Design and Development of Microfluidic Lab-On-Chip Bioimpedance Analyzer

Thesis submitted in partial fulfillment of the requirements for the degree

of

Bachelor of Technology

In

Biomedical Engineering

By

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CERTIFICATE

This is to certify that the thesis entitled, "**Design and Development of Microfluidic Lab-On Chip Bioimpedance Analyzer**" submitted by Mr. Samyak Mohanty (Roll No-111BM0009) in partial fulfillment of the requirements for the award of degree of Bachelor of Technology in Biotechnology & Medical Engineering with specialization in "Biomedical Engineering" at National Institute of Technology, Rourkela is an authentic work carried out by him under my supervision and guidance. To the best of my knowledge, the matter embodied in the thesis has not been submitted to any other university/institute for the award of any Degree.

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ABSTRACT

Impedance analysis for biological samples has proved to be one of the most powerful non-invasive techniques developed so far, for understanding the electrophysiological properties of the tissues. Presently all the impedance analyzer systems that are available in the market are large and expensive. There is a need of small, portable, low-cost system which can be used commercially. In this context, an attempt has been made to design and develop a Lab-On-Chip Bioimpedance Analyzer System. For this purpose, the portable microfluidic platform for impedance analysis was prepared on cupper print laminated board by chemical etching. The device was successfully operated and had a sensitivity output value in terms of frequency ranging from 50 Hz to 10 KHz. The impedance analysis was done for various samples such as PBS, NaCl solution, cell culture medium(DMEM) and bacterial cell culture. However, all the samples were shown a capacitive response. Optimization of the platform was done on the basis of the electrode spacing, diameter and flow rate so as to bring accuracy in impedance analysis measurement. The device was designed in such a way so that the analysis can be made even at a reduced sample volume, moreover portability of the device makes it stand out among commercially available systems in the market.

Keywords: -electrophysiological, portable, Lab-On-Chip, optimization, DMEM culture medium

CHAPTER-1

INTRODUCTION

1. INTRODUCTION

Electrical Bioimpedance (EB) may be defined as the passive electrical property of cells/tissues under the application of applied external Electric field [1]. Bioimpedance of a living tissue may also otherwise be defined as the ability to oppose to a specific electric current flow. The biological materials, typically the tissues constitutes millions of cells and each cell holds many organelles and intracellular conducting fluids inside a lipid bi-layer membrane called as the "cell membrane". And in addition to that there is also an extracellular fluid medium encompassing the cells. This shows that the electrical conductivity of any living tissues is determined by the electrical characteristics of its constituents [2]. The lipid bilayer membrane, in general, acts as a dielectric medium while the intracellular and extracellular media for having been conferred a noticeable ionic conductivity acts as electrolytes. Therefore the bi-layer lipid membrane, the intracellular fluid, and extracellular fluid behaves like a protein-lipid-protein system or, In other words, like a capacitor circuit contributing to the frequency-dependent response. But this impedance analysis on the living tissues is only possible when an alternating analyzing current is passed on to the tissue surface resulting in the charge dispersion across the cell membrane and ultimately resulting in conductivity of tissues/cells [3].

Recent advancements have shown that the development of powerful informative techniques such as the Bioelectric impedance analysis of living tissues/cells has been a subject of increasing importance. Electrical Bioimpedance Analysis(BIA) is one of the most reliable non-invasive techniques developed so far which has shown tremendous applications in the field of medicine, as well as in biochemistry and modern biology. Through this technique, we can continuously

monitor all the physiological properties of the living tissues thereby analyzing on different body compositions and other clinical conditions [4]. However, the results are measured on the basis of the frequency response. The response of the bioelectric impedance analysis is changed depending on the physiological, anatomical and pathological conditions of the living tissues. Thus, the studies on the impedance analysis of tissues can provide a lot much information on the anatomical and physiological state of any particular sample. The bioimpedance data has in fact been quite productive in the characterization of tissues on the basis of valuable parameters like cell dimensions, the state of the cell membrane and the status of the intracellular and extracellular media. However, there is always a certain demand for a monitoring system through which the impedance analysis can be carefully investigated. Therefore, it is extremely important to design and develop such impedance monitoring systems where we can successfully study the electrical properties of the tissues and through them their physiological properties, a culmination of both simultaneously, otherwise known as the **electrophysiological properties [5]**.

It has earlier been observed that all living tissues and cells shows capacitive response and for that reason tissues show a decrease in impedance value with increasing current in the analyzing circuit. However, the structure of the biological material and its frequency dependence is rather complex, similarly the analysis of bioimpedance information may be complicated as millions of different cells contribute into a specific bioimpedance measurement.

CHAPTER-2

LITERATURE REVIEW

2.1 Dielectric properties of biological tissues

The biological tissues of all the living subjects like the plants or animals comprise of millions of cells arranged in a definite three-dimensional(3-D) construct. Each of the cell comprises of Intracellular fluid(ICF) and bilayer lipid membrane surrounding the medium, which is again encompassed on another fluid medium called as the Extracellular fluid(ECF). Since all the three components are made from a different material, it was usual that they would respond uniquely to the alternating current signal [6].

The ICF consists of the nucleus and the cytoplasm, which are made up of proteins, chemicals, salts with K+ ions having the highest concentration and hence, conducting. Similarly, the ECF also has high concentration of Na+ and Cl- ions making it electrically conducting. These ions can move freely and are able to transport electrical charge and provide highly conducting paths to the applied source. But, however, the plasma membrane which is sandwiched between two conducting medium is made up of electrically non-conducting lipid molecules and will act like a dielectric medium. Thus, it leads to the formation of a protein-lipid-protein(P-L-P) structure behaving like a capacitance when an alternating current is applied against it [7]. So it has been proved that all living tissues and cells shows capacitive response and for that reason tissues show a decrease in impedance value with increasing current in the analyzing circuit. At low frequency, the biological tissue may be thought of as an ionic conductor. But the situation is different in case of high frequency for the cell membrane electrically separate from the cell because of its low conductivity and thus acts as a dielectric medium [8].

According to **Fricke's Model** it can be seen that the ionic current flowing through the biological tissues passes through two different pathways with respect to the frequency.

a) At very low frequency alternating current will behave as direct current and since the plasma membrane is made up of lipid molecules is electrically non-conductive, no current will pass inside of the cell and will only flow in the extracellular medium as shown in the fig(1)

b) However, as the frequency increases the cell membrane acts as capacitor and charging and discharging of the current takes place with respect to the frequency rate. Thus, the study of displacement of the charges in the plasma membrane is extremely important since current flows through entirely through the cell membrane by displacement in both the extra- and intracellular fluid. At very high frequencies, the displacement process is so fast that membrane impact is almost negligible and all the current goes through the cell in similar a fashion as in outside of the cell almost unhindered [9].

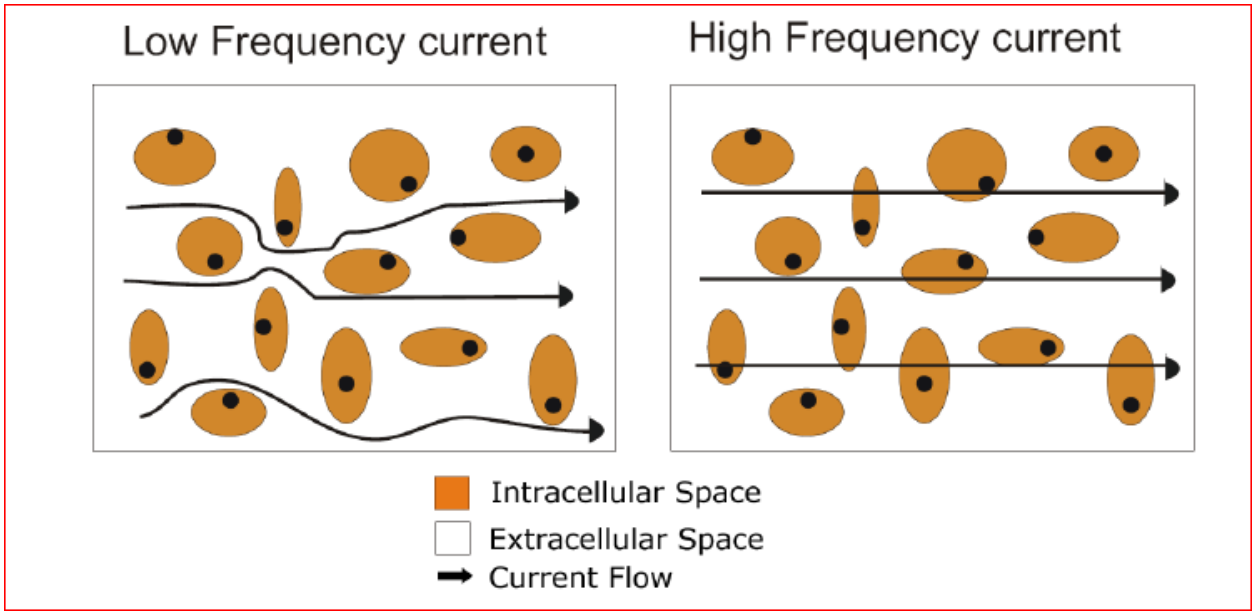


Fig-1 Current flow in tissues at low and high frequencies

2.2 Lab-On-Chip Device

A **Lab-On-Chip(LOC)** device also known as **microfluidic** device may be defined as a powerful non-invasive **microelectromechanical systems(MEMS)** that can easily integrate one or several miniaturized laboratory functions on a single chip ranging from few millimeters to square centimeters in size [9]. These devices are extremely small, portable, reconfigurable and cost effective with high power consumption, therefore making it affordable for high mass production. Few of the distinct features of LOCs which makes it one of the most productive devices in semiconductor industry is as follows:-

- Very high throughput processing
- Very high obtainable temperature homogeneity due to a small volume in picolitre or milliliter range.
- Maintains continuous and segmented flow of samples in microfluidics.
- Increases chemical reactions time for quickly desired output.
- And Relatively short quality tests times [10].

In the present scenario, the LOCs have huge commercial applications in semiconductor industry specifically in miniaturization of systems such as in Diagnostics, Biochemistry, Bioanalysis, volume sensing, biocomputing, drug-testing, cytometry. Hence, they have created a major revolution in the fields of biomedical and biotechnology from eliminating the limitations of room-sized laboratories to microchip-based portable devices [11].

