Development of low Cost Portable Platform for Bioimpedance Based Diagnostics

Thesis submitted in partial fulfillment of the requirements for the degree

Of

Master of Technology
In
Biomedical Engineering

By

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NATIONAL INSTITUTE OF TECHNOLOGY, ROURKELA

CERTIFICATE

This is to certify that the thesis entitled, "**Development of low Cost Portable Platform for Bioimpedance Based Diagnostics**" submitted by Mr. MD. SARFARAZ ALAM in partial fulfillment of the requirements for the award of degree of Master 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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Table of Contents

LIST OF FIGURES……V

LIST OF TABLES

ACRONYMS

PCB …….……Print Circuit Board

E. Coli……..…….Escherichia Coli

EB……...….Electrical Bioimpedance

BIA…….……...Bioelectrical Impedance Analysis

VCCS……..Voltage Controlled Current Source

EDTA……Ethylene Diamine Tetra Acetic acid

MEM……..Minimal Essential Media

PBS…….Phosphate Buffer Solution

OD…….Optical Density

CRO……...Cathode Ray Oscilloscope

RPI…….…Relative Proliferation Index

ABSTRACT

Analyzing the impedance of biological samples has gained importance in the last decade. Presently all the bioimpedance analyzer available in the market are heavy and costly. In this context, an attempt has been made to develop a portable, low cost bioimpedance analyzer. For this purpose, the portable cartridges for sample analysis were prepared on cupper print circuit board by chemical etching. The developed device was successfully operated in a frequency range of 50 Hz to 20 KHz to measure impedance of various samples such as glucose, NaCl solution, bacterial cell culture and xanthan gum. All the measured samples were shown capacitive dominance. Optimization of electrode spacing and area was done to improve its efficacy in measuring the bioimpedance. Furthermore, the device was also employed for screening of anticancer drugs like cis-platin using HT-29 colon cancer cell line. The device was found operational for analyzing small sample volume (50-200µl) , moreover portability of this developed device makes it a special among commercially available instruments used for measuring bioimpedance.

Key words: Bio-impedance, portable, anticancer drug, biological sample, HT-29 cell line

CHAPTER # 1

INTRODUCTION

1.1 INTRODUCTION

Electrical bioimpedance (EB) may be defined as the passive electrical property of cell/tissue when energized with an electric potential [1]. The passive electrical properties were determined by the observation of tissue electrical response to the injection of external electrical energy. Some biological tissues also show active electrical properties since they are capable of generating currents and voltages (e.g. the nerves). In the present scenario, bioelectrical impedance examination is the strategy used most often, because of its low expense, simple to operate and its convenience. Bioelectrical impedance measures electrical parameters like resistance & reactance. Ionic salts help in carrying the current in ionic membranes. Small openings in the membrane called ion channels are selective to specific ions and determine the membrane resistance. The biological tissue generally shows capacitive impact. Due to this the cell and tissues shows a decrease in impedance as the frequency of analyzing current is increased [2].

1.2 Dielectric properties of biological tissue:

Biological tissue mainly constitutes have extracellular fluid and cells. The cells hold organelles and intracellular fluid inside a lipid bi-layer membrane known as the cell membrane. The extracellular fluid is the medium encompassing the cells. The electrical conduct of the tissue is determined by the electrical characteristics of its constituents [2]. Because both intra- and extracellular fluids hold ions, they are considered to be electrolytes. These ions can move freely and are able to transport electrical charge. Therefore, from an electrical perspective, biological tissue may be considered an ionic conductor at low frequencies [3]. The cell membrane electrically separate from the cell because its conductive properties are very low. Thus, a conductor-dielectric-conductor structure is made by the

intracellular space, the lipid bi-layer membrane and the extracellular space. The most common ions contained in the extracellular fluid are Na+ and Cl- the potassium ion K+ has the largest concentration in the intracellular fluid. The existence of charges free to move at both sides of the cell membrane allows the accumulation of charges, which gives rise to the most noticeable dielectric properties of the cell. The dielectric properties of tissues are given by the dielectric conduct not only of the cell membrane and the surrounding ionic fluids. Also in the intra- and extracellular spaces there are polar molecules, proteins and macromolecules that are too large to move in the presence of an electric field. However, these ions can move and align the dipole along the gradient of the electrical field [4]. Consequently, the low frequency properties of biological tissue and the electrical behavior exhibited at higher frequencies make biological tissue to be considered as an ionic dielectric conductor[5].

