Frequency characterization of dielectric parameters and impedance of irradiated diabetic blood using He-Ne laser and non-diabetic human blood samples

Sylvester J. Gemanam1,2*, Nursakinah Suardi1, Barnabas A. Ikyo2, Samson Damilola Oluwafemi1.

1 School of Physics, Universiti Sains Malaysia (USM), Pulau Pinang, Penang, 11800, Malaysia.
2 Department of Physics, Faculty of Science, Benue State University, Makurdi, 102119, Nigeria.

Email: gemanamsly@gmail.com; nsakinahsuardi@usm.my

Abstract. The research study elucidates the dielectric parameters of both diabetic and non-diabetic human blood relatives to frequency which are paramount for blood study and functional therapeutic mechanisms using He-Ne laser irradiation. In the work, the Agilent 4294A impedance analyser within frequency range 40 Hz – 30 MHz and designed cuvette cells with two electrodes each were used for the analysis of 48 human blood samples (24 diabetic blood of glucose level 9.28 mmol/L and 24 non-diabetic blood) collected through venepuncture. Irradiations were done with 532 nm wavelength He-Ne laser of power output of 60 mW for 5, 10, 15 and 20 minutes’ durations. The studied dielectric parameters (dielectric constant, dielectric loss and electric conductivity) demonstrated significantly high values in the control diabetic blood with low impedance level compared to the control non-diabetic blood which demonstrated higher impedance level. The irradiated diabetic blood shows reduced values of the dielectric parameters after irradiation exposure durations of 5 and 10 minutes with significant increase in impedance level. While the dielectric parameters significantly increased when exposed diabetic blood for 15 and 20 minutes became crenated therefore abruptly experienced a dropped in the impedance level. The observed significant variation in dielectric parameters and impedance levels of both blood could be recognised as the result of cellular morphology and the physiological conditions of erythrocytes membranes and other constituents which characterizes its electric response. The irradiated diabetic blood demonstrates positive biostimulation effects due to the enhance stimulation ability of the He-Ne laser irradiation as a safe and effective therapeutic modality for diabetes mellitus.

Keywords: Dielectric parameters, Diabetic blood, Impedance analyser, Human blood, Laser power, He-Ne laser.

1. Introduction.
The dielectric parameters of the human blood have been a recent challenge over this century as it is a painstaking researching studies with alternating current in the frequency domain, intending to elucidates the clinical applications of most medical equipment (Beving, et. al., 1994; Rauf, 2013). These dielectric parameters of substances are that inherent molecular properties in all substances capable of impeding the movement of electrons when there is an applied electric field. There are important in many scientific techniques, such as dosimetry, research tools design, understanding of electromagnetic medical concepts and studying mechanism of photons interactions with biological tissues. The analyses of the
fundamental dielectric occurrence enable the possibility of studying the behaviour and impedance levels of biological membrane and that of blood (Suardi, et. al., 2016; Gemanam, et. al., 2020).

The human blood is a heterogeneous mixture that constitutes of erythrocytes, leucocytes, plasma etc. The plasma probably constitutes the most diverse varieties of biological constituents among tissues, some being intrinsic to the organs (lipsids, hormones, amino tissues, proteins, antibodies, ions and adjusting factors etc.), exogenous substances or component substances (clothing and defense enzymatic systems, such as compliment, etc.) (Raaf, 2013; Gemanam, et. al., 2020). Blood serves as the primary transport medium of the body, conveying oxygen, and nutrients, messages to the different tissues and the same time waste products and CO₂ to the excretion organs (Raaf, 2013). It is also known as a fluid connective tissues that plays numerous important roles in coordinating the discrete cells into a common complex organism. Any ailment such as diabetes mellitus, anemia, HIV, cancer, etc., that affects the human body is intertwined between the blood and the cells (tissues) therefore affecting its dielectric parameters (Ansari, et. al.,2015).

Diabetes mellitus is an intricate, dreadful ailment as well as progressively health problem that increased with incidence worldwide. By the year 2030 the World Health Organization (WHO) estimated 366 million people will be diagnosed with diabetes (5% of the world population) (Desouky, 2009).