Some of few different types of Lab-On-Chips devices are as follows-Micro pumps and Microvalves, Fluidic mixers, Electro-wetting chips, Electrophoretic chips, Magnetophoretic chips, **Bioimpedance chips,** Microbioreactors, Cytometers, Polymerase chain reaction (PCR) chips, Immunoassay chips, Cell Sorters, Microarrays etc.

However, our subject of interest is limited to understanding the working principle and applications of **Bioimpedance Analyzer Circuit [12]**

2.3 Bioimpedance Analyzer Circuit

Bioimpedance Analyzer Circuit is a **Lab-on-Chip(LOC)** measuring instrument or device which can measure the bioelectrical impedance of the tissues/cells produced in the biological sample when an alternating current is passed through it. One of the major applications of such small scale impedance measuring device is it can increase the spatial resolution which in turn can limit the probability of dielectric breakdown by reducing the strength of required Electric field [12]. It is one of the most profound analysis technique in which the body composition and the physiological properties of any biological sample can be found out in terms of the frequency response. The frequency depends on the anatomical, pathological and physiological status of the biological tissues. Thus, the studies on impedance analysis can provide a lot of information about both its anatomical and physiological characteristics [13]. Since the response for electrical bioimpedance is directly dependent on frequency, multifrequency impedance analysis can provide better information in terms of the tissue characterization than the single frequency

analysis. Therefore, multifrequency impedance analysis has been found as one of the most effective non-invasive techniques of impedance measurement. Therefore, a lot of research has been carried out on both the techniques in order to diagnose a large number of diseases [14].

2.4 Bioimpedance Analysis & Measurement

Bioimpedance of a biological tissue can be measured when a low frequency alternating current is applied to the tissue or any biological sample through an array of electrodes connecting the tissue or sample surface. The alternating current is usually applied to avoid the tissue damage and is never conducted with a direct current signal. It has been observed that all living tissues/cells show capacitive reactance and due to which there is a decrease in the impedance value with increasing alternating current [15].

Mathematically, the Impedance(Z) of any biological sample can be calculated by dividing the measured voltage signal (V in rms) to the applied current signal (I in rms).

i.e **Impedance(Z) = Vrms/Irms.....(1.1)**

Since Z is a complex quantity, it can also be expressed in terms of real and imaginary part-

$$\mathbf{Z = R+jX} \quad .....(1.2)$$

$$\mathbf{Re[Z] = R = Resistance}$$

$$\mathbf{Img[Z] = X = Reactance}$$

where Z is called as the impedance of the sample, Real part of Z is the Resistance and Imaginary part of Z is the Reactance. Then again, Reactance can be divided into two types- Capacitive Reactance(Xc) and Inductive Reactance(XL). Since it has been mentioned biological sample show a capacitive response, we are more interested in capacitive reactance(Xc) [16].

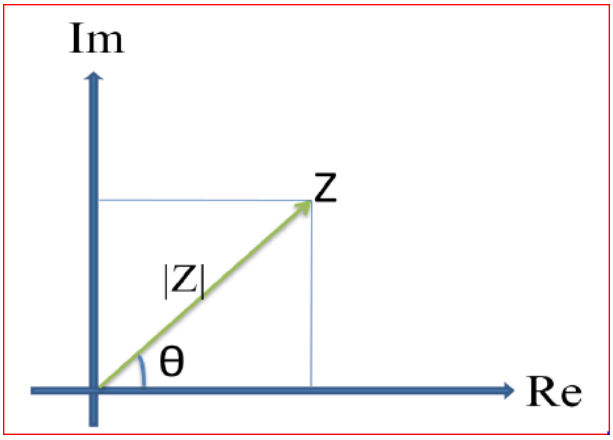


Fig-2 Schematic representation of impedance in complex plane

2.5 Major parts of Bioimpedance Spectroscopy

2.5.1 Electrodes:

An **Electrode** in bioimpedance analyzer functions as a **transducer** which converts the ionic current (such as Na+/K+/Ca2+/Cl- ions etc). present in the electrolyte into electronic current. Thus, in short, it acts as a bridge between the biological sample and electronic measuring device [17]. Electrodes can broadly be classified into two types:-

1. Polarizable Electrodes, in which the electrode-electrolyte interface acts like a capacitor with ideally zero DC biased current flow. Ex- Platinum in NaCl

2. Non-Polarizable Electrodes, in which the electrode-electrolyte interface acts like a resistor with free current flow throughout. Ex-Platinum Hydrogen Electrode [18].

2.5.2 Voltage Buffer:

The Voltage Buffer circuit used in Bioimpedance analysis is a non-inverting unity gain voltage buffer circuit. It makes a copy of the input voltage at the output without drawing any current from where the input terminal is connected. It prevents the loading of the input circuit from the output stage and also helps in energy transfer between the circuits. One of the distinctive advantages of Voltage Buffer-it helps in Impedance Matching [19].

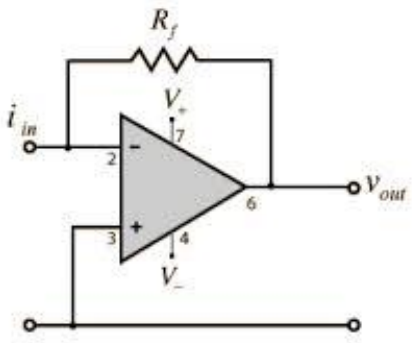


Fig-3 Schematic Representation of a Non-Inverting Voltage Buffer Circuit

2.5.3 Printed Circuit Board(PCB):

Printed Circuit Board is one of the most simplest electronic platforms which has a dual property for providing firm mechanical support and better electrical connections to the electronic

components. However, in Bioimpedance analysis we are more interested at electrodes formed from the etched Copper laminates of a non-conducting substrate [20].

2.5.4 Microchannels:

The microchannel is defined as a channel which has at least one of its sides in the micrometer range .These channels are more often used in impedance analyzer circuits and are usually preferred over microwells. The advantages over microwells is as follows:-

- Dynamic flow characterization of a medium
- Study of variation in physiological properties through impedance measurement by mixing more than one solution
- Study of variation in impedance by changing the design geometry