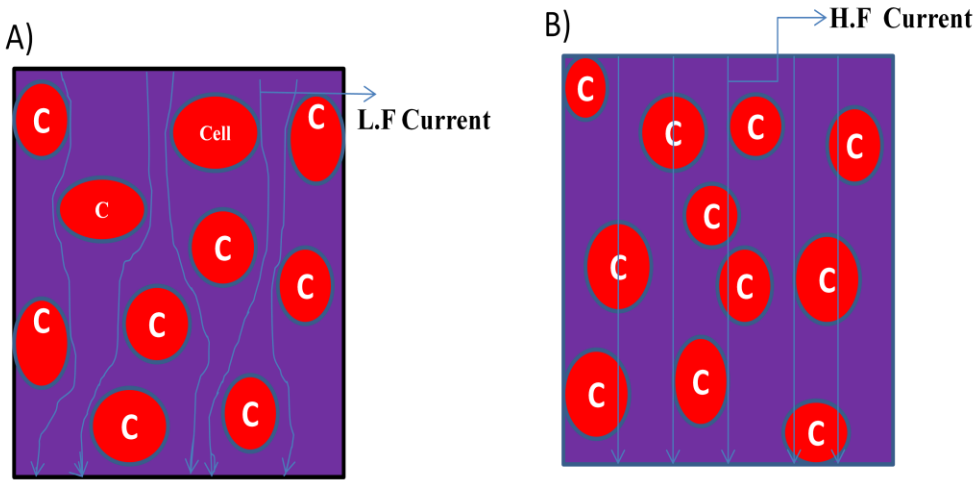


Figure 1.1: Current flow in tissue at low and high frequencies as shown in A and B respectively. Cells were represented with C

As Fricke's model recommends, the ionic current flowing through biological tissue follows distinct paths at different frequencies. As shown in Figure 1.1 the current flowing at very low frequencies does not pass through the cells; rather, current flows through the extracellular

medium. However, as the frequency increases in the β dispersion frequency range, the cell membrane capacitor charges and discharges the current at the frequency rate. Thus, the charge displacement in the cell membrane becomes important and current flows through the cell membrane by displacement in both the extra- and intracellular fluid. At very high frequencies, the charge-discharge process is so fast that the cell membrane impact is very small, and nearly all the current goes through the cell in the same path as outside the cell [6].

1.3 Origin of bioimpedance:

Electrical behavior of tissues was described for the first time in 1871. Furthermore, electrical behavior was explained for a high range of frequencies on bigger extent of tissues, including damaged or experiencing changes after death. Segal et. al [7] conducted the original studies with the help of electrical impedance measurements as an index of total body water (TBW), by inserting two subcutaneous needles. For the first time, four-surface electrode bioimpedance analyzer (BIA) technique was introduced by Kushner et al. [8]. A major handicap of surface electrodes is usage of high current (800 mA) and voltage in decreasing the instability of injected current relating to cutaneous impedance. By the end of 1970's the establishment of BIA was at the pinnacle, including those that support the connections between the impedance and the body water substance. Single frequency BIA analyzers were found to be potential in industrial access and by 1990s; the business sector has incorporated a few multi-frequency analyzers. The utilization of BIA as a bedside technique has enhanced the equipment in convenient and safe mode, due to its simplicity, non invasive nature and able to produce quick reproducible outcomes [9]. All the more as of late, segmental BIA was created to overcome inconsistencies between resistances (R) and body mass of storage unit. Further, a few gatherings (Rigaud et al 1995) were utilized in commercial purpose impedance

analyzers for research activities. This equipment, in any case not suitable for genuine four-point bioimpedance estimation and do not meet well being necessities and the estimation rate is not sufficient for a percentage of the pertinent provisions. Furthermore, the information exchange to the PC is somewhat abate. An alternate methodology is to create proper indicator molding fittings a bioimpedance connector that is suitable for genuine four-point estimation (Y'elamos et al 1999)—and use it with business impedance analyzers to measure living specimens. These connectors are not took into account use on human subjects. A few methodologies to making precise bioimpedance and transimpedance estimations with extraordinarily created frameworks have been distributed (Milnes et al 1999, Nebuya et al 1999,waterworth et al 2000), however these frameworks have a restricted frequency extend up to 1.25 MHz and a low accuracy [10]. The information rate at high frequencies is frequently a restricting variable in these frameworks. Thus, further studies must concentrate to make execution of exact bioimpedance and transimpedance estimations when frequencies extend up to 10 MHz possible. The early digitization idea utilized the advantage of high stability and low noise. Moreover, another methodology utilizing the under sampling method was employed and tested in impedance spectroscopy, which makes it likely of extending to the frequency range to higher frequencies and to decrease the costs of the system with no loss of measurement accuracy [11]. To validate the system properties, a straightforward tissue phantom was utilized.

1.4 Major part of bioimpedance spectroscopy:

1.4.1 Electrode:

Electrode provides the interface between the sample and electronic measuring devices [12]. It changes the ionic current into electronic currents.

Electrodes have two types

a) Polarizable electrodes:

In polarizable electrodes, if current is applied to the circuit no charge crosses to the interface. It behaves like a capacitor.

b) Non-polarizable electrodes:

In non-polarizable electrodes, if current is applied to the circuit charges freely crosses the interface [13]. It behaves like a resister. Example of good non-polarizable electrode is silver/silver chloride.

