A Study on Cell Membrane Physiology of Human Erythrocytes due to Intracellular Tonicity through Dielectrophoresis

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ABSTRACT:

To get an insight into the physiological realty of biological cells it is necessary to obtain knowledge regarding their electrical make. It is in this view an attempt has been made to study the dielectric property of human erythrocytes suspended in solutions of different tonicity solutions (Isotonic, Hypertonic, and Hypotonic) using the technique of Dielectrophoresis. This technique is concerned with the electrical constitution of the cell and is sensitive to small perturbations in cell physiology. In the present study, Erythrocytes suspended in different prepared solutions are subjected to Non-Uniform Electric Field produced by Pin-Pin Electrode configuration. Dielectrophoretic Collection Rate of Human Erythrocytes was measured at a constant frequency, time, applied voltage, and concentration of cell. The present study reveals that the tonicity of the medium affects the membrane physiology of human erythrocytes which at the cellular level is assessed using the technique of Dielectrophoresis.

Keywords: *Human Erythrocytes, Tonicity, Dielectrophoresis.*

1.INTRODUCTION:

Blood is made up of two parts. One is a liquid part. It is called plasma. It is composed of water, salts, and protein. Plasma accounts for more than half of all blood. The second one is the solid part of the blood that contains RBCs, WBCs, and platelets. Red blood cells carry oxygen from the lungs to various organs and tissues. Through the action of white blood cells, the human body is protected from various diseases, infections, and foreign bodies. RBCs are easily obtained in large amounts, comparatively simple to manipulate, and have the least comprehended electro-physical properties of any biological cell. Erythrocyte dielectric properties may provide important insights into membrane physiology [1].The capability of an extracellular fluid to maintain its tonicity is that it can move water into or out of a cell via osmosis. Tonicity is measured by the osmotic pressure of two solutions divided by a semi-permeable membrane. A good understanding of tonicity provides insight into how dehydration can affect human body cells. Tonicity is classified into three types: Isotonicity, Hypotonicity, and Hypertonicity i) Isotonicity(similar concentration to human blood): If the concentration of solutes outside and within the blood cell is identical, and if the solutes are unable to pass through the membrane, this solution is isotonic for the blood cell. Healthy people come under isotonicity. ii) Hypotonicity (lower concentration than human blood): When human blood cells are placed in the presence of a hypotonic solution, the cell expands due to a net flow of water into it. If the concentration of solutes outside the cell is less than the concentration of solutes inside the cell, the solution is hypotonic to the cell because the solutes are unable to cross the membrane. A cell swells as a result of this. People who are ill (such as

those with, anemia, or premature babies) are affected by hypotonicity. iii) Hypertonicity (higher concentration than human blood): When human blood cells are immersed in a hypertonic solution, they will lose volume as a result of a net outflow of water from the cell. The solution is hypertonic to the cell if the solute concentration in it is greater than that inside the cell and the solutes are unable to cross the membrane. This causes the cell to shrink. People who are unhealthy are prone to hypertonicity. Neutral matter translational motion is caused by polarisation effects in a NUEF, as opposed to electrophoresis, which is motion caused by the reaction of a body in an electric field to a free charge, whether uniform or not.

