Indian Journal of Fundamental and Applied Life Sciences ISSN: 2231-6345 (Online)
An Online International Journal Available at http://www.cibtech.org/jls.htm
2012 Vol. 2 (2) April-June, pp. 75 -78 /Kumar et al.

Research Article

STUDY ON EXCESS PERMITTIVITY OF HUMAN ERYTHROCYTES THROUGH DIELECTROPHORESIS

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ABSTRACT

The main aim of this article is to develop the ideas at cellular level by studying the electrical properties of human erythrocytes of different physiological conditions by using the technique of Biological cell dielectrophoresis. The papers report the data on excess permittivity (K_e) and Dielectrophorertic Collection Rate (DCR) of erythrocytes of healthy persons. In this study, erythrocyte suspension of normal blood is subjected to non-uniform electric field produced by pin-pin electrode configuration. The parameter DCR is measured at constant cell concentration, frequency and applied voltage. From the knowledge of DCR, K_e is calculated. The study of the parameter Excess permittivity gives an insight to understand the electrical make up of biological cells and electrophysiological alterations.

Key Words: *Dielectrophoresis, DCR, Excess permittivity, Erythrocytes.*

INTRODUCTION

As is known blood performs several functions in the body such as transportation of oxygen from lungs to various parts of the body and carbon dioxide from various parts of the body to lungs, transportation of water, salts and digested food materials from intestine to various parts of the body and waste materials from various parts of the body to kidney and skin etc. Dielectrophoretic technique is a sensitive tool which detects subtle changes in physiology of erythrocytes. In the past extensive work was done on dielectric behaviour of biological tissues, cells and macromolecules.

Gopala Krishna *et al*., (1993) reported the Dielectrophoresis behaviour of blood drawn from the patients suffering from thrombosis. The magnitude of the Dielectrophoretic Collection Rate (DCR) of the samples was compared at on of the characteristic frequency 2 MHz. The DCR was found to be low in diseased sample as compared with that of the normal sample. Kularni et al (2005) reported the osmotic fragility of human adult and umbilical cord blood. Their study suggests that the osmotic fragility is slightly increased in neonatal RBCs.

Abdul Hameed *et al*., 92011) studied the structured exercise in interventions for type 2 diabetes mellitus. Their paper reviews the epidemiology of diabetes and problems of physical functions associated with type 2 diabetes. Kaleem Ahmed Jaleeli ,(1996) reported the Dielectrophoretic behaviour of normal and diseased human erythrocytes. The DCR and threshold voltage of diseased blood was compared with that of normal blood. Gopala Krishna *et al*., (1993) reported the alterations in DCR of human red blood cells in isotonic solution (2.1 % glycine and 5.5 % glucose) in the volume ratio of 9:1 under spherical field geometry in the β-dispersion region for every 10 minutes. The DCR is found to decrease by a factor of 80 % due to one hour storage in isotonic solution and 10 % increase in conductivity is also noted from their study. Suresh Kumar *et al*., (2011) presented the data on excess permittivity of human erythrocytes through dielectrophoresis. The data reveals significant variations in the excess permittivity.

Ramakrishna (Rao *et al*., 2009) reported the data on excess permittivity (K_e), DCR and threshold voltage (V_{th}) of erythrocytes of healthy persons and patients suffering from different types of cancer. The study reveals significant differences in DCR, K_e and V_{th} of erythrocytes of cancer patients when compared with that of healthy persons.

A survey of literature reveals that a large number of investigations have been carried out to explain the behaviour, alterations, dielectric properties and dielectrophoretic nature of red blood cells under different

experimental conditions by adopting different methods. But information on excess permittivity of red blood cells of the healthy persons of different groups of age 20 to 25 years through dielectrophoretic technique is scanty.
Hence, an attempt has been made to study excess permittivity of human erythrocytes of healthy persons and its effect on physiology of red blood cells by dielectrophoretic technique.

MATERIALS AND METHODS

Sample collection
Fresh samples of human blood of volume 5 ml were collected from healthy persons of age group 21-25 years and different blood groups. The blood samples then transferred to a siliconised glass bottle and a anticoagulant EDTA (Ethylene di-amine tetra acetic acid) was used at the rate of 300 µ lit. per 20 ml of blood sample. These dielectrophoretic studies were carried out within one hour of the collection sample.

Sample preparation
The normal healthy human blood cells were isolated from plasma by centrifuging the blood at the rate of 1500 rpm for 15 minutes. Then they were mixed with the isotonic glycine, glucose solution. Glycine is 2.1 % and glucose 5.5 % in the volume ratio 9:1. The concentration of the cells was determined using a RBC counting chamber and a spectrocalorimeter with optical density as a guide.

Description of dielectrophoretic setup
Non uniform electric field in dielectrophoretic studies is produced by employing different field geometries such as spherical, cylindrical and isomotive. The field between pair of rounded tips of electrodes is approximated to the spherical field geometry. An electrode chamber with a pair of platinum wires placed parallel to each other known as the wire-wire electrode chamber. The chamber is called pin-pin electrode chamber if the wires are placed along the straight line with their tips facing each other and separated by a small distance. Whatever may be the field geometry, the most delicate part of the experimental technique is construction of electrode chamber.
In the present investigation, erythrocytes were subjected to NUEF produced by spherical field geometry. A pair of platinum wires of diameter 400 µm was placed 1 mm above the surface of a glass slide in such a way that these axes lie along the same straight line with the grounded tips facing each other and were separated by a distance 520 µm. The wires were passed through a non-conducting ring of 1 cm internal diameter. When this ring was cemented on a glass slide it forms pin-pin electrode chamber, which can produce NUEF when a.c. voltage is applied between the electrodes. The chamber can hold about 0.3 ml of cell suspension.