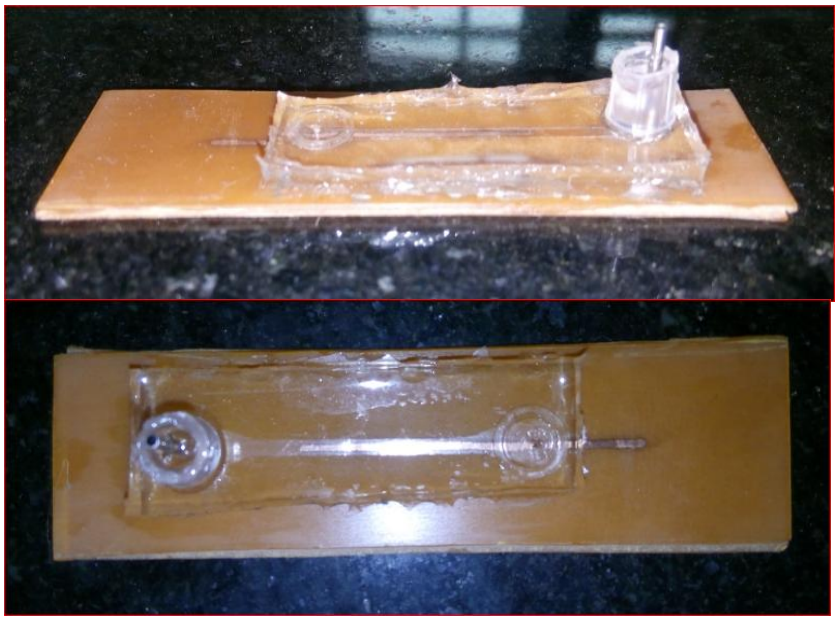


Fig-4 Microchannel Bioimpedance Analyzer Circuit

CHAPTER-3

OBJECTIVE & WORKPLAN

3.1 Scope of the present investigation:

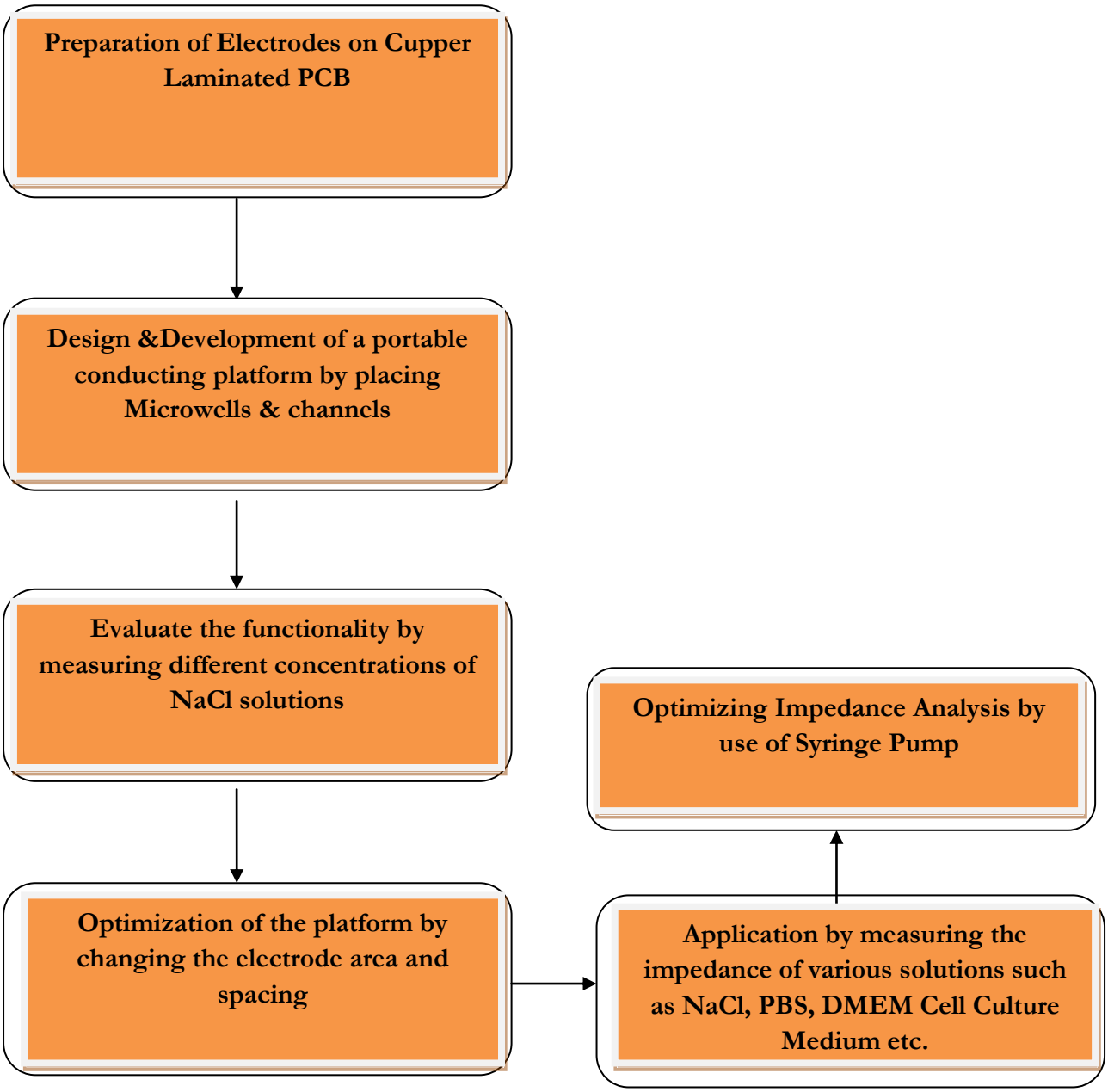
Bioelectrical impedance analysis is one of the most powerful non-invasive techniques used so far in monitoring body compositions and various clinical conditions. However, for this analysis to take place we need an analyzer circuit which can work as a platform for impedance measurement. But the existing techniques are costly, larger in size and somewhat more complicated on the basis of their working principle. Therefore, there is a need to design and analyze an Impedance Analyzer circuit which is portable, cost-effective and at the same time can work in a real-time monitoring system. Keeping that perspective in mind, we have aimed to **"Design and Develop a Microfluidic Lab-On-Chip Bioimpedance Analyzer Circuit"**

3.2 Objective:

The project entitled "**Design and Development of Lab-on-a-Chip Bioimpedance Analyzer"** can be worked upon in three major phases:-

- Design & Development of a portable conducting analyzer platform.
- Sample Preparation and Impedance Analysis under static or flow condition

3.3 Work Plan:


Preparation of Electrodes on Cupper Laminated PCB
Design &Development of a portable conducting platform by placing Microwells & channels
Evaluate the functionality by measuring different concentrations of NaCl solutions
Optimization of the platform by changing the electrode area and spacing
Application by measuring the impedance of various solutions such as NaCl, PBS, DMEM Cell Culture Medium etc.
Optimizing Impedance Analysis by use of Syringe Pump

CHAPTER-4

MATERIALS &METHODS

4.1 Aids & Materials Required:

4.1.1 Software Required-

- EAGLE 5.6.0 (Schematic Software)

4.1.2 Required Instruments-

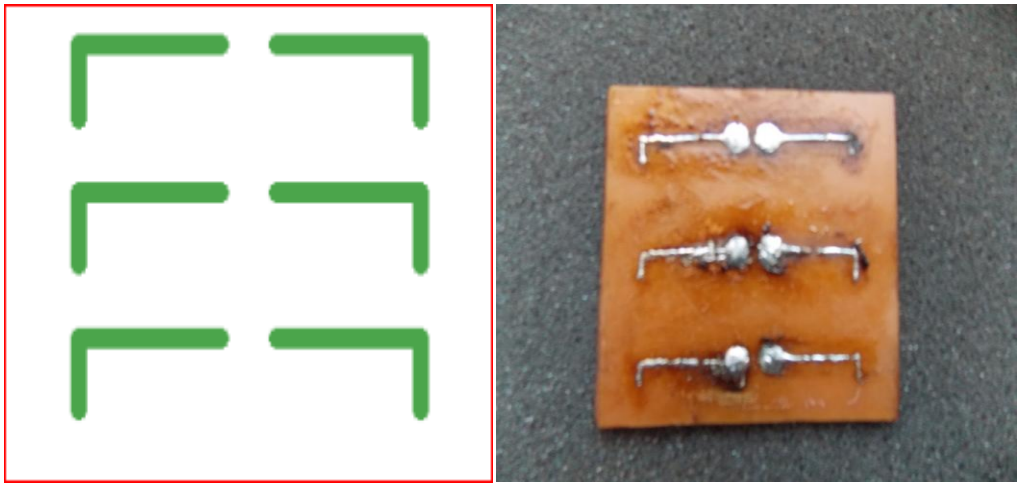
- Function Generator
- Digital Oscilloscope
- Digital Multimeter
- Syringe Pump
- CO2 Incubator
- Magnetic Stirrer
- Orbital Shaker

4.2 Preparation of Electrodes

Electrodes can be prepared by using two methods:-

a) Electrode prepared via Printing using EAGLE 5.6.0 (Schematic Software) or

b) Electrode prepared via manually using Marker [21]



(A) (B)

Fig-5 A)Electrodes designed from EAGLE Software & B) PCB Chip Electrode

Table-1 Flow Chart for preparation of Electrodes

PCB Design using EAGLE (Schematic Software)
Masking (Heating using Electric Iron)
Etching (using Ferric Chloride Solution)
Demasking
Attachment of Microchannels upon Electrodes using PDMS
Hot air oven treatment (45-60 minutes)

4.3 PCB Designing:

Designing of Printed Circuit Boards (PCB) is one of the most important phases in the development of the Bioimpedance Analyzer Circuit. It is usually developed in a series of four steps [22-23]-

Step 1: Washing and Cleaning: - It involves cleaning the surface of the PCB board, thereby making it free from the contaminants. It is very important for the surface to be cleaned so that the masking material can have good adherence to the surface. The materials which are basically used are de-oxidizing agents and Alcohols such as Ethanol and other chemicals.

Step 2: Masking: - It is a methodology that involves deposition of the masking material on the Cu laminated surface so as to assure prevention of the required area that is to be etched [24]. As already mentioned, this can be done by two ways via EAGLE software and also via Marker.

Step 3: Etching:- It is a chemical procedure technique that involves immersion of the masking Cu laminated sheet in the Ferric Chloride Solution for a stipulated time period(10 minutes), resulting in the removal of the unmasked part of the Copper Laminate.

$$\mathbf{FeCl3 + Cu \rightarrow FeCl2 + CuCl}$$

$$\mathbf{FeCl3 + CuCl \rightarrow FeCl2 + CuCl2}$$

The CuCl2 formed as the end product is used for the designing of the electrodes [24].

Step 4: Demasking: -This technique is used for the removal of masking material by normal water or by applying detergents/scrub on the material.

4.4 Development of Bioimpedance Analyzer Circuit :-

The main criteria for the development of Bioimpedance Analyzer Circuit are-

a)Sinusoidal wave generator

b) Voltage controlled constant current source(VCCS) [13].

And here we considered exactly the same by using the Function generator for the sine wave signal generation. The output terminal of the generator was again fed into a non-inverting unity gain voltage buffer circuit as shown in the Fig-7 below. The voltage buffer connected to the system acts as feedback loop system which makes a copy of the input voltage at the output without drawing any current from where the input terminal is connected. It prevents the loading of the input circuit from the output stage and also helps in energy transfer between the circuits. One of the distinctive advantages of Voltage Buffer-it helps in Impedance Matching. The output of voltage buffer was connected into the non-inverting VCCS. Therefore, this combination of voltage buffer and VCCS contributes to the impedance analysis. Finally AC current injected into the circuit can help in impedance variations of any biological samples [25].

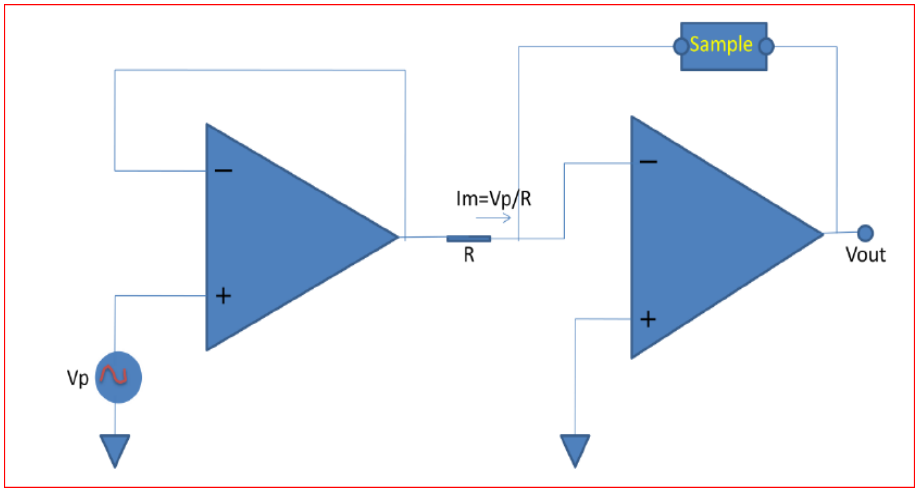


Fig-6 Bioimpedance Analyzer Circuit

4.5 Sample Preparation and its Impedance Analysis

4.5.1 Study of Impedance Analysis in Microwells Analyzer platform

Three different varieties of solutions were made in the laboratory. The 1st was Normal Distilled Water, 2nd was 10ml Apple Juice Solution and the 3rd was 5ml Broth in 10ml of Apple Juice Solution. Each of the solutions was added to each one of the microwells separately as shown in Fig-7 and finally connection was made. The diameter and spacing of the electrodes were 0.13" and 0.05" respectively. An Electric field was generated across the sample and the corresponding changes in voltage were recorded with respect to varying frequency with the help of DSO. But, it should be remembered that all the readings were made under conditions with Input Voltage(Vpp) of 1V, R=9.1 kΩ and frequency range from 50Hz to 10 kHz.

