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TRANSFORMATION OF FREQUENCY DISPERSION OF ELECTRICAL PARAMETERS OF LIVER TISSUES DEPENDING ON STORAGE TEMPERATURE

Introduction. *Presented article discusses the temporal transformation of frequency characteristics of the few of studied electrical parameters of liver tissues during their storage at temperatures ranging from 2 °C to 35 °C.*

Methods. *The cells for the studies were made from the plastic body of a 2 ml medical syringe. The ends of the cylindrical sample were in contact with a nickel mesh. Nickel conductors welded to the mesh served as a current collector. Cylindrical samples with a height of 1.2 cm, a diameter of 2 cm and a mass of 1–1.5 g were placed in a specially designed, hermetic thermostat 1/120 SPU under the influence of temperatures of 2 °C, 8 °C, 12 °C, 25 °C and 35 °C for 2, 5, 8, 10 and 14 hours.*

Results. *The study examines and provides the description of changes in direct current conductivity, the frequency dispersion of the real component of conductivity, tangent of the dielectric loss angle within the frequency range of 0.01 Hz to 100 kHz (the beta-dispersion range) and resonance frequency. The functional relationship between the transformation of the thermal-time dependence of electrical parameters and the degree and nature of morphological changes in biological tissues has been established.*

Conclusions. *Local values and the direction of monotonicity of the polarization relaxation time function in the studied frequency range can be considered as a criterion for determining the degree and type of destructive changes in experimental tissues which were stored in the air at different temperatures and time periods.*

Keywords: *impedance spectroscopy, liver tissue, conductivity, temperature, resonant frequency, loss tangent, beta-dispersion, relaxation time.*

Introduction

Physical methods for studying biological tissues are effective ways to establish the relationship between their electrical properties and physical parameters. One of the fast, non-invasive, easy-to-use, and reliable methods for analyzing the structural integrity of biomaterial is the method of determining the complex electrical impedance over a wide frequency range (Schmidt et al., 2016, p. 110–118). The

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evaluation of tissue structural integrity under pathological processes or harmful effects is also within the scope of impedance spectroscopy (Morgan et al., 2019). For example, in the study (Kenworthy et al., 2018), based on the results of measuring electrical impedance, the authors constructed equivalent electrical circuits of liver tissues damaged by aggressive factors, including gamma radiation and thiourea. Comparing the parameters of the constructed circuits with the results of microscopic examination of the samples allowed the authors to establish the potential correspondence of the circuit model elements to specific cellular environments and structures. Calculating the parameters of the circuits and observing the transformation of the electrical circuit structure, along with their comparison with the results of microscopic analysis, allowed the authors to conclude the probable dependence of structural and parametric study results on the intensity of influencing factors. In the proposed work, an attempt was made to analyze the frequency dispersion of complex electrical parameters of liver tissues under the influence of temperature. The aim was to determine the peculiarities of charge transfer in the electrolyte subsystem of tissues under different degrees of structural degradation.

Methods

Cells for research were made from a plastic body of a 2 ml medical syringe. The ends of the cylindrical sample contacted with a nickel mesh. Nickel conductors, welded to the mesh, served as current conductors. The structure of the experimental equivalent scheme was chosen similarly to the one we provided in previous works (Pryimak et al., 2021, pp. 1–11). Cylindrical samples, 1.2 cm high, 2 cm in diameter, and weighing 1–1.5 g, were placed in a specially designed, hermetic thermostat 1/120 SPU under the influence of temperatures 2 °C, 8 °C, 12°C, 25 °C, and 35°C for 2, 5, 8, 10, and 14 hours. The acquisition of electrical impedance spectra was performed using a standard AUTOLAB PGStat 30 spectrometer in the frequency range of 0.01 Hz – 100 kHz. To reduce the impact of amplitude values of the measurement signal voltage on the condition of organic tissues, the peak potential was reduced to 0–5 mV. The signal was recorded automatically, and the dependencies of the real and imaginary values of specific conductivity (σ' and σ''), dielectric permittivities (ξ' and ξ''), and the loss tangent were constructed and processed using the Origin software. The frequency dispersion of electrical conductivity and dielectric permittivity is determined from Nyquist diagrams using formulas (4, 5). Electrical conductivity is determined by the formula:

