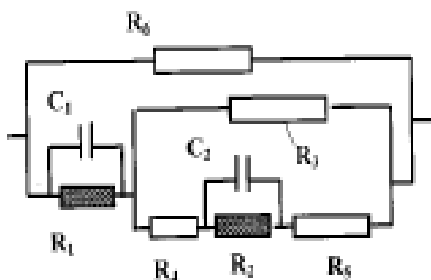


Fig.1 Equivalent circuit of intact cell

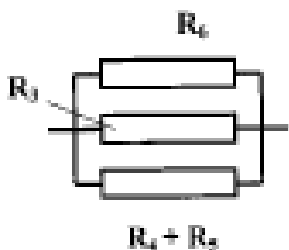


Fig.2 Equivalent circuit of ruptured cell

The frequency-dependent electrical conductivity of a tissue system may be defined as

$$\sigma(\omega)^s = \frac{l}{A |Z(j\omega)^s|} \quad (1)$$

where l is the length of the sample, A is the area perpendicular to the electrical field, and $Z(j\omega)^s$ is the system impedance, where

$\omega=2\pi f$ is the angular frequency. The model considered here is valid if the intact and ruptured cells as well as in homogeneities in initial intact and processed cell systems are regularly distributed.

Using this approximation, the impedance of a homogeneous tissue sample containing different portions of intact cells as well as non-cellular compartments can be represented by eq 2,

$$Z(j\omega)^s = \frac{n}{m} \left(\frac{i}{Z(j\omega)^s} + \frac{p}{Z^p} + \frac{g}{Z^g} \right)^{-1} \quad (2)$$

where i and p are the ratios of intact and ruptured cells to the total number of cells in an elementary layer, g is the ratio of the in homogeneity inclusion unit to the total number of cells in an elementary layer, $i + p + g=1$, Z_g is the resistance of additional intracellular volume elements and Z_p is the resistance of the elementary unit with ruptured membranes.

For homogeneous samples consisting of intact cells only ($i=1$; $p=0$; $g=0$), equation 2 can be simplified to

$$Z(j\omega)^s = \frac{nZ(j\omega)^i}{m} \quad (3)$$

The rearrangement of equation 3 using $m = A/A_c$ and $n=l/d$ yields

$$Z(j\omega)^s = \frac{A_c}{A} \frac{l}{d} Z(j\omega)^i \quad (4)$$

This implies that the impedance of the intact tissue is a linear function of the impedance of one elementary unit. Due to this fact, the specific conductivity of tissue sample containing intact cells, $\sigma(\omega)^s$, and the specific conductivity of the elementary unit with intact membranes, $\sigma(\omega)^i$, are equal. In the high frequency range, the conductivities of intact cells and of cells with ruptured membranes are practically not different. This can be explained by the fact that, within this frequency range, the intact cell membrane has a negligible impedance (membrane reactance $X1(j\omega)$; $X2(j\omega) \rightarrow 0$). A decrease in the difference of conductivity in high and low-frequency ranges in β -dispersion is the result of cell rupture [2].

1. Calculations

In plant tissue with a density-packed cell system, each cell can be treated approximately as a cube. The relative volume of vacuole within a cell (V) and the relative conductivity (S) can be expressed as

$$V = V_{\text{vacuole}} / V_{\text{cell}} \quad (5)$$

$$S = \sigma_{\text{plasma}} / \sigma_{\text{vacuole}} \quad (6)$$

where V_{vacuole} and V_{cell} are the vacuole and cell volumes and σ_{plasma} and σ_{vacuole} are the plasma and vacuole specific conductivities respectively. In the high-frequency range, the

correlation between intact cell impedance (Z_h^i), vacuole (R5) and plasma resistances (R3, R4) can be demonstrated by eqs 7-9:

$$R_3 = \frac{R_6 Z_h^i [S + V^{1/3} (1 - V^{2/3})^{-1} + 2(1 - V^{1/3})]}{(R_6 - Z_h^i) [S + 2(1 - V^{1/3})]} \quad (7)$$

$$R_4 = R_3 (V^{2/3} - V) (1 - V^{2/3}) \quad (8)$$

$$R_5 = R_3 S (V^{-2/3} - V^{1/3}) \quad (9)$$

Tonoplast resistance (R2) is calculated from the following correlation between plasma membrane resistance (R1) and the relative volume of the vacuole in the cell:

$$R_2 = R_1 V^{-2/3} \quad (10)$$

Equations 7-10 are derived from correlation among Z_h^i , R1-6, V, and S under the assumption that the vacuole in cubic form is centrally positioned in a cell.