Dielectrophoresis is said to be positive when neutral matter is attracted to it and negative when it is repelled from the stronger field region (Pohl, H.A., et al., 1978). Pohl investigates biological matter at the cellular and molecular levels in its early stages[2].Herbert A. Pohl et al. (1971) studied the dielectric properties of yeast cells using dielectrophoresis and discovered that the yield of collected yeast cells increased linearly with increasing field strength and was directly proportional to cell concentration[3]. Gopala Krishna et al. investigated the effect of voltage, frequency, suspension conductivity, cell concentration, and exposure time on yeast cells in the 3 kHz to 1.5 MHz frequency range (1989) [4]. Gopala Krishna et al [5] determined the dielectrophoretic collection rate (DCR) of human, frog, chicken, and pigeon erythrocytes, using spherical field geometry. The variations in DCR spectra were attributed to variations in the cell's electrical make-up. Using the single cell levitation technique, Gopala Krishna et al [6] reported excess dielectric constant for mitotic and non-mitotic S. cerevisiae cells in the frequency range of 100 Hz to 20 MHz. Significant differences in the characteristic frequencies of DEP spectra have been attributed to changes in molecular composition and cellular electric fields. Gopala Krishna et al [7] reported the findings of a systematic dielectrophoretic study on human erythrocytes from the A, B, AB, and O blood groups treated with citrate phosphate dextrose-adenine (CPD-Adenine) as an anticoagulant in the frequency range of 0.1 MHz to 10 MHz using spherical field geometry under a set of experimental conditions. The findings demonstrated that the DCR versus frequency relationship is group specific. Gopala Krishna et al [8] investigated the effect of thrombosis on human erythrocyte dielectrophoretic collection. The DCR of normal and diseased human erythrocytes was investigated by varying the frequency of the applied field from 1 MHz to 10 MHz while holding all other parameters constant. Dielectrophoretic spectra were used to determine characteristic frequencies. Gopala Krishna et al [9] described the dielectrophoretic characterisation of normal and diseased blood. The dielectrophoretic behaviour of human RBCs from diabetes mellitus and jaundice patients was studied. Heller and colleagues (1960)[10] investigated the response of various organisms to a high field strength in the frequency range of 105 to 108. We observed pearl chain formation, orientation, rapid rotation, and frequency-dependent orientation as a result of the high field strength. Abdul Hameed et al. (2011) investigated the role of structured exercise in type 2 diabetes mellitus interventions. this paper discusses diabetes epidemiology as well as physical function issues associated with type 2 diabetes. Dielectrophoretic behaviour of normal and diseased human erythrocytes was described by Kaleem Ahmed Jaleeli (1996).The DCR and threshold voltage were compared to normal blood with diseased blood. Gopala Krishna et al., (1993)[11] reported changes in DCR of human red blood cells in isotonic solution under spherical field geometry in the -dispersion region every 10 minutes. The DCR is found to decrease by 80 percent after one

hour of storage in isotonic solution, and a 10% increase in conductivity is also observed in their study. Zia Ur Rahaman et al.(2021)[12] investigated the membrane physiology of different fishes erythrocytes using DCR method and compared it with human erythrocytes.

A review of the literature shows that studies have been conducted using various methods to describe the behaviour, alterations, dielectric properties, and dielectrophoretic nature of erythrocytes under various experimental conditions. However, due to extracellular tonicity, information obtained via dielectrophoretic technique from red blood cells of healthy people aged 20 to 30 years is sufficient. Dielectrophoretic analysis is a delicate tool for detecting subtle changes in erythrocyte physiology. As a result, an attempt was made to use this method to study the membrane physiology of various tonicity solutions (isotonicity, hypotonicity, and hypertonicity) and compare them to different human erythrocytes suspended in tonicity solutions. The dielectrophorosis technique was used to study the membrane physiology of human erythrocytes from healthy people due to intracellular tonicity.So we attempted to investigate the membrane structure of human erythrocytes using various experimental methods. Using the dielectrophoresis technique, the current study investigates the dielectric behaviour of human red blood cells in the presence of tonicity.

2.MATERIAL AND METHODS:

2.1.Collection of sample

Fresh samples of human blood at a volume of 3 mL were collected from 20 healthy people in the age group of 20–30 years and from different blood groups traditional volunteers United Nations agency were free from any medication. After collection, the blood samples were stored in a heparin anticoagulant to prevent them from coagulating. These dielectrophoretic studies were carried out within two hours of the collection of the sample.

2.2Sample Preparation

Healthy human blood cells are suspended in different tonicity solutions. Then they were washed with the isotonic, hypotonic, and hypertonic glucose solutions. Glucose concentrations of 2.5% for hypotonic, 5% for isotonic, and 10% for hypertonic. The cell density was determined using a spectrocalorimeter and an RBC counting chamber with optical density as a guide.