$$\sigma = \frac{1}{Z^*} = \frac{S}{\rho^* \cdot l}, \quad (1)$$

where Z^* , ρ^* are the complex impedance and specific electrical resistance, respectively, and S , l are the area and thickness of the sample, respectively; $\omega = 2\pi f$, ε_0 is the electrical constant, and $j = \sqrt{-1}$. From the diagram, $\sigma' - (\sigma'' - \varepsilon_0 \varepsilon_\infty \omega)$ (ε_∞ is the dielectric permittivity at high frequencies) in the low-frequency region, the value of specific direct current conductivity σ_{dc} was determined at different temperatures. The value of ε_∞ is determined from the Cole-Cole diagram (Tarasov, & Titov, 2013,

p. 352–356) $\varepsilon'' - \varepsilon'$ by approximating the experimental curve to the intersection with the abscissa axis (Olekhnovich, Radyush, & Pushkarev, 2012, pp. 2236–2242).

The real ε' and imaginary ε'' dielectric permittivities are determined by the formulas:

$$\varepsilon' = \frac{\rho''(\omega)}{(\rho''(\omega))^2 + (\rho'(\omega))^2 \cdot \varepsilon_0 \cdot \omega}, \quad (2)$$

$$\varepsilon'' = \frac{\rho'(\omega)}{(\rho''(\omega))^2 + (\rho'(\omega))^2 \cdot \varepsilon_0 \cdot \omega}. \quad (3)$$

The frequency dependence of the real component of complex conductivity is determined by the formula

$$\sigma'(\omega) = \frac{\rho'(\omega)}{(\rho''(\omega))^2 + (\rho'(\omega))^2}. \quad (4)$$

The frequency dependence of the imaginary component of complex conductivity is determined by the formula

$$\sigma''(\omega) = \frac{\rho''(\omega)}{(\rho''(\omega))^2 + (\rho'(\omega))^2}. \quad (5)$$

Results

Experimentally obtained Nyquist diagrams of the studied samples for different temperatures stored for 2–14 hours are shown in Fig. 1. The frequency range of the obtained spectra is 0.01 Hz to 100 kHz. Z' and $-Z''$ are the real and imaginary components of the impedance, respectively. The high-frequency sections of the spectra are shown in the insets, with arrows indicating the directions of frequency increase.

Decrease in both the real and imaginary parts of the complex impedance was observed across the entire range of measurement frequencies (Fig. 1). The overall appearance of the impedance spectrum, with a clearly defined polarization branch whose slope decreases with decreasing frequency, corresponds to the equivalent circuit model described in previous work (Pryimak et al., 2021, pp. 1–11). This model includes three R-CPE elements in the initial stages of destruction or two R-CPE elements in the final stages, which aligns well with the charge ion transport model in an ordered system containing a number of equivalent elements corresponding to the number of homogeneous regions.

Using relation:

$$R = R' + iR'', \sigma = \frac{1}{R} = \frac{1}{R' + iR''} = \frac{R' - iR''}{R'^2 + R''^2} = \frac{R'}{R'^2 + R''^2} - i \frac{R''}{R'^2 + R''^2};$$

where $\sigma' = \frac{R'}{R'^2 + R''^2}$ and $\sigma'' = \frac{R''}{R'^2 + R''^2}$ are the real and imaginary parts of the system's complex electrical conductivity, respectively. The dependencies $\sigma'(\omega)$ for the selected group of temperatures are shown (Fig. 2). It can be noted that there is a gradual increase in conductivity with increasing temperature and frequency (Fig. 3a). On the other hand, there is a sharp increase in conductivity at 308 K during the 14-hour exposure, with the conductivity level remaining stable for other temperatures within the same time interval (Fig. 2b). The selected frequency range of 0.01 Hz to 100 kHz corresponds to low-frequency ion polarization mechanisms depending on their location relative to the plasma membrane (Abasi et al., 2022,

pp. 495–516) and the passage of current through it (Dean et al., 2008, pp. 165–177), which corresponds to α -dispersion. This result can be caused by an increased concentration of ions accumulated over a long period of temperature exposure in the membrane damage zone.

