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A STUDY ON EXCESS PERMITTIVITY OF ERYTHROCYTES OF PATIENTS SUFFERING FROM IRON DEFICIENCY ANEMIA THROUGH DIELECTRODYNAMIC TECHNIQUE

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Abstract: The paper presents data on excess permittivity (K_e) of erythrocytes of the patients suffering from iron deficiency anemia. For the calculation of excess permittivity, dielectrodynamic collection rate (DCR) of erythrocytes is measured when erythrocytes are suspended in the solution of 2.1 % glycine and isotonic 5.5% glucose mixed in the volume ratio of 9:1 and subjected to non-uniform electric field (NUEF) produced by pin – pin electrode configuration. The measurements are made in the frequency range of 1 – 10 MHz at constant applied voltage, cell concentration, elapsed time and temperature. The K_e data is 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 are less in anemia when compared to that of normal irrespective of frequency of applied electric field.
Keywords: Anemia; Erythrocytes; Dielectrodynamic collection rate; Excess permittivity; Non- uniform electric field; Pin – pin electrode configuration.

1. Introduction

Anemia is a particular situation of blood in which it does not have sufficient number of erythrocytes or hemoglobin content. As is known, hemoglobin is a main part of erythrocytes and binds oxygen. If erythrocytes are too few or abnormal, or hemoglobin is abnormal or low, the cells in human body will not get enough oxygen. The body becomes fatigue, because of the fact that organs were not getting oxygen what they need to function properly. The anemia can be classified based on the size of erythrocytes. If the size is smaller than the normal, called microcytic, it means there is deficiency of iron. The larger size of erythrocytes, called macrocytic, reveals the possibility of deficiency of Vitamin B12 orfolate. In a particular anemia (normocytic) in which size of erythrocytes is normal, but number of cells per unit volume is less. Mostly anemia is being investigated pathologically and biochemically. But biophysical studies are scanty in literature. In view of this, an attempt is made to study anemia through dielectrodynamics of erythrocytes.

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Pohl [1] was the first to apply non – uniform electric fields (NUEF) to biological matter. Further, Pohl [2] described the phenomenon of dielectrophoresis ie the motion of neutral matter suspended in a suitable medium and subjected to NUEF. Pohl and coworkers
[3-5] did a good amount of research on dielectrophoresis at cellular, particulate and molecular levels.

Gopala Krishna and his associates [6-12] did extensive work on dielectrophoresis of human erythrocytes of different pathological, physiological and environmental conditions.

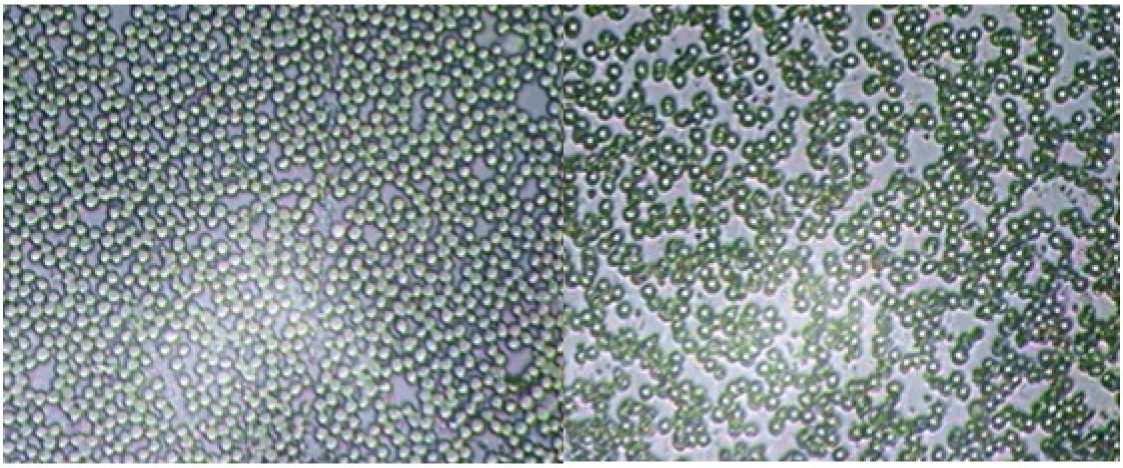
Adeel Ahmad and researchers [13-18] improved the technique of dielectrodynamics employing image capturing devices and interfacing with computer, and did extensive work on dielectrophoretic collection rate (DCR) of erythrocytes of patients suffering from tuberculosis, cancer, and diabetes mellitus. Also, they examined the influence of cigarette smoking on the physiology of human body through dielectrodynamics of erythrocytes. Their contribution established usefulness of dielectrodynamic technique in medical discipline for diagnosis of physiological abnormalities and diseases; to detect malfunctioning of erythrocyte membrane; administration and monitoring a drug.

