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STUDY OF HUMAN ERYTHROCYTES EXCESS DIELECTRIC CONSTANT

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ABSTRACT
Dielectrophoresis, a new technology, was used in this work to measure the Excess Dielectric Constant of Human blood samples. Using a wire-wire electrode configuration, the erythrocyte samples are suspended in glycine-glucose solution and subjected to 1 MHz Non-Uniform Electric Field. DCR is determined for the erythrocyte sample based on the applied frequency and voltage. The Excess Dielectric Constant (K) is determined using DCR. A better knowledge of human cell dielectric behavior and other electrophysiological alterations may be gained via research into the excess Dielectric Constant (EDC).

KEYWORDS: Excess Dielectric Constant, Dielectrophoresis, Wire-Wire Electrode Configuration, Human Erythrocytes, Glycine-Glocose Medium.

INTRODUCTION

In a non-uniform electric field, the polarization effects lead matter to move translationally, and this is known as Dielectrophoresis or Di electrodynamics (a.c or d.c). Different field geometries may be used to produce an electric field that is not uniform. Pohl and Pylmale were the first to describe the use of non-uniform electric field effects on biological materials, often known as biological dielectrophoresis. Pohl and Hawk were the first to separate live cells from dead cells. Mason and Twonsley used the DEP approach to differentiate live cells and organelles of the same species that differed only in nutrition. To discover a single cell's excessive permittivity, Chen and Pohl devised a method known as single cell dielectrophoresis. The yield spectra of single cell organisms and organelles were used by Pohl to identify them. Diffusion coefficients of proteins in solution were calculated by Eisenstadt and Schienberg. Dielectrophoretic examination of biological matter at the cellular, particle, and molecular levels was performed by Pohl and his colleagues. For the yeast organism saccharomyces cervisiae, Gopala Krishna and his colleagues calculated the dielectrophoretic collection rate (DCR). Dynamic and static single cell dielectrophoresis were used to measure the excess permittivity of individual yeast cells. Using dielectrophoretic spectroscopy and spherical field geometries, Gopala Krishna et al. investigated the behavior of erythrocytes from several locomotional animals.

Various biochemical and biophysical features of human red blood cells have been studied by scientists. Blood cells transfer oxygen from the lungs to the cells of the body and remove carbon dioxide. Human erythrocytes' Dielectrophoretic characteristics have been thoroughly investigated. Excess permittivity, among other biophysical features, has also been examined in several studies. The membrane structure of human red blood cells has been studied using a variety of experimental methods. Through the use of the Dielectrophoresis method, the current innovative computer-aided approach studies the dielectric behavior of human red blood cells.

LITERATURE REVIEW

S. Abdalla et. al, (2022): In order to accurately identify diabetes, the glycated hemoglobin (HbA1c) is widely acknowledged as an effective marker of glucose accumulation over a given length of time. HbA1c levels have been measured using broadband dielectric spectroscopy (BBDS) on human blood samples, both normal and diabetic (NGBSs). This methodology has improved our knowledge of glycation processes in both academic and medical contexts, such as the quest for a novel method of treating diabetes by reducing the additional electric charges that are generated by glycation processes, for example. With the use of BBDS, the dielectric characteristics of NGBSs are examined, focusing on how BBDS changes with the glycation growth rate (GG). From 101 Hz to 3.2 107 Hz, we've covered a wide spectrum. Strong beta-relaxation (Debye-relaxation) has been clearly found as a result of the RBCs induced oscillations in that frequency band. As an added bonus, we've developed a theoretical model to help explain our findings. As a result, we have several theoretical (and experimentally validated) relationships that describe the temporal course of HbA1c growth: Different HbA1c concentrations were studied for 140 days to determine the ac-electrical conductivity and dielectric constant as a function of frequency. The results of the experiments show that glycation increases the electric charges on RBCs. Glycated blood might be treated with this technology, which would reduce the additional charges on RBCs that build owing to their glycation, and so completely abolish

glycation. The wide frequency range provides important new information about dynamic processes in NGBSs.

