

A Study on Excess Dielectric Constant of Human Erythrocytes through a Novel Technique of Dielectrophoresis

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ABSTRACT:The present study shows the Excess Dielectric Constant of Human blood group samples using a novel technique of Dielectrophoresis. The erythrocyte samples are suspended in glycine-glucose medium and are exposed to Non-uniform Electric Field at a frequency of 1 MHz which is produced by the wire-wire electrode setup. For the applied frequency and voltage, the Dielectrophoretic Collection Rate (DCR) is calculated for the erythrocyte sample. With the help of DCR, the Excess Dielectric Constant (ϵ^k) is calculated. The study of the excess Dielectric Constant gives an understanding on the dielectric behaviour of the human cells and its other electrophysiological changes.

KEYWORDS:Novel technique, Excess DielectricConstant, Human Erythrocytes, Dielectrophoresis, Computer aided setup, Dielectrophoretic Collection Rate.

I. INTRODUCTION

The human red blood cells have been scientifically investigated for various bio-chemical and bio-physical properties. Human erythrocytes play a vital role in transporting oxygen from lungs to the body cells and carry away carbon dioxide. An extensive study has been done on the Dielectrophoretic properties of human erythrocytes. Various bio-physical properties like excess permittivity have also been studied upon through various investigations. Different experimental techniques were used to study the membrane structure of the human red blood cells. The present novel technique with a computer aided setup is used to study the dielectric behaviour of the human red blood cells through the Dielectrophoresis technique.

II. RELATED WORK

Shikha Rathore and Basharath Ali [1] studied the effect of Laser radiation on electrical conductivity of human erythrocytes. Digital conducting meter was used in this study for measurement of electrical conductivity. Blood samples were radiated using He-Ne laser. This method thus provides information about the changes in the biophysical parameter with the effect of laser radiation.

Suresh Kumar et al. [2] investigated on the electrical properties of human red blood cells of different physiological conditions using the Dielectrophoresis technique. Parameters like excess permittivity and Dielectrophoretic Collection Rate (DCR) were determined to understand the makeup of biological cells and the electrophysiological alterations.

Ateeba Shazi et al. [3] studied on the excess permittivity of diabetic human erythrocytes using dielectrodynamical technique. The diabetic erythrocyte sample was subjected to the non-uniform electric field using a pin-pin electrode setup. The dielectrodynamical collection rate (DCR) is measured for the sample and the excess permittivity is measured using a computer aided set up. The data obtained for the diabetic human erythrocyte is then compared with the normal erythrocyte. The study suggested that the diabetic erythrocytes were less dielectric as compared to the normal erythrocytes.

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Abdul Malik et al. [4] determined the excess dielectric constant of human erythrocytes of different human blood groups using the technique of lone cell dielectrophoresis. The erythrocyte sample was suspended in the glycine-glucose medium and subjected to non-uniform electric field. The velocity of the single cell is then measured using the technique. The study concluded that the excess dielectric constant was more or less the same for the human erythrocytes irrespective of the blood groups.

Adeel Ahmad and researchers [5-8] have further improved the techniques by introducing the image capturing devices and interfaces with computerised set up. An extensive scientific investigation was done on dielectrophoretic collection rate (DCR) of human erythrocytes of patients suffering from diabetes, tuberculosis and cancer. The influence of cigarette smoking on the physiology of human body was studied using the dielectrodynamics studies of erythrocytes. This dielectrodynamics technique contributed a lot in the medical field for the diagnosis of various diseases and physiological abnormalities. It further helped in detecting the malfunctioning of human erythrocyte membrane.

Gopala Krishna et al. [9] used the laser diffraction technique to determine the radius of normal and anaemic erythrocytes. Gopala Krishna and his research associates [10-16] have done an extensive research work on human erythrocytes of different pathological and physiological conditions using the dielectrophoresis technique.

III. MATERIALS AND METHODS

1-2 ml of human blood samples from healthy donors are collected in a siliconised glass bottles. EDTA (Ethylenediaminetetraacetic acid) is added as an anticoagulant to these. The samples are then stored at a temperature of 4°C until the experimental investigations are carried out, which is usually done within one hour after the sample collection.

As a part of sample preparation, the human erythrocytes from the plasma are isolated by centrifuging the blood sample at the rate of 1500 RPM for about 15 minutes. The blood cells are then washed in an Isotonic glycine, glucose solution. The isotonic glycine is 2.1 % and isotonic glucose is 5.5 % in the volume ratio of 9:1. The preferred dilution is about 500 times. Using an RBC counting chamber and a Spectrophotometer, Systronics 106 the concentration of the cells are determined with Optical density as a guide.

