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Dielectrophoretic Study Of Fish And Human Erythrocytes

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Abstract: Among the various biological cells, the Red Blood cells are readily obtained in large quantities and least understood interms of their physiology. In view of this, an attempt has been made to study the dielectric properties of fish and Human erythrocytes using the technique of Dielectrophoresis. The present study reports the data on the Dielectrophoretic Collection Rate (DCR) and Threshold Voltage (V_{Th}) of fish and Human erythrocytes. In this study, Erythrocyte suspension of fish and Humans is subjected to a Non-Uniform Electric field produced by Pin-Pin electrode configuration. The parameters DCR and $V_{Th}$are measured at constant voltage, frequency, and cell concentration. The study reveals a significant difference in the values of DCR and $V_{Th}$of fish Erythrocytes when compared with that of Human Erythrocytes.

However special importance is given in the present study regarding the application of the Dielectrophoretic technique in fisheries research. This technique offers a fruitful method for learning the cellular as well as electrical makeup of biological cells.

Keywords: Erythrocytes. Fish, Human, Dielectrophoretic Collection Rate (DCR), Threshold Voltage (V_{Th})

1. INTRODUCTION

Blood is a vital fluid found in Human Being and Other animals. It is a tissue made up of cells in suspension and plasma containing substances in solution. The miles of arteries, veins, and capillaries are the paths through which blood flows to and fro and transports oxygen from lungs to tissues. Among various biological cells, the Red Blood Cells are readily obtained in large quantities, relatively easy to manipulate, and least understood in terms of their electro-physical properties. The dielectric properties of erythrocytes may provide an important insight into its membrane physiology.

Fish live in very intimate contact with their environment and are therefore very susceptible to physiochemical changes, which may be reflected in their blood. Fishes might be exposed to various toxicants in aquatic environments for example Sewage and other wastes of domestic nature, Agricultural discharge, Industrial effluents, Wastes from thermal and nuclear power plants, Pesticides added in the water, etc. This exposure to various types of chemical pollutants can induce changes in the membrane physiology of Fish Erythrocytes.

Dielectrophoresis is the translational motion of neutral matter caused by the polarization effects in a non – uniform electric field which is different from electrophoresis - motion caused by the response to a free charge on a body in an electric field whether uniform or non-uniform. Dielectrophoresis is said to be positive when the neutral matter is attracted towards and negative when repelled from the stronger field region respectively (Pohl, H.A., et al., 1978). Analysis of biological matter at cellular and molecular levels are studied at the initial stages by Pohl. Herbert A. Pohl and Joen S.Crane (1971) studied the dielectric properties of Yeast cells using the Dielectrophoresis technique and they observed that yield of collected yeast cells increased linearly with increasing field strength and was directly proportional to the cell concentration. The effect of voltage, frequency, suspension conductivity, cell concentration, and exposure time of non-uniform electric field on yeast cells was determined in the frequency range of 3 kHz to 1.5 MHz by Gopala Krishna et al. (1989). Heller and co-workers (1960) studied the response of various organisms to a high field strength in the frequency range of 10^5 to 10^8. Pearl chain formation, orientation, rapid rotation, and frequency-dependent orientation were observed due to this high field strength. Y Kikuchi (1991) subjected the RBC of different species of mammals and fish to a Nuclepore filtration testand suggested that the RBC filterability may not differ greatly in mammals and fish when compared at a comparative temperature. Kaleem Ahmed Jaleeli (1996) studied the behavior of normal and diseased human RBC using the Dielectrophoretic Technique. The DCR and V_{Th} of diseased blood were compared with that of normal blood. Ramakrishna et al., (2009) obtained the data on DCR and V_{Th} of cancer and healthy persons using the technique of Dielectrophoresis. Results revealed a significant difference in the values of DCR and $V_{Th}$of normal and cancer blood. Suresh Kumar et al. (2011) determined the excess permittivity of Normal human erythrocytes as a function of time at a constant frequency of 1 MHz. Generalov, V.M., et al. (2020) studied the dielectric properties of human red blood cells. They found out that the values of the complex permittivity, electrical capacitance, and dielectric loss tangent almost remain constant despite the changes in conductivity and the content of NaCl in the cell suspension.

The dielectrophoretic technique is a sensitive tool that detects subtle changes in the physiology of erythrocytes but has not yet been explored in the field of fisheries research even though it is more than three decades old. So here we attempted to study the membrane physiology of different fishes using this technique and compared it with human erythrocytes.

2. MATERIALS AND METHODS:

In the present study, live Fishes: *Clarias Batrachus, Roho Labeo,* and *Channa Punctatus* were brought to Biophysics Research Laboratory, Department of Physics, Nizam College, Osmania University from one of the famous fish markets in Hyderabad.