2. Materials and Methods

Blood samples each of 2 ml from normal people and patients suffering from sulfur deficiency anemia were collected in siliconised glass bottles, adding the anticoagulant EDTA and stored at 4^0C until use. The experimental observations were completed within one hour after the collection of the sample.

Erythrocytes of normal blood and of the patients suffering from anemia wereisolated 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 same glycine –glucose medium at desired concentration. The concentration of the cells was determined using a red blood cell counting chamber and a spectrophotometer with optical density as a guide.

The slides of blood of healthy persons and anemic patients were prepared for microscopic observation (Fig.1) and also for the determination of size.

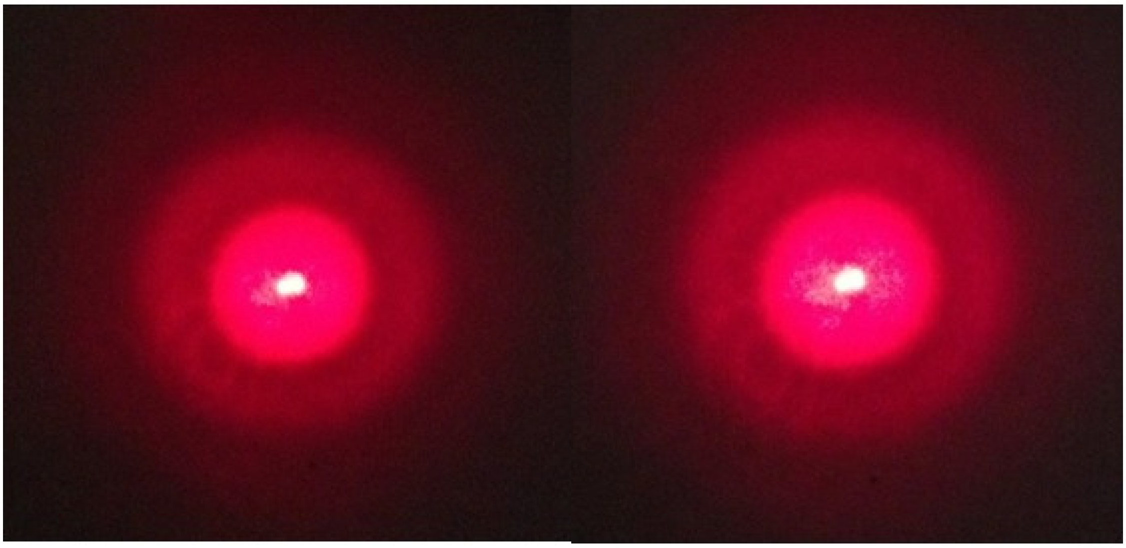


Normal Anemia

Fig. 1. Micrographs of erythrocytes of healthy donors and patients suffering from anemia

The radius of erythrocytes of normal and anemia was determined using the technique of laser diffraction developed in the laboratory, the details of which were mentioned elsewhere [19]. The diffractograms of erythrocytes for both normal and anemia are shown in Fig. 2.

Dielectrodynamic collection rate (DCR) is the observed parameter for the calculation of excess permittivity (K_e) of erythrocytes, which was measured by suspending erythrocytes in the medium of 2.1 %glycine and 5.5% glucose mixed in the volume ratio of 9: 1 and subjecting them to non – uniform electric field produced by pin – pin electrode configuration. The dielectrodynanic setup is shown in Fig. 3.



Normal Anemia

Fig. 2. Diffractograms of erythrocytes of healthy donors and patients suffering from anemia