Fig-7 Microwell Bioimpedance Analyzer platform

. **Calculations:**

$$Z = V_{rms}/I_{rms} \quad \text{.....(1)}$$

$$I_{rms} = (V_{pp} * 0.707)/R \quad \text{.....(2)}$$

where, Z=Impedance of the sample

Vrms=Voltage Drop

Irms=Current Injected at Frequency, f

Vp = Supply voltage of function generator

4.5.2 Impedance Measurement of NaCl Solution :

From the above analysis, it has also been observed that impedance measurement using microwells could only be able to measure static solutions. However, it has its limitations for the measurement of dynamic flow. Another drawback of this technique is that for accurate measurement, a large volume of solution is required which may not be feasible all the time. Therefore, in order to eradicate such limitations, from now on we will be using microchannel conducting platforms.

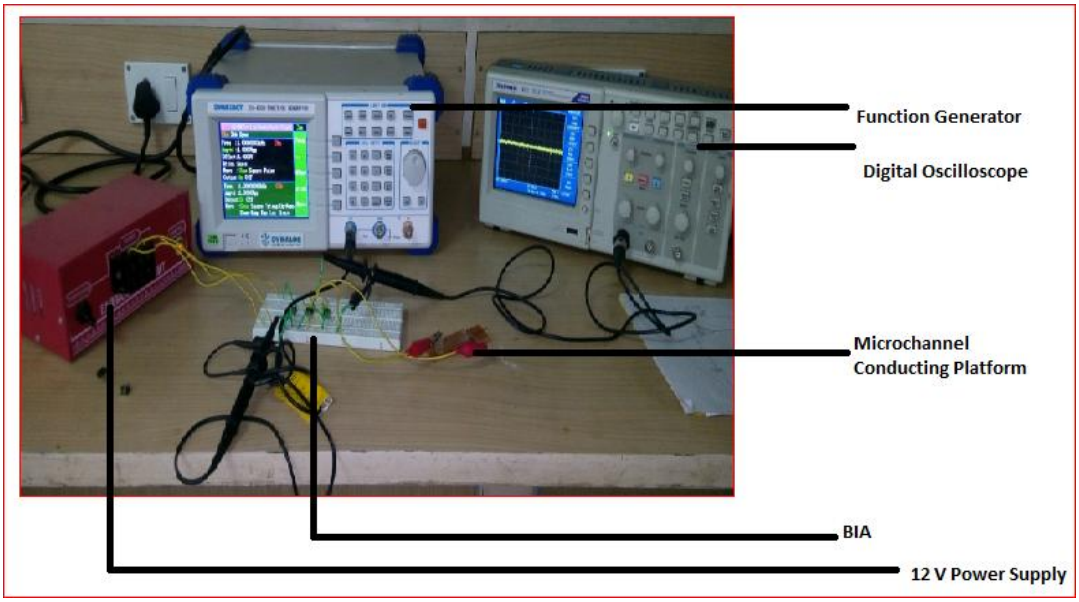
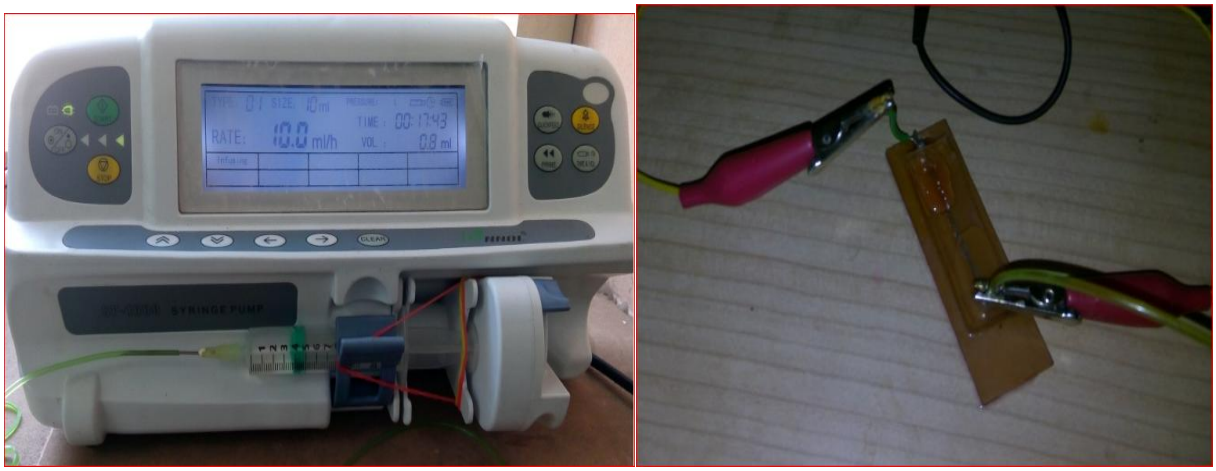


Fig-8 Experimental Setup for Impedance Analysis

Different concentrations of NaCl solutions were prepared using distilled water. Initially, the solutions were seeded onto the surface of the electrode using micropipette and then current was passed through the solution. Again the changes in the voltage value were analyzed with varying frequency. The measurements were performed with the conditions of input voltage(Vpp)=1V and frequency ranging from 50 Hz to 100 kHz.

4.5.2 (A) Optimized Impedance Analysis with help of Syringe Pump:-

Maintaining a controlled amount of flow rate for any biological sample during Impedance analysis is of primary importance. Earlier we were using micropipette for the flow analysis. But, however, we couldn't able to maintain a steady flow rate that is required for the optimized result. Therefore, in order to overcome this limitation, the use of Syringe Pump is very much desirable [26].



(A) **(B)**

(C)

Fig-9 (A) Syringe Pump (B) Connection of the pump to the conductive platform

(C) Experimental Set-up for Impedance Analysis with the help of Syringe pump

In this experimental set-up, the syringe pump was first made to connect with the conducting platform. The aim was to measure impedance values at three different flow rates such as 2ml/h, 4ml/h, and 6ml/h. Keeping the flow rate constant, for each NaCl solution 5 readings were recorded at a subsequently fixed time interval of 2 minutes. Hence. this method is by far, one of the best techniques of Impedance analysis

4.5.3 Impedance Measurement of PBS Solution:-

1X PBS solution was prepared in the laboratory. The composition is as stated below-

$CaC_2.2H_2O$ =0.33gm

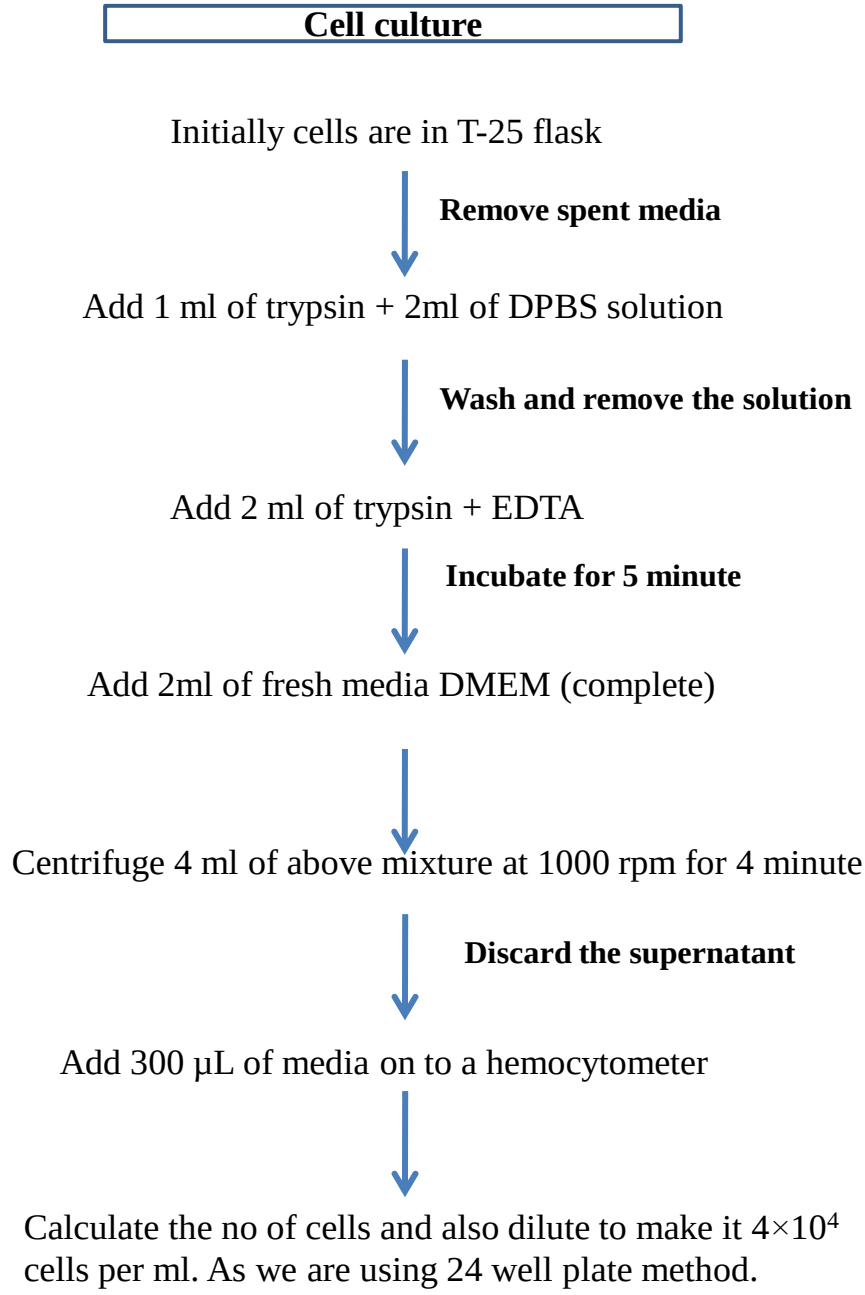
$MgCl_2.6H_2O$ =1.0gm(1 litre)

