UACEE International Journal of Advancements in Electronics and Electrical Engineering – IJAEEE
Volume 2 : Issue 3 [ISSN 2319 – 7498]
Publication Date : 09 September 2013

Comparative study of Excess Permittivity in Normal and Diabetes Mellitus of Human Erythrocytes

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Abstract: **This paper reveals the data on excess permittivity of normal and diabetes mellitus of human erythrocytes. In the present investigation, erythrocytes suspension of normal and diabetic blood is subjected to Non Uniform Electric Field (NUEF) produced by pin-pin electrode configuration. At least 40-45 samples were taken the data of excess permittivity of normal blood and diabetic mellitus were taken into consideration. The results shows that the variation in the dielectric properties affects the whole body with respect to excess permittivity as in table 1.3.The average data reveal significant variation in excess permittivity of erythrocytes of diabetic patients. When compared with that of healthy persons as shown in graphical representation of 2.1 & 2.2. (1).**

Keywords: **Erythrocytes, Diabetes mellitus, Pin-Pin electrode Configuration, Normal blood.**

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Introduction

Every one's blood has some glucose in it. In people who do not have diabetes, the normal range is about 70 to 120. Blood glucose goes up after eating, but returns to the normal range 1 or 2 hours later. A desirable blood glucose range for most people with diabetes is from about 80 to 120 hrs. This is before a meal, before breakfast or 4 to 5 hours after last meal. For most people the target for 2 hours after meal is 180 or less. Before bed time, blood glucose should be between 100 and 140. In the present investigation, the effect of diabetes on the physiology of red blood cells has been studied by dielectrophoretic technique, when they are subjected to non-uniform electric fields of different strengths and frequencies. Dielectrophoresis is a very sensitive tool to detect subtle changes in cell physiology in terms of electrical behavior of cells. In the past, large number of investigations was carried out to explain fusion, size and shape of red blood cells of healthy persons under different experimental conditions by adopting on dielectriphoretic behavior of human red blood cells of the persons suffering from diabetes is scanty.

The term dielectrophoresis was first used by pohl [1], which he described as the translational motion of neutral matter caused by polarization effects in a non-uniform electric field originally,

this term was strictly referred to the phenomena of induced dipole on particles due to a non-uniform field. However the other electro kinetic phenomena arise from non-uniform electric fields, in particular, traveling wave dielectrophoresis was termed using pin-wire electrode. Manipulation is limited to large particle like cells. Using this electrode configuration. Pohl [2] has demonstrated the separation of viable and non-viable yeast cells in 1966, Pohl has been extended the experiments to separate other biological cells, including canine.

Biological cell dielectrophoresis offers a novel approach for cellular characterization, which may be of value in the field of clinical medicine and cell biological research. Finally it is also concluded that electrical properties of erythroustes are important for the understanding of the functioning of various systerns, which carry out life process, thrombocytes, chloroplasts, mitochondria and bacteria Virsaladze, et al[3] investigated the contribution of studies in endothelial dysfunction in Diabetic vasculopthy and elucidated the present understanding of the major role of the endothelial dysfunction in the pathogenesis of diabetic vascular lesions. Le Devehat, et. al..[4] provided evidence for and against early hemorheological abnormalities in Diabetic Mellitus (DM).

Sample preparation

A satisfactory suspension of erythrocytes can be made by directly diluting whole blood with isotonic glycine-sucrose or glyeine-glucose solution. The preferred dilution is 500 times. Finding a suitable medium for the cells to be stable is a time and error procedure. Not only are the erythrocytes active metabolically and generally deteriorate rather rapidly in put in an unfavorable medium, which does not constantly furnish the requisites ions and other metabolites in a rather narrowly defined concentrations range, but hemolysis or rupture can occur. If diluted in isotonic sucrose or glucose erythrocytes gradually lies over a period of an hour. It was found that blood diluted with isotonic glycine was resistant to lyses.

Glycine, an amino acid can exist as Zwitterions but conducts little current. Since most dielectriophoresis experiments requires suspensions having rather high resistivities media with low ionic conduction are desirable. Isotonic gluyciner 2.1% has a resistivity approximately $5x10^4$ ohm-cm. When a sample of whole blood is diluted fold in it, the resistivity falls to about $2.3x10^4$ ohm-cm, still satisfactory for many experiments on their dielectriophoresis.To accommodate the metabolic need of the erythrocytes for glucose a standard medium consisting of 90% (by volume) isotonic glycine (2.1%) and is isotonic glucose (5.5%) was used in this experiment.

The expression to calculate excess permittivity is

$$K_e = \frac{9d_1(t)B\,(r_2-r_1)^2\, r_1^2\, y^2}{64\pi E_0\, \Phi a^6\, C^2}$$

Where

r_1 = radius of the electrode (m)

r_2 = distance between the axes of the electrodes(m)

d1= density of the suspending medium (kg/m^3)

a = radius of the cell (m)

t = time of applied voltage (sec)

Φ = voltage of applied alternating field (volt)

C = Concentration of cell Suspension ($cells/m^3$)

E0 = permittivity of free space (F/m)

B = micro polar parameter;Y = yield (m)

Materials and methods

Fresh samples of normal blood of volume nearly 2 ml were collected from Maderesh Inderesh Hospital, Dehradun. The blood samples from the patients suffering from Diabetic were collected from Apollo nursing Centre, Dehradun. EDTA was used as an anticoagulant at the rate of 300 µl for 20 ml of blood samples. The dielectrophoretic studies were carried out within one hour of collection of samples. Red blood cells of normal and diseased blood were isolated from plasma by centrifuging the blood at the rate of 1500 rpm for about 15 minutes. Then the packed cells were washed in isotonic, glycie-glucose solution (2.1% glycine and 5.5% glucose in the volume ratio of 9:1). The packed cells, when washed, were then mixed with isotonic solution. The concentration of cells was determined using a red blood cell counting chamber and spectro-colorimeter, with optical density as a guide.

