International Journal of Science, Environment and Technology, Vol. 4, No 3, 2015, 864 – 869

ISSN 2278-3687 (O)
2277-663X (P)

A STUDY ON EXCESS PERMITTIVITY OF DIABETIC HUMAN ERYTHROCYTES USING COMPUTER AIDED DIELECTRODYNAMIC TECHNIQUE

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Abstract: In the present study, suspension of diabetic erythrocytes in glycine – glucose solution is subjected to non - uniform electric field (NUEF) produced by pin – pin electrode configuration. Yield or dielectrodynamic collection rate (DCR) of erythrocytes is measured as a function of frequency at constant voltage, elapsed time and cell concentration. Excess permittivity of erythrocytes is computed, knowing yield and micropolar parameter. The data is compared with that of normal erythrocytes. The study suggests that diabetic erythrocytes are less dielectric compared to normal erythrocytes.
Keywords: Dielectrodynamics, Erythrocytes, Diabetes mellitus, NUEF, Dielectrodynamic Collection Rate (DCR), Excess permittivity.

1. Introduction

Diabetes mellitus is being studied in the disciplines of biochemistry, genetics, pharmacology, medicine, biomedical engineering etc. But the study on biophysical aspects of diabetic blood has not drawn much attention. Most of the investigations are on diabetic blood serum; however, erythrocytes are badly neglected. In view of this, dielectrodynamics of diabetic erythrocytes has been studied in order to understand their electrical makeup.

The first application of non-uniform electric field effects on biological matter or in other words biological dielectrophoresis was described by Pohl & Hawk [1]. Chenand Pohl [2] described a technique known as single cell dielectrophoresis to find excess permittivity of a single cell. Pohl [3, 4] did extensive work on dielectrophoresis at cellular and particulateand molecularlevels.

Gopala Krishna and his coworkers [5-12] were the first in the country to establish the research on cellular dielectrophoresis and did extensive work on human and animal erythrocytes, and yeast cells of their different physiological and environmental conditions.

*Received May 21, 2015 * Published June 2, 2015 * www.ijset.net*

Jafer Sadiq et.al [13-15] developed mathematical models for the calculation of dielectrophoretic collection rate (DCR) and excess permittivity (K_e) of human erythrocytes for the techniques of dielectrophoretic collection rate and single cell dielectrophoresis. They showed the agreement between calculated and experimental values. However, in the past, the DCR and K_e values obtained from Pohl's theory were not in agreement with experimental values.

Recently Rauf, et al [16] presented a comparative account on dielectrophoretic behavior of normal and diseased erythrocytes at a frequency of 1 MHz of applied voltage. Suresh Kumar et al [17] calculated excess permittivity of normal erythrocytes as a function of elapsed time at constant frequency of 1 MHz.

Ramakrishna *et al* [18] 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}.

A survey of literature reveals that number of investigations has 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 diabetic erythrocytes is lacking in literature. In view of this an attempt is made to study dielectric behavior of diabetic erythrocytes by using the technique of dielctrodynamics and determining the parameter, *Excess Permittivity*.

2. Materials and 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 4^0C until use. The experimental investigations were completed within one hour after the collection of the sample.

RBCs of normal and diabetic 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 280μm 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 310μm. 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 into100 divisions/cm, each division corresponds to 10μm at ×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 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 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 linear relation between frequency (ν) and B in the frequency range of 100 kHz – 10 MHz reported by Jafer Sadiq, et al [13].

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 diabetic erythrocytes is calculated using the relation

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

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.

3. Results and Discussion

Table 1 presents the data on yield (Y) and excess permittivity (K_e) of normal and diabetic human erythrocytes as a function of frequency in the range of 1 to 8 MHz at room temperature. The K_e values are average for 10 samples each of normal and diabetic human erythrocytes. It is evident from the table that K_e of diabetic erythrocytes is less, when compared to that of normal, irrespective of frequency of applied electrical field.

Figure 1 shows dielectrophoretic spectrum ie 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) diabetic blood samples. Two peaks are observed in dielectrophoretic spectrum for both normal and diabetic blood samples. It is interesting note, from the figure, the peaks at the frequencies 4 and 6 MHz; and 3.5 and 6 MHz for normal and diabetic blood respectively.

The relatively low values of DCR in the case of diabetic blood reveal the fact that the presence of glucose in the blood plasma makes the erythrocytes more conductive and hence less dielectric. The frequency shift of 0.5 MHz in first peak of the spectrum indicates that the erythrocyte membrane is perturbed due to the presence of glucose in the case of diabetic mellitus as the peak is concerned with dielectric relaxation.

The present study suggests that dielectrodynamic technique can serve as a potential tool for detecting; diagnosing and analyzing as well as drug administration; and monitoring *Diabetes mellitus*.