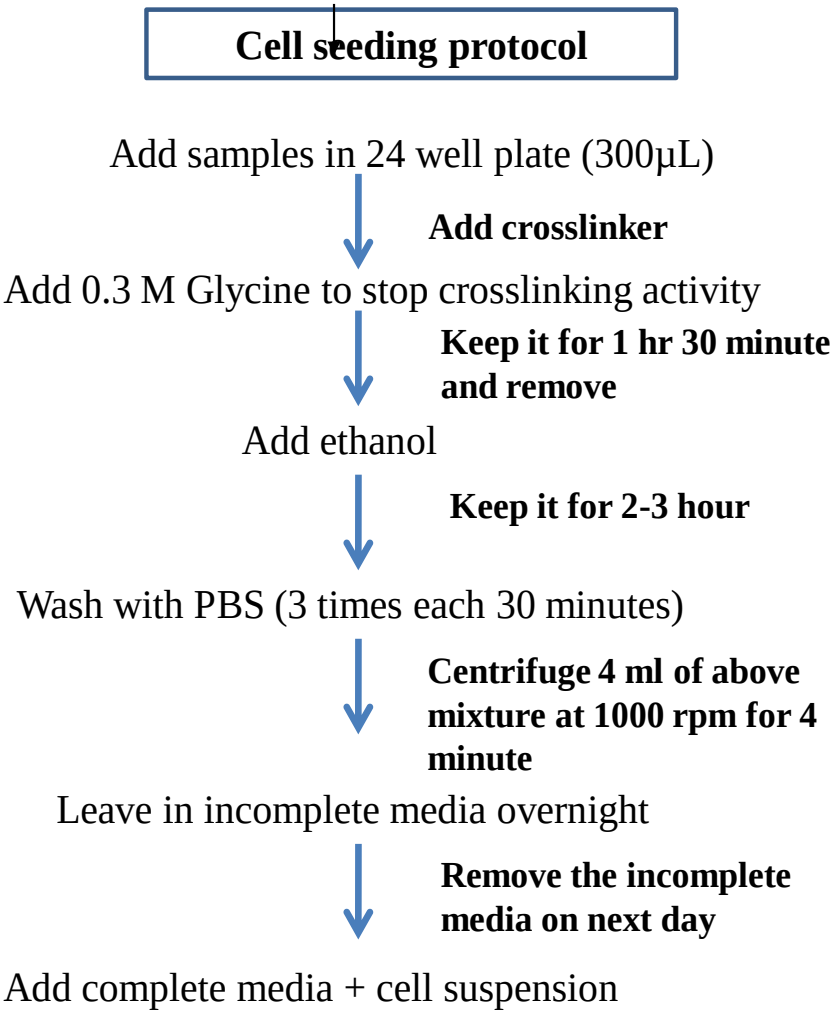
The solution was added to the microchannel connected to the current injection electrodes. Current passed through the 1X PBS solution and the corresponding voltage was observed with the help of CRO at varying frequency. All the measurements were performed with the input voltage of 1V and frequency range from 50 Hz to 20 kHz.

4.5.4 Impedance Measurement of DMEM Solution:-

5ml of DMEM cell culture solution was prepared in the laboratory. The solution was added to the microchannel connected to the current injection electrodes. Current passed through the solution and the corresponding voltage was observed with the help of CRO at varying frequency. All the measurements were performed with the input voltage of 1V and frequency range from 50 Hz to 20 kHz.

4.5.5 Impedance analysis of HaCaT cell culture solution:-




Cell seeding protocol
Add samples in 24 well plate (300µL)
Add crosslinker
Add 0.3 M Glycine to stop crosslinking activity
Keep it for 1 hr 30 minute and remove
Add ethanol
Keep it for 2-3 hour
Wash with PBS (3 times each 30 minutes)
Centrifuge 4 ml of above mixture at 1000 rpm for 4 minute
Leave in incomplete media overnight
Remove the incomplete media on next day
Add complete media + cell suspension

CHAPTER-5

RESULTS AND DISCUSSIONS

5.1 Impedance Analysis in Microwell Analyzer Platform

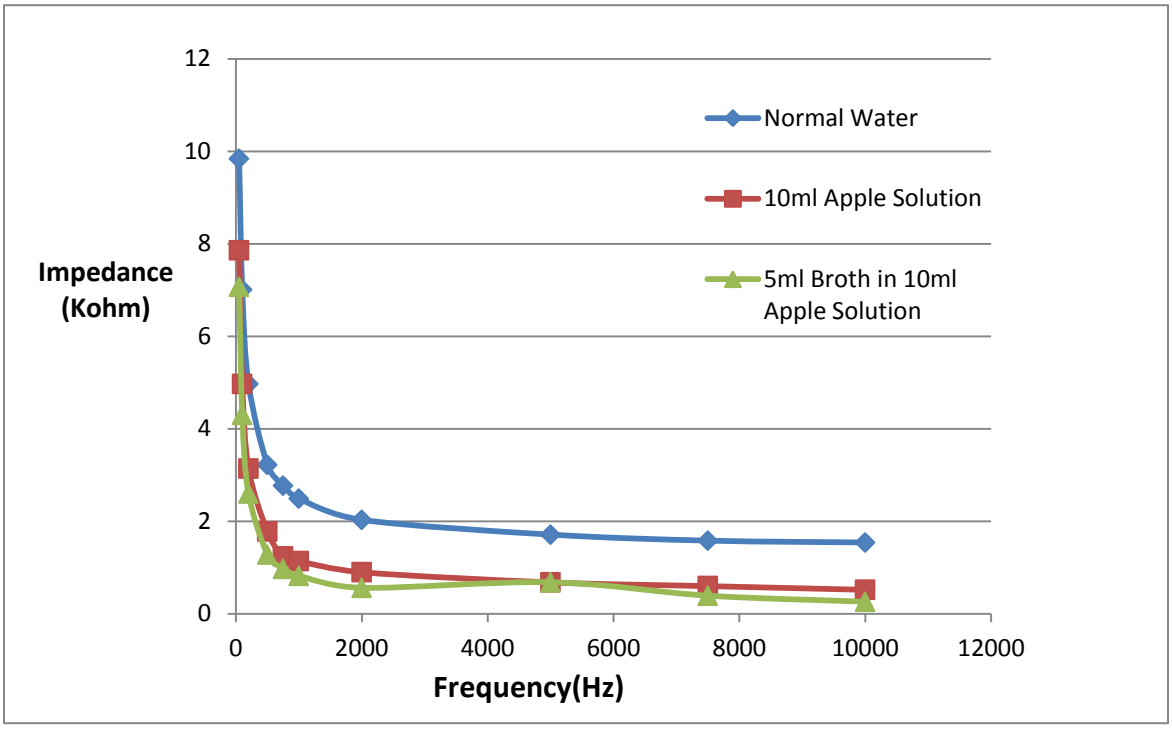


Fig-10 Impedance Vs Frequency plot of a) Normal Water b) Apple Solution c) 5ml Broth in 10ml Apple Solution

The above impedance analysis has been done in the frequency range of 50Hz to 10KHz. The Voltage Constant Current Source(VCCS) gives a constant current of 0.7 mA as input to the sample for analysis. So, as a result, the Impedance of the sample can be shown as in Fig-10 . From the graph, it can be observed that there is a steep decrease in the impedance value at the initial stage, but then it remained almost constant when the frequency was increased. This clearly indicates the capacitive response.

5.2 Impedance Measurement of NaCl Solution:

5.2.1 Static Condition

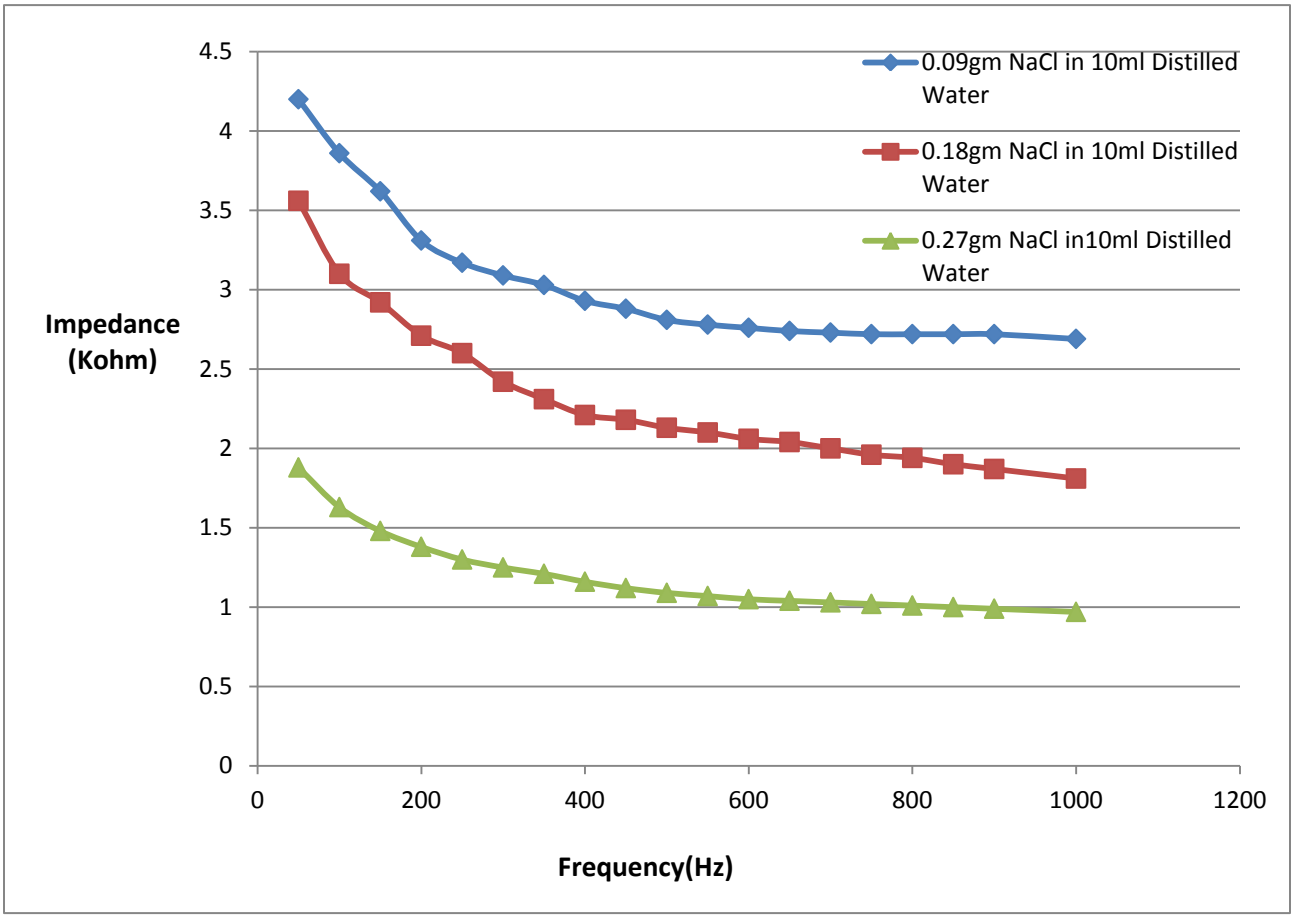


Fig-11 Impedance Vs Frequency plot of a) 0.09gm NaCl b) 0.18gm NaCl c) and 0.27gm NaCl in 10ml Distilled Water

The above impedance analysis has been done in the frequency range of 50 Hz to 100 kHz. The Voltage Constant Current Source(VCCS) gives a constant current of 0.087 mA as input to the sample for analysis. So, as a result, the Impedance of the sample can be shown as in Fig-11 . From the graph, it can be observed that there is a decrease in the impedance value at the initial

stage with increasing frequency but then in the later part it remained almost constant. This clearly indicates the capacitive response.