1.4.2 Voltage buffer:

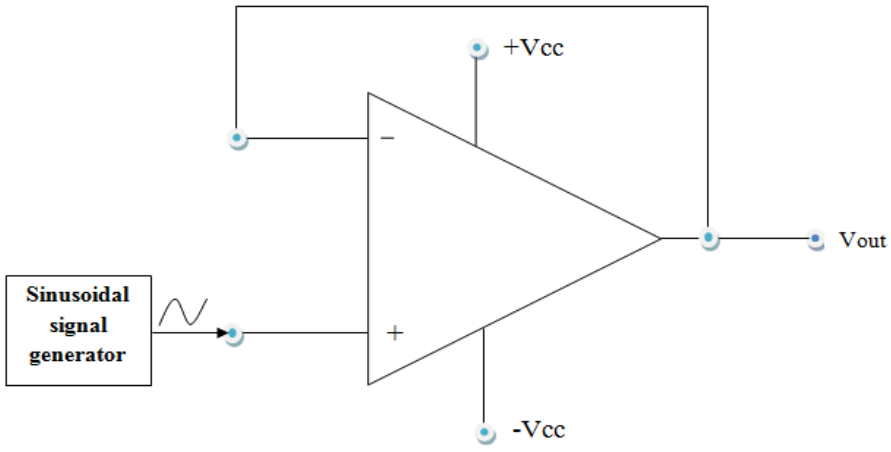


Figure 1.2: Schematic representation of non-inverting voltage buffer

- It is unity gain voltage buffer.

- It is used for impedance matching.
- It transform the electrical impedance from one circuit to other
- Benefit to maximize energy transfer between circuit or systems
- The purposes of each to isolate the mentioned characteristics to avoid loading the input circuit or source from the output stage.

1.4.3 PCB (Printed circuit board):

It supports mechanically and electrically join with electronic part having conductive tracks cushions and different characteristics etched from copper sheets laminated onto a non-conductive substrate [14]. It could be single sided (one copper layer) or may be twofold sided (two copper layers)

1.4.4 Soldering:

It is a methodology in which two or more metal things are joined together by melting and streaming a filler metal (solder) into the joint. It is differ from welding in that soldering does not include the melting the work pieces [15]. Soldering iron or soldering gun is uses for manual soldering. The temperature between 180-190°c is the most usually utilized for melting the alloy. Soldering performed utilizing combinations with a melting point over 450°c is called "hard welding" or brazing [16]. Sn/Pb(63/37) utilized as a part of electrical/electronic work. Solder's tensile and shear strengths is greater when tin concentration is greater.

1.5 Existing bioimpedance measuring methods:

EBI measurements can be used in several applications and several methods exist to measure the impedance of biological sample but here we described widely used method.

1.5.1 Single Frequency and Spectroscopy Measurements:

In the history of EBI single frequency has been used the longest to monitor changes such as those caused by breathing or produced by cardiac activity in impedance cardiograph [17]. Single frequency electrical bioimpedance (SF-EBI) measurements at 50 kHz have also been used for TBC, giving rise to single frequency bioimpedance analysis (SF-BIA), which is a widely used method [18]. These measurements can be accomplished with wrist-to-ankle configurations or body segments. EBIS measurements require information for the whole spectrum or at least enough frequencies to perform spectroscopy analysis. Thus, the term spectroscopy (Kuhlmann *et al.*, 2005) is used. Until now, the most accepted applications of EBIS measurements are skin cancer screening (Aberg *et al.*, 2005; Aberg *et al.*, 2004) and BCA. The measured frequencies for BCA and all the applications related to body composition and hydration status typically range from a few kHz to upper frequencies in the range of hundreds of kHz or up to 1MHz. In BCA, EBIS measurements allow direct differentiation between intra-extracellular water compartments, which allows ECW and ICW to be estimated. EBIS is a commonly used method for the early detection of several diseases when hydration monitoring is used.

1.5.2 Two and Four-Electrode Measurements method

Bioimpedance may be measured by connecting terminals of two electrodes to the surface of sample. Both electrodes can be utilized to inject the current and to detect the voltage drop in the sample. The electrode-electrolyte interface impedances are in series arrangement with the specimen impedance [19]. At low frequency these parasitic impedance are not healthy as it affects the impedance measurement. In the present context, an alternative detecting method is utilized: the current is infused with several electrodes and ensuing voltage drop is measured

with an alternate couple of electrodes. This system known as four-electrode method or tetrapolar method has been utilized for since 6-decades (Boulier et. al, 1996) and it usually cancels the impact of the electrode-electrolyte interface impedance [20]. It is well known that, the four-electrode system is not completely free from errors. So, other parasitic elements (i.e. capacitances of wires and instrumentation input impedances) combined with the electrode-electrolyte impedances cause some errors at high and low frequencies and that constrains the useful measurement range of the this method from few Hz to several MHz [21]. Under particular circumstances, the measurements were done with two electrodes can also be used because the electrode impedance is very low as compared to the impedance under test so it was neglected. In those cases, electrode areas have large and frequencies above 10 kHz are normally used.

