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A Study on Excess Dielectric Constant of Human Erythrocytes through Lone Cell Dielectrophoresis

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ABSTRACT: The paper reports excess dielectric constant of human blood of groups A, B, AB and O using the technique of loan cell dielectrophoresis. In this technique, velocity of selected single cell is measured when erithrocytes are suspended in glycine-glucose medium and subjected to non- uniform electric field (NUEF) at 1 MHz, produced by wire-wire electrode configuration or cylinderical field geometry. The slope of straight line between square of the applied voltage (on X-axis) and velocity (on Y-axis) of erythrocytes gives the value of excess dielectric constant, knowing other parameters concerned with electrode chamber and electric field strength. Excess dielectric constant of erythrocytes is more or less the same, irrespective of blood groups.

KEY WORDS: Excess Dielectric Constant, Loan Cell, Dielectrophoresis, Wire-Wire Electrode Configuration, Human Erythrocytes, Glycine-Glocose Medium.

I. INTRODUCTION

The blood and its erythrocytes have been put for a number of diverse scientific investigation like active transport of ions and non ionic compounds , because of the importance as an active transport system of oxygen, nutrients , anti bodies and other chemical compunds to the cells of vital organs through circulatory system and the fact that the non nucleated human erythrocytes are easily obtained, manipulated and metabolically more simple than other body cells. The electrophoretic properties of human red blood cells have been extensively investigated and the red cell has also been used, before for the study of electrofusion. Further the membrane structure of the red cell is very well known and also membrane permeability to cation transport in human red blood cells. The present study is an attempt to understand dielectric behaviour of erythrocytes through a novel method developped which is termed as lone cell dielectrophoresis, which concentrates on single cell alone.

II. RELATED WORK

In the past, extensive work has been done on biological cell dielectrophoresis with special reference to human erythrocytes. Different dielectric techniques were used to study the electrical properties of erythrocytes in their normal physiological conitions. The first application of non uniform electric field effects on biological matter or in other words biological dielectrophoresis was described by Pohl and Hawk [1]. The separation of living cells from dead was made by Pohl and Hawk [2] and Mason and Towasley [3]. Gopala Krishna et al. [4] reported the dielectric collection rate (DCR) of human erythrocytes considering cylindrical field geometry.

Dimitrov et al. [5] used axi-symmetric electric field to get preliminary experimental data for erythrocytes adhesion and fusion they concluded that cell membrane fusion can be induced without DC pulse and merely using dielectrophoretic effect. Dimitrov et al. [6] measured the specific cell polarisation (dielectrophoretic coefficients divided by the cell volume) of human erythrocytes suspended in isotonic media (glycine – glucose and sorbital

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solutions) at the frequency of 2.0 MHz. Tsoneov et al. [7] measured dielectrophoretic velocities of human erythrocytes in axi-symmetric fields as a functions of applied voltage (varied up to 19 volts) and distance from the axis of symmetry at 10 MHz. The velocity of cell is proportional to the square of the applied electric field and cube root of distance from axis of symmetry as predicted by theory. Dimitrov and Zhelev [8,9] studied dynamic cell dielectrophoretic mobilities of chloroplasts, erythrocytes and lymphocytes etc., in the frequency range of 1.0 KHz to 12 MHz at different iron concentrations.

Gopala Krishna et al. [10] studied the behaviour of erythrocytes belonging to animals of different locomotions using dielectrophoretic spectroscopy subjecting them to spherical field geometry. Neuman et al. [11] studied the fusion of human cells of cerebrospinal fluid and peripheral blood. The cells were fused by applying short duration electric pulses after dielectrophoretic collection .The importance of this method for diagnosis and therapy was discussed and applications were mentioned.

Gopala Krishna et al. [12] determined DCR of human, frog, chiken and pigeon erythrocytes using spherical field geometry. The difference in DCR spectra were attributed to the variations in electrical makeup of the cells.
Gopala Krishna et al. [13] investigated the influence of physical variables such as frequency, voltage, of the applied electric field, suspension conductivity, cells concentrations and exposure time of the cell to the non-uniform electric field on DCR spectra of human erythrocytes in cylindrical field geometry in the frequency range of 30 KHz to 1.5 MHz.their results were in conformity with the theory of dielectrophoresis.