5.2.2 Dynamic Flow Condition using Syringe Pump

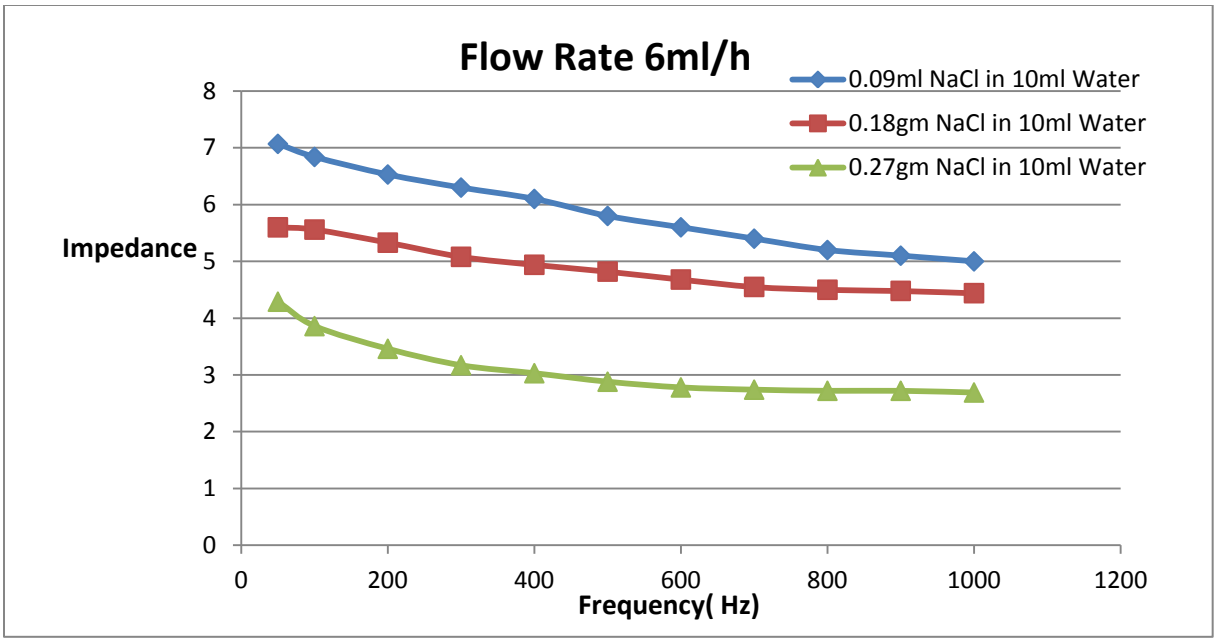
Flow Rate(F) (in ml/h)	Concentration(C) x gm NaCl in 10ml Water	Time Interval (t)	Frequency (Hz)	R1 kΩ	R2 kΩ	R3 kΩ	R4 kΩ	R5 kΩ
6	0.09	2	50	6.63	6.5	6.2	6.43	6.5
			300	5.09	5.03	5.1	5.31	5.2
			1000	2.8	2.9	2.3	2.43	2.1
	0.18	2	50	5.63	5.93	5.7	5.34	5.2
			300	3.5	3.23	3.5	3.76	3.5
			1000	2.03	1.99	2.1	2.19	2.2
	0.27	2	50	2.93	2.99	3	3.04	2.7
			300	2.43	2.56	2.4	2.6	2.7
			1000	1.8	1.23	1.1	1.24	1.2
4	0.09	2	50	5.64	5.8	5.2	5.69	5.7
			300	4.12	4.29	4.1	4.26	4.3
			1000	2.62	2.93	2.7	2.81	2.9
	0.18	2	50	4.57	4.5	4.2	4.2	4.3
			300	2.3	2.54	2.4	2.76	2.3

Flow Rate(F) (in ml/h)	Concentration(C) x gm NaCl in 10ml Water	Time Interval (t)	Frequency (Hz)	R1	R2	R2	R4	R5
4	0.27	2	1000	0.98	1.12	1.0	1.05	1.0
2	0.09		50	4.29	4.22	4.1	4.08	4.3
			300	3.09	3.3	3.1	3.17	3.1
			1000	2.2	2.39	2.5	2.42	2.6
	0.18	2	50	3.43	3.41	3.5	3.58	3.7
			300	2.19	2.26	2.1	2.29	2.2
			1000	1.73	1.79	1.6	1.72	1.7
	0.27	2	50	1.89	1.91	1.9	1.78	1.8
			300	1.12	1.22	1.3	1.16	1.2
			1000	0.92	0.98	0.8	0.96	0.8

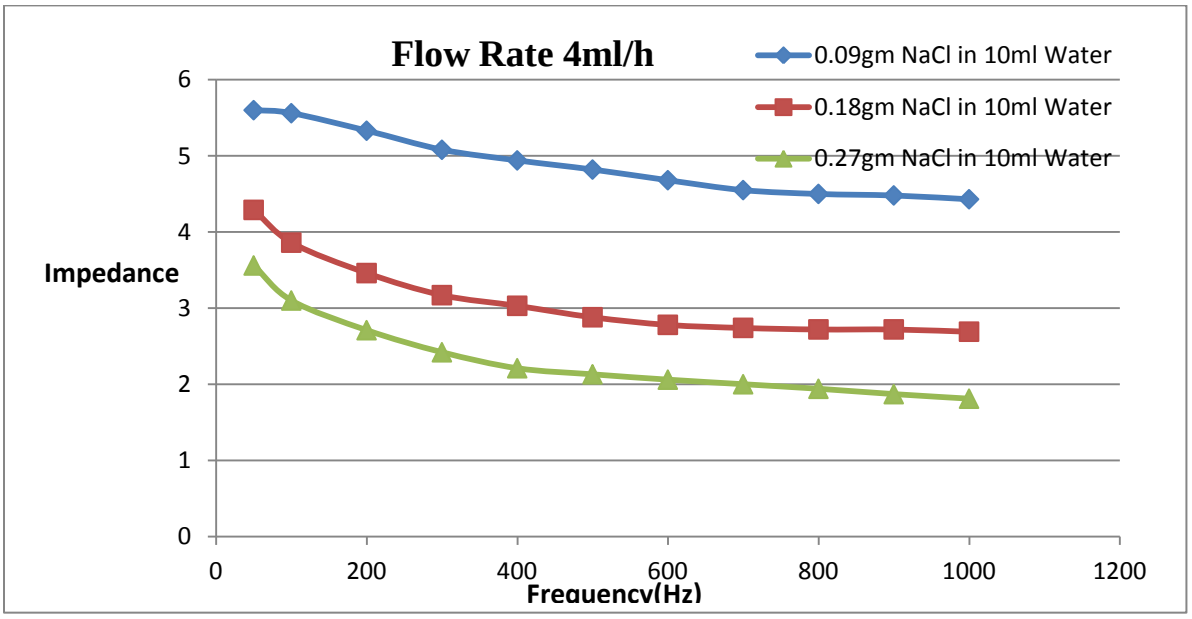
Table-2 Measurement of Impedance value of different concentration NaCl solutions at different flow rates

From the above table, it is observed that, while increasing the flow rate of any particular NaCl solution, the resulting Impedance value also increases. This shows that the Impedance is directly proportional to the flow rate at a constant frequency.

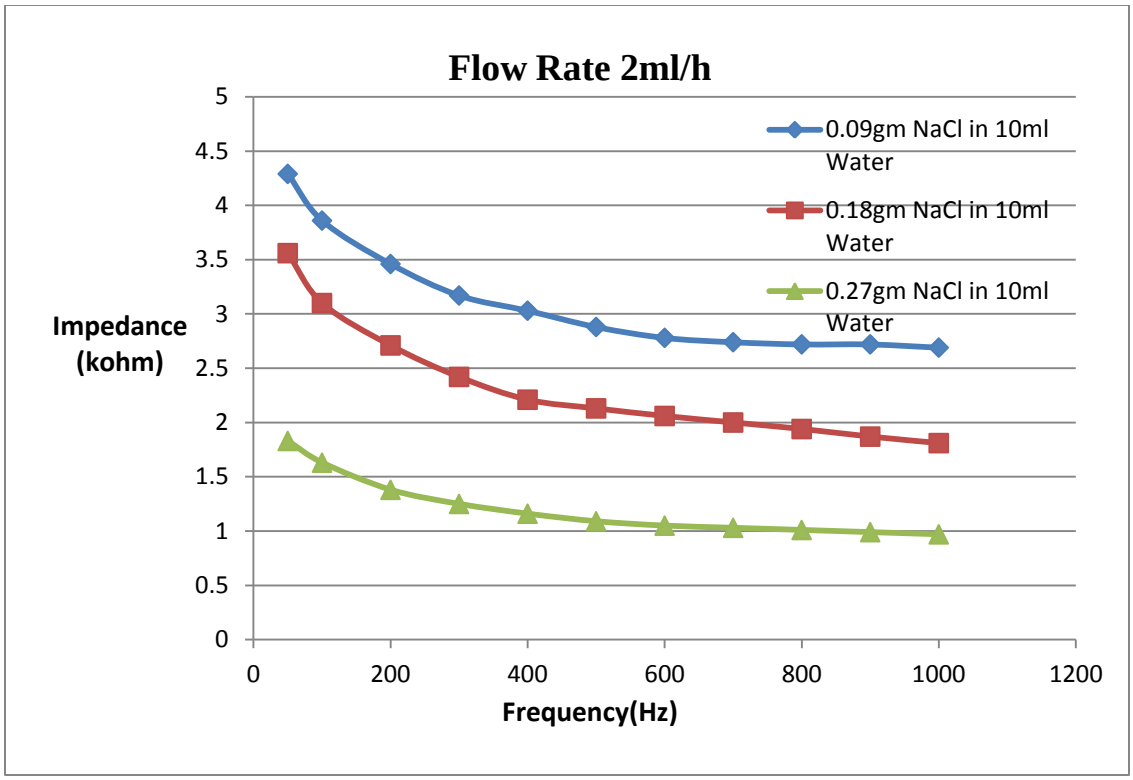
The mean impedance value of each of the measurements of different concentration NaCl solutions were taken for each independent frequency and the results were again analyzed at different flow rates as shown in the graph Fig-12 plotted below.