Sylvester J. Gemanam et. al, (2021): Diabetic and non-diabetic human blood relatives' dielectric properties are clarified in the research study, which is crucial for blood studies and functional treatment processes employing He-Ne laser irradiation. The study. Impedance analyzers and cuvette cells with two electrodes each were used for the examination of 48 human blood samples (24 diabetes blood with a glucose level of 9.28 mmol/L and 24 non-diabetic blood) taken by venipuncture in this study. The 532 nm He-Ne laser of 60 mW power output was used for irradiations lasting 5, 10, 15, and 20 minutes. Dielectric constant, dielectric loss, and electric conductivity were all shown to be much greater in diabetes blood than non-diabetic blood in the control group, despite the fact that the diabetic blood had a lower impedance. After 5 and 10 minutes of irradiation exposure, the dielectric properties of diabetic blood exhibit lower values with a considerable increase in impedance. A sudden decline in impedance was seen when diabetes blood was exposed for 15 and 20 minutes because the dielectric properties greatly increased. When it comes to erythrocyte membrane dielectric characteristics and impedance levels, the observed wide range of values may be explained by erythrocyte shape, physiological circumstances, and other aspects of the erythrocyte's electric response. He-Ne laser irradiation is a safe and effective treatment for diabetes mellitus, as shown by the beneficial biostimulation effects of the irradiated diabetic blood.

Mohd. Abdul. Malik et. al, (2017): Loan cell dielectrophoresis was used to study the dielectric constant of human blood samples from groups A, B, AB, and O. A non-uniform electric field (NUEF) at 1 MHz, either by wire-wire electrode configuration or cylindrical field geometry, is used to detect the velocity of an individual erythrocyte suspended in glycine-glucose solution. Erythrocyte velocities and applied voltages are used to calculate excess dielectric constant. Other factors, such as electrode chamber size and electric field intensity, are also taken into consideration. All blood types have about the same amount of extra dielectric constant in erythrocytes.

Ateeba Shaz et. al, (2015): Researchers in the current work used a pin – pin electrode arrangement to create a non-uniform electric field (NUEF) to test the effects of diabetic erythrocyte suspensions in glycine–glucose solution. At the same voltage, the elapsed time, and the cell concentration, the yield or Di electrodynamic collection rate (DCR) of erythrocytes is measured. Knowing the yield and the micropolar parameter, one may calculate the erythrocyte excess permittivity. When compared to normal erythrocytes, the data is analyzed. According to the findings, diabetic erythrocytes have a lower dielectric constant than healthy ones.

Kotb MA et. al, (2014): Understanding the dielectric characteristics of cells and their structural components (membrane, cytoplasm, etc.) is critical for understanding cell structure, function, and metabolic pathways. Workers who had been exposed to benzene had their RBCs isolated by centrifugation and rinsed with phosphate buffer and adjusted to pH = 7.4, 290+ 3 mg/kg, and 50 percent hemoglobin value in their blood. A-c voltage in the 50Hz-1MHz range was used to study the relative permittivity ', dielectric loss ", dielectric strength, relaxation duration, and the ac conductivity of these cells. The data obtained show that the RBCs of the benzene-exposed employees showed substantial alterations in all of the examined electric parameters, including real conductivity, real and imaginary relative permittivity, relaxation time, dielectric loss, and dissipation factor.

Ajay Vasishth et. al, (2013): Human erythrocytes with normal and diabetes mellitus excess permittivity is studied in this research. Pin-pin electrode design produces a non-uniform electric field (NUEF) on erythrocyte suspensions from normal and diabetic blood in the current study. Samples were obtained from at least 40-45 people with normal blood and diabetes, and the results were taken into account. Excess permittivity is affected by changes in dielectric characteristics throughout the body, according to the data. Excess permittivity of diabetes individuals' erythrocytes varies widely, on average.

METHODOLOGY

A siliconized glass vial is used to collect 1-2 ml of blood samples from healthy volunteers. The anticoagulant EDTA (Ethylenediaminetetraacetic acid) is included in these medications. A temperature of 4°C is then used to keep samples safe until the experiments can be performed, which is typically within an hour of when they are collected. The human erythrocytes are extracted from the plasma by centrifuging the blood sample at 1500 RPM for around 15 minutes as part of sample preparation. An isotonic glycine and glucose solution is used to cleanse the blood cells. In a 9:1 volume ratio, isotonic glycine makes up 2.1% while isotonic glucose makes up 5.5%. A 500-fold dilution is the most common. RBCs are counted using a Spectrophotometer, Sysronics 106, which measures the optical density of the cells. Dielectrophoretic studies often utilize Spherical, Isomotive, or Cylindrical geometrical fields to generate NUEF (Non-Uniform Electric Field). Field geometry is spherical for electrodes with round tips. Regardless of the field shape, the electrode chamber setup is the most

critical and sensitive element of this experiment. Spherical field geometry is the focus of the present investigation. To test its effectiveness, human erythrocytes are incubated with the NUEF produced by this field. There are two types of electrode chamber: wire-wire and pin-pin. A pair of Platinum wires are arranged in a wire-wire electrode chamber and separated by a little distance. A wire-wire electrode chamber is employed in this scenario. One millimeter above the glass slide's surface, a pair of 400-m-diameter platinum wires are positioned such that their axes are parallel and their tips face one another. They are separated by 750 micrometers. A non-conducting ring is used to guide the platinum wires (with internal diameter of 1cm). The electrode chamber is made up of a glass slide on which the ring is mounted. The blood cell suspension will fit in this chamber to a depth of around 0.3 milliliters (Fig.1.). Applying an alternating current (AC) voltage between these electrodes will result in the NUEF.