Burt et al. [14] used dielectrophoretic optical chamber method to characterise friend murine erythroleukamia cells as afunction of hexamethylene bisactamide (HMBA) treatment. The effect of saponin treatment on RI cells and neuraminidase on human erythrocyteswas also studied. They have shown that the dielectrophoretic behaviour could be interpreted in terms of cell membrane conductivity effects.

Gopala Krishna et al. [15] determined DCR of human erythrocytes of A, B and O blood groups as a function of frequency using spherical field geometry for different tonicities. Their results indicate significant variation in the hypo-tonic and hyper-tonic conditions compare to isotonic behaviour. Gopala Krishna et al. [16] reported electro-physiological behaviour of HRBC by lone cell dynamic technique at 17th Annual Conference of Indian Society of Comparative Animal Physiology GO. He has also reported the influence of malaria on dielectrophoretic behaviour of human erythrocytes.

III. MATERIALS AND METHODS

Blood samples of 1 ml of A, B, AB and O from healthy donors were drawn in the anticoagulant EDTA, brought to the laboratory in siliconised bottles and stored at 4^0C until use. The experimental investigations were completed within one hour after the collection of the sample. Erythrocytes were isolated from plasma by centrifuging the blood at the rate of 1500 rpm for about 15 minutes. The cells were washed in isotonic glycine (2.1 %) and isotonic glucose (5.5%) solution in the volume ratio of 9 : 1. The preferred dilution is 500 times. The packed cells after washing, were then mixed with the isotonic medium at desired concentrations. The concentration of the cells was determined using a red blood cell counting chamber and a spectrophotometer (Systronics 106) with optical density as a guide.

The conductivity of isotonic solution was determined using digital conductivity meter (Systronics 304). If the erythrocytes are not put in a proper isotonic medium, the cells deteriorate metabolically and hemolysis or rupture occurs.
The experimental setup for the present study of dielectrphoresis of erythrocytes consists of wire-wire electro chamber, compound microscope, signal generator of frequency ranging from 1KHz to 20 MHz, frequency counter and digital storage oscilloscope. The electro chamber was mounted on aconventional microscope stage and the observations were made with an eyepiece micro meter marked into 100 divisions per second, each division corresponds to 10 microns at 10x of the objective. Velocity of HRBC was measured, when subjected to non uniform electric field.

Excess dielectric constant of erythrocytes of different groups was calculated using the relation,

$$K_e = \left\{\frac{(d_1-d_2)B\omega}{12\epsilon_0 x}\right\}\left(log_e \frac{r_1}{r_2}\right)^2 (R_1^4 - R_2^4)\left(\frac{v}{V^2}\right)$$

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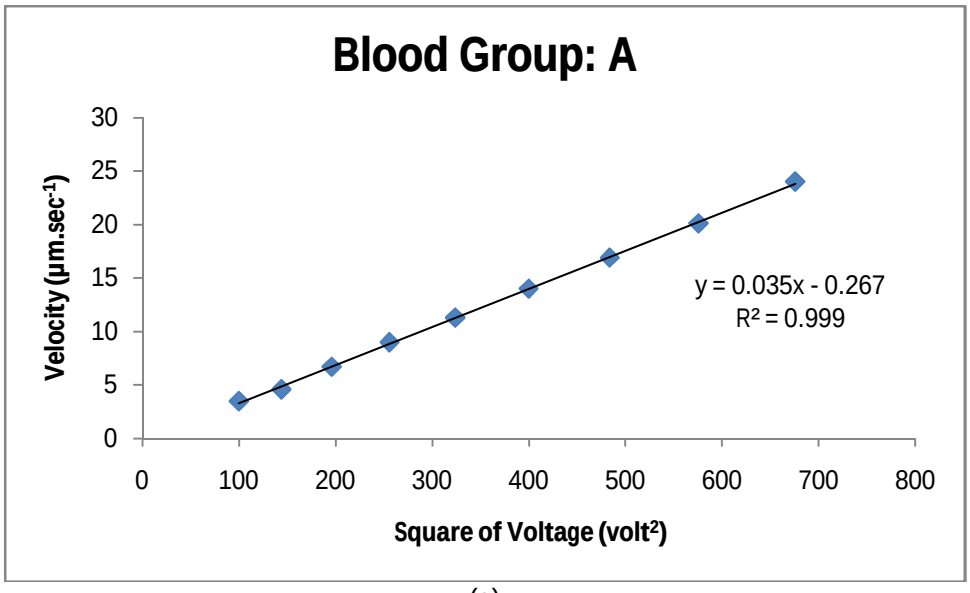
Where R_1 : Initial Position of the erythrocytes = 210um
R_2 : Final Position of the erythrocytes = 110um
x: Travelling Distance of the erythrocytes for the time t =100um
v: Velocity of erythrocytes
ω: Angular Frequency of the Applied Field
B: Micro Polar Parameter related to medium and applied field =1.64
d_1: Density of the Medium =1048 Kgm^{-3}
d_2: Density of the erythrocytes
a: Radius of the erythrocytes
V: Applied Voltage
ϵ_0: Permittivity of free Space
r_1: Radius of the Electrode =80um
r_2: Space between two electrodes =320um
ν : Frequency of Alternative Electric Field =1 MHz