In the Dielectrophoretic Studies, NUEF (Non Uniform Electric Field) is generally produced by employing Spherical, Isomotive or Cylindrical geometrical fields. For a pair of electrodes with rounded tips, spherical field geometry is produced. Irrespective of the field geometry, the most important and delicate part of this experimental technique is the electrode chamber setup. The current study involves spherical field geometry. This field produces NUEF which is subjected to the human erythrocytes. The electrode chamber may be a wire-wire or pin-pin electrode one. In a wire-wire electrode chamber, a pair of Platinum wires is placed parallel to each other separated by a small distance. In this case, a wire-wire electrode chamber is used. A pair of platinum wires of diameter 400 μm is placed 1mm above the surface of a glass slide in such a way that these axes lie along the same straight line with the tips facing each other. These are separated by a distance 750 μm . These platinum wires are passed through a non-conducting ring (with internal diameter of 1cm). This ring is fixed on a glass slide which forms the electrode chamber. This chamber holds about 0.3 ml of the blood cells suspension (Fig.1.). These electrodes can produce the NUEF when an A.C voltage is applied between them.

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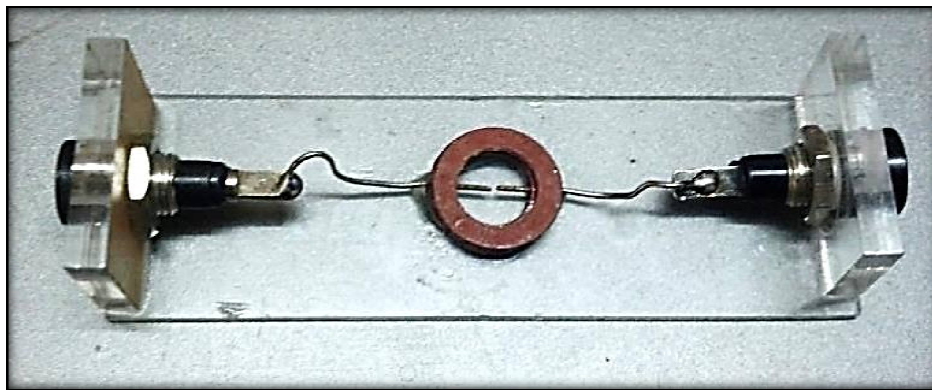


Fig.1. Electrode Chamber of Dielectrophoresis Studies.

The experimental setup of the human blood cells dielectrophoretic study is shown in Fig.2. The wire-wire electrode chamber is mounted on a trinocular microscope (Labo Triumph 90066) to observe the collection of erythrocytes or formation of pearl chains at the electrode tips. A CCTV system is connected to this microscope with a video camera and a video monitor. This CCTC system facilitates the microscopic observations and measurements. The motion of the erythrocytes is easily observed and measurements are done through this system for duration of time.

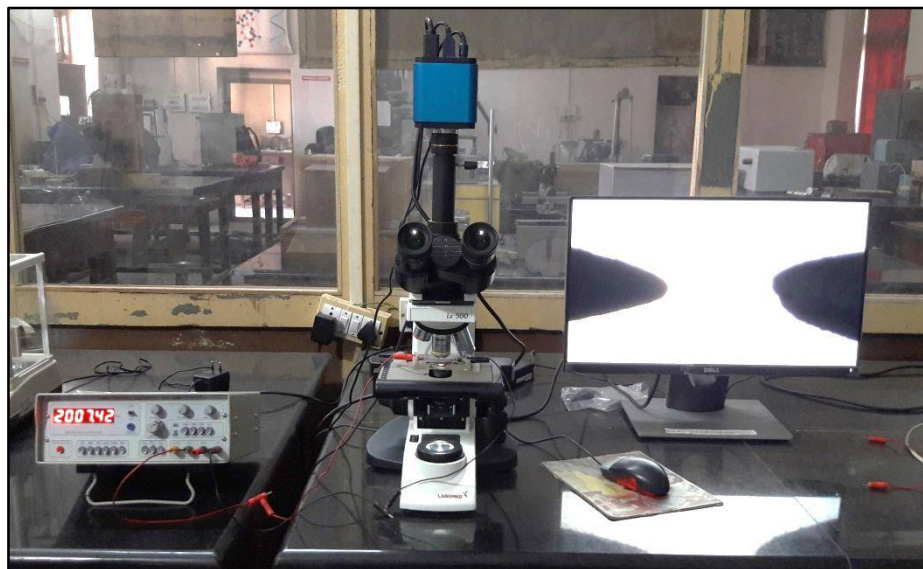


Fig.2. Experimental Setup of Dielectrophoresis of human erythrocytes.