Diabetes mellitus results in impaired lipid profile, especially increased susceptibility to lipid peroxidation, which is responsible for the increased incidence of atherosclerosis, a main complication of the diseases (Desouky, 2009). It can usually be referred to as diabetes and known as abnormal glucose in the blood. The modality for diabetes diagnosis are symptoms of hyperglycemia and plasma glucose value of Fasting plasma glucose ≥7.0 mmol/l (total blood glucose ≥11.1 mmol/l or 2-h plasma glucose ≥11.1 mmol/l during oral glucose tolerance test (OGTT) (Thoren, et. al., 2014). According to Desouky (2009), high glucose values cause a toxic effect on erythrocytes, which leads to restructuring of erythrocytes membranes and electrolytes imbalance cell membrane. This has some consequential changes on the blood impedance levels and its dielectric parameters.

According to Zhao, (1996), the blood electrical impedance can reject certain diseases that involve abnormal compositions of certain plasma proteins, therefore results in impedance change which might be a useful index for evaluating blood physiology and the quality of stored blood. Dielectric spectroscopy (DS) is very sensitive to small changes. Ansari, et. al., (2015), reported that blood exposure to lower level laser irradiation has the medical effects which are mainly systemic healing mechanisms such as biostimulative, antibacterial, analgesic, immune corrective, anti-allergic, antitoxic, vasodilative, antiarrhythmic, antihypoxic, spasmylytic, anti-inflammatory, and other properties. It is safe and effective therapeutic modality for decreasing blood sugar level in diabetic mellitus (Ansari, et. al., 2015; Soheila, et. al., 2011).

The term blood irradiation therapy is considered as a method whereby blood is being exposed to mostly low level laser light for the purpose of treatment thru biostimulation. Low level laser light irradiation (LLL) has the ability to be non-thermal and non-destructive cell function changes and also known as laser biostimulation which is fundamentally used in some medical field cases (Moshkovska and Mayberry, 2005). The effects of laser biostimulation are based on the promotion of both weak anti-inflammatory cellular activity and poor spontaneous regenerative activity. The basic mechanism is the increased synthesis of adenosine triphosphate (ATP). Lowering ATP synthesis early results in a higher positive laser effect. Laser biostimulation improves fatigue cells repair and improves the microscopic deficit for skin-resistant therapies and mucosal ulcer; arteries, veins, microcirculatory, and lymphatic circulation deficit; metabolic and neurogenic insufficiencies; and infection resistant (Sowers, et. al., 2017 and Soheila, et. al., 2011).

The experimental results from different studies on the frequency-dependent dielectric parameters of cell suspensions are normally interpreted in terms of simple electrical components giving rise to a β-dispersion due to the Maxwell-Wagner effect (Beving, et. al., 1994). The erythrocytes suspension in the simplest model is treated as an equivalent circuit for the blood measured capacitance and conductance (Davey and Kell 1989; Kell and Davey 1990). According to Kim, et. al., (2012), the blood impedance reveals the heterogeneous characteristics of the blood, such as β-dispersion. Within the measured range of blood impedance, the magnitude of the impedance decreased owing to the passage of electrical current through the RBC membranes and the physiological condition of the blood. Beving, et. al., (1994), studied the dielectric properties of human red blood cells (erythrocytes) suspended in whole blood and in isotonic media at various volume fractions (haematocrits) have been studied in the
frequency range of 0.2-10 MHz, in which the so-called β-dispersion due to the Maxwell Wagner effect is known to occur. The capacitance and conductance at 25 °C were measured by an instrument interfaced to a computer. The conductivities behaved in a predictable manner with a mean of 0.458 S.m⁻¹ whereas the value of membrane capacitance (Cm) (and indeed the actual capacitance of the suspension) was dependent on the amount of plasma present. Hence, in stationary normal (anticoagulated) whole blood samples, Cm was as high as 2.98 μF.cm⁻² in contrast to about 0.9 μF.cm⁻² in blood diluted more than two-fold (to less than 20% hct) in isotonic media. The high apparent Cm value in stationary samples to be a result of rapid cell aggregation in the presence of plasma, where rouleaux formation takes place before visible sedimentation sets in.