Velocity of erythrocytes was determined at constant frequency of 1 MHz at different voltages ranging from 10 volt to 26 volt rms with an interval of 2 volt.

IV. RESULTS & DISCUSSION

Fig. 1. Presents plots between square of voltage on X-axis and velocity of the selected cell for human blood of groups A, B, AB and O on Y-axis. The plots are straight lines, the slopes of which give excess dielectric constant of erythrocytes, knowing the parameters of electrode configuration and also of the fluid that suspends erythrocytes. Here electrode configuration is wire - wire type and the fluid is considered to be micropolar. The data on excess dielectric constant along with reported data for the comparision. It is to be noted that there is no significant change in excess dielectric constant of erythrocytes belonging to blood of groups A, B, AB and O. It seems the antigens present on erythrocytes membrane have not much role to play in influencing the dielectric behaviour of erythrocytes of different blood groups. As far as technique is concerned, it is a novel and unique technique which measures excess dielectric constant of a lone cell suspended in an isotonic solution.



(a)

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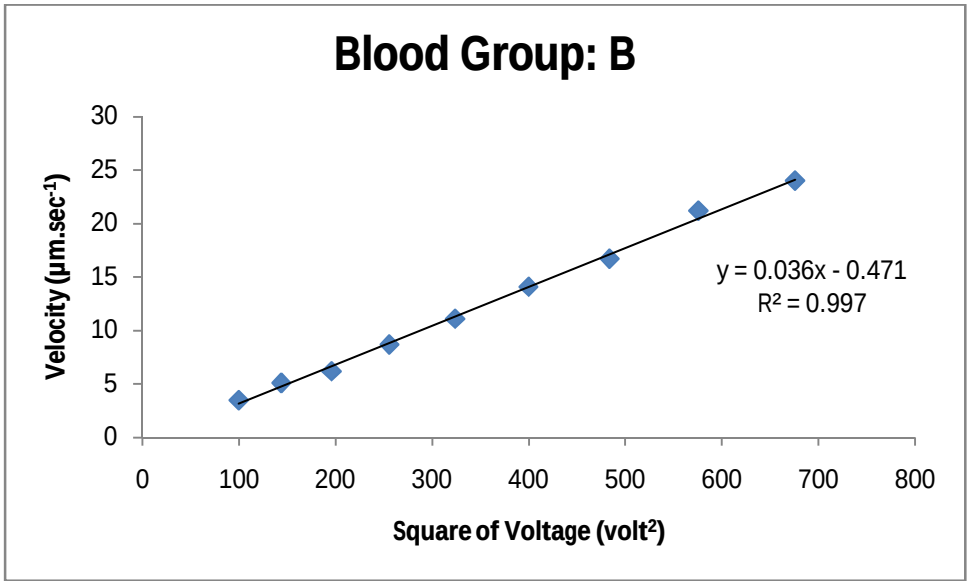