Table 1 - Data on Yield and Excess Permittivity of erythrocytes as function of frequency for Normal and Diabetic blood

Frequency (MHz)	*Yield*		*Excess Permittivity*	
	Y_N *(μm/min)*	Y_D *(μm/min)*	K_{eN}	K_{eD}
1	380	307	3.53	2.30
3	307	293	3.73	3.40
4	347	273	5.40	3.34
5	280	200	3.88	1.98
6	233	207	4.30	3.39
7	160	113	1.47	0.73
8	93	87	0.53	0.46

Y_N: Yield of normal erythrocytes; Y_D: Yield of diabetic erythrocytes; K_{eN}: Excess permittivity of erythrocytes of normal blood; K_{eD}: Excess permittivity of erythrocytes of diabetic blood.

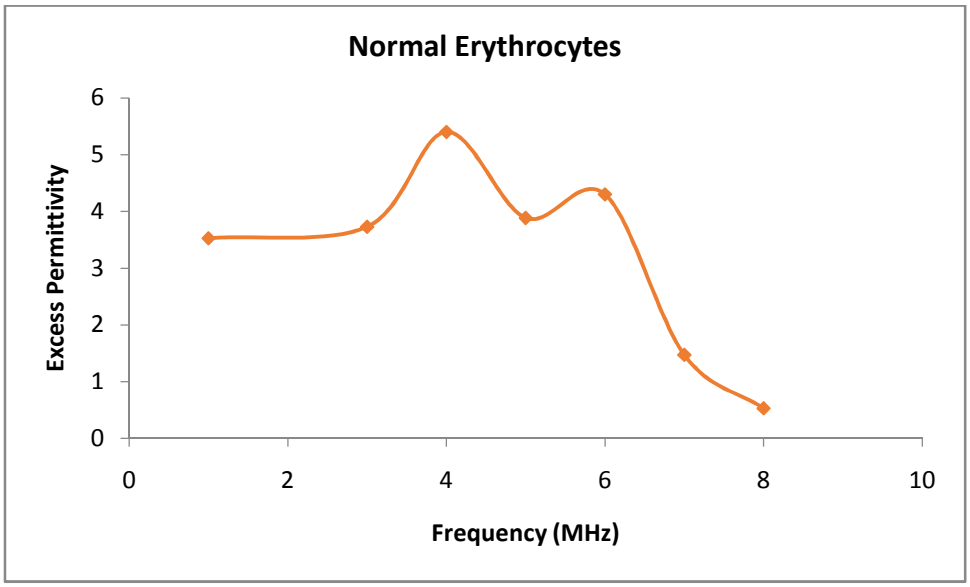

Fig. 1(a)

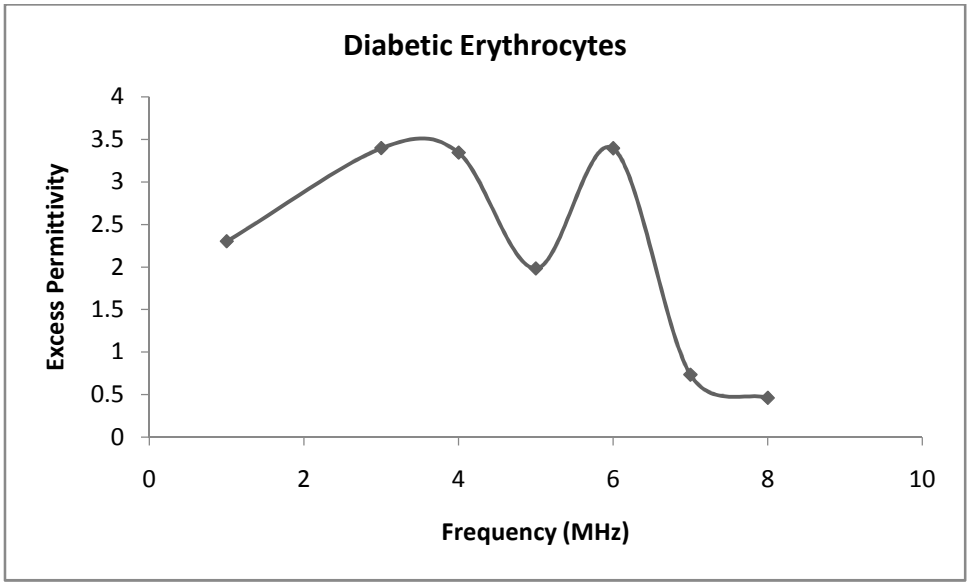

Fig. 1(b)

Figure 1. Dielectrodynamic spectrum (K_e versus Frequency) of (a) normal and (b) diabetic human erythrocytes

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