A signal generator with frequency ranging from 1 KHz to 20 MHz is connected to these electrodes. The signals are stored and analysed through Digital Storage Oscilloscope. The human blood cell suspension sample is dropped into the electrode chamber and the signals are applied to the electrodes. Due to dielectrophoresis phenomenon, the cells are collected at the electrode tips and form a pearl chain.

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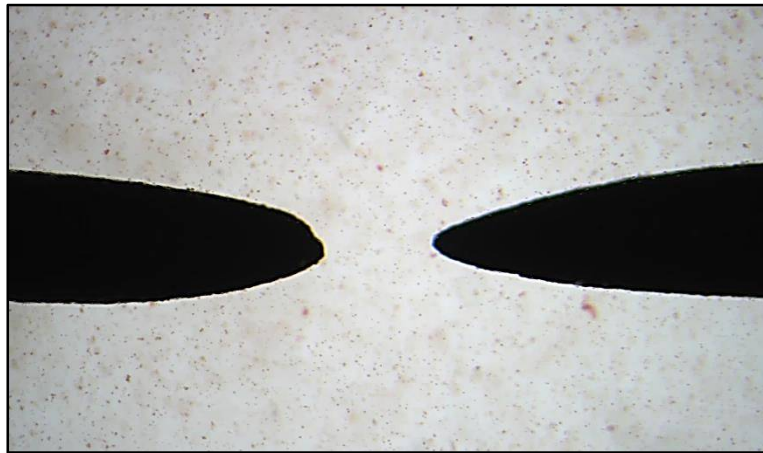


Fig.3. (a) Human erythrocytes at the electrodes without applying Electric Field

Fig.3. (a). Electrodes at the electrode chamber with cell suspension sample without the application of Electric Field. The baseline readings are taken with the help of computer aided setup. On the application of Electric Field, the blood cells are collected at the tips of the electrodes in the form of pearl chain due to mutual dielectrophoresis.

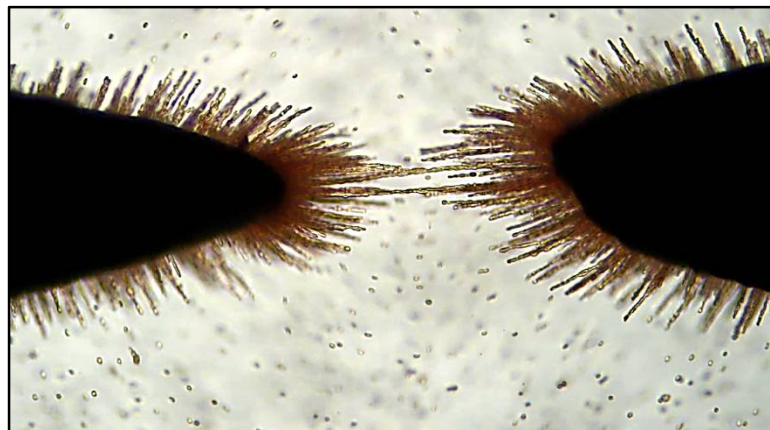


Fig.3. (b) Human erythrocytes forming pearl chains at the electrodes on application of Electric Field.

(Fig.3. (b). Due to dielectrophoresis, the cells are collected at the tips of the electrodes in systematic fashion. These are monitored through the monitor connected to the microscope. With the help of computer aided setup, the average chain length is measured for 1min. This gives the Yield or DCR. Electrical conductivity of the blood cell suspension, its concentration, applied voltage, frequency and the elapsed time is kept constant. A computer program is written in 'C' language which is used for calculating and analysing the results obtained. The Yield or DCR is thus measured.

The DCR or Yield for different blood samples obtained with the help of this technique is used in calculating the Excess Dielectric Constant (ϵ^K) using the relation,

$$\epsilon^K = y^2 \frac{dB\omega 9r_1^2(r_2 - r_1)^2}{R^6 64\pi^2 V^2 r_2^2 TC^2}$$

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Where,

r_1 = Radius of the electrode = 125×10^{-6} m

r_2 = Distance between the tips of the electrode = 750×10^{-6} m

d = Density of the suspension = 1027 kg/m^3

ω = Angular Frequency = $2\pi\nu$

B = Micro Polar parameter = 1.64

R = Radius of erythrocyte = 3.5×10^{-6} m

V = Applied Voltage = 30 vrms

T = Time = 60 sec

C = Cell concentration = 8485.9 cells/m^3

y = Yield or DCR

IV. RESULTS & DISCUSSION

Table-1 gives the data on the DCR (Yield) and Excess Dielectric Constant of human erythrocytes for different blood samples. It is observed from the Table and from the graph (Fig.4.) that there is no significant change in the excess dielectric constant of erythrocytes belonging to different human blood samples.