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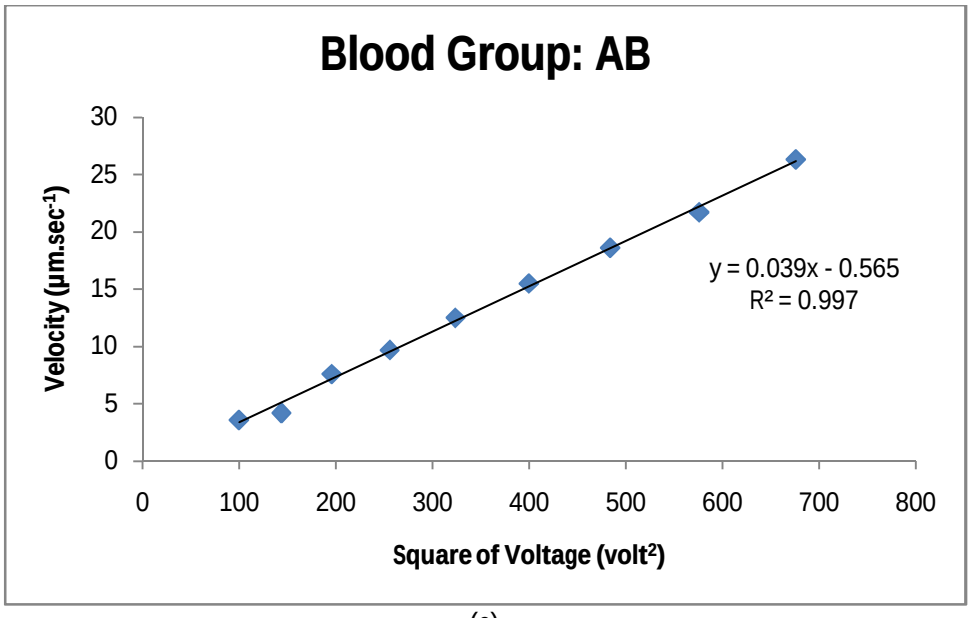

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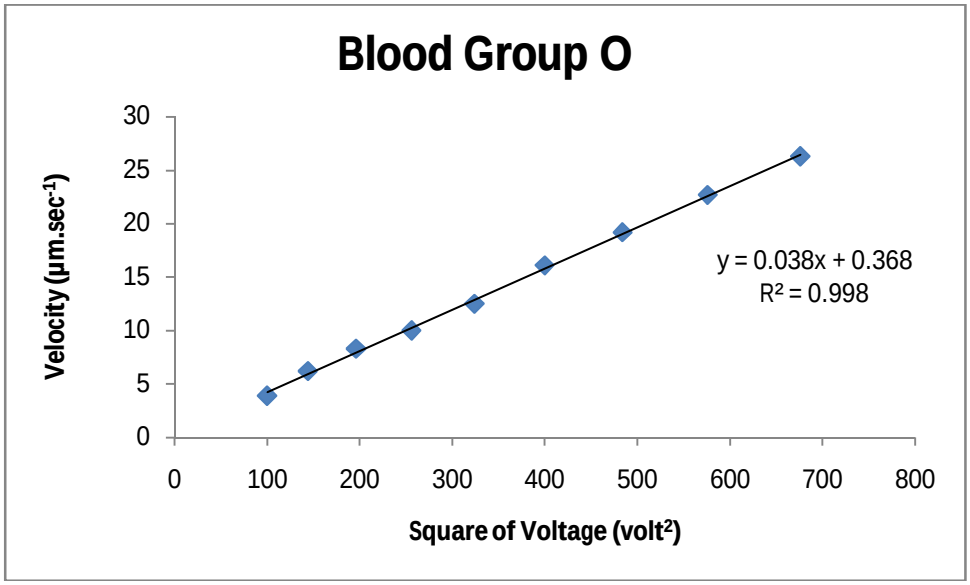

(d)

Fig .1. plots between square of voltage and velocity of erythrocytes for the blood groups (a) A Group, (b) B Group, (c) AB Group and (d) O Group.

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