*Collection of Blood***:**

A blood sample of fish was collected from the caudal vein by introducing a disposable sterile syringe (2.5 ml) and the blood was then transferred into an anticoagulant tube containing EDTA. Here in the present study, EDTA was used as an anticoagulant. The same collection procedure was followed for three different fishes. A Human Blood sample of volume 2.5ml was collected from a Healthy donor and was stored in a heparin anticoagulant to prevent it from coagulation.

Experimental setup:

The dielectrophoretic studies were carried out within one hour of the collection of samples. Fish and human erythrocytes were isolated from plasma by centrifuging the blood at 1500 rpm for 15 minutes. The erythrocytes were then washed in isotonic, glycine 2.1% – glucose

5.5% solution in the volume ratio of 9:1. Washed erythrocytes were then mixed with the isotonic solution. The concentration of cells was determined using an RBC counting chamber and Spectro colorimeter, with an optical density as a guide (G. Gopala Krishna et al. (1989)). The same procedure was followed for human blood.

In this study, a Non-Uniform Electric Field is produced using Pin – Pin electrode configuration. It consists of a pair of platinum wires with a diameter of 280µm that is positioned 1mm above the surface of the glass slide in such a way that their axes aligned along the same straight line and their grounded tips facing each other. The two platinum wires are separated by a distance of 310µm. The wires were passed through a non-conducting ring with an internal diameter of 1cm. This ring was cemented on a glass slide and forms a pin-pin electrode chamber.This electrode chamber was mounted on a Digital microscope and observations were made on a Digital LCD Screen connected to Microscope as shown in Figure 1**.** The a. c. signals were drawn from the RF oscillator of 1 MHz frequency. When a.c voltage is applied between the electrodes it producesa non-uniform electric field.Erythrocyte suspension of volume 2ml was 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 a Non-Uniform Electric Field (NUEF). The cells were in motion and were collected at the electrodes, forming a pearl chain (Figure 2), which is observed on the LCD computer screen connected to a microscope. The length of the chain for a fixed time of 1 min, called Dielectrophoretic collection rate (DCR) is measured. The detailed procedure about the dielectrophoretic setup is mentioned somewhere else (G. Gopala Krishna et al. (1989), Kaleem Ahmed Jaleeli, 1996).

Further for the first time threshold voltage (V_{Th}) of fish erythrocyte was calculated. It is the minimum voltage at which the collection of erythrocytes takes place at the electrode.

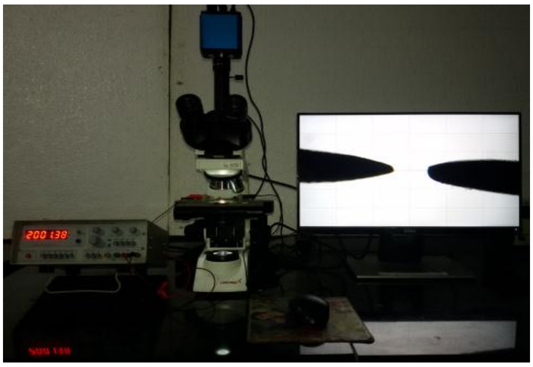

Figure 1: Experimental setup for Dielectrophoretic study

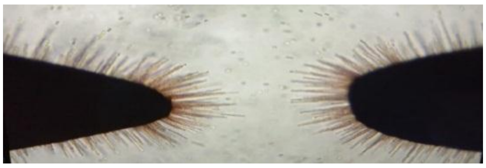

Figure 2: Image of pearl chain formation

3. RESULT:

Table 1: Data on Threshold Voltage and Dielectrophoretic Collection Rate of Human and Fish Erythrocytes

Sample	**Threshold Voltage (V_{Th})**	**Dielectrophoretic Collection Rate (DCR) in µm**
Human Erythrocytes	20.33	92.35
Fish Erythrocytes (Fresh Water)		
1. Clarias Batrachus	12.03	127.15

2. Roho Labeo	10.43	133.27
3. Channa Punctatus	10.30	137.33

Table 1 presents the data on Threshold Voltage (V_{Th}) and Dielectrophoretic Collection Rate (DCR) of Human and Fish Erythrocytes. Dielectrophoretic Collection Rate and the Threshold voltage of fish and human erythrocytes differ significantly. In comparison to Human erythrocytes, the DCR value of Fish erythrocytes is very high.