Experimental setup
The electrode chamber was mounted on a conventional microscope stage and observations were made with an eyepiece micrometer marked into 100 divisions/cm, each corresponds to 10 µm at x10 of the objective. The a.c. signals were drawn from R. F. oscillator. The cell suspension of fixed volume 0.2 ml was dropped into the chamber and the electrical signals were applied between the platinum electrodes for 1 minute for a fixed voltage 30 V_{pp}. The cells were collected at round tips of the electrodes along the field lines in the form of pearl chain due to mutual dielectrophoresis. The average chain length was measured for 1 min, which gives yield or DCR. Keeping electrical conductivity of cell suspension, concentration, applied voltage, frequency and elapsed time to be constant, DCR of human erythrocytes was measured.

Calculations of excess permittivity (K_e)
Knowing dielectrophoretic collection rate (DCR) or yield (y), the excess permittivity (K_e) is calculating using the relation,

$$K_e = \frac{9\, r_1^2\, d\, \omega\, B\, (r_2 - r_1)^2}{64\, a^6\, \pi^2\, \varphi^2\, r_2^2\, t\, C^2}\, y^2$$

Where r_1 = radius of the electrode = 0.00025m
r_2= distance between the electrodes = 0.00075m

ω= angular frequency	= 2π ν
ν= frequency of applied voltage	= 1 MHz
B= micropolar parameter	= 1.64
Φ= applied voltage	= 30 V
a= radius of erythrocytes	= 3.5 x 10^{-6}m
C= cell concentration	= 8.4859 x 10^{9} cells/cm^{3}
t= time	
y= yield or DCR	
d= density of the medium	= 1027 kg/m^{3}.

RESULTS AND DISCUSSION

Table-1 presents the data on dielectrophoretic collection of erythrocytes as a function of time and calculated values of excess permittivity for 10 normal persons. It is evident from the Table-1 that a considerable variation is observed in dielectrophoretic collection rate (DCR) and excess permittivity (K_e). All biological cells are lossy dielectric, partly polarisable and partly conductive. The dielectric measurements of biological cells are rather difficult. Usual techniques of dielectric measurements cannot be applied to living cells. Hence the dielectric studies on suspended biological cells in suitable medium can be possible by using the technique of dielectrophoresis.

Table 1: Data on DCR as function of time and excess permittivity values for different samples

S. No.	Time (sec)	√t	Sample 1		Sample 2		Sample 3		Sample 4		Sample 5	
			DCR (μm/min)	K_e	DCR (μm/min)	K_e	DCR (μm/min)	K_e	DCR (μm/min)	K_e	DCR (μm/min)	K_e
1	30	5.47	50	2.94	40	1.88	20	0.57	20	0.47	70	5.75
2	60	7.47	60	2.11	50	1.47	40	0.94	30	0.53	90	4.76
3	90	9.48	70	1.92	60	1.41	50	0.98	50	0.98	120	5.64
4	120	10.95	80	1.88	70	1.44	60	1.06	60	1.06	130	4.96
5	150	12.24	90	1.9	80	1.5	70	1.15	70	1.15	140	4.6
6	180	13.41	100	1.96	90	1.59	70	0.96	70	0.96	150	4.4
7	210	14.49	100	1.68	100	1.68	70	0.82	70	0.82	150	3.37
8	240	15.94	100	1.47	110	1.78	70	0.72	70	0.72	150	3.3
S. No.	Time (sec)	√t	Sample 6		Sample 7		Sample 8		Sample 9		Sample 10	
			DCR (μm/min)	K_e	DCR (μm/min)	K_e	DCR (μm/min)	K_e	DCR (μm/min)	K_e	DCR (μm/min)	K_e
1	30	5.47	50	2.94	50	2.94	60	4.23	70	5.75	40	1.88
2	60	7.47	60	2.11	60	2.11	70	2.88	80	3.76	50	1.47
3	90	9.48	70	1.92	70	1.92	90	3.17	90	3.17	60	1.41
4	120	10.95	80	1.88	80	1.88	100	2.94	100	2.94	70	1.44
5	150	12.24	90	1.9	90	1.9	110	2.84	110	2.84	80	1.5
6	180	13.41	100	1.96	100	1.96	110	2.37	120	2.82	90	1.59
7	210	14.49	120	2.42	100	1.68	110	2.03	130	2.83	90	1.36
8	240	15.94	120	2.11	100	1.47	110	1.78	140	2.88	90	1.49

In dielectrophoresis the electrical status of the cell is assessed by studying its mechanical behaviour, when subjected to NUEF. A parameter called excess permittivity is considered as a marker of dielectric behaviour of the cell. The parameter takes care of permittivity of the suspending medium and permittivity of the suspended cell. There are several dielectrophoretic methods to determine excess permittivity of

cells such as DCR, critical balance voltage and dielectrophoretic velocity. In the present investigation excess permittivity is calculated from the knowledge of DCR. It is observed that the value of excess permittivity is different for different samples.
The study of this parameter excess permittivity gives an insight to understand the electrical make up of biological cells and also electrophysiological alterations.

ACKNOWLEDGEMENTS
The authors thank Head Department of Physics, Nizam College for providing laboratory facilities and support. Special thanks to Principal, Nizam College, Hyderabad for his encouragement.

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