

International Journal of Innovative Research in Science, Engineering and Technology

(An ISO 3297: 2007 Certified Organization)

Website: www.ijirset.com

Vol. 6, Issue 6, June 2017

Dielectrophoresis of Erythrocytes of Patients Suffering From Jaundice

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ABSTRACT: Paper reports dielectrophoretic data on erythrocytes of blood of patients suffering from Jaundice. Dielectrophoretic collection rate (DCR) or yield is measured suspending the red cells in isotonic medium of glycine – glucose and subjecting them to non – uniform electric field (NUEF) produced by pin – pin electrode configuration ie spherical field geometry at the frequency range of 1 MHz to 10 MHz. The bilirubin content in blood samples ranges between 1 and 18%. DCR decreases with the bilirubin content in blood samples. DCR spectrum reveals *Two* relaxations at 4 MHz and 7 MHz. Data is compared with normal blood samples. There is a shift in the dielectrophoretic relaxation time when compared with normal erythrocytes. The study suggests that

KEYWORDS: Jaundice, erythrocytes, Non uniform electric field (NUEF), pin – pin electrode configuration, DCR, relaxation time, bilirubin.

I. INTRODUCTION

Jaundice is the condition in which excessive bile pigment is produced and deposited in different types of tissues and give the colour of yellow. It may be because of the production of bile pigment more than liver can excrete in normal course. In diseases of the liver, bilirubin excretion is accumulated in the blood. In some cases, obstruction in the transport system of the bile may cause jaundice.

Jaundice itself not a disease, but it is a symptom of abnormality in pathological processes that occur along physiological pathway of the metabolism of bilirubin in blood. When matured erythrocytes are damaged, their membranes become fragile and are ruptured. Then cellular contents and also hemoglobin from erythrocytes are released into plasma. The hemoglobin is phagocytized by macrophages and split into its heme and globin portions. The globin portion, a protein, is regarded into amino acids and plays no role in jaundice. But two reactions take place with heme. The first is oxidation reaction results in a green color pigment *biliverdin*, iron and carbon monooxide. The next step is the reduction of biliverdin to a yellow color pigment *bilirubin*. This bilirubin is unconjugated, free or indirect bilirubin. Each day, approximately 4 mg of bilirubin per kg of blood is produced from the break down of heme from hemolysed erythrocytes. About 20% bilirubin comes from other heme sources such as ineffective erythropoiesis, myoglobin and cytochromes [1]. The cause of jaundice is classified into *Three* main groups which are: haemolytic jaundice, hepatic jaundice and obstructive jaundice. Jaundice is being diagnosed pathologically and biochemically. The present study is an attempt to understand jaundice through dielectrophoresis of erythrocytes.

II. RELATED WORK

Pohl [2] was the first to expose biological matter to non – uniform electric fields (NUEF) at a wide range of frequency. Further, Pohl [3] described the phenomenon of dielectrophoresis ie the motion of neutral matter suspended

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in a suitable medium and subjected to NUEF. Pohl and coworkers [4-6] did extensive research on dielectrophoresis at cellular, particulate and molecular levels. Gopala Krishna et al [7] studied the behavior of erythrocytes belonging to animals of different locomotion by using dielectrophoretic spectroscopy by subjecting them to spherical fielded geometry. They measured dielectrophoretic collection rate (DCR) of human, frog, chicken and pigeon erythrocytes by subjecting them to spherical field geometry. The differences in DCR spectra were attributed to the variations in electrical makeup of the cell. Gopala Krishna et al [8] investigated the influence of physical variables such as the frequency, voltage of the applied electric field, suspension conductivity, cell concentration and exposure time of the cell to the non-uniform electric field on DCR spectra of yeast cells by using the technique of static and dynamic dielectrophoresis in the frequency range of 3 kHz – 1.5 MHz. Their results were in the conformity with theory of dielectrophoresis. Gopala Krishna, et al [9] investigated alterations in electrical double layer of yeast cells of different stages. Suresh Kumar et al [10] calculated excess permittivity of normal erythrocytes as a function of elapsed time at constant frequency of 1 MHz. Rama Krishna et al [11] 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 revealed significant differences in DCR, K_e and V_{th} . Ateeba et al [12] studied excess permittivity of diabetic erythrocytes suspended in glycine – glucose solution and subjected to non - uniform electric field (NUEF) produced by pin – pin electrode configuration. Yield or dielectrodynamical collection rate (DCR) of erythrocytes was measured as a function of frequency at constant voltage, elapsed time and cell concentration. Excess permittivity of erythrocytes was computed, knowing yield and micropolar parameter. The data was compared with that of normal erythrocytes. The study suggested that diabetic erythrocytes were less dielectric compared to normal erythrocytes. Ateeba et al [13] presented data on excess permittivity (K_e) of erythrocytes of the patients suffering from iron deficiency anemia. The K_e data was compared with that of normal erythrocytes. The K_e spectra exhibit peaks at about 5 MHz for anemia, while 4 MHz in the case of normal blood. Further, K_e values were less in anemia when compared to that of normal irrespective of frequency of applied electric field.