2.3.Dielectrophoresis:

Dielectrophoresis analysis as an instrument for characterization of the dielectric properties of erythrocytes from humans The Dielectrophoresis Principle : When a particle or cell is exposed in relation to an electric field, it becomes polarised. If there is an inhomogeneous electric field, the electrostatic forces at the dipole's two ends are not equal, and movement is induced. When a polarisable substance is suspended in a non-uniform E.F, it undergoes

dielectrophoresis. The electric field polarises the particles, causing the poles to feel a force along the field lines that can be either attractive or repulsive depending on the dipole's orientation.

In the current study, healthy donors' new samples of human blood from various groups were used. To prevent coagulation, the samples were collected and stored in a heparin anticoagulant. Within two hours of sample collection, dielectrophoresis experiments were carried out. To begin, different concentrations of tonicity solutions (isotonic, hypotonic, and hypertonic) were prepared. The stored red blood cells were combined with isotonic, hypotonic, and hypertonic solutions.

2.3.1.Configuration of the experiment:

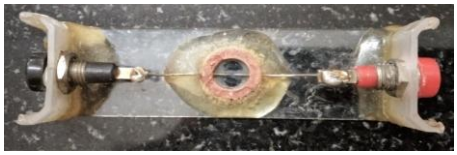


Figure.1. Electrode Chamber of DCR Studies

The chamber for electrodes was placed on a standard microscope platform, and measurements were taken using a micrometre. The alternate current signals were obtained from a radio frequency oscillator. A 0.199 mL cell suspension was dropped into the chamber, and electrical signals were applied between the two electrodes for 30 seconds at a voltage of 30 V. Due to mutual dielectrophoresis, the cells were collected at the electrodes along the field in the formation of a pearl chain. The average pearl chain length was measured for 30 seconds, yielding the DCR value. The DCR of human erythrocytes was measured while keeping the electrical conductivity of cell suspension, frequency, and elapsed time constant, but at different concentrations.The experimental arrangement of dielectrophoresis shown in below figure. (2).

2.3.2.Experimental Setup of Dielectrophoresis :

Figure 2 depicts the experimental setup for the human erythrocyte dielectrophoretic study. The wire-wire electrode chamber is mounted on a microscope with three lenses to observe the formation of pearl chains at the electrode tips. A video camera and a video monitor are connected to this microscope as part of a surveillance system. This monitoring system makes microscopic observations and measurements easier. The movement of the erythrocytes is easily observed, and measurements of time are taken using this system.

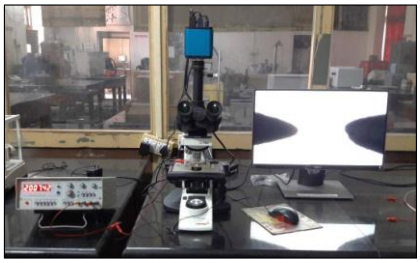


Figure 2: Experimental setup for DCR study

A signal generator with a frequency of 2000 kHz is connected to these electrodes. The human blood cells are suspended in different tonicity solutions. These samples are dropped into the electrode chamber one by one, and the signals are applied to the electrodes. The cells form a pearl chain due to the dielectrophoresis phenomenon.