All the biological cells are partly polarised and partly conductive and are lossy dielectric. It is very difficult to measure the dielectric properties of these living cells and the usual techniques cannot be applied. Hence, the Dielectrophoresis technique can be the possible option for the dielectric studies on suspended biological cells in the suitable medium. Also, this novel technique with computer aided setup is useful in measuring the excess dielectric constant of a lone cell suspended in an isotonic solution. In this investigation, the excess dielectric constants of human erythrocytes are calculated from the knowledge of DCR (Yield). It is also observed that the antigens present on the human erythrocytes membrane do not seem to have much influence on the dielectric behaviour of the erythrocytes of different blood samples.

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

S.No	Sample Code	DCR($\mu\text{m}/\text{min}$)					Excess Dielectric Constant (ϵ^k)
		1	2	3	Mean	S.D (\pm)	
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

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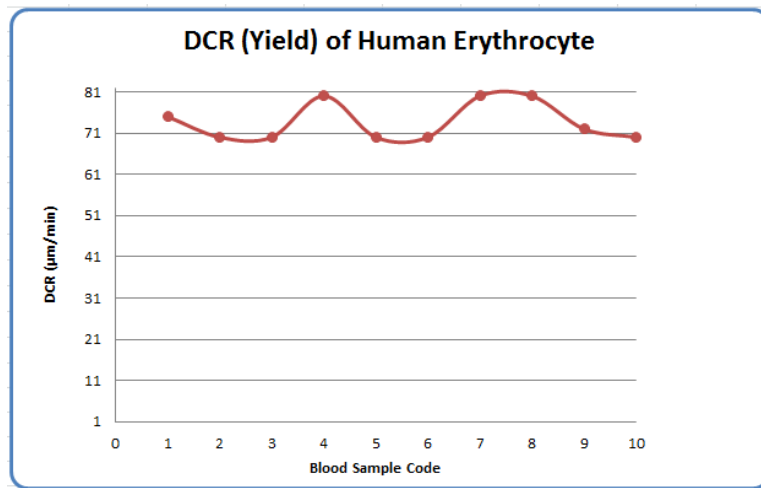


Fig.4 (a). DCR spectrum of Human Erythrocytes of different Blood samples.

Fig.4 (a) shows plot for the Dielectrophoretic collection of human erythrocytes for different blood samples for the applied voltage. There is no significant change seen in the DCR values for different sample codes. It can be inferred that the antigens present on the human erythrocytes membrane does not seem to have much influence on the dielectric behaviour of the erythrocytes of different human blood samples.

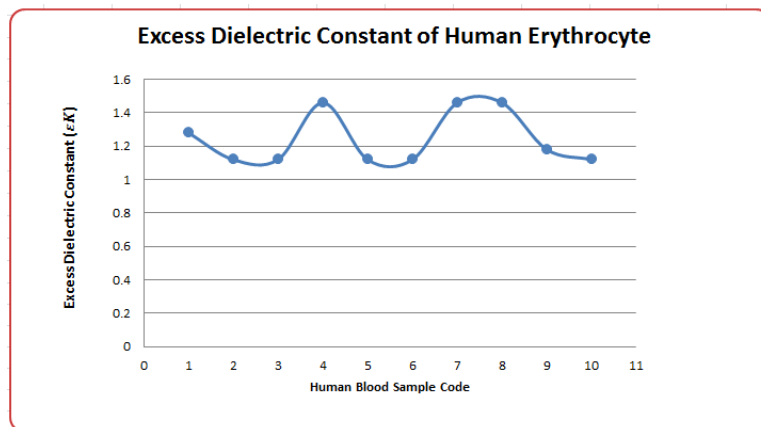


Fig.4 (b). Excess dielectric constant spectrum of Human Erythrocytes of different Blood samples

Fig.4 (b) shows the dielectrophoretic spectrum, i.e., the plot between Excess Dielectric Constant (ϵ^K) on Y-axis and the human blood sample codes on X-axis. It is observed from the figure that there is no significant change in the excess dielectric constant of erythrocytes belonging to different human blood samples.

The study of this parameter, Excess Dielectric Constant therefore gives an insight in understanding the dielectric behaviour of the human blood cells and its other electrophysiological changes.

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