1.6 Bioimpedance Spectroscopy measurement:

Impedance is a common word in electrical and electronics. Precisely, impedance is known as the opposition of flow of alternating current through a conductor. Generally biological tissues are complex as they include dielectrics and show frequency dependent responses [22]. Geometrical dimensions and tissue properties have a great impact on Bioimpedance.

$$Z = R + jX \quad \ldots\ldots\ldots\ldots (1.1)$$

Where, $Re\{Z\} = R$ = Resistance

$Im\{Z\} = X$ = Reactance

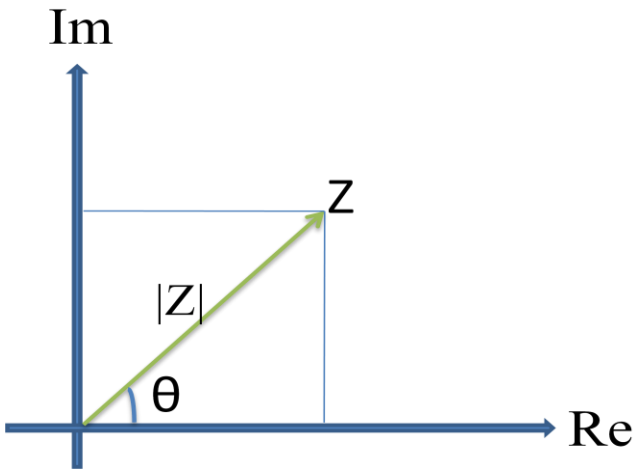


Figure 1.3: Schematic representation of impedance in complex plane.

Where Z is called impedance of the sample, real part of impedance (Z) is called resistance and imaginary part of impedance (Z) is called reactance. Reactance (X) have two types 1st is capacitive reactance and 2nd is inductive reactance [23]. Generally biological samples have capacitive in nature.

Capacitive reactance:

$$X_c = -j(1/2\pi fC) \ldots\ldots\ldots \quad (1.2)$$

Inductive reactance:

$$X_L = j(2\pi fL) \ldots\ldots\ldots\ldots (1.3)$$

Where X_L and X_C are capacitive and inductive reactance respectively, f is frequency of the signal, C and L are the capacitance and inductance of the solution.