Figure 1. Electrode Chamber of Dielectrophoresis Studies.

Figure 2 depicts the experimental set-up used to conduct the dielectrophoretic investigation on human blood cells. It's possible to see the erythrocyte collection or pearl chain creation at the electrode tips using a wire-wire electrode chamber fitted on a trinocular microscope (Labo Triumph 90066). A video camera and a video monitor are attached to this microscope as part of a CCTV system. The microscopic observations and measurements are made easier with the help of this CCTC system. Erythrocyte motility may be readily visualized, and observations can be made over extended periods of time with this technology.

Figure 2. Experimental Setup of Dielectrophoresis of human erythrocytes.

These electrodes are attached to a signal generator with a frequency range of 1 kHz to 20 kHz. Using a Digital Storage Oscilloscope, the signals may be stored and analyzed. In the electrode chamber, a human blood cell suspension sample is placed, and the signals are applied to the electrodes. Dielectrophoresis collects cells at the electrode tips, forming a pearl chain.

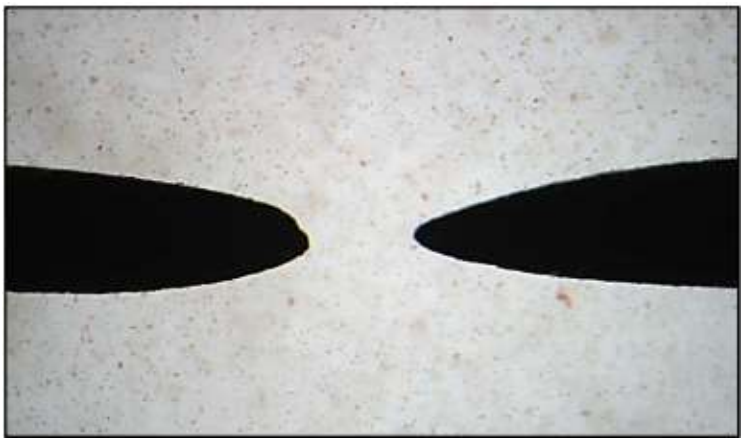

Figure 3. (a) Human erythrocytes at the electrodes without applying Electric Field

Figure 3. (a). There is no electric field applied to the electrodes at this point in the experiment. The computer-aided setup is used to obtain the baseline readings. The blood cells create a pearl chain at the electrode tips when an electric field is applied, owing to the phenomenon known as mutual dielectrophoretic.

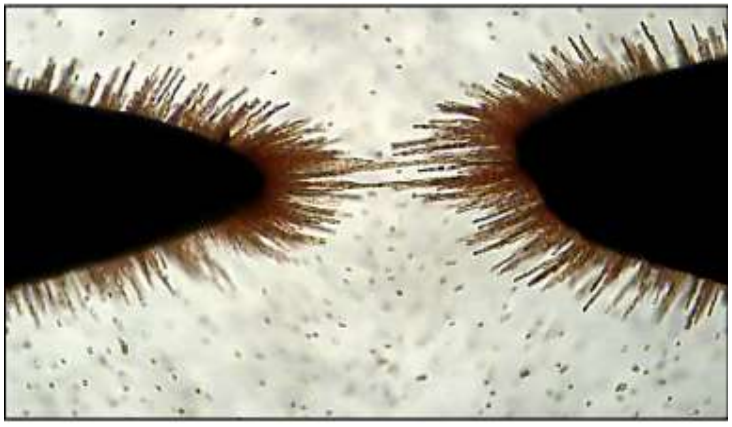

Figure 3. (b) Human erythrocytes forming pearl chains at the electrodes on application of Electric Field.

ANALYSIS

DCR (Yield) and Excess Dielectric Constant (EDC) values for human erythrocytes are shown in Table-1 for various blood samples. The excess dielectric constant of erythrocytes belonging to various human blood samples may be seen in the Table and graph. A lossy dielectric is found in all biological cells, which is why polarization and conductivity are found in all cells. The dielectric characteristics of these live cells are very difficult to test, and the normal methods cannot be used. Dielectrophoresis, thus, may be a viable choice for conducting dielectric tests on biological cells that are suspended in a suitable media. With this new computer-aided setup, you can also measure the excess dielectric constant of a single cell hanging in an isotonic solution. Human erythrocyte excess dielectric constants are determined using DCR information in this study (Yield). The dielectric properties of erythrocytes from various blood samples seem to be unaffected by antigens found on the membrane of human erythrocytes.