Flow Rate 6ml/h
0.09ml NaCl in 10ml Water
0.18gm NaCl in 10ml Water
0.27gm NaCl in 10ml Water
Impedance
Frequency(Hz)
0
1
2
3
4
5
6
7
8
0
200
400
600
800
1000
1200

(A)


Flow Rate 4ml/h
0.09gm NaCl in 10ml Water
0.18gm NaCl in 10ml Water
0.27gm NaCl in 10ml Water
Impedance
Frequency(Hz)
0
1
2
3
4
5
6
0
200
400
600
800
1000
1200

(B)



(C)

Fig-12 Frequency Vs Impedance plot analysis of different concentration NaCl solutions at different flow rates-(A) 6ml/h (B) 4ml/h (C) 2ml/h

5.3 Impedance Measurement of PBS Solution:-

(i) Static flow

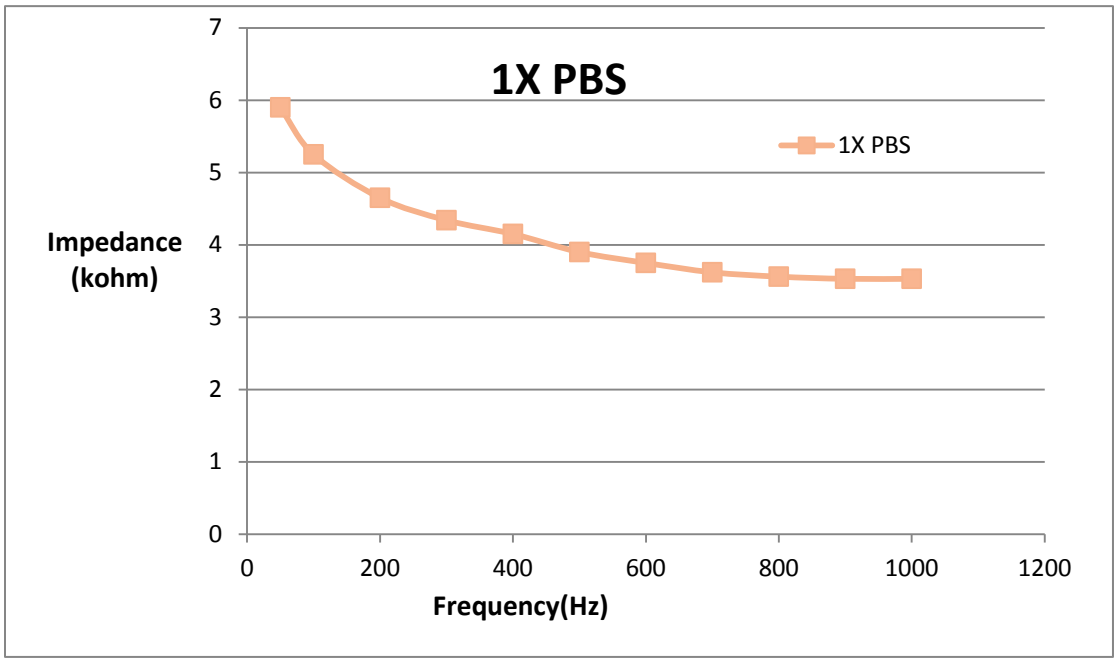


Fig-13 Frequency Vs Impedance plot of 1X PBS

The above impedance analysis has been done in the frequency range of 50 Hz to 10 kHz. The Voltage Constant Current Source(VCCS) gives a constant current of 0.087 mA as input to the sample for analysis. So, as a result, the Impedance of the sample can be shown as in Fig-13 . From the graph, it can be observed that there is a decrease in the impedance value at the initial stage with increasing frequency but then in the later part it remained almost constant. This clearly indicates the capacitive response.

5.3(i) Dynamic flow Impedance Analysis-

Flow Rate(F) (in ml/h)	**Concentration(C)**	**Time Interval (t)**	**Frequency (Hz)**	**R1** kΩ	**R2** kΩ	**R3** kΩ	**R4** kΩ	**R5** kΩ
6	1X PBS	2	50	5.90	5.34	5.7	5.87	5.9
			300	4.34	4.22	4.5	4.1	4.2
			1000	3.51	3.41	3.5	3.76	3.6
4	1X PBS	2	50	4.20	5.93	5.7	5.34	5.2
			300	3.17	3.22	3.6	3.09	3.0
			1000	2.68	2.32	2.1	2.9	3.1
2	1XPBS	2	50	3.58	3.51	3.2	3.6	3.9
			300	2.42	2.43	2.4	2.12	2.6
			1000	1.21	1.32	1.2	1.28	1.2

Table 3-Frequency Vs Impedance analysis at different flow rates.

From the above table, mean impedance value of each of the measurements were taken of a particular frequency of different flow rates and finally a graph was plotted as shown in the figure-14 below.

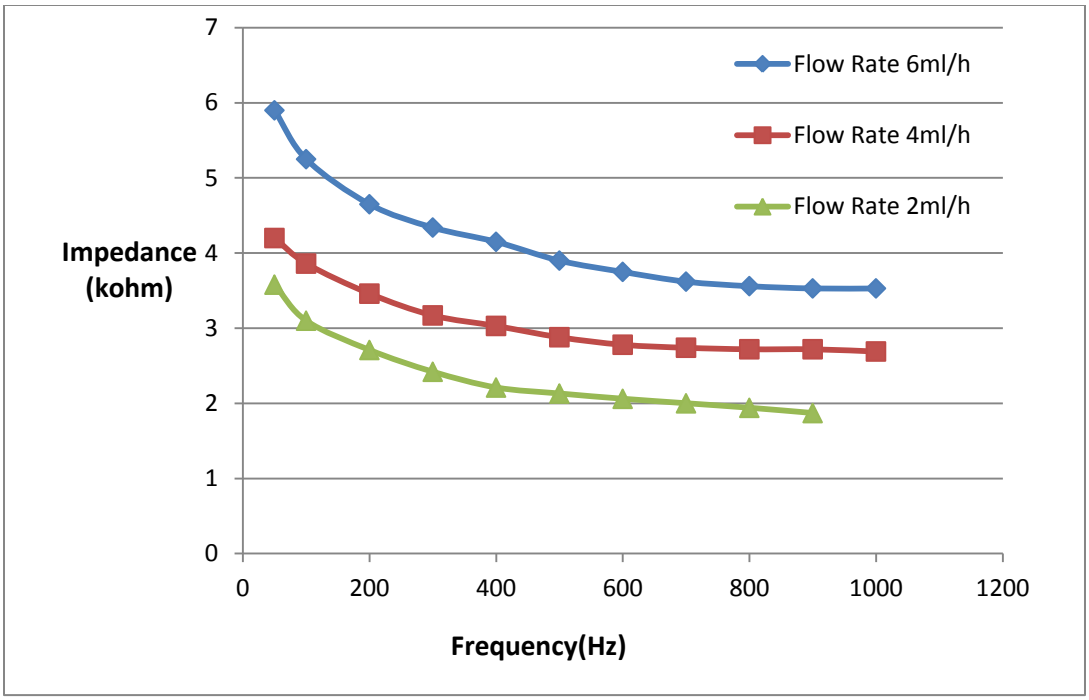


Fig 14- Plot shows Frequency Vs Impedance of 1X PBS solution at different flow rates

Impedance analysis of IX PBS solution was done with the help of a syringe pump. Impedance values at different flow rates of 6ml/h, 4ml/h, and 2ml/h were observed and analyzed. From the above graph, it can be concluded that, while increasing the flow rate of 1X PBS solution at a constant frequency, the resulting Impedance value also increases. This shows that the Impedance is directly proportional to the flow rate at a defined frequency.

