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# DIELECTRIC PROPERTIES OF HUMAN ERYTHROCYTES IN NORMAL AND CARCINOGENIC STATE

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The temperature dependence of the complex dielectric permittivity and the frequency of water molecules dielectric relaxation in the erythrocyte suspension and erythrocyte membranes of donors and oncological patients within the temperature range of 2-50 °C has been studied by the microwave dielectric method. The values of water molecules dielectric relaxation activation energy in these systems were calculated. It is supposed that the observed features of the dielectric parameters dependence on temperature accompanied by the change of activation energy are related to the dehydration of erythrocyte membranes of oncological patients.

**KEY WORDS:** erythrocyte, erythrocyte membranes, microwave dielectric method, dielectric permittivity, frequency of water molecules dielectric relaxation, activation energy.

At present numerous studies show that the basic symptoms of tumor growth in a human body at an early stage of the disease can be revealed by test results showing change of biochemical, structural and functional features of blood [1-4]. The quantitative and qualitative variations of the erythrocyte membrane composition are often found in malignant neoplasm [5, 6], they can in their turn cause changes in membranes organization and, as a consequence, changes in physical and physicochemical properties of membranes and entire cells [1, 3].

Since an erythrocyte is a fluid hydrophilic system, 65 % of its mass being water [7], structural reorganizations of membranes entail the change of free - bound water correlation and are accompanied by the change of the dielectric parameters of the cells. These changes are registered by the microwave dielectric method within the frequency range of water molecules dispersion and make it possible to use the measurements of dielectric permittivity of the biological objects as a physical criterion for the estimation of structural disturbances caused by various factors [8-11].

In this work the temperature dependence of the complex dielectric permittivity of erythrocyte suspension and erythrocyte membranes of healthy donors and oncological patients within the temperature range of 2-45 °C have been studied by the microwave dielectric method.

## MATERIALS AND METHODS

The erythrocyte suspension and erythrocyte membranes were obtained from the blood of healthy donors and oncological patients. The blood was taken by a puncture of a vein during the diagnostic period before any medical treatment. The studied group was composed of the patients with breast cancer (II stage) and lung cancer (II stage). The blood of healthy donors was taken as a control. The erythrocytes were trice centrifuged (3000g, 10 minutes) in an isotonic Na - phosphate buffer (pH 7,4), after which the cell suspension was prepared in the same buffer in the proportion of 1:2.

White erythrocyte membranes (i.e. those with a very low hemoglobin content) were obtained by the method described in [12]. The erythrocytes washed several times in the isotonic solution of chloride sodium (pH 7,4 was achieved by adding the Na – phosphate buffer), were subjected to hemolysis for 7 minutes at 0 °C in the hypotonic 5 mM Na – phosphate buffer containing 1mM EDTA, the erythrocytes – buffer relation being 1:40. The erythrocyte membranes were washed 3-4 times in hypotonic buffer by means of centrifugation at 15000g for 15 minutes. The centrifugate of the erythrocyte membranes was used for dielectric parameters measurements.

The real ε' and imaginary ε" parts of the complex dielectric permittivity $\varepsilon^* = \varepsilon' - i\varepsilon''$ of the erythrocytes suspension and erythrocyte membranes were measured by the microwave dielectric method at a frequency of 9,2 GHz [13] within the temperature range of 2-50 °C. The values of ε' and ε" were determined by using the calibration curves [14]. The corrections based on measurements of conductivity contribution were made for inorganic ions presence. The conductivity σ was measured at a frequency of 1 kHz [8].

On the basis of obtained values of ε' and ε" the frequency of water molecules dielectric relaxation $f_d$ in the erythrocytes suspension and erythrocyte membranes was calculated by using equation: $f_d = f\,(\varepsilon' - \varepsilon_\infty)/\varepsilon''$ (1), where $f_d$ - and f – are the frequency of water molecules dielectric relaxation and the frequency of microwave field, correspondingly, and $\varepsilon_\infty = 5{,}5$ – is the dielectric constant of water over the infrared frequency range [8,15]. The frequency of water molecules dielectric relaxation $f_d$ is a parameter which characterizes the mobility of water molecules in microwave field and, consequently, the degree of their interaction with surrounding

molecules. The temperature dependence of $f_d$ satisfying the equation $f_d = A \cdot e^{-\Delta E/RT}$ (A – temperature independent coefficient, R - absolute gas constant) makes it possible to determine the values of water molecules dielectric relaxation activation energy $\Delta E$ (or activation enthalpy) of studied systems by means of calculating the slope ratio in coordinates $\ln(f_d)$ - 1/T [16].