A perusal of literature reveals that several investigations have been carried out to explain the alterations, dielectric properties and dielectrophoretic nature of red blood cells under different experimental conditions by adopting different methods. But, the study on excess permittivity of erythrocytes of blood drawn from the patients suffering from jaundice is lacking in literature. In view of this, an attempt is made to investigate dielectric behavior of erythrocytes of jaundice patients by measuring dielectrophoretic collection rate (DCR) using the technique of dielectrodynamics.

III. MATERIALS & METHODS

Blood samples each of 2 ml from normal people and patients suffering from diabetes mellitus were drawn in anticoagulant EDTA and then brought to the laboratory in siliconised glass bottles and stored at 4°C until use. The experimental investigations were completed within one hour after the collection of the sample.

RBCs of normal and jaundice blood samples were isolated from plasma by centrifuging the blood at the rate of 1500 rpm for about 15 minutes. The cells were washed in the solution of 2.1 % glycine and isotonic 5.5% glucose mixed in the volume ratio of 9: 1. 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 with optical density as a guide.

In the present investigation NUEF is generated using pin - pin electrode configuration. A pair of platinum wires of diameter 280 μm was placed 1mm above the surface of the glass slide in such a way that their axes lie along the same straight line with the grounded tips facing each other and were separated by a distance of 310 μm . The wires were passed through a non-conducting ring of 1cm internal diameter. This ring was cemented on a glass slide and forms pin-pin electrode chamber, which produces non uniform electric field, when a. c. voltage is applied between the electrodes. Electrode chamber was mounted on a microscope and observations were made with an eyepiece micrometer marked into 100 divisions/cm, each division corresponds to 10 μm at $\times 10$ of the objective. The a. c. signals were drawn from RF oscillator. One or two drops of erythrocyte suspension were placed in the chamber. When the signal generator is switched on, a non-uniform electric field is produced in the chamber and erythrocytes were subjected to NUEF. The

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cells were in motion and were collected at the electrodes, forming a chain, which is observed under the microscope employing CCTV. The length of the chain for a fixed time say 1 min, called Dielectrodynamics collection rate (DCR) is measured using CCTV camera and interfacing with computer. Observations were made as a function of frequency and at constant voltage (Φ): 30 volt peak to peak, cell concentration (C): 8.48×10^9 cells/m³; density of erythrocyte suspension (d): 1027 kg/m³; the mean radius of erythrocyte is 3.5 μ m.

IV. RESULTS & DISCUSSION

Fig. 1. Is a plot between dielectrophoretic collion rate (DCR) in μ m/min on y - axis and percentage of bilirubin in mg on x – axis. It is a straight line with negative slope. It means DCR decreases linearly with the increase of bilirubin content. Linear regression equation alongwith $R^2 = 0.92$.