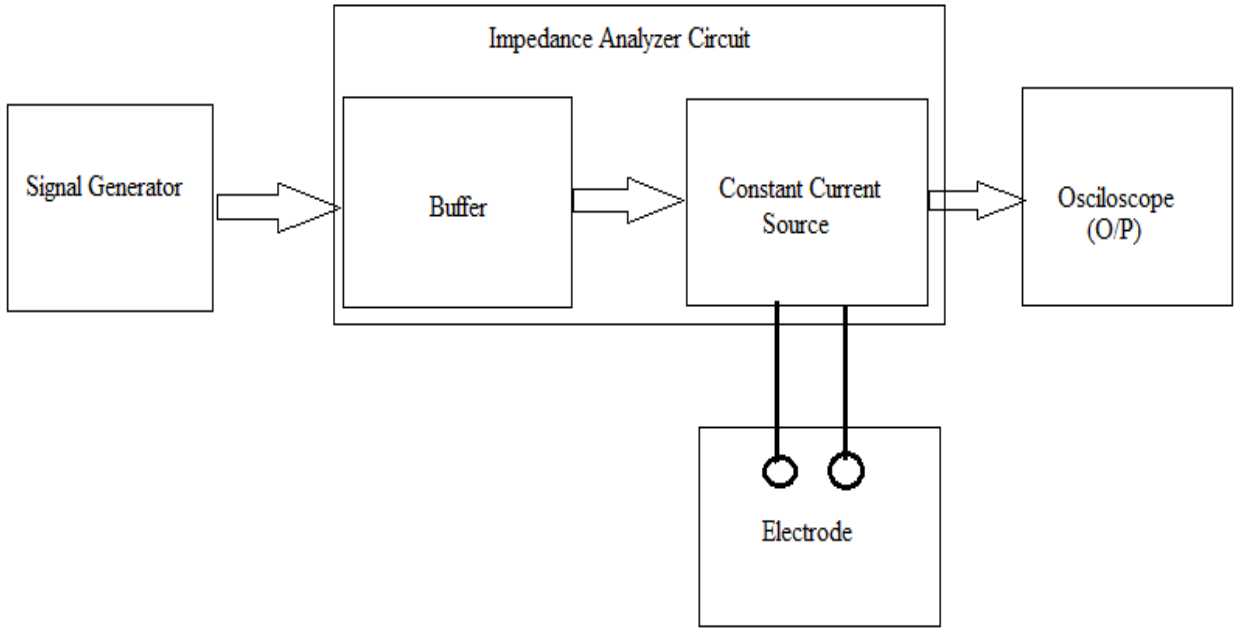


Figure 1.4: B.D. of bioimpedance analyzer set-up

In the figure 1.4 the signal generator is used for generating sine wave signal. The output of this generator was goes into buffer working in the non-inverting mode. This voltage buffer reduces the loading effect of signal generator. The output of voltage buffer connected into VCCS, which working on the inverting mode. The regulated constant AC current injected into a sample whose impedance was to be analyzed. Oscilloscope records the output data in the form of Vrms. The combination of voltage buffer and VCCS provides as an impedance analyzer. Subsequently, the developed impedance analyzer was used for the analysis of electrical behavior of biological sample.

1.7 Potential application:

S.NO.	Types of Application	Description	References
1	Impedimetric investigation of blood	This impedance sensing devices were created on 2 inch width Pyrex wafers utilizing metal photolithography	Pradhan et al.: Quantitative evaluation

	glucose concentration using impedance sensing devices with different electrode area	procedures. This three-anode technique with a few measurements of electrodes are utilized, the proportion of working electrode area(WE) range and reference electrode(RE) region and WE and counter electrode(CE) region were set at 0.01.the CE and RE were put at a separation of 100µm from WE position in all the 4 plans to avoid cross contamination. This manufactured device associated with machine controlled electrochemical workstation Sp 150(bio-rationale France) for measuring impedance. These investigational data was exported to ZsimpWin software for further study with equivalent circuit simulation.	of blood glucose concentration. J Electr. Bioimp. 2013;4:73-77.
2	Impedimetric characterization of human blood using three-electrode based ECIS devices	Same as above	Pradhan et al.: Impedimetric characterization of human blood . J Electr. Bioimp. 2012;3:12-19.
3	Measurement of the electrical conductivity of water	The system is focused around the estimation of the resistance of a column of water of given dimensions. The estimation cell comprises of two end supplies with platinum electrodes are put. These are differentiated by a removable focus segment 100" long and 10 mm width. The resistance measured between the electrodes as a function of frequency with, and without, the core segment set up. The distinction gives the resistance of the centre section. Conductivity of the fluid computed by the assistance of known dimensions of centre section. The cell is created out of demountable glass segments which have even surfaces so that when in contact	Jones: Mesurement of the electrical conductivity of water. IEE Proc.-Sci. Meas.Technol.2002; Vol. 149, No. 6.

		there is leakage of the fluid.	
4	Electrical conductivity measurement method in seawater desalination based on variable frequency excitation	This online monitoring method of a conductivity measurement method based on the changing the frequency in desalination seawater. In this method, microcontroller controlled the auto adaptive variable frequency excitation source. A microcontroller has a specified voltage, and brings variable frequency sine wave. An adaptive frequency will be utilized for measuring the different frequency range	Hu et al.: Electrical conductivity measurement method in seawater desalination.IEEE,ICEMI:2009
5	Bioelectrical impedance spectroscopy for the assessment of body fluid volumes in term of neonates	The method followed was based on the current response to a voltage step excitation. In the mentioned method, a data acquisition card (National Instrument, PCMCIA DAQ Card model AI-16E-4, Autix, TX, USA) was installed in laptop for the generation of the step voltage excitation and for data acquisition. A specific program was developed (Lab View, National Instruments) to handle data acquisition and to estimate of bioelectrical impedance parameters.	Ferreira and Souza: BIS for the assessment of body fluid volumes. Brazilian journal of medical and research. 2004;37:1595-1606
6	Impedimetric investigation of calf's femur tissue using impedance sensing device with variable frequency range.	Authors focused on developing a method based on impedance spectroscopy where the sensing electrode was attached to the tool. In this described method, electrical impedance probe (consisting of sense and bulk electrode) was used by an Agilent 4294A precision impedance Analyzer. During operatin the device, the insertion electrode consisting of a spring wire, was stuck deeply into the soft-tissue sample attached to the bone. The second (sense) electrode consisting of a rounded tip with 2 mm diameter was attached to a stage that could be displaced along two axes. This sense electrode is inserted into the processed area, by moving the sense tip across the cut surface of the bone	Schaur et al.: Characterization of bone tissue with EIS. IEEE.2012; 978-1-4577-1767-3/12