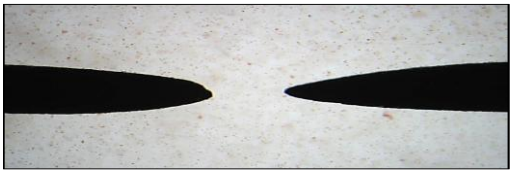


Fig.3. (a) Image of electrodes without E.F

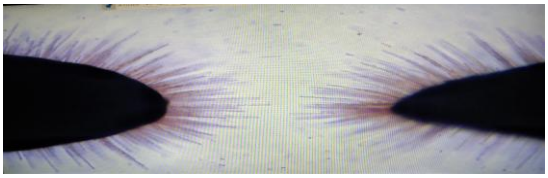


Fig.3(b): Image of pearl chain formation of Isotonic

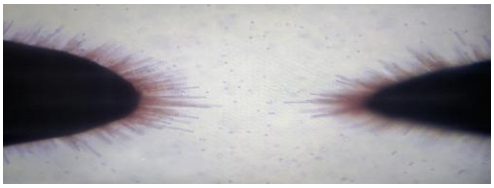


Fig.3(c): Image of pearl chain formation of Hypotonic

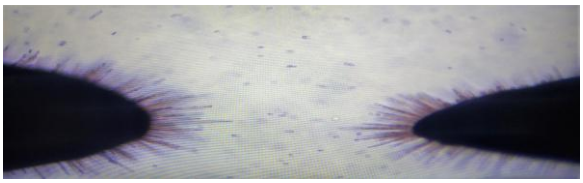


Fig.3(d): Image of pearl chain formation of Hypertonic

Figure 3. (a). Electrodes in an electrode chamber with a cell suspension sample but no electric field applied. When an electric field is applied, blood cells form a pearl chain at the tips of the electrodes due to mutual dielectrophoresis.

Figure 3. (b), (c), and (d)). Due to dielectrophoresis, the cells are collected in a systematic fashion at the electrode tips. These are monitored using the microscope's monitor. The average chain length is measured for 30 seconds using a computer-assisted setup. This results in the DCR. The electrical conductivity of a blood cell suspended in isotonic, hypotonic, and hypertonic solutions, as well as its concentration, applied voltage, frequency, and elapsed time, are all kept constant. The DCR is thus calculated.

3.RESULT :

Table 1 shows the information on dielectrophoretic erythrocyte collection as a function of square root of time, with DCR values for 20 healthy people. Table 1 shows that there is a significant variation in dielectrophoretic collection rate and time with different tonicity solutions (isotonic, hypotonic, and hypertonic) effects on human erythrocytes. Standard dielectric measurements cannot be performed on living cells. As a result, using the dielectrophoresis technique, dielectric studies on suspended biological cells in appropriate solutions (such as different tonicities) are possible. In the present study, we compared the DCR values of erythrocytes of healthy human blood at corresponding time interval values (√T) for isotonic, hypotonic, and hypertonic solutions.

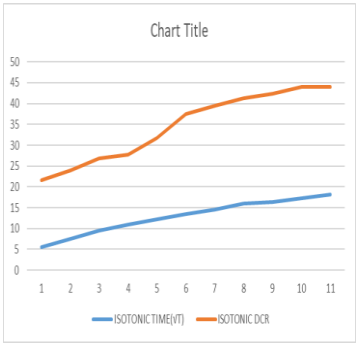
TABLE 1 : Dielectrophoretic Collection Rate (DCR) And √T Of Erythrocytes Of Healthy Human Blood suspended in Isotonic, Hypotonic And Hypertonic Solutions.

S.NO	TIME(√T)	ISOTONIC DCR	HYPER TONIC DCR	HYPOTONIC DCR
1	5.47	15.35±4	8.35±2.2	11.32±2
2	7.47	18.38±5	11.57±2	12.43±2
3	9.48	19.37±3	12.32±3	13.23±4
4	10.95	23.47±2	13.81±3	15.38±3
5	12.24	34.28±2	16.62±2.5	18.49±3
6	13.41	37.46±1	17.34±4	25.55±2
7	14.49	44.32±4	18.54±5	28.88±3
8	15.94	52.51±6	18.91±4	32.12±1
9	16.43	52.64±6	19.12±3	33.34±2
10	17.3	53.34±5	19.34±4	35.86±3
11	18.16	53.54±2	19.34±5	35.86±4

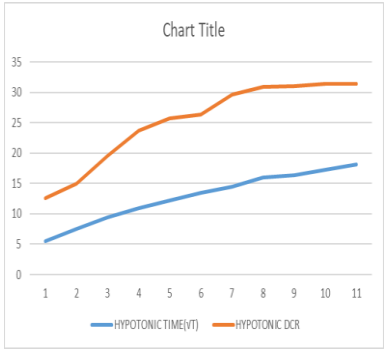
It is clear from Table.1 that DCR's magnitude (data chain pearl collection rate) for hypertonic solution is less compared with the DCR (data chain pearl collection rate) for isotonic solution, which is high with healthy donor

blood at 2 MHz. Tonicity affects the volume of a cell. An isotonic solution results in no net movement of water. For this reason, data chain pearl formation is greater than both hypotonicity and hypertonicity (shown in figure (3(b))). A cell swells when it is exposed to a hypotonic solution. For this reason, data chain pearl formation is greater than the DCR values of hypertonicity and less than the DCR values of isotonicity (shown in figure(3(c)) , As a result, data chain pearl formation is less than the sum of isotonicity and hypotonicity DCR values (as shown in figure 3(d)).