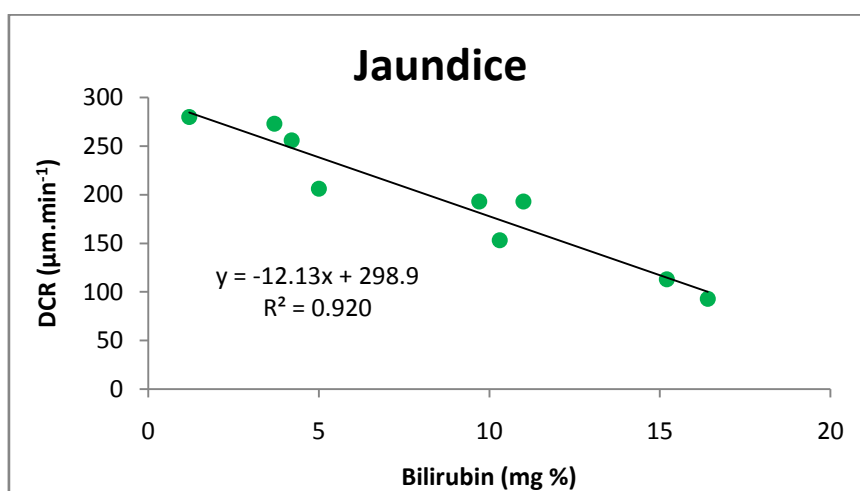


Fig. 1. A plot between DCR and bilirubin content

It is evident from Fig. 1. that erythrocytes of jaundice blood are less dielectric and more conductive than normal erythrocytes as DCR is related to dielectric constant. In the case of jaundice, blood contains both normal and hemolised cells. The red blood cell becomes ghost cells, once hemoglobin comes out ie hemolysis. The concentration of cells (No. of cells/ml) in the blood decreases and bilirubin ontent increases, thereby causing decrease in DCR. Fig. 2. is a grph between DCR and frequency of a.c. field ranging from 1 to 9 MHz. Figure shows peaks at 4 MHz and 7 MHz. The peaks are related to dielectrophoretic relaxation and the frequencies are relaxation frequencies. The reciprocal of relaxation frequencies 4 MHz and 7 MHz gives relaxation time 0.25 and 0.14 μ sec. The two peaks in DCR spectrum may perhaps reveal Maxwell – wagner or interfacial polarisation of erythrocyte membrane.

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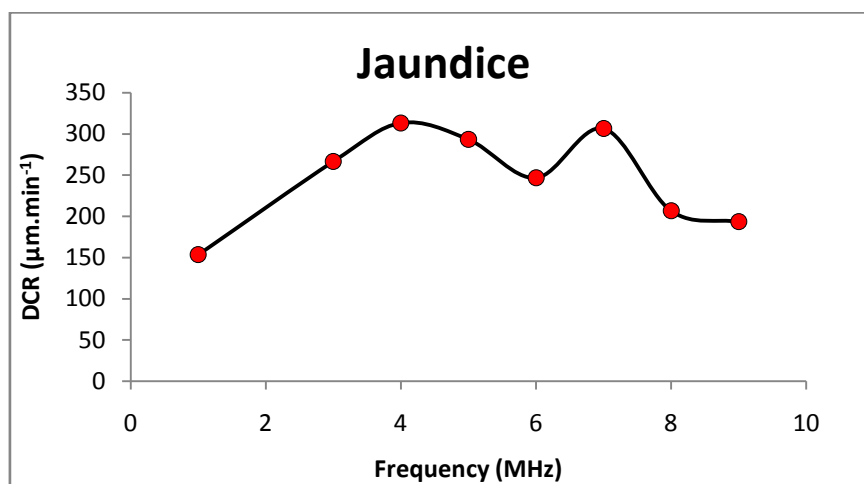


Fig. 2. A plot between DCR and Frequency of applied a.c. voltage

The present study suggests that the erythrocyte membrane physiology may be perturbed due to a disease like jaundice. The erythrocyte seems to be very sensitive to electrical makeup of the cell, altered due to a disease. So in the language of Dielectrophoresis, the disease is sensed at the erythrocyte membrane level and the disease seems to drive the membrane towards the more dielectric and less conductive state or vice-versa.

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