		and the complex impedance between the two inserted electrodes was recorded as a function of frequency and position.	
7	Design and testing of an impedance-based sensor for monitoring drug delivery	MEMS devices were fabricated in the Microsystems Technology Laboratory at MIT for drug delivery applications. Silicon wafers with a dimension of 4 inch, double-side polished, 300 mm thick, 1-10Ω cm were used. A 100nm thermal oxide layer was grown on both surfaces and covered with a 150 nm low pressure chemical vapor deposition (LPCVD) nitride layer to act as an etch stop. Wafers were immersed in 5.17 M KOH at 85°C until the silicon was etched through the entire thickness of the wafers to form an array of pyramidal reservoirs capped by nitride/oxide membranes.	Johnson et al.: Design and testing of an impedance based sensor for monitoring drug delivery. Journal of the electrochemical society.2005; 152(1):H6-H11
8	The application of bioimpedance method for foot sole blood perfusion characterization	This principle of the method is as follows. Initially, MCU receives command from PC and then the specified channel of multiplexer is switched on by MCU. MCU sets up the applied output frequency of applied signal for the impedance measuring circuit as well as monitors the temperature at the same time. Finally, MCU transmits the measured raw data to PC for demodulation.	Cheng et al.: Application of bioimpedance method for blood perfusion characterization. IEEE, Computer society.2012
9	Portable bioimpedance monitor evaluation for continuous impedance measurements. towards wearable plethysmography application	The AD5933-based bioimpedance meter (AD5933-EBIM) used in this work, it is a battery operated portable tetrapolar spectrometer controlled by a Microchip PIC24FJ microcontroller. It uses Bluetooth technology to transfers control messages and data between device and a PC station. The AD5933-EBIM dimensions are 50x90x15 mm, and 70gram of weight including the battery and a plastic box.	Ferreira et al.: Continuous impedance measurements towards wearable plethysmography application. IEEE EMBS. July 2013

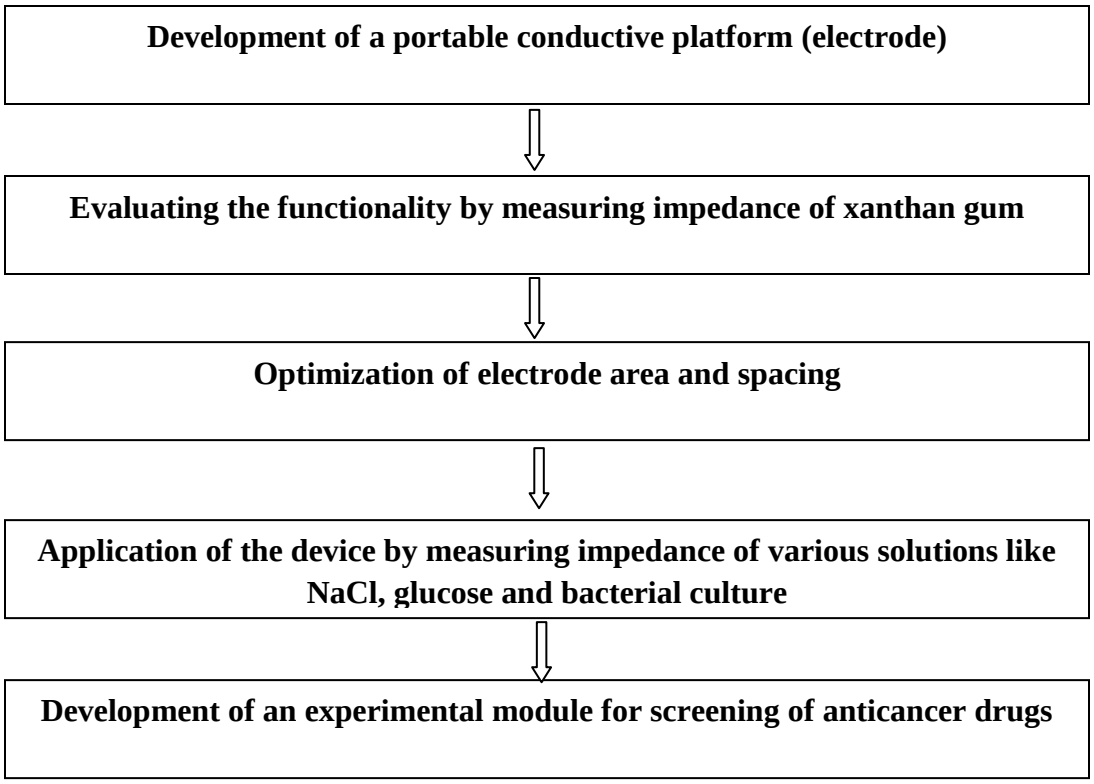
1.8 Scope of the present investigation:

Presently there is a need for non-destructive, real time monitoring system for quality controls of biological samples. In this regards bioimpedance based analysis can be need. Existing systems that work on this principle are big, cumbersome and costly. So there is a need to prepare a portable bioimpedance analysis system. Keeping that view in mind here we have aimed to "**Development of low Cost Portable Platform for Bioimpedance Based Diagnostics".**

1.9 Objectives:

- To prepare a portable conductive platform and its testing
- To optimize electrode area and spacing.
- To develop an experimental module for impedance based anti cancer drug screening.