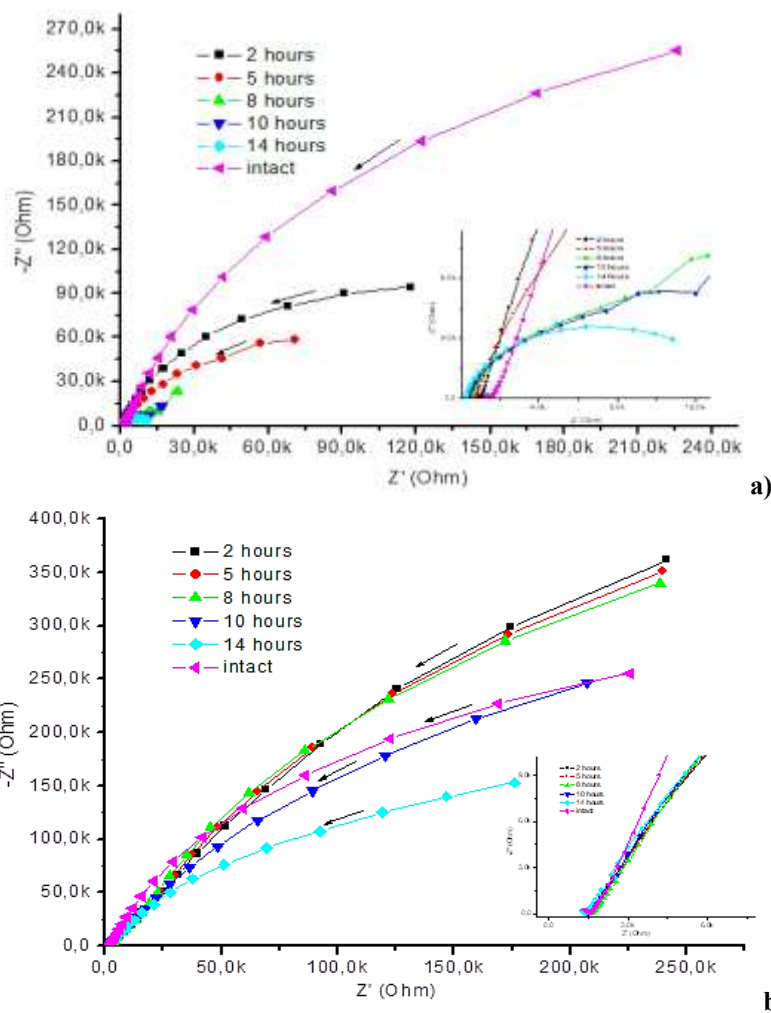


Fig. 1. Impedance spectra of liver tissues stored at a) 271 K, b) 308 K

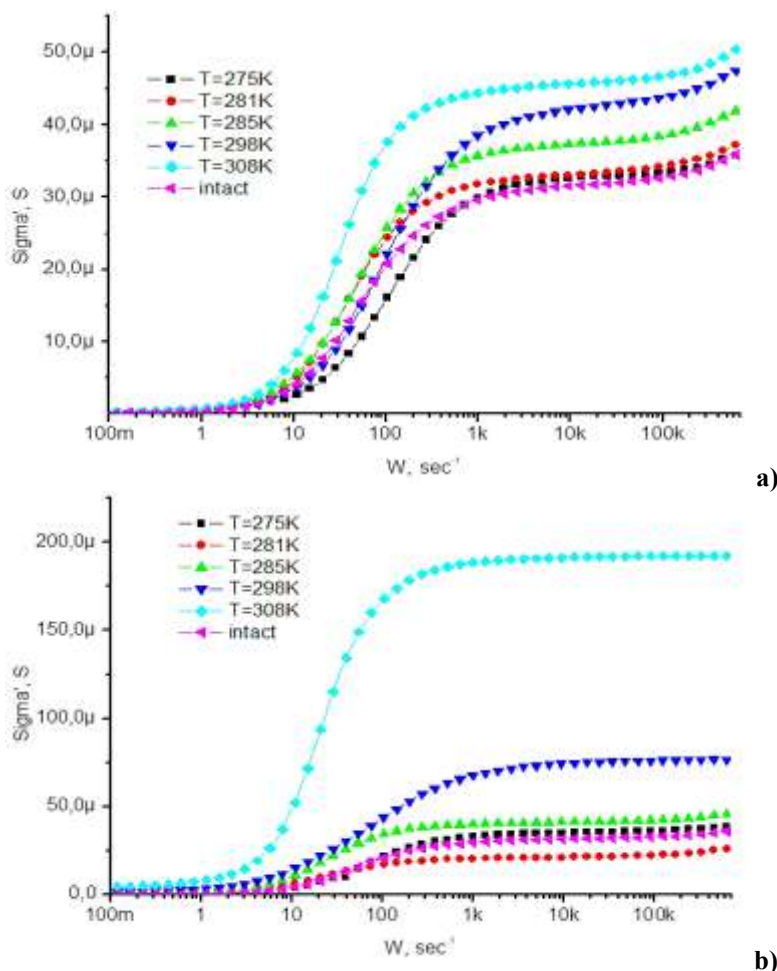


Fig. 2. Conductivity spectra dependence on the frequency of liver tissue samples exposed to temperature for a) 2 hours, b) 14 hours