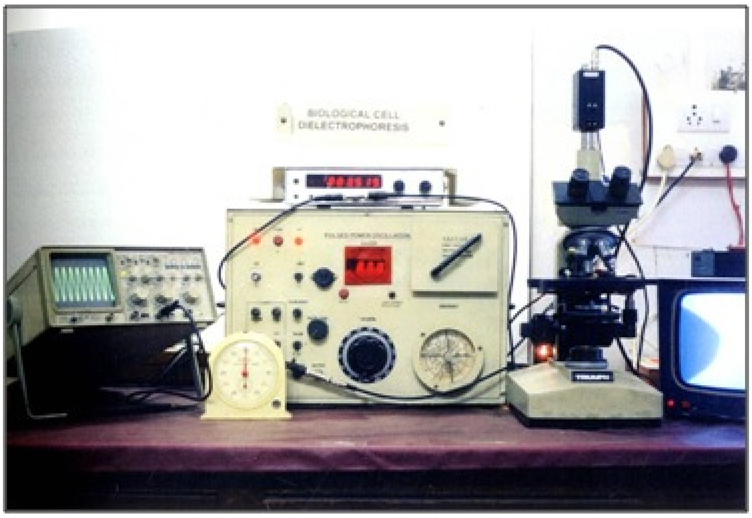


Fig 3. Dielectrodynamic setup

The details of production of NUEF, construction of cell chamber, dielectrodynamic setup and measurement DCR were mentioned elsewhere [6,7]. Observations onDCRwere made under the optical microscope with an eyepiece micrometer marked into100 divisions/cm, each division corresponds to 10 µm at ×10 of the objective.

The length of the chain formed by erythrocytes for a fixed time say 1 min, called Dielectrodynamics collection rate (DCR), was measured as a function of frequency and at constant voltage (Φ): 30 volt peak to peak, cell concentration (C): 8.48 x 10^9 cells/m^3; density of erythrocyte suspension (d): 1027 kg/m^3; the mean radius of erythrocyte is 3.5 µm. The micropolar parameter (B) is calculated using the relation between frequency (ν) and B in the frequency range of 100 kHz – 10 MHz reported by Jafer Sadiq, et al [20-22].

The parameter, excess permittivity of erythrocyte is the permittivity of erythrocyte suspended in a physiological solution. It involves the permittivity of erythrocytes and suspending medium. Excess permittivity of erythrocytes of anemic blood is calculated using the relation

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

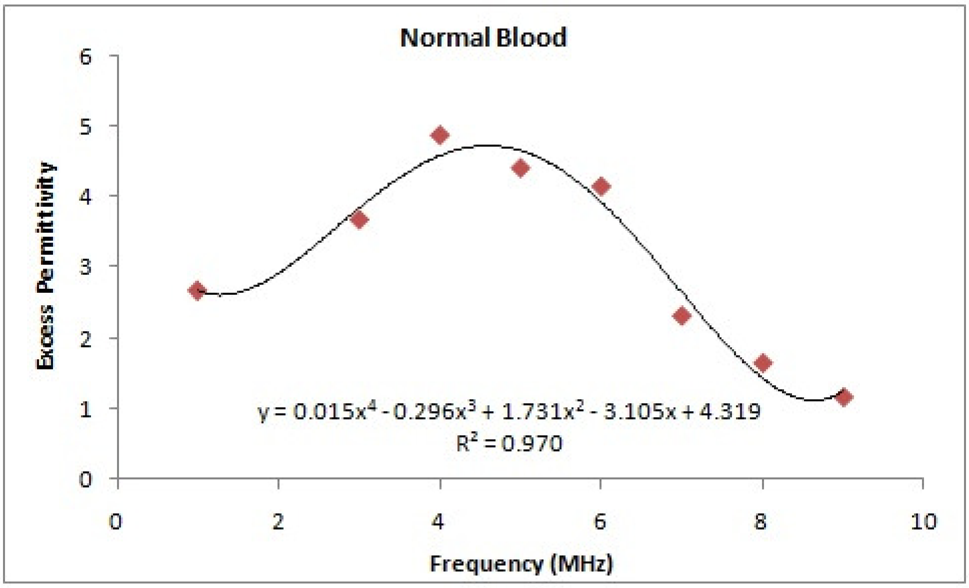
where r_1: radius of the electrodes; r_2: distance between tips of the electrodes; ω angular frequency of applied voltage; B: Micropolar parameter; a: radius of the erythrocytes, C: concentration of erythrocytes; $\emptyset^2$: square of applied voltage; t: elapsed time; Y: yield or DCR; d: density of the medium.

Excess permittivity (K_e) was calculated for erythrocytes of the blood of healthy persons (normal) and the persons suffering from anemia as a function of frequency in the range of 1 to 10 MHz at room temperature. The average of excess permittivity (K_e) was obtained for 10 samples each of normal and of anemia.