1.10 Work plan:



CHAPTER # 2

MATERIALS & METHODS

Materials and Methods:

2.1 Materials:

The IC OP-07 was obtained from Analog devices, Noorwood USA. The PCB sheet, resistors, normal saline was procured from local market. Glucose and Luria broth bertani were procured from Himedia, Mumbai, India. Ethanol was obtained from Honyon International Inc., Hong Yang Chemical Corpn., China. Distilled water was used throughout the study.

2.2 Methods:

The project entitled with "Development of Low cost portable platform for bioimpedance based diagnostics" has two major part, 1st is preparation of electrode 2nd is circuit development of impedance analyzer device and 3rd is sample preparation and its impedance analysis.

2.3 Required instruments:

- Function generator
- Oscilloscope
- Cooling centrifuge-REMD (C-24BL)
- Double beam UV spectrometer
- Magnetic stirrer
- Orbital shaker
- Digital multimeter

2.4 Preparation of electrodes:

Electrode prepared by two methods, 1st is electrode prepared via printing using eagle and 2nd is electrode prepared via manually using marker.

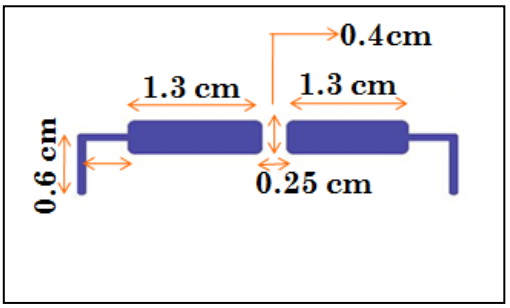


Figure 2.1: Design of conductive channel using eagle 5.6.0

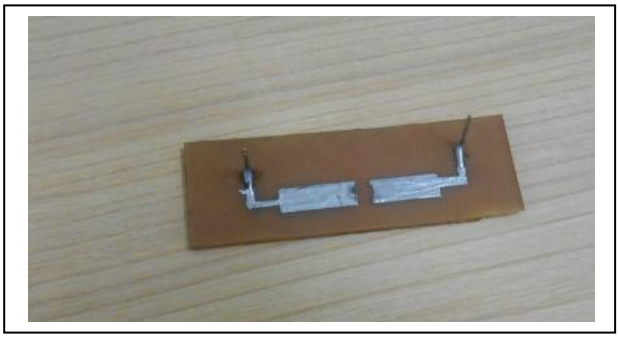


Figure 2.2: PCB chip (Electrode)

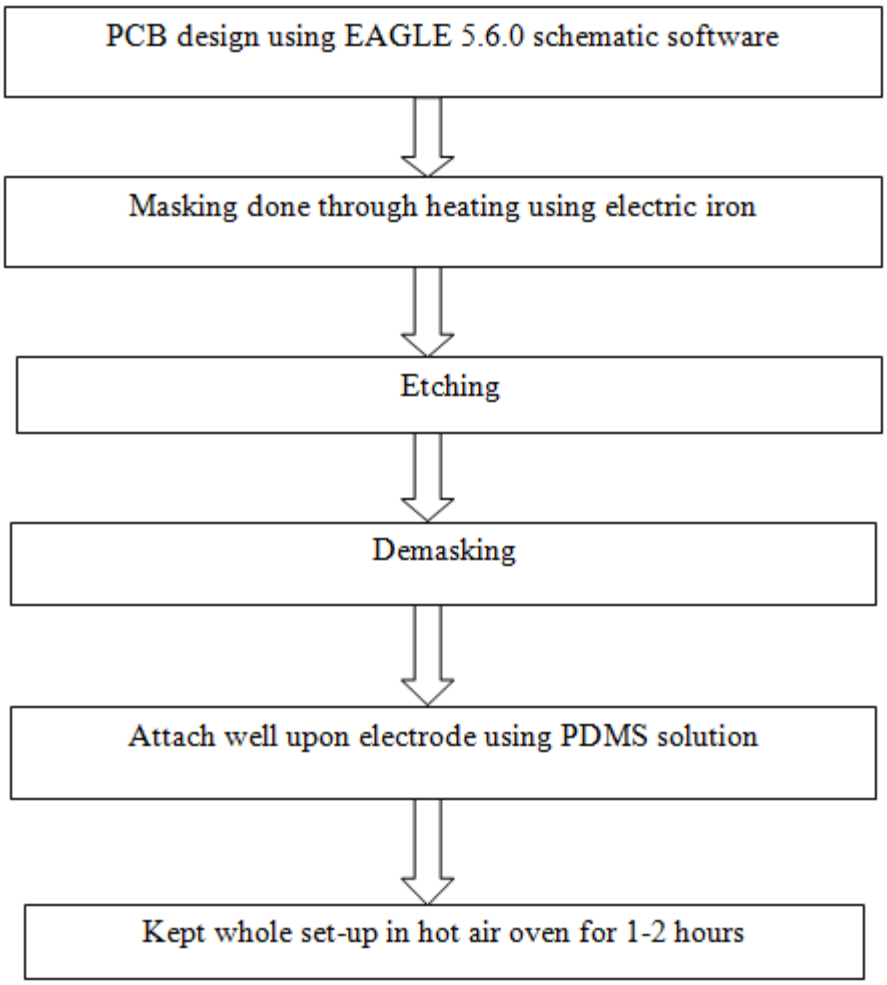


Figure 2.3: Flow chart for preparing of electrode.