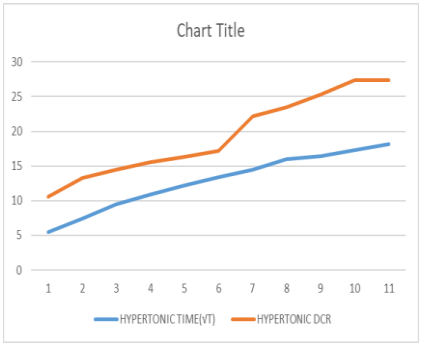
Graph: 1.(a)



Graph: 1.(b)

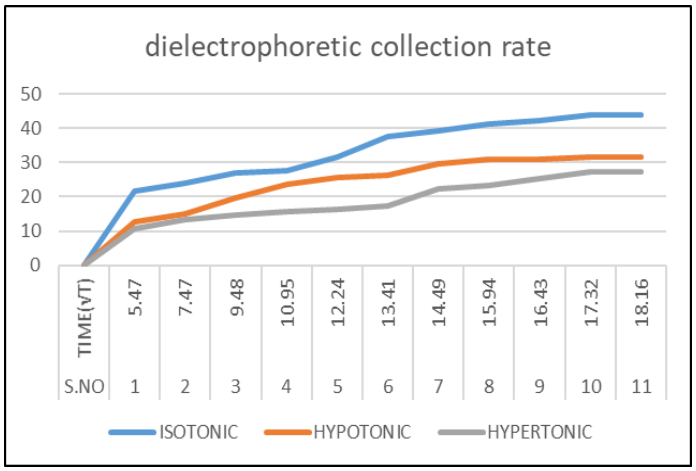


Graph: 1.(c)



Graph: 1.(a),(b) & (c) Graphs represents the isotonic, hypotonic and hypertonic of the DCR with √T of healthy human blood.

Graph:(2).



Graph.2 represents the different tonicity comparison of the DCR with √T of healthy human blood

Graph:(2) depicts the dielectrophoresis spectrum, which is the plot of Time(√T) and DCR values with tonicity solutions of human blood samples. The above graphs show that the data chain pearl formation of erythrocytes of different tonicities (isotonic, hypotonic, and hypertonic) varies with different human healthy blood samples. Dielectric behaviour of the tonicity effect on human blood cells as well as other alterations in electrophysiology

4. Discussion and Conclusion:

The current study is primarily concerned with NUEF and its interaction with human blood cells in order to comprehend how the dielectrical properties of tonicity affect human erythrocytes**.** Human erythrocytes are non-nucleated. In the DCR, spectra of human erythrocytes have been obtained using the method of dielectrophoresis. It can be observed that the DCR in human erythrocytes suspended in different tonicity solutions and compared here with DCR values of isotonicity, hypotonicity, and hypertonicity.

These variations in the DCR of human erythrocytes could be due to the fact that in the physiology of membranes as well as changes in their biological surroundings. Here, for the first time, we study human erythrocytes suspended in different tonicity solutions at a frequency of 2000 kHz the erythrocyte appears to act as an extremely sensitive sensor for signal detection and keep them in the membrane. As a result, it may cause the membrane to become more dielectric and less conductive, or vice versa. By demonstrating the molecular structure and interaction of electrostatic charges, there is also a function among the molecules of the erythrocyte surroundings.

The following conclusions are drawn from the current study regarding tonicity-induced changes in erythrocyte physiology: Tonicity refers to the non-cellular solution that can affect osmosis and alter the size of a cell using the dielectrophoresis technique.