In order to understand the characteristics of dipole reorientation processes under the influence of an alternating external sinusoidal electric field, the values of the dielectric loss tangent were calculated. The graphs of the dependence $\text{tg } \delta(\omega)$ are presented in Fig. 3. Nature of the dependence of the loss tangent on frequency is extreme. The increase in the minimum value of the loss tangent over time is due to the formation of a large number of dipoles of a certain size due to extensive destruction (Fig. 3a-b). Histological analysis of the samples under such temperature shows the formation of local tissue destruction, blurring of hepatocyte contours in liver samples, and an increase in intercellular spaces, which may indicate plasma membrane destruction and, consequently, a decrease in resistance and increased permeability. This is reflected in the change in the structure of the equivalent circuit from six- to five- and four-element models, particularly at 308 K (Pryimak et al., 2021, pp. 1–11).

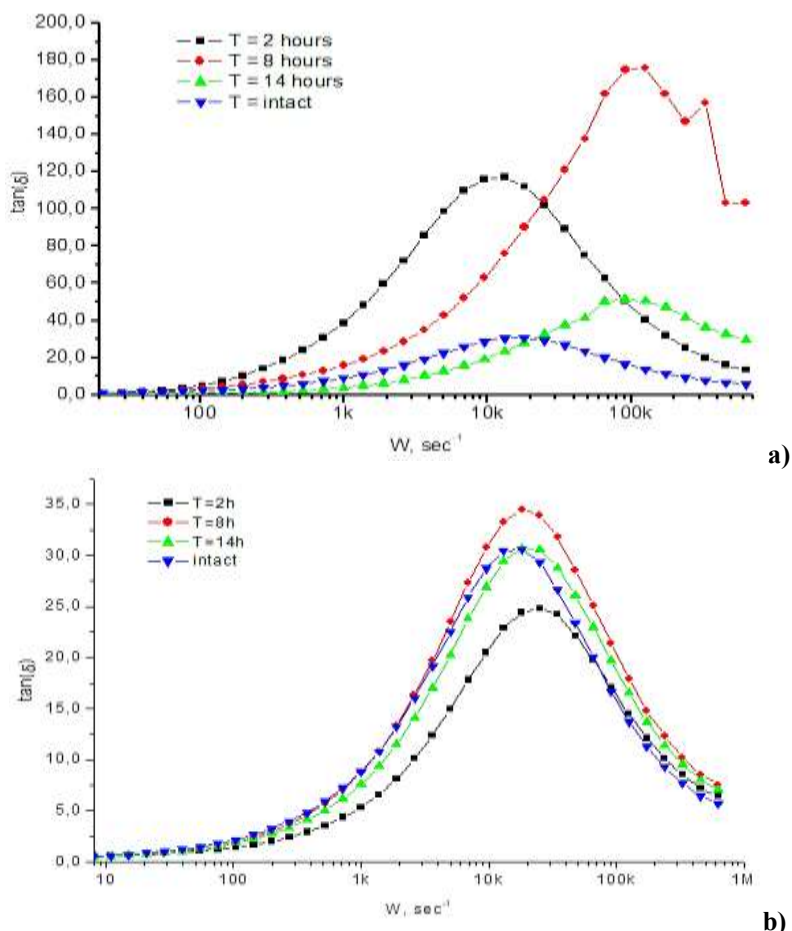


Fig. 3. Dependence of the loss tangent on frequency at a) 275 K, b) 308 K. The high-frequency region of the spectrum is shown in the inset