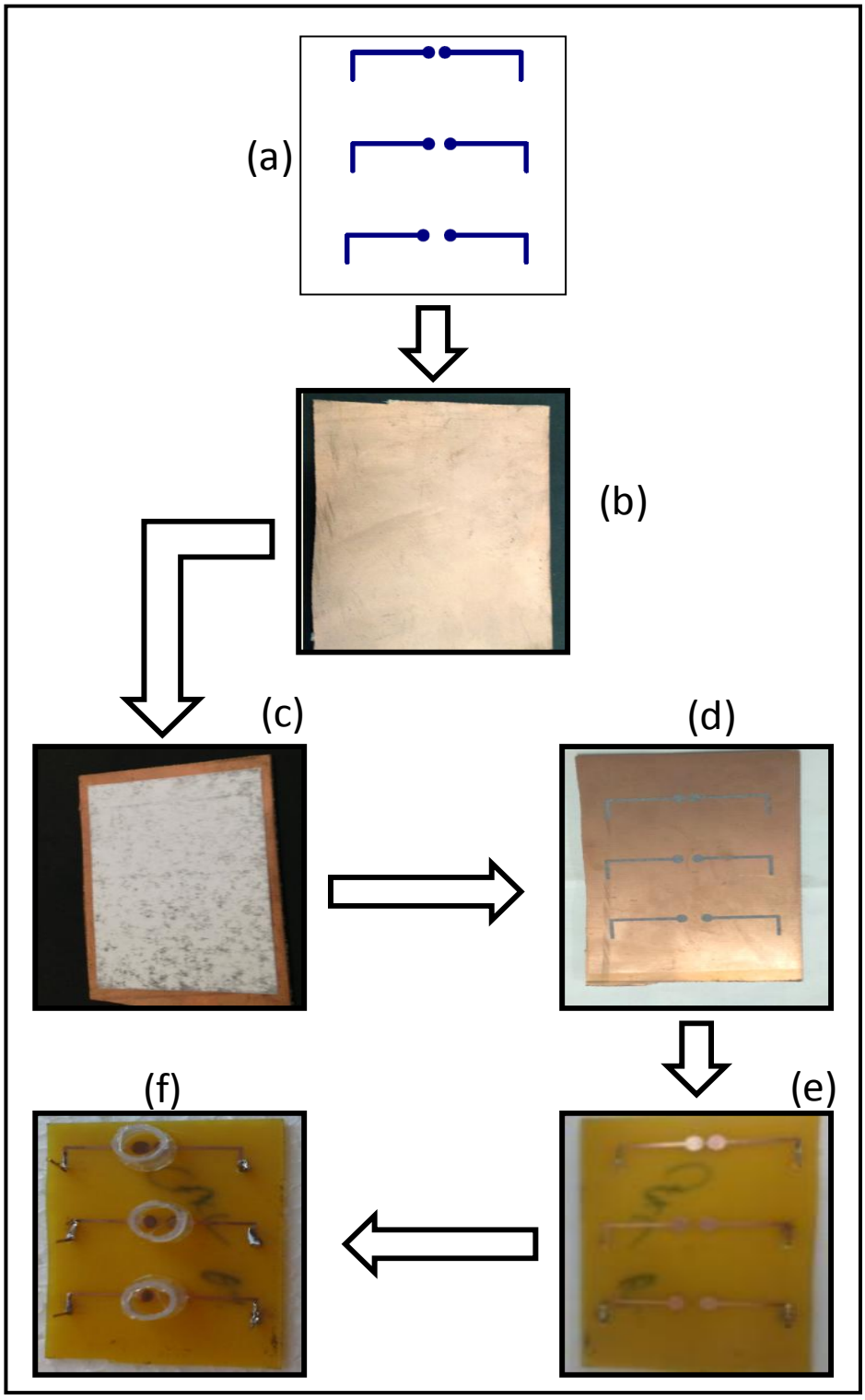


Figure 2.4: Pictorial representation of different steps of electrode preparation (a) Design of electrode using eagle (b) Fresh PCB sheet (c) Impregnated PCB (d) Masking (e) Demasking (f) 3-well final electrode.

2.5 PCB design:

It is usually done in a series of four steps, cleaning, masking, etching, and demasking [24].

2.5.1 Cleaning:

It is the first step that the surface to be cleaned is free of contaminants. A proper cleaned surface may get