1. The tonicity of a solution frequently correlates with its osmolarity. The term osmolarity describes the entire solute concentration in a solution.
2. When used in a hypotonic solution, the ectoplasmic fluid has less osmolarity greater than the fluid within the blood cell; as a result, water (H_2O) enters the cell and causes it to swell.
3. When used in a hypotonic solution, ectoplasmic fluid has a higher osmolarity than intracellular fluid. Water escapes from the blood cell, causing it to contract..
4. The extracellular fluid in when used in a isotonic solution has the same osmolarity as the cell. The cell will not have any net water movement into or out of it. These are seen in a study of dielectrophoresis.
5. The dielectrophoresis method can be utilised to some extent in the treatment monitoring of hypotonic and hypertonic people through the dielectrodynamic behaviour of erythrocytes.

The comparatively lower values of DCR in the case of hypertonic people's blood show the fact that the presence of $C_6H_{12}O_6$ (glucose) in the blood makes the human erythrocytes conductive and hence low dielectric. The human erythrocyte membrane is perturbed due to the presence of glucose ($C_6H_{12}O_6$) in the case of hypertonic people as the peak is concerned with dielectric relaxation. The present study suggests that the dielectrophoresis method has the potential to be used for detecting, diagnosing, and analysing diseases as well as drug administration, and monitoring hypotonic and hypertonic patients

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4.1.1Author contributions:

Jogu Sumathi: Research planning, material collecting, characterizations, writing, Result analysis and Result editing, Adeel Ahmad: Results fitting, Kaleem Ahmed Jaleeli: Supervision

Declarations:

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Conflict of Interest: The authors declare that they do not have any conflicts of interest that could have influenced the work presented in this paper.

Ethical Approval: This study was performed following the procedures formulated by National Ethical Guidelines for Biomedical and Health Research by Indian Council of medical Research, New Delhi, and certifies that the studies on human blood in vitro were carried

5.References:

[1] Farha Isna,Raisa Faheem,Kaleem Ahmed Jaleeli,Bilirubin content and dielectrophoresis of erythrocytes of patients suffering from Jaundice,Medplus International Journal of Biochemistry,Volume 3,Issue 3.September 2017 PP 50-51.

[2] VikrantVerma,RenuKChandha,ManishaMehta,AnkitaKapoor,Tonicitydetermination:Hypertonicity,Hypotonicity and Isotonicity, Method to adjust the Isotonicity, Method to know the degree of tonicity,Research Article,Volume 6,Issue No.9

[3] N.Manohar Reddy,S.Chandra Lingam and Adeel Ahmad,A Study on osmotic fragility of erythrocytes of tuberculosis patient's blood,CIBTech Journal of Microbiology ISSN:2319-3867(online)2012 Vol.1(2-3).

[4] Gopala Krishana G., Ram Moahan D and Adeel Ahmed, Indian J. comp. AnimalPhysiol, Vol. 7, No. 1(1989a), pp. 39

[5] Gopala Krishna G., Siddambary P., and Adeel Ahmed, (1990), Proc. 17th National conf. on fluid Mechanics and fluid power, R.E.C, Warangal, India.

[6]] Gopala Krishana G, Siddiambary P and Adeeel Ahmed, Indian J. Phys, Vol. 65B (1991),pp. 469.

[7] Gopala Krishana G., Kaleem AhmedJaleeli, Adeel Ahmed and Rama Krishna P. ,Indian J. Comp. Anim. Physoil., Vol. 12(1993), pp. 523.

[8] Gopala Krishna.G,Jaleeli K.A, Adeel Ahmed and Rama Krishna P,Indian J.Comp Animal Physiol, Vol. 12(1994), pp. 23.

[9] G. Gopala Krishna, A.K.W. Anwar Ali, D. Ram Mohan and Adeel Ahmed (1989) Dielectrophoretic study of Human erythrocytes. Journal of Biomedical Engineering, Volume 11, Issue 5, pages 375-380, https://doi.org/10.1016/0141-5425(89) 90099-X.