Koht, et. a., (2014), studied the dielectric properties of cells and the structural parts of the cell (membrane, cytoplasm, etc.) for the benzene exposed workers and that of the normal blood (control). The relative permittivity ε'c, dielectric loss ε"c, dielectric strength Δεc, relaxation time τ and the ac conductivity σac of these cells were studied using an a-c voltage in the frequency range 50Hz -1MHz. The results obtained indicate that all the studied electric parameters, including real conductivity, real and imaginary relative permittivity in addition to the relaxation time, dielectric loss and the dissipation factor suffered significant changes in the RBCs of the benzene exposed workers than that of the control subjects.

According to experimental results by Asami, et. al., (1976), the electrical properties such as Cm, dielectric increment (ΔK), of suspended yeast cells in potassium chloride (KCl) solution by the 'bridge' method in within the frequency range of 1 kHz -100 MHz. The obtained Cm was 1.1 μF/cm². The authors also developed the yeast cell model to support their results. Research by Rauf, (2013), reported the electrical properties of human blood and plasma for different physiological and environmental conditions. The dielectric parameters include dielectric constant, dielectric loss and electrical conductivity of human blood and plasma of groups A, B, AB and O been measured at the frequency of 1kHz with digital LCZ meter. They found high values of these parameters in plasma, low in 90% packed erythrocytes and hemolysed blood, and in between in whole blood irrespective of blood group. Groupwise differences in dielectric parameters in the case of plasma are not evident, but could be found in whole blood, hemolysed blood and 90% packed RBC. Their work reveals the significant variation in dielectric parameters could be attributed to the cellular concentration and also due to the presence of erythrocyte membrane.

Research by Santorelli, et. al., (2018), measured dielectric properties of whole blood samples for the investigation of anaemia at microwave frequencies (500 MHz–8.5 GHz) and reported hemoglobin (Hgb) as the component of the blood that show to be the biggest predictor of changes in complex permittivity, demonstrating that permittivity measurements at a single frequency can potentially be used to detect anaemia. In this present work we have sought to unravel how high blood glucose influence diabetic blood cells, the cellular structures effect on dielectric parameters due to the insulin conditions of the blood. As it is known to affects the cell structure and proteins which may directly or indirectly affects the dielectric parameters of the blood. Also the LLLI biostimulation effects on diabetic blood as a therapy for diabetes mellitus.

2. Experimental Research Materials and Methods.

2.1. Samples collection
A total number of 48 human blood samples (24 diabetic blood of glucose level 9.28 mmol/L and 24 non-diabetic blood), all from 38 males and 16 non-pregnant females collected through venepuncture. The work approval was given by the University clinic management and controls as well as Human Ethics approval from JEPeM USM, under study protocol code of USM/JePeM/16060208. The blood samples were collected inside anticoagulant Ethylene Diameate Tetra Acetic acid (EDTA) treated tubes through venous blood from well informed participants of total average of 35.3 ±3 years old.

2.2. Samples preparation and Experimental Evaluations
The blood samples morphology and physiology conditions of the two grouped samples (diabetic and non-diabetic blood) were properly examined using optical microscopy connected to the desktop scanner (Suardi, et. al., 2016; Gemanam, et. al., 2020). The control and irradiated groups for both of the blood
samples were identified and the irradiation was carried out for the irradiated group using He-Ne low level laser of 532 nm wavelength. The laser power of irradiation was 60 mW (intensity of 4761.905 W/m²) for 5, 10, 15 and 20 minutes’ exposure duration.

The research work used the Agilent 4294A impedance analyser within frequency range of 40 Hz – 30 MHz at oscillation level of 500 V and designed cuvette cells with two electrodes each for the analysis. The electrodes of area “A” 0.36 cm² and separating distance “d” of 0.59 cm from each other were loaded with the 1millimetre blood samples. The instruments were used to measure the capacitance, $C_p$ and dissipation factor $D_f$ of the blood samples with a delayed time of 0.05 sec as shown in Figure 1.