Diabetes is a group of metabolic disorders with in common manifestation: hyperglycemia. Chronic hyperglycemia causes damage to the eyes, kidneys, nerves, heart and blood vessels. The DCR and excess permittivity of human erythrocytes of diabetic patients are low when compared with the normal.

In case of diabetes, the human erythrocytes lose its original dielectric property and starts exhibiting more conductive behavior, when compared to normal erythrocytes. The dislectrophoretic investigation suggests that the physiology of human erythrocytes is perturbed due to diabetes and these perturbations in cell physiology are monitored in DCR data. The erythrocytes seems to behave as a very sensitive to pick up signals and store excess permittivity of erythrocytes is calculated for both normal and diabetic blood. The data is tabulated in Table 1.3.

TABLE 1.1 DATA ON EXCESS PERMITTIVITY OF ERYTHROCYTE OF NORMAL BLOOD

Sample Code	Condition	Glucose Concentration (mg/dl)	Excess Permittivity (Ke)
N-1	FBS	100	1.70
N-2	FBS	86	4.23
N-3	FBS	98	2.11
N-4	FBS	86	4.23
N-5	FBS	86	1.467
N-6	FBS	81	1.775
N-7	FBS	89	0.938
N-8	PLBS	128	0.178
N-9	FBS	96	3.30
N-10	FBS	87	3.75
N-11	PLBS	109	1.77
N-12	FBS	81	3.75
N-13	FBS	89	2.47
N-14	FBS	89	3.30
N-15	FBS	89	4.23
N-15	FBS	83	1.77

TABLE 1.2 DATA ON EXCESS PERMITTIVITY OF ERYTHROCYTE OF DIABETIC BLOOD

Sample Code	Condition	Glucose Concentration (mg/dl)	Excess Permittivity (Ke)
DM-1	FBS	113	0.52
DM -2	FBS	116	1.18
DM -3	FBS	116	0.93
DM -4	FBS	116	1.46
DM -5	FBS	119	1.18
DM -6	FBS	120	0.36
DM -7	FBS	120	0.05
DM -8	PLBS	123	0.71
DM -9	FBS	135	1.18
DM -10	FBS	135	1.77
DM -11	PLBS	140	0.60
DM -12	FBS	148	1.77
DM -13	FBS	150	0.73
DM -14	FBS	160	0.70
DM -15	FBS	167	1.77

DM -16	FBS	183	1.18
DM -17	FBS	183	1.77
DM -18	FBS	193	0.06
DM -19	FBS	203	0.04
DM -20	FBS	212	0.05
DM -21	FBS	212	0.09
DM -22	PLBS	137	1.16
DM -23	PLBS	176	1.16
DM -24	PLBS	210	0.07
DM -25	PLBS	230	1.18
DM -26	PLBS	232	1.18
DM -27	PLBS	232	0.09
DM -28	PLBS	235	0.07
DM -29	PLBS	244	0.07
DM -30	PLBS	258	0.05
DM -31	PLBS	296	0.07
DM -32	PLBS	312	0.03
DM -33	PLBS	351	0.07

TABLE 1.3

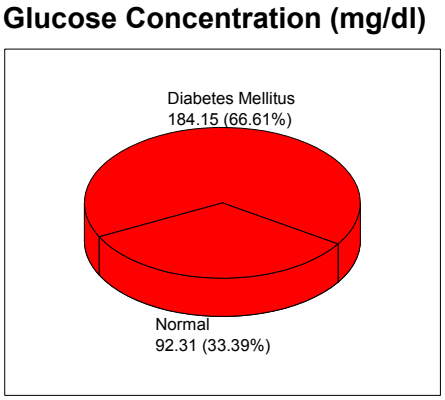
A COMPARISON OF AVERAGE VALUES OF EXCESS PERMITTIVITY FOR NORMAL AND BLOOD OF DIABETIC PATIENT WITH RESPECT OF GLUCOSE CONCENTRATION.

Condition of the sample	Glucose Concentration (mg/dl)	Excess Permittivity
Normal	92.31	2.41 $\pm$.22
Diabetes Mellitus	184.15	0.705 $\pm$ 0.63

Result and discussion

The study demonstrates that high glucose concentration, when compared with normal glucose level, influences the dielectric nature of the erythrocyte membrane. The mean value of 33 samples of diabetes mellitus was taken for comparison with normal blood to have excess permittivity.

The percentage variation for excess permittivity in case of diabetes mellitus is 70.74% less as compared with the normal blood. Hence by comparing the dielectric behavior of erythrocytes of the normal and diseases blood, it is possible to monitor the treatment.



Graph 2.1 Glucose concentration of diabetes mellitus and normal blood

Conclusion

1. HRBC's have been taken as typical cells for the study of dielectric behavior of biological cells.
2. The electric field to which cells are subjected is non-uniform alternating electric field, produced by pin-pin electrode configuration.
3. The medium in which cells are suspended and free to move is assumed to be micro polar, when the cell moves under the action of the dielectriporetic force, it will also experience a viscous force opposing the motion. When the cell is in dynamical equilibrium.

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