R6 is calculated under the condition that the extracellular resistance is very low, as is plasma membrane resistance (as approximation $R_6 < 0.01R_1$). The electrical resistances R3, R4 and R5 are calculated from 7-9 using the impedance of intact cells at high frequencies (Z_h^i) and variation of parameters V and S. The characteristic cell impedances Z_1^i and Z_h^i were calculated according to equations using specific conductivities σ_1^i and σ_h^i determined from tissue in the intact state at 3 kHz and 12.5 MHz, respectively:

$$Z_1^i = d / A_c \sigma_1^i \quad (11)$$

$$Z_h^i = d / A_c \sigma_h^i \quad (12)$$

By variation of the relative volume V from 0 to 1, of relative conductivity S from 0.5 to 1.5, and of membrane capacities C1 and C2 from 1 to 50 pF, the parameters necessary for best fit for rebuilding a measured conductivity spectrum, $\sigma(\omega)$ s, were determined.

The equivalent circuit parameters are derived from the relative volume of the vacuole in a cell and the relative conductivity. The dimensions of an endosperm cell present in rice are given below in the table 1 [7].

TABLE 1 : CELL DIMENSIONS IN RICE

Area of the cell $A_c = 614 \pm 19 \times 10 \mu m^2$
Thickness of the cell $d = 80 \mu m$
Cell membrane thickness = 7 nm

The characteristic cell impedance Z_1^i and Z_h^i are calculated using specific conductivities and at 3 kHz and 12.5 MHz respectively. The specific conductivities are calculated from the dielectric properties of rice. The table 2 gives the dielectric properties of rice at β -dispersion range [6].

TABLE 2 : DIELECTRIC PROPERTIES OF RICE

S.No	Frequency	ϵ'	ϵ''
1	3 kHz	7	2
2	12.5 MHz	5	0.3

The relative complex permittivity of a dielectric material is given as

$$\epsilon_r = \epsilon' - j\epsilon'' \quad (13)$$

where ϵ' - dielectric constant of the dielectric material

ϵ'' - dielectric loss factor of the material

$$\text{Loss tangent } \delta = \epsilon'' / \epsilon' \quad (14)$$

where δ - loss angle of the dielectric material

The conductivity is given by

$$\sigma = \omega \epsilon_0 \epsilon_r'' \quad (15)$$

where $\omega = 2\pi f$ - angular frequency

ϵ_0 - permittivity of free space

Substituting the values

$$\sigma = 55.63 \epsilon_r'' \times 10^{-12} \text{ Siemens/m} \quad (16)$$

The specific conductivities are calculated using the equations (15) and (16). The calculated conductivities are used to calculate the characteristic impedances of the cell at β - dispersion range. Substituting the values of specific conductivities, cell dimensions and characteristic impedances in the equations from 5 to 12, the equivalent circuit parameters are calculated and the values are given below in the table 3.

TABLE 3 : EQUIVALENT CIRCUIT PARAMETERS OF CELL IN RICE

R_1	R_2	R_3	R_4	R_5	R_6	C_1	C_2
45 M Ω	63.26 M Ω	1.693 M Ω	54.4 k Ω	399.7 5k Ω	430 k Ω	10 pF	10 pF

The total impedance of the circuit is 10.679M Ω at 50Hz frequency.

RESULT AND DISCUSSION

1. Impact of Electric Field Intensity on Paddy Seeds

The paddy seeds were grouped and they were treated in pulsed electric field at different electric field intensities. The applied impulse voltage levels were 10, 20, 30 and 40kV and the number of pulses applied were 50 for each group. After the application of electric field, impedance and capacitance each group of treated seeds were measured by using precision impedance analyser 6500B.

The effect of electric field treatment on seeds can be studied by measuring the impedance and conductance of the seeds before and after the electric field treatment. The variation of impedance and conductance with respect to applied voltage to the seed groups at the frequencies of 50Hz, 10 kHz and 1 MHz were plotted. The impedance of treated seeds was 133MΩ and was lesser than that of untreated seeds. When the impulse voltage of 10kV, 50 pulses was applied, the impedance of the seeds no longer remained 133MΩ and it dropped to 0.149MΩ.