The double layered cell membrane’s insulation property is considered equivalent to the dielectric material of a capacitor in an electric circuit, while the conductive cytoplasm recognised as a resistor. The interfacial phenomena serve as an electrical double layer between an electrode and electrolytic solution (plasma), which as well modelled as a capacitor. Thus combining the mechanisms of an electric double layer, cell membrane, and cytoplasm, formed the equivalent electrical circuit and was used in modelling and impedance reading and evaluations (Pradhan, et. al., 2012).

The blood samples impedance values, $Z$ and conductance were calculated to determine the blood samples dielectric response using a designed blood cuvette in Figure 1 as sample holder with two opposite pure copper electrodes connected to the impedance analyser (Bortner, et. al., 1997; Collins, et. al., 2004; Cotler, et. al., 2016; Farkas, et. al., 2013; Kelemen, et. al., 2001; Adam, et. al., 2016).

The dielectric permittivity, $\epsilon^*(\omega)$ is expressed as a complex number,  
\[ \epsilon^*(\omega) = \epsilon'(\omega) - i\epsilon''(\omega), \]  
where $\epsilon'(\omega)$ is the real part known as relative permittivity or dielectric constant and $\epsilon''(\omega)$ the imaginary part, represents the loss factor as functions in angular frequency, $\omega$. The dielectric constant $\epsilon'$ was evaluated using (Kotb, et. al., 2014; Gemanam, et. al., 2020; Collins, et. al., 2004) 
\[ \epsilon'(\omega) = \frac{C_p d}{\epsilon_o A}, \]  
where, $d$ is the distance between the two electrodes, $\epsilon_o$ is the permittivity of free space, and $A$ is the electrode cross-sectional area.

The imaginary part of the dielectric constant, $\epsilon''$, was ascertained using the relations (Bhat, et. al., 2016; Sowers, et. al., 2018; Soheila, at. al., 2011; Matthias and Andrew, 2013). 
\[ \epsilon''(\omega)=\epsilon'\tan\delta \]
\[
\sigma = \varepsilon'' \omega \varepsilon_0 \quad (4),
\]
\[
\sigma = \varepsilon'' \omega \varepsilon_0 \quad (5),
\]
where, \( \sigma \) is the conductivity of the medium or tissue (S/m), \( \varepsilon_0 = 8.854 \times 10^{-12} \text{F.m}^{-1} \), \( \omega = 2\pi f \) (where \( f \) is the frequency in Hz) (Jawad, et al., 2011; Rosenthal, 1948; Bhat, et al., 2013).

Therefore, the real and imaginary impedance (\( Z' \) and \( Z'' \)) are obtained from equations 6 and 7 respectively,
\[
Z' = \frac{G}{\sigma^2 + C_2 \omega^2} \quad (6)
\]
\[
Z'' = \frac{\omega C}{\sigma^2 + C_2 \omega^2} \quad (7),
\]
What is \( G \) and \( C' \)?

whereas, the symbols retain their usual meanings as above (Bhat, 2016; Sowers, 2018).

3. Results and Discussion.

It is observed that the magnitude of the blood impedance decreases with the increasing frequency as there is passage of electric current through the Red Blood Cells (RBC’s) membranes and other blood constituents. These revealed the heterogeneous characteristics of blood therefore demonstrating mostly \( \alpha \) and \( \beta \)-dispersion at these lower frequency regions (Yuan et al., 2009; Kim, et al., 2012). The result of the two electrodes measurement of the blood impedance and characteristics is shown in Figure 2(a), where both the imaginary and the real part of the impedance in the frequency range 40 Hz up to 45.4 kHz for the non-diabetic blood and 40 Hz up to 48.6 kHz for the diabetic blood decrease with the square root of the frequency. This occurred because of the polarization impedance which is a frequency-dependant (Chang, et al., 2008; Pradhan. et al, 2012). Here exist the \( \alpha \)-dispersion which is resulted from the effects of the electrode-electrolyte interface. The non-diabetic impedance is observed to demonstrate higher impedance compared to the diabetic blood which shows more stressed range for the electrode polarization. The results agree with the findings by Kamat, et al., (2014), which concluded that the blood impedance decreases when blood glucose level increases and the impedance module decreases when blood glucose level decreases. At higher frequencies of > 45.5 kHz for non-diabetic blood and 48.6 kHz for the diabetic blood where formed a valley, the nonlinear effects caused by the magnitude of the applied current or voltage are minimal. Then occurred a depressed semicircle which is typical for heterogeneous medium such as the human blood. From the valley to the frequency of 18.6 MHz, the blood impedance decreases as the frequency increases, which is due to the fact that at these frequencies the electrical current passes through the cell membranes of the erythrocytes. This frequency dependency is known as the \( \beta \)-dispersion.
Figure 2. Shows the plots of compared values of a) impedance and dielectric parameters; b) dielectric constant, c) dielectric loss and d) conductivity of non-diabetic blood with that of the diabetic blood.