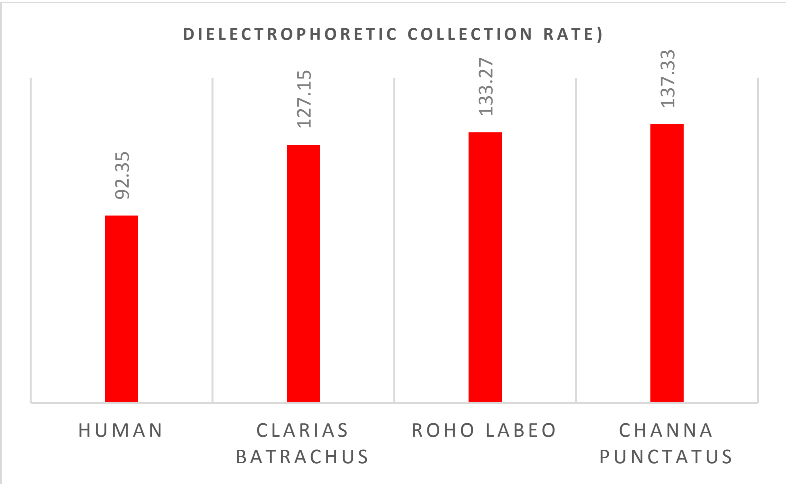

Figure 3: Comparison of Dielectrophoretic Collection Rate of Human and Fish Erythrocytes

Figure 3 represents the comparison of the Dielectrophoretic Collection Rate (DCR) of Human and Fish Erythrocytes (of three fishes: Clarias Batrachus, Roho Labeo, and Channa Punctatus).

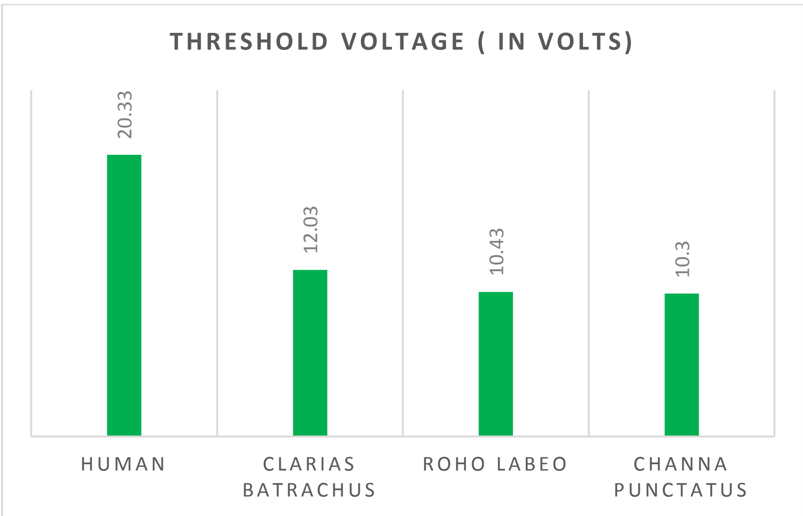

Figure 4: A Comparison of Threshold Voltage of Human and Fish Erythrocytes

Figure 4 represents the comparison of the Threshold Voltage (V_{Th}) of Human and Fish Erythrocytes (of three fishes: Clarias Batrachus, Roho Labeo, and Channa Punctatus).

4. DISUCSSION AND CONCLUSION:

It is a well-known fact that Human erythrocytes are Non – Nucleated whereas fish erythrocytes are nucleated**.** Dielectric measurements of biological cells are very challenging and standard techniques of dielectric measurements cannot be applied to living cells. Hence, dielectric studies on suspended biological cells in a suitable medium can be possible only by using the technique of Dielectrophoresis. The subtle changes in the erythrocyte membrane can be sensed in a better way by using the technique of Biological cell Dielectrophoresis.
In the present investigation, DCR spectra of fish and human erythrocytes have been obtained using the technique of Dielectrophoresis. It can be observed that the DCR of Fish Erythrocytes is large in comparison to Human erythrocytes. Further, the DCR values of three fishes i.e., *Clarias Batrachus, Roho Labeo,* and *Channa Punctatus* are nearly the same. These changes in the Dielectrophoretic Collection Rate (DCR) of fish and human erythrocytes may be attributed to the changes in their membrane physiology along with the changes in their living environment. Here for the first time threshold voltage(V_{Th}) of fish erythrocyte has been determined at a frequency of 1 MHz. The threshold voltage (V_{Th}) is the minimum applied voltage for which the erythrocytes start to get collected at the electrodes. Threshold voltage can be seen to vary inversely with DCR i.e., with the increase in the value of DCR, V_{Th} is decreasing.
This technique can be used as an important and easy tool in Fisheries research to detect the minute changes in the membrane physiology of fish due to various factors. So in the language of Dielectrophoresis, the changes in the aquatic environment which may cause disease in the Fish can be sensed at the erythrocyte membrane level using this technique.The erythrocyte seems to behave as a very sensitive sensor to pick up signals and store them in its membrane due to which it may drive the membrane towards a more dielectric and less conductive state or vice – versa, thereby showing that the molecular organization and electrostatic interaction among the molecules is also a function of erythrocyte environment.

Conflict of Interest:
The authors declare that they have no conflict of interest that could have influenced the work reported in this paper.
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