## RESULTS AND POINTS FOR DISCUSSION

The temperature dependence of the dielectric parameters $\varepsilon'$ and $\varepsilon''$ of erythrocyte suspension and erythrocyte membranes obtained from the donors' blood and the blood of oncological patients is shown in Fig. 1 and Fig. 2. The temperature dependence of frequency of water molecules dielectric relaxation $f_d$ in studied systems are shown in Fig. 3 and Fig. 4. The data on water molecules dielectric relaxation activation energy $\Delta E$ are given in tables 1 and 2.

The temperature dependences of $\varepsilon'$ and $\varepsilon''$ of the erythrocyte suspension and erythrocyte membranes have a number of particular features. The step changes of $\varepsilon'$ are observed in the erythrocyte membranes (Fig. 2) and suspension of erythrocytes in NaCl (Fig. 1) at temperatures 6-12 °C. The temperature dependence of dielectric losses $\varepsilon''$ for all samples is sigmoid (Fig. 1 and Fig. 2).

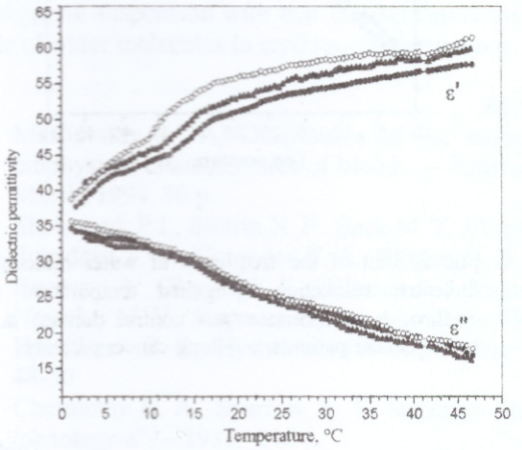


Fig. 1. Plot of ε' and ε" against temperature for erythrocyte suspension: ● – control donors; ▲ – breast cancer patients; o – lung cancer patients.

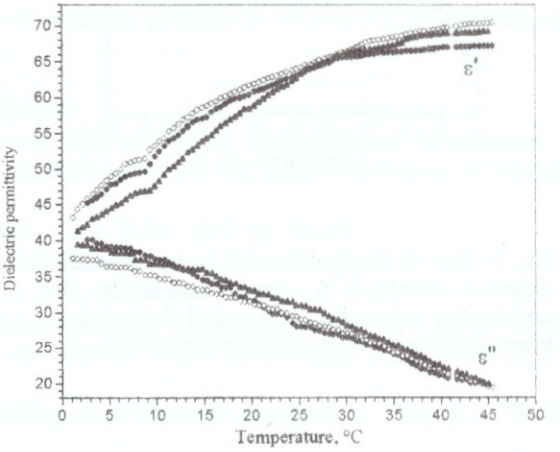


Fig. 2. Plot of ε' and ε" against temperature for erythrocyte membranes: ● – control donors; ▲ – breast cancer patients; o – lung cancer

The dielectric permittivity $\varepsilon'$ of the erythrocyte suspension of oncological patients exceeds the control values of the dielectric permittivity $\varepsilon'$ within the entire temperature range. It should be noted that the dielectric permittivity $\varepsilon'$ of the erythrocytes at temperature above 10 °C in case of lung cancer is much higher than $\varepsilon'$ of the erythrocytes in case of breast cancer (Fig. 1). The plot of the temperature dependence of $f_d$ demonstrates the rise in the frequency of the water molecules dielectric relaxation in the suspension of erythrocytes of oncological patients in comparison with control donors within the whole temperature range (Fig. 3). The decrease of $f_d$ at 6-12 °C in donors' erythrocyte suspension in comparison with $f_d$ of oncological patients can be explained by the appearance of additional hydrogen bonds which must mean that water molecules rotation slow done (Fig. 3) accompanyied by the change of the dielectric relaxation activation energy. In case of the donors' erythrocyte suspension, the increase in the activation energy by 18,8 kJ/mole at 12 °C and its decrease at 17 °C by 15,2 kJ/mole are observed (table 1). In the oncological patients' erythrocyte suspension the change of the activation energy is observed at temperatures 9-18 °C (breast cancer) and 23-36 °C (lung cancer).