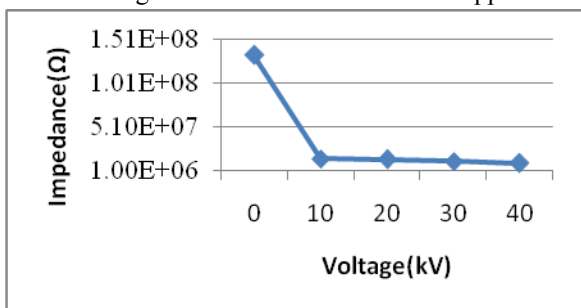


Fig.3 Voltage vs. Impedance at 50Hz

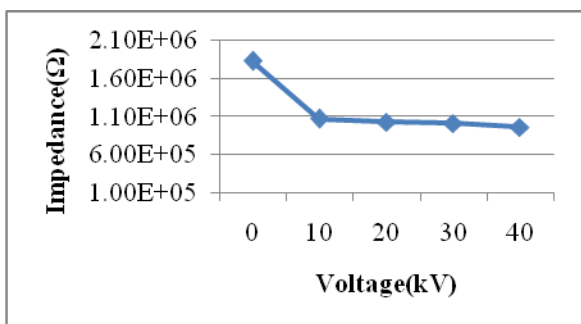


Fig.4 Voltage vs. Impedance at 10kHz

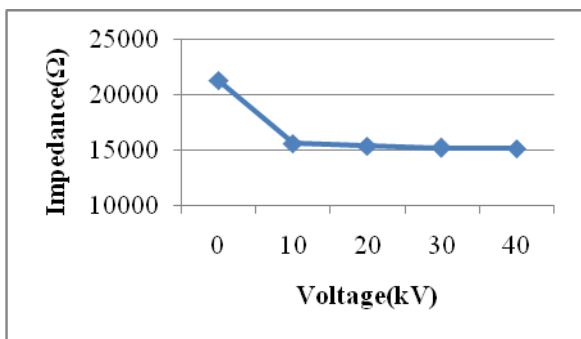


Fig.5 Voltage vs. Impedance at 1MHz

As the applied voltage was increased the magnitude of applied electric field increased which led to further linear decrease in the impedance of the seeds. Alternatively, the conductance of the seeds increased with respect to applied electric field. Initially, the conductance of the untreated seeds was very low in the order of 10⁻⁹ Siemens. As the applied voltage to the seeds increased, the conductance of the seeds also increased linearly.

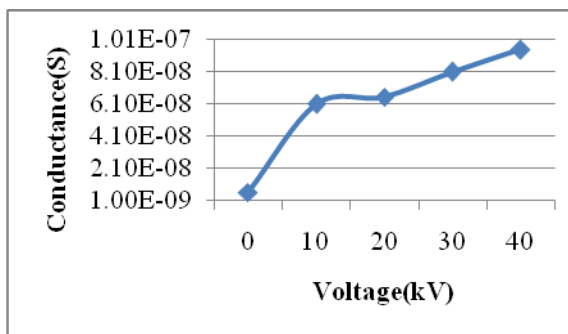


Fig.6 Voltage vs. Conductance at 50Hz

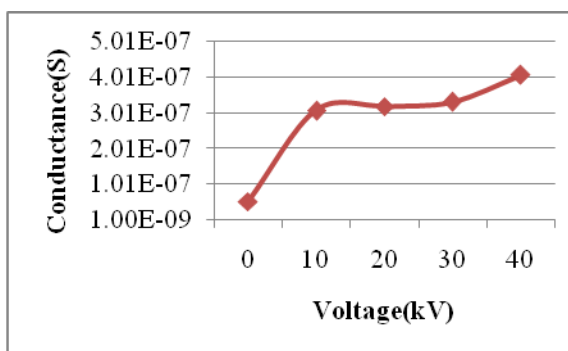


Fig.7 Voltage vs. Conductance at 10kHz

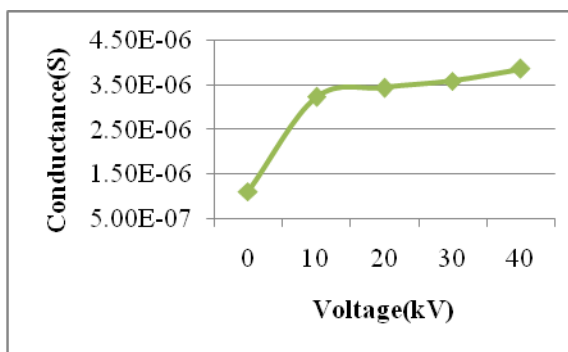


Fig.8 Voltage vs. Conductance at 1MHz

If the applied electric field has enough strength to break the seeds, the impedance of the seeds will be reduced to a very lower value and the cell components of the seed will become to conductive state. This electrical breakdown of the cell is termed as cell rupture which stops the seed germination. The breakdown electrical breakdown of the cells due to electric field treatment should be avoided to prevent the cell rupture. This impedance analysis method will be a useful tool for determining the maximum safest voltage or optimum voltage to be applied for seeds for improving the germination of the seeds.

CONCLUSION

A study of the treatment of seeds in higher electric field intensities leads to an electrical modelling of the plant cell

present in the seeds. Impedance analysis method is presented for determining the changes in electrical properties of seeds due to electric field treatment. The optimum or maximum safest electric field to be applied to the seeds for improving the germination can be determined by measuring the impedance and conductance of the seeds in a wide range of frequencies. An accurate and simplified method to find out the seed breakdown, cell rupture and to optimize the electric field treatment of seeds was presented.

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