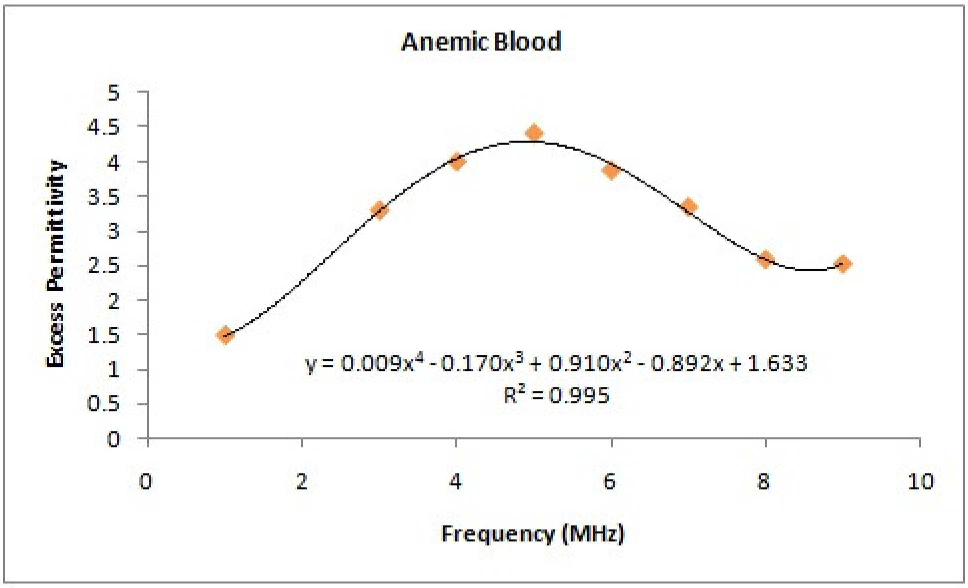
3. Results and Discussion

Fig. 4.shows dielectrodynamic spectrum i.e. the plot between K_e of erythrocytes on Y - axis and frequency of applied a.c. field on X - axis in case of (a) normal and (b) anemia blood samples. A peak is observed in dielectrodynamic spectrum for both normaland anemia blood samples at 4 MHz and 5 MHz respectively. It is interesting to note from Fig. 4 that K_e decreases sharply after attaining maximum value for normal erythrocytes and it is slow in the case of anemic erythrocytes as a function of frequency ranging from 1 MHz to 10 MHz.

The maximum value of K_e is 4.9 for erythrocytes of normal blood and 4.4 for erythrocytes of anemic blood (Fig. 5). The low value of K_e of erythrocytes of anemic blood in comparison with that of normal can be attributed to decrease in size of erythrocytes and concentration of hemoglobin.The low value of K_e suggests that the membrane of erythrocyte of anemic blood may perhaps be more conductive than that of normal blood.



(a)



(b)

Fig. 4. Excess permittivity vs Frequency for erythrocytes of (a) Normal (b) Anemia human blood

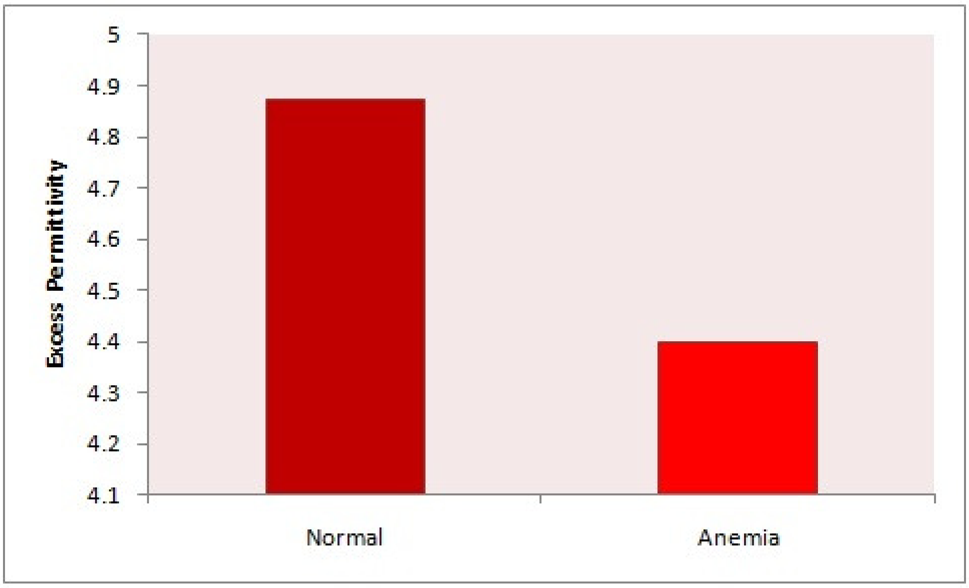


Fig. 5. A comparison between excess permittivity of normal and anemic erythrocytes

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