Increase in temperature, besides causing membrane destruction, is reflected in the increase in the loss tangent and resonant frequency, which is due to the increased number of small-sized dipoles, which can be parts of membrane components and elements of intracellular organelles. This corresponds to the reduced values of the CPE and R parameters of the samples under the influence of 298 K and 308 K, compared to intact samples (Pryimak et al., 2021, pp. 1–11). Additionally, at high temperature, membrane destruction results in a large number of dipole polarization sources, which also contributes to the increase in ω_{res} . Thus, the processes of tissue destruction under the influence of temperature, as a whole, are reflected in the decrease of the complex impedance in both its real and imaginary parts, which can be related to structural membrane destruction, thereby increasing its permeability (Fig. 2a-b). In turn, increasing of the concentration of charge carriers, both external

and internal, in the destruction areas contributes to the increase in conductivity (Fig. 2a-b) in alternating current, and the disappearance of distribution boundaries, which causes greater mobility of the accumulated ions. Elements of the destroyed membrane and the high concentration of ions leads to the formation of a large number of dipoles (Fig. 3a-b) of sufficiently small size, observed when an external alternating electric field is applied.

Discussion and conclusions

The study of the Nyquist diagram parameters of liver tissues stored over a certain time range at different temperatures confirms a two-stage destruction process. In the first stage, there is a change in the concentration and composition elements of charge carriers without prominent signs of morphological structure destruction. The second stage involves structural changes and evidently leads to irreversible destruction. Storing liver tissues at low temperatures (275–285 K) leads to minor changes in the ion subsystem, whereas increasing the temperature and duration of storage leads to significant tissue changes, which, in turn, is reflected in the kinetics of impedance spectrum parameters. The main indicators of the nature and degree of destruction can be the resonant frequency determined from the frequency dependence of the dielectric loss tangent. In the initial stages of destruction, the time dependence of ω_{res} has a decreasing character. The second stage of destructive changes is accompanied by a temporal increase in this value. This characteristic may, in the future, become a technologically controllable parameter for developing a rapid and non-invasive method for characterizing overall damage, potential changes in ion composition, and the degree of destruction of both cells and organs as a whole.

Authors' contribution: Ivan Gasiuk – conceptualization, methodology; Taras Pryimak – methodology, data validation, writing original draft, writing – revising and editing; Dmytro Chervinko – formal and data analysis, writing – revising and editing.

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ТРАНСФОРМАЦІЯ ЧАСТОТНОЇ ДИСПЕРСІЇ ЕЛЕКТРИЧНИХ ПАРАМЕТРІВ ТКАНИН ПЕЧІНКИ ЗАЛЕЖНО ВІД ТЕМПЕРАТУРИ ЗБЕРІГАННЯ

Вступ. Метод створення схем заміщення, який активно використовується в біоімпедансних дослідженнях, незважаючи на відносну простоту використання, не дозволяє сформувати загальну характеристику процесів транспортування іонів у тканинному середовищі при використанні змінного електричного струму та за умови наявності руйнівного впливу. У пропонованій роботі здійснено спробу аналізу частотної дисперсії комплексних електричних параметрів тканин печінки під впливом температури (2–35 °) з метою встановлення особливостей перенесення зарядів в її електролітній підсистемі тканин за різних ступенів структурної деградації.

Методи. Комірки для досліджень виготовляли з пластикового корпусу медичного шприца об'ємом 2 мл. Торці циліндричного зразка контактували з нікелевою сіткою. Струмовідводом слугували нікелеві провідники, приварені до сітки. Циліндричні зразки висотою 1,2 см, діаметром 2 см та масою 1–1,5 г поміщались у спеціально сконструйований, герметичний термостат 1/120 СПУ під вплив температур 2 °C, 8 °C, 12 °C, 25 °C та 35 °C протягом 2-х, 5-ти, 8-ми, 10-ти та 14 год.

Результати. Розглянуто зміни провідності за постійного струму, з частотною дисперсією дійсної складової провідності та тангенсом кута

діелектричних втрат у діапазоні частот 0,01 Гц – 100 кГц (діапазон β -дисперсії). Встановлено зв'язок трансформації термочасової залежності електричних параметрів від ступеня та характеру морфологічних змін у біологічній тканині.

Висновки. Показано, що локальні значення та напрямки монотонності функції часу поляризаційної релаксації у досліджуваному діапазоні частот вимірювання спектрів електричного імпедансу може слугувати критерієм ступеня та типу деструктивних змін тканин печінки при її зберіганні в атмосфері повітря за різних температур.

Ключові слова: *імпедансна спектроскопія, тканини печінки, провідність, температура, резонансна частота, тангенс кута втрат, бета-дисперсія, час релаксації.*

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