The dielectric parameters (dielectric constant, dielectric loss and conductivity) in Figures 2b, 2c and 2d demonstrated higher values in diabetic blood compared to the non-diabetic blood. There is a similar trend observed for the both blood in all the Figures. The dipole orientation and ionic conduction that are known to takes place within the blood thereby interacting strongly at the microwave frequencies but exhibited a sharp declined as the frequency increased for the dielectric constant and loss (Abdalla, 2011a; Beving, et. al., 1994. The blood dielectric constant and loss maintained a horizontal path after a sharp declined (Jaspard, et. al. 2003). This may be that the cell membranes and its interiors (the cytoplasm and other components) at higher frequency provides less resistance and the blood capacitance reduced therefore the cells and the plasma ensures the higher conduction process as in Figure 2d. The calculated and experiment data results by Abdalla, (2011b), showed the dielectric constant values of the blood to be constant only at the orientational stage (ionic polarization) of frequency from 1 Hz to around 40 Hz thereafter declined rapidly to a steady path within the frequency range of 10 to 100 kHz (Jaspard, et. al.,2003; Abdalla, 2011b).

The linear regression model had an $R^2$ values of 0.5419 and 0.5366 for the nondiabetic and diabetic blood respectively in dielectric constant in Figure 2a, demonstrating that there is a fair linear relationship (weaker) between variations in the blood components and changes in the frequency range. The linearity relationship is strongly observed between the variations in blood conductivity (Figure 2c) and changes in the frequency which shows $R^2$ values of 0.9713 and 0.9412 for the non-diabetic and diabetic blood respectively demonstrating its frequency-dependent.

The results also showed how the diabetic blood became highly conductive throughout the frequency range, this corroborates to Abdalla, et al. (2010) findings which stated that diabetic blood is more
conductive than normal blood. The resulted increase in the diabetic conductivity with frequency is probably due to the change in the surface charges of the RBCs membrane and/or the increasing penetration of the applied electric field through the plasma membrane since there is less resistance within the diabetic blood (Koth, et. al., 2014; Hölzel, 1997). The significant values of the dielectric parameters in the diabetic blood may also be due to the bloats RBCs, immature RBCs and some blast cells presents in the blood (in Figure 3) as a result of the effects of the diabetic mellitus (Kazemikhoo, et. al., 2015). According to Rauf, et. al., (2013), it is being the cell membranes which characterizes the dielectric parameters of the blood and most other biologically important tissues. The consequent elevation of glucose in the blood plasma affecting primarily the RBCs and the vascular endothelial cells, including the walls of capillaries. Therefore, the uncontrolled blood glucose or the impaired glucose tolerance or normally results in diabetes microvascular complications (Cho, et. al., 2008).