5.4 Impedance Analysis of DMEM solution:-

Flow Rate(F) (in ml/h)	**Concentration (C)**	**Time Interval (t)**	**Frequency (Hz)**	**R1** kΩ	**R2** kΩ	**R3** kΩ	**R4** kΩ	**R5** kΩ
6	5ml DMEM	2	50	21.49	21.14	20.45	20.5	20.5
			300	18.39	18.39	18.62	18.9	18.2
			1000	12.87	14.02	12.41	12.4	12.6
4	5ml DMEM	2	50	20.34	20.45	19.88	19.7	18.3
			300	17.58	17.58	16.20	15.4	15.7
			1000	14.02	14.01	14.25	14.2	14.3
2	5ml DMEM	2	50	18.62	19.65	18.96	18.73	18.7
			300	16.65	17.93	17.01	16.43	16.5
			1000	12.41	13.21	12.52	13.90	12.5

Table 4-Frequency Vs Impedance analysis of DMEM Solution at different flow rates

5.5 Study of Impedance Analysis with and without HaCat Cells

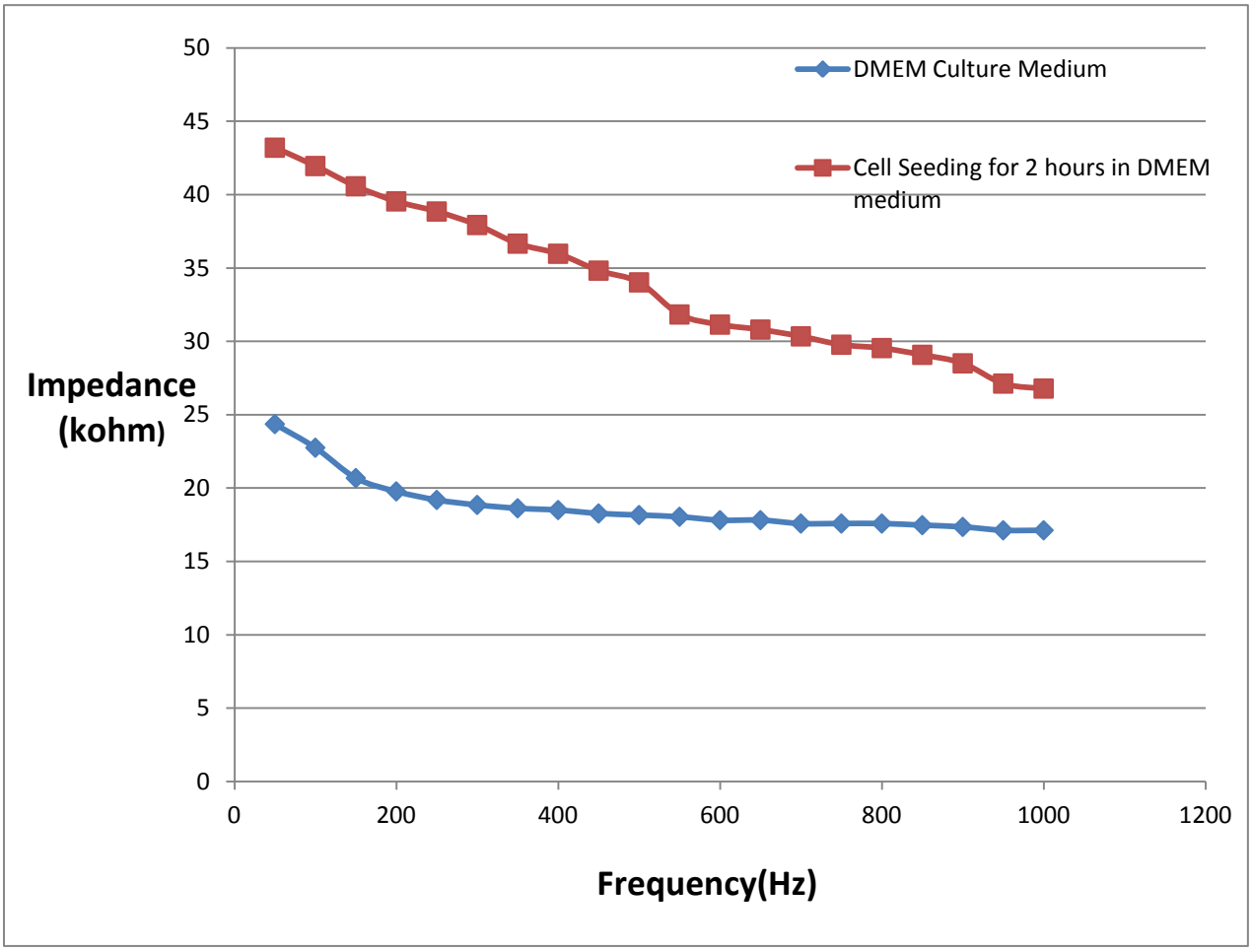


Fig 15-Frequency Vs Impedance plot of DMEM Solution with and without HaCaT cells

A 5ml DMEM solution was prepared in the laboratory. Then, HaCaT cells were seeded on the microchannel platform in DMEM Culture medium. A comparative impedance analysis was done of both the solutions with and without HaCaT cells, and the frequency range was 50 Hz to 10 kHz. The changes in the impedance value with frequency is as shown in the figure above Fig-14.

From the graph, it was observed that in both the cases the solution is decreased and then it remains almost constant with increasing frequency. This clearly shows the capacitive response. It was also observed that impedance value increases when concentration of the HaCaT cells in the DMEM culture medium is increased. This experiment was repeated several times with a time interval of 2 hours and the results showed similar trends. Therefore, it was evident that increased HaCaT cell concentration in the culture medium showed increased impedance at a particular frequency.

CHAPTER-6

CONCLUSION AND FUTURE WORK

CONCLUSION:

A suitable Lab-On-Chip analyzer device was successfully developed for measuring the impedance of biological samples. The sensitivity of designed instrument efficiently works on the range of 50 Hz to 10 KHz and in some cases to even 100 KHz. To improve the efficacy of the analysis, the electrodes spacing and diameter were also optimized. Moreover, the developed device was successfully tested for measuring the impedance of various biological samples such as NaCl solution, Apple Juice solution, PBS and impedance analysis was also done with and without HaCaT cells in DMEM culture medium. However, all the recorded analysis had shown the capacitive response. The device was also made to measure the impedance of different biological samples through continuous flow mechanism using a syringe pump and from that, the optimized data were recorded. Hence, this experimental set-up was quite a success and one of the major advantages of this platform over commercially available instruments is its portability.

FUTURE WORK:

A simple and portable platform for impedance analysis of biological samples is of primary importance. There were problems regarding measuring values at high frequency due to a lot of noise getting added into the analysis. But, still that can be overcome by digitalization. Therefore sensitivity and operating range of the developed instrument can be improved by either digitalizing the entire process or by interfacing the system with software like MATLAB etc.

REFERENCES:

1. Miklavčič, D., N. Pavšelj, and F.X. Hart, *Electric properties of tissues.* Wiley encyclopedia of biomedical engineering, 2006.
2. Gabriel, C., A. Peyman, and E. Grant, *Electrical conductivity of tissue at frequencies below 1 MHz.* Physics in medicine and biology, 2009. **54**(16): p. 4863.
3. Gabriel, C., S. Gabriel, and E. Corthout, *The dielectric properties of biological tissues: I. Literature survey.* Physics in medicine and biology, 1996. **41**(11): p. 2231.
4. Basser, P.J. and B.J. Roth, *New currents in electrical stimulation of excitable tissues 1.* Annual review of biomedical engineering, 2000. **2**(1): p. 377-397.
5. Sanchez, B., et al., *Novel approach of processing electrical bioimpedance data using differential impedance analysis.* Medical engineering & physics, 2013. **35**(9): p. 1349-1357.
6. Foster, K.R. and H.P. Schwan, *Dielectric properties of tissues and biological materials: a critical review.* Critical reviews in biomedical engineering, 1988. **17**(1): p. 25-104.
7. Pethig, R. and D.B. Kell, *The passive electrical properties of biological systems: their significance in physiology, biophysics and biotechnology.* Physics in medicine and biology, 1987. **32**(8): p. 933-970.
8. Gabriel, S., R. Lau, and C. Gabriel, *The dielectric properties of biological tissues: III. Parametric models for the dielectric spectrum of tissues.* Physics in medicine and biology, 1996. **41**(11): p. 2271.
9. Chen, J., et al., *Classification of cell types using a microfluidic device for mechanical and electrical measurement on single cells.* Lab on a Chip, 2011. **11**(18): p. 3174-3181.

10. Bera, T.K., *Bioelectrical Impedance Methods for Noninvasive Health Monitoring: A Review*. Journal of Medical Engineering, 2014. **2014**.

11. Trojanowicz, M., *Recent developments in electrochemical flow detections—a review: Part I. Flow analysis and capillary electrophoresis.* Analytica chimica acta, 2009. **653**(1): p. 36-58.

12. van den Berg, A. *Labs on a chip for health care applications*. in *14th International Conference on Miniaturized Systems for Chemistry and Life Sciences*. 2010.

13. Gómez Abad, D., *Development of a capacitive bioimpedance measurement system.* 2009.

14. Giurgiutiu, V. and B. Xu. *Development of a field-portable small-size impedance analyzer for structural health monitoring using the electromechanical impedance technique*. in *Smart Structures and Materials*. 2004: International Society for Optics and Photonics.

15. Ezenwa, B.N. and W.P. Couch, *Multiple frequency bio-impedance measurement system*. 1991, Google Patents.

16. Ayliffe, H.E., A. Bruno Frazier, and R. Rabbitt, *Electric impedance spectroscopy using microchannels with integrated metal electrodes.* Microelectromechanical Systems, Journal of, 1999. **8**(1): p. 50-57.

17. FitzGerald, J.J., et al., *Microchannel electrodes for recording and stimulation: in vitro evaluation.* Biomedical Engineering, IEEE Transactions on, 2009. **56**(5): p. 1524-1534.

18. Khalil, S.F., M.S. Mohktar, and F. Ibrahim, *The Theory and Fundamentals of Bioimpedance Analysis in Clinical Status Monitoring and Diagnosis of Diseases.* Sensors, 2014. **14**(6): p. 10895-10928.

19. Hsu, C.-C., *Voltage buffer circuit*. 2006, Google Patents.

20. He, J., et al., *Characterization of Leaf-Inspired Microfluidic Chips for Pumpless Fluid Transport.* Journal of Bionic Engineering, 2014. **11**(1): p. 109-114.

21. McAdams, E., et al., *The linear and non-linear electrical properties of the electrode-electrolyte interface.* Biosensors and Bioelectronics, 1995. **10**(1): p. 67-74.

22. Lin, Y.-H., S.-T. Yeh, and C.-B. Li, *Printed circuit board with differential vias arrangement*. 2006, Google Patents.

23. Ball, M.O. and M.J. Magazine, *Sequencing of insertions in printed circuit board assembly.* Operations Research, 1988. **36**(2): p. 192-201.

24. Saubestre, E., *Copper etching in ferric chloride.* Industrial & Engineering Chemistry, 1959. **51**(3): p. 288-290.

25. Griep, J.P., *Voltage controlled current source*. 1984, Google Patents.

26. Sia, S.K. and G.M. Whitesides, *Microfluidic devices fabricated in poly (dimethylsiloxane) for biological studies.* Electrophoresis, 2003. **24**(21): p. 3563-3576.