In the erythrocyte membranes lung cancer patients the value of $\varepsilon'$ approaches the one of control patients while in the erythrocyte membranes of the patients with breast cancer the decrease of $\varepsilon'$ at temperatures 2 - 25 °C is observed (Fig. 2). The process is accompanied by a decrease in the frequency of the water molecules dielectric relaxation within the whole temperature range (Fig. 4). Such changes may be evidence of the increase in the amount of bound water in the erythrocyte membranes, possibly as a result of the membrane loosening in case of cancer disease. At the further temperature growth the increase in $\varepsilon'$ in oncological patients' erythrocyte membranes in comparison with that of healthy donors is observed. There is a decrease in the activation energy (table 2) by 10,4 kJ/mole for control patients at the temperature range 6-8 °C, and also by 1,4 kJ/mole for the suspension of the erythrocyte membranes of the patients with breast cancer and by 4,2 kJ/mole for the suspension of the erythrocyte membranes of the patients with lung cancer.

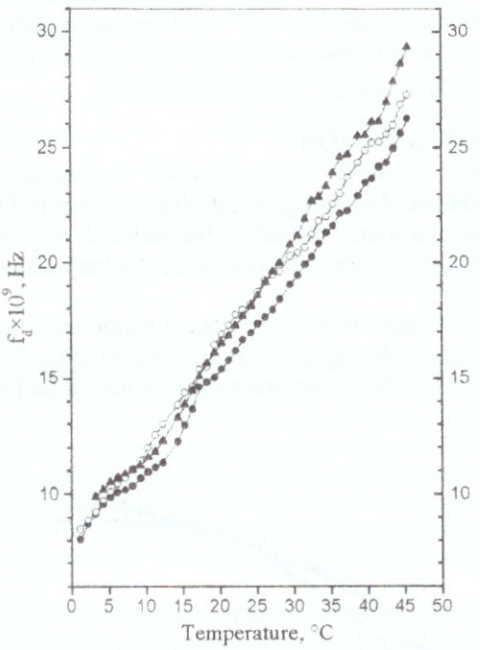


Fig. 3. Plot of the frequency of water molecules dielectric relaxation $f_d$ against temperature for erythrocytes suspension: ● – control donors; ▲ – breast cancer patients; o – lung cancer patients.

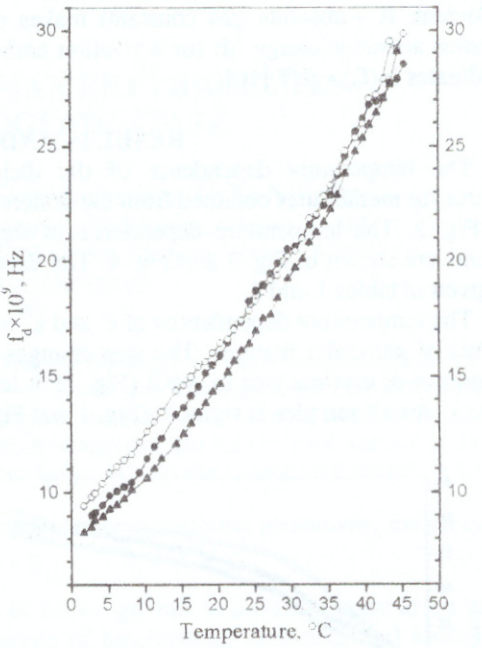


Fig. 2. Plot of the frequency of water molecules dielectric relaxation $f_d$ against temperature for erythrocyte membranes: ● – control donors; ▲ – breast cancer patients; o – lung cancer patients.