[10] Generalov, V.M., Safatov, A.S., Kruchinina, M.V., A.A.Gromov, G.A.Buryak, K.V.Generalov and V.N.Kruchinin (2020) Dielectric Properties of the Human Red Blood Cell. Meas Tech 63, 580-586,https://doi.org/10.1007/s11018-020-01826-9

[11] Herbert A. Pohl and Joe S. Crane (1971) Dielectrophoresis of cell. Biophysical Journal, 11(9): 711-727.

[12] Zia Ur Rahaman,Mohd Abdul Saleem,Kaleem Ahmed Jaleeli(2021) Dielectrophoretic Study of fish and Human Erythrocytes,International Journal of Aquatic Science,vol 12,Issue 02,2021

[13] Kaleem Ahmed Jaleeli (1996) Dielectrophoretic studies on normal and diseased human erythrocytes. Ph.D. Thesis, submitted to Osmania University, Hyderabad, India.

[14] Ramakrishna Rao, Kaleem Ahmed Jaleeli, Bellubbi BS and Adeel Ahmad (2009) Dielectric nature of Cancerous erythrocytes. Journal of pure and applied physics, Vol.21, No.2, pp.201-203.

[15] Suresh Kumar D, Kaleem Ahmed Jaleeli, and Adeel Ahmad (2011) Study on Excess permittivity of human erythrocytes through dielectrophoresis. Indian Journal of Fundamental and Applied Life

science, Vol.2, No.2, pp.75: 864-869.

[16] Jafer Sadiq M., Waheedullah A., and Adeel Ahmed, Bulletin of pure and AppliedScience, Vol. 14 D(1995), pp. 83.

[17] Khan Tabassum Tanweer, Gopala Krishna and Kaleem Ahmed Jaleeli, J. Pure & Appl.Phys., Vol. 22, No. 1(2010), pp.1.

[18] Mohammed Abdul Razack Maniyar, Dr. Mohd Khalid Mubashir Uz Zafar[1] A Study on Excess Dielectric Constant ofHuman Erythrocytes through a Novel Technique of Dielectrophoresis.

[19] Ateeba Shazi, Chandra Lingam S and Adeel Ahmad, "A Study on Excess Permittivity of Diabetic Human Erythrocytes using Computer Aided Dielectrodynamic Technique", International Journal of Science, Environment and Technology, Vol.4, No.3, pp. 864-869, 2015.

[20] Abdul Malik Mohd and M K M Zafar, "A Study on Excess Dielectric Constant of Human Erythrocytes through Lone Cell Dielectrophoresis", International Journal of Innovative Research in Science, Engineering and Technology, Vol.6, Issue 5, May, pp. 9318- 9322, 2017.

[21] M K M Zafar, Kaleem Ahmed Jaleeli and Adeel Ahmad, "A Dielectrophoretic study on erythrocytes of human blood stored in the preservative solutions CPD-A, ACD and EDTA", J.Pure & Appl. Phys., Vol. 17, No.2, pp. 75-78, 2005

[22] Ramakrishna Rao, Kaleem Ahmed Jaleeli, Bellubi BS and Adeel Ahmad, "Dielectric nature of cancerous erythrocytes", Journal of Pure & Applied Physics, Vol 21, No. 2, pp. 201-203, 2009.

[23] N. Manohar Reddy, "Biophysical Investigations on Blood of Patients Suffering from Tuberculosis", Ph.D. Thesis,Jawaharlal Nehru Technological University, Hyderabad, 2010.

[24] Gopala Krishna G and Anwar Ali AKW and Adeel Ahmad, "Average Size & Shape of Blood Cells by Laser diffraction", Indian Journal of Experimental Biology, Vol. 21, pp. 677, 1983.

[25] Gopala Krishna G, Kaleem Ahmed Jaleeli and Adeel Ahmad (1993). Influence of thrombosis on dielectrophoretic behaviour of human erythrocytes. Indian journal of comparative Animal physiology proceedings of 15th annual conference of Indian society for comparative physiology Gulberga,Karnataka,India 23-26