Table 1: Data on DCR (Yield) and Excess Dielectric Constant of human erythrocytes for different blood samples

S.No	Sample Code	DCR(μm/min)					Excess Dielectric Constant (ε^K)
		1	2	3	Mean	S.D (±)	
1	SC1	75	75	75	75	0.00	1.28
2	SC2	70	70	70	70	0.00	1.12
3	SC3	70	70	70	70	0.00	1.12
4	SC4	80	80	80	80	0.00	1.46
5	SC5	70	70	70	70	0.00	1.12
6	SC6	70	70	70	70	0.00	1.12
7	SC7	80	80	80	80	0.00	1.46
8	SC8	80	80	80	80	0.00	1.46
9	SC9	70	70	75	72	2.89	1.18
10	SC10	70	70	70	70	0.00	1.12

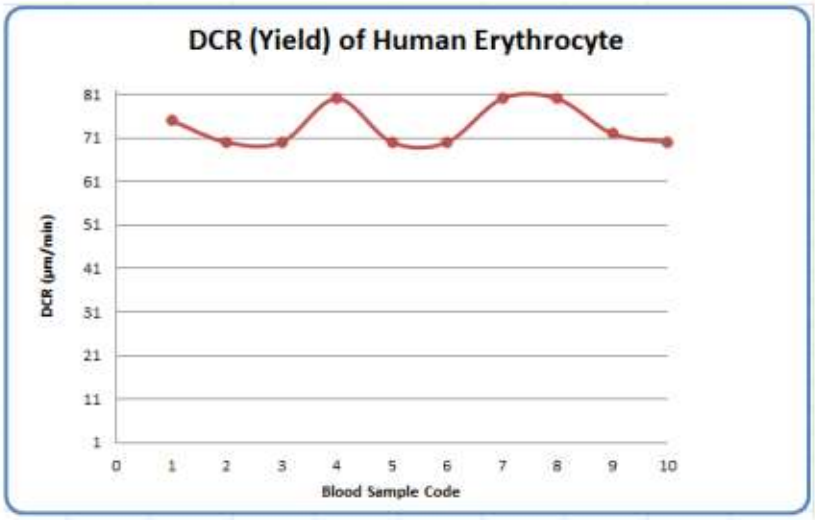

Figure 4 (a). DCR spectrum of Human Erythrocytes of different Blood samples.

For various blood samples, Fig.4 (a) depicts the plot for the Dielectrophoretic collection of human erythrocytes for the applied voltage. The DCR values for various sample codes don't vary much. The dielectric properties of erythrocytes from various human blood samples were shown to be unaffected by antigens found on the membrane of human erythrocytes.

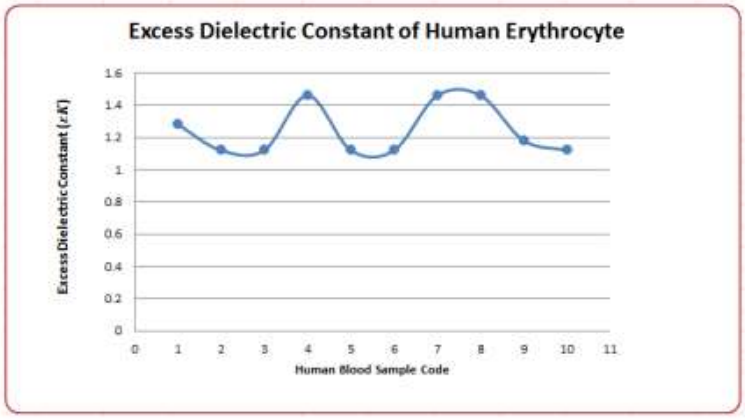

Figure 4 (b). Excess dielectric constant spectrum of Human Erythrocytes of different Blood samples

Excess Dielectric Constant (K) on the Y-axis and the codes for human blood samples on the X-axis are shown in Fig.4 (b). According to the graph, erythrocytes from various human blood samples have about the same excess dielectric constant.

CONCLUSION

Understanding the dielectric behavior of human blood cells and other electrophysiological changes is made possible by studying this parameter, Excess Dielectric Constant. Non-diabetic blood had greater impedance levels and lower dielectric properties compared to the control diabetic blood. When irradiated diabetes blood was subjected to irradiation for 5 and 10 minutes, the dielectric properties of the irradiated diabetic blood were greater than when the blood was treated for 15 and 20 minutes. Diabetes mellitus may be treated safely and effectively with low-level laser irradiation (LLLI).

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