The low level laser devices have been shown to offer possibilities for both diagnostic and therapeutic medical applications (Santorelli, et. al., 2018). The results of this research work demonstrated a positive biostimulation of the laser irradiation of the diabetic blood as demonstrated in Figures 4 and 5. In Figure 4a is the graph of dielectric constant against frequency where $\varepsilon'$-Diabetic represents the dielectric constant curve for the control diabetic blood. The $\varepsilon'$-5 mR is the dielectric constant curve for the diabetic blood irradiated for 5 minutes duration using low level laser, $\varepsilon'$-10 mR is for 10 minutes, $\varepsilon'$-15 mR is for 15 minutes and $\varepsilon'$-20 mR is for that of 20 minutes irradiation durations. The same applies for the dielectric loss in Figure 4b and that of the conductivity in Figure 4c. The dielectric constant and dielectric loss demonstrated a similar trend in all the curves, which shows higher ionic polarization and dipole orientation at $\alpha$-dispersion region exhibiting a sharp declined at increased frequency.
The irradiated diabetic blood for 5 (ɛʹ-5 mR, ɛʺ-5 mR and σ-5 mR) and 10 (ɛʹ-10 mR, ɛʺ-10 mR and σ-10 mR) minutes duration showed a dropped curved compared to the control diabetic blood curve. This illustrates the biostimulation effects of the blood-laser irradiation by decreasing the diabetic blood dielectric parameters. According to Kazemikhoo, et al. (2015), the intravenous laser therapy decreases blood glucose level significantly which seems laser irradiation may have an effect on arginine and increase the production of the nitric oxide (NO). Therefore, shows that it decreases the tissue hypoxia, oxygenation stimulation and tissue metabolism normalization (Kazemikhoo, et al., 2015; Cieslar, et al., 1995). They also went further that the ATPase was observed to significantly lower in diabetic patients after laser irradiation and stimulated the Mg⁺-ATPase, Ca²⁺, Na⁺/K⁺ - ATPase (Kazemikhoo, et al., 2015; Soheila, et al., 2011). This may also have resulted to the activation of the ATP synthesis and energy formation in the blood cells (Kazemikhoo, et al., 2015). Whereas when irradiated for 15 (ɛʹ-15 mR, ɛʺ-15 mR and σ-15 mR) and 20 (ɛʹ-20 mR, ɛʺ-20 mR and σ-20 mR) minutes showed a significant increase that got the diabetic blood deteriorated and resulted to crenation and bio-inhibition. The diabetic blood showed bloat cells as a result of the excessive irradiation duration that causes the erythrocytes membranes to haemolysed as in Figure 5.

Figure 4. Shows the frequency characteristics behaviour of dielectric properties such as a) dielectric constant, b) dielectric loss and c) conductivity of control diabetic blood and after low level laser irradiation at 60 mW power output for different exposure durations.
Figure 5 a) Smearred non-diabetic blood irradiated for 15 minutes showing bloated cells circled in blue and cells crenation in red circles. b) Smearred non-diabetic blood stimulated >15 minutes durations using laser at 50 mW power output showing cell crenation. Magnifications 100X.

The electrical impedance of the diabetic and non-diabetic blood and those irradiated at different durations of 5 (D-5m), 10 (D-10m), 15 (D-15m) and 20 (D-20m) minutes were obtained within the frequency range from 40 Hz to 30 MHz using the electrochemical impedance spectroscopy. Generally, blood and its various constituents demonstrated higher impedances at lower frequencies that steadily decrease to lower values with the increase of frequency as in the Cole-Cole plot in Figure 6. Although, the control non-diabetic blood (non-D (control)) demonstrated higher impedance compared to the control diabetic blood and those irradiated blood using different exposure durations. It is observed that all the blood has nearly the same impedance as from 10 kHz upward. This agreed with Pradhan, et. al., (2012), that it is evident from their experimental results that human blood and its components have definite impedance signatures that decrease with the increase of frequency.

The impedance levels plot in Figure 6, also demonstrated the biostimulation effects of the laser irradiation when exposed under different exposure time. The irradiation for 5 as (5 (D-5m)) and 10 as (10 (D-10m)) minutes exposure durations demonstrated an improve impedance compared to its control diabetic blood while the irradiation for 15 and 20 minutes (shown as 15 (D-15m) and 20 (D-20m) respectively) rather decreases the diabetic blood impedance. This might be due to excessive irradiation which affects the blood cell membranes therefore caused crenation and rather reduced the diabetic blood impedance at the lower frequencies (Pradhan. et. al, 2012; Chang, et. al., 2008; Kamat, et. al., 2014).