Table 1

Activation energies of water molecules dielectric relaxation in erythrocyte suspension

| Erythrocyte suspension (control donors) | | Erythrocyte suspension (breast cancer patients) | | Erythrocyte suspension (lung cancer patients) | |
|---|---|---|---|---|---|
| T, °C | ΔE, kJ/mole | T, °C | ΔE, kJ/mole | T, °C | ΔE, kJ/mole |
| 2-6 | 19,8±0,8 | 3-9 | 14, 7±0,6 | 2-23 | 21,95±0,5 |
| 6-12 | 12,6±0,8 | 9-18 | 24,2±0,8 | 23-36 | 13,39±0,5 |
| 12-17 | 31,4±0,6 | 18-32 | 19,3±0,6 | 36-42 | 18,29±0,6 |
| 17-42 | 16,2±0,6 | 32-42 | 13,7±0,6 | | |

Table 2

Activation energies of water molecules dielectric relaxation in erythrocyte membranes

| Erythrocyte membranes (control donors) | | Erythrocyte membranes (breast cancer patients) | | Erythrocyte membranes (lung cancer patients) | |
|---|---|---|---|---|---|
| T, °C | ΔE, kJ/mole | T, °C | ΔE, kJ/mole | T, °C | ΔE, kJ/mole |
| 2-6 | 21,7±0,5 | 2-8 | 19,3±0,5 | 2-5 | 22,0±0,5 |
| 6-8 | 11,3±0,6 | 8-15 | 17,9±0,5 | 5-8 | 17,8±0,5 |
| 8-25 | 24,0±0,5 | 15-42 | 21,5±0,6 | 8-15 | 21,9±0,6 |
| 25-36 | 13,5±0,5 | | | 15-32 | 15,9±0,6 |
| 36-42 | 31,0±0,6 | | | 32-41 | 22,2±0,6 |

The change of the inverse temperature function of $\ln f_d$ within 6-12 °C range, characteristic of suspension containing erythrocytes or their membranes, can mean structural reorganization of the erythrocyte membranes.

The increased value of $\varepsilon'$ of oncological patients' erythrocytes is obviously caused by a decrease in the amount of bound water in the systems, i.e. by the dehydration of the erythrocyte membranes. With the increase in amount of free water in erythrocytes, the change of its structure takes place, which becomes more similar to the structure of bulk water with its characteristic hydrogen bonds. Probably, one of the reasons for such differences is the physicochemical properties of native circulating erythrocytes of the patients with breast cancer and lung cancer. In particular, the changes in a lipid composition of their membranes can cause such differences [2-4].

## CONCLUSIONS

The increase of the frequency of water molecules dielectric relaxation in the erythrocytes suspension of oncological patients has been revealed.

It has been shown that the structural transitions of erythrocyte membranes at 12 °C are accompanied by the change in the proportion of free and bound water in erythrocytes.

It has been found that in the oncological patients' erythrocyte suspension the change of the activation energy is observed at temperatures 9 °C (breast cancer) and 23 °C (lung cancer).

So, comparison of the dielectric permittivity and frequency of water molecules dielectric relaxation in erythrocyte suspension with that in erythrocyte membranes make its possible to receive the information about state of water molecules in erythrocyte membranes.

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## ДИЭЛЕКТРИЧЕСКИЕ СВОЙСТВА ЭРИТРОЦИТОВ ЧЕЛОВЕКА В НОРМЕ И ПРИ КАНЦЕРОГЕНЕЗЕ

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Методом СВЧ-диэлектрометрии исследованы зависимости диэлектрической проницаемости и частоты диэлектрической релаксации молекул воды в суспензии эритроцитов и эритроцитарных мембранах онкологических больных от температуры в области 2-45 °С. Вычислены значения энергии активации диэлектрической релаксации молекул воды в данных системах. Предполагается, что наблюдаемые особенности зависимостей диэлектрических параметров от температуры, сопровождающиеся изменением энергии активации, связаны с дегидратацией мембран эритроцитов онкологических больных.

**КЛЮЧЕВЫЕ СЛОВА:** эритроциты, эритроцитарные мембраны, СВЧ-диэлектрометрия, диэлектрическая проницаемость, частота диэлектрической релаксации, энергия активации.