Figure 6. The Cole-Cole plot of the impedance level of non-diabetic blood and diabetic blood irradiated using different exposure durations.
At lower frequency levels, the blood cell membranes insulated effectively the cytoplasm therefore current only flows around the cells, but at increased frequencies, the cell membranes became more conductive and easily current passed through the cells. The cells themselves effectively behaves as a resistive path to the flow of current. However, the flow of current depends on the cytoplasm complex impedance ratio to the system total solution present (Pradhan, et. al., 2012). Therefore, the physiological condition of the blood has much on the blood impedance level.

4. Conclusion.
This particular study observed the nature of impedance variation with respect to frequency increase remains the same for the analysis of all the considered blood and for the different irradiated exposure durations, the impedance magnitude varies with the blood physiology and morphology. The electrical impedance of the non-diabetic blood, demonstrated higher impedance levels with reduced dielectric parameters compared to the control diabetic blood counterpart. Whereas the irradiated diabetic blood demonstrated higher values of the dielectric parameters after irradiation exposure durations of 5 and 10 minutes but dropped significantly when the exposed diabetic blood became crenated for 15 and 20 minutes’ exposures. There is an observed biostimulation effects of the diabetic blood using LLLI that rejuvenates the blood cells therefore encourages the safe and efficient low level laser therapy for diabetes mellitus.

References
[1] Abdalla S (2011a) Effect of erythrocytes oscillations on dielectric properties of human diabetic-blood American institute of Physics (AIP) Adv. 1(1) 012104, 1-11
[2] Abdalla, S. (2011b). Low frequency dielectric properties of human blood. IEEE transactions on nanobioscience, 10(2), 113-120.
[3] Abdalla, S., Al-ameer, S. S., & Al-Magaishi, S. H. (2010). Electrical properties with relaxation through human blood. Biomedicalics fluidics, 4(3), 1–15.
[4] Adam G.O., Park, B-Y., Choi, K-M., Kang, H-S., and Kim, G-B., (2016), Effects of Ultraviolet Blood irradiation in a Diabetes Rabbit Model. Journal of diabetes and obesity, 3, 2.
[5] Ansari, F. (2015). The Hypoglycemic Effect of Intravenous Laser Therapy in Diabetic Mellitus Type 2 Patients; A Systematic Review and Meta-analyses. ARCHIVOS DE MEDICINA, 1(1), 7.
[6] Asami, K., Hanai, T., & Koizumi, N. (1976). Dielectric properties of yeast cells. The Journal of membrane biology, 28(1), 169-180.
[7] Beving, H., Eriksson, L. E. G., Davey, C. L., & Kell, D. B. (1994). Dielectric properties of human blood and erythrocytes at radio frequencies (0.2–10 MHz); dependence on cell volume fraction and medium composition. European biophysics journal, 23(3), 207-215.
[8] Chang, Z., Pop Gheorge A. M. and Meijer Gerard C. M. (2008), A Comparison of Two- and Four-Electrode Techniques to Characterize Blood Impedance for the Frequency Range of 100 Hz to 100 MHz, IEEE Transactions on Biomedical Engineering, Vol. 55, No. 3, 1247-1249.
[9] Cho, Y. I., Mooney, M. P., & Cho, D. J. (2008). Hemorheological disorders in diabetes mellitus. Journal of diabetes science and technology, 2(6), 1130-1138.
[10] Cieslar, G., Sieron, A., Jaskolski, F., Turczynski, B., & Adamek, M. (1995, March). Changes of electrical conductivity and relative permittivity of blood in guinea pigs irradiated with low-energy lasers. In Laser Technology IV: Applications in Medicine (Vol. 2203, pp. 162-164). International Society for Optics and Photonics.
[11] Cotler, H. B., Chow, R. T., Hamblin, M. R., Carroll, J., & Hospital, M. G. (2016). HHS Public Access. MOJ Orthop Rheumatol, 2(5), 1–16.
[12] Collins, C. M., Liu, W., Wang, J., Gruetter, R., Vaughan, J. T., Ugurbil, K, And Smith, M.B., (2004), Temperature And Sar Calculations For A Human Head Within Volume And Surface Coils At 64 And 300 Mhz, Journal Of Magnetic Resonance Imaging 19:650–656.
[13] Desouky, O. S. (2009). Rheological and Electrical Behavior of Erythrocytes in Patients With Diabetes Mellitus. Romanian J. Biophys, 19(4), 239–250.
[14] Farkas, J. P., Hoopman, J. E., & Kenkel, J. M. (2013). Five Parameters You Must Understand to Master Control of Your Laser / Light-Based Devices. Aesthetic Surgery Journal, 33(7), 1059–1064.

[15] Gemanam, S. J., Suardi, N., Mokmeli, S. and Mustafa, I.S. (2020), Evaluation of the proper level of specific absorption rate of human blood for 532 nm laser in blood low-level laser therapy, Laser Phys. 30 (2020) 035601, 1-9.

[16] Jaspard, F., Nadi, M., & Rouane, A. (2003). Dielectric properties of blood: an investigation of haematocrit dependence. Physiological measurement, 24(1), 137.

[17] Jawad, L. A., Al-Mukhtar, M. A., & Ahmed, H. K., (2011), The relationship between haematocrit and some biological parameters of the Indian Shad, Tenalosa ilarsha (Family Clupeidae), Aminami Biodiversity and conservation journal, 27(2) p47-52.

[18] Kamat, D. K., Bagul, D., & Patil, P. M. (2014). Blood glucose measurement using bioimpedance technique. Advances in Electronics, 2014.

[19] Kelemen, C., Chien, S., & Artmann, G. M. (2001). Temperature Transition of Human Hemoglobin at Body Temperature: Effects of Calcium. Biophysical Journal, 80(6), 2622–2630.

[20] Kim, M., Kim, A., Kim, S., & Yang, S. (2012). Improvement of electrical blood hematocrit measurements under various plasma conditions using a novel hematocrit estimation parameter. Biosensors and Bioelectronics, 35(1), 416–420.

[21] Kotb, M. A., Ramadan, H. S., Shams El Din, R., Motaweh, H. A., & El-Bassiouni, E. A. (2014). Dielectric properties of red blood corpuscles of workers chronically exposed to benzene in workplace. European Scientific Journal, 10(18), 365-378.

[22] Pradhan, R., Mitra, A., & Das, S. (2012). Impedimetric characterization of human blood using three-electrode based ECIS devices. Journal of Electrical Biomedicine, 3(1), 12-19.

[23] Rauf, A. (2013). A dielectric study on human blood and plasma. International Journal of Science, Environment and Technology, 2(6), 1396-1400.

[24] Rosenthal, T. B. (1948). The effect of temperature on the pH of blood and plasma in vitro. Journal of Biological Chemistry, 173(1), 25-30.

[25] Santorelli, A., Abbasi, B., Lyons, M., Hayat, A., Gupta, S., O’Halloran, M., & Gupta, A. (2018). Investigation of anemia and the dielectric properties of human blood at microwave frequencies. IEEE Access, 6, 56885-56892.

[26] Soheila M., Daemi M.,Shirazi Z. A.,Shirazi F. A., et al. (2011). Evaluating the efficiency of low level laser therapy (LLLT) in combination with intravenous laser therapy (IVL) on diabetic foot ulcer, added to conventional therapy. Journal of Lasers in Medical Sciences, 2(1):18-25.

[27] Sowers, T., Vanderlaan, D., Karpiouk, A., Donnelly, E. M., Smith, E., & Emelianov, S. (2018). Laser Threshold and Cell Damage Mechanism for Intravascular Photoacoustic Imaging. Lasers in Surgery and Medicine, (September), 1–9. https://doi.org/10.1002/lsm.23026.

[28] Thorens, B. (2014). Innovating in Type 2 Diabetes. Medicographia, 36(3), 384–390.

[29] Bhat M A 2016 Health hazardous of specific absorption rate (SAR) of mobile phone tower waves Am. Res. J. Phys., Volume 20(6), 1–7.

[30] Yuan, X. Z. R., Song, C., Wang, H., & Zhang, J. (2009). Electrochemical impedance spectroscopy in PEM fuel cells: fundamentals and applications. Springer Science & Business Media.

[31] Zhao, T. X. (1996). New applications of electrical impedance of human blood. Journal of medical engineering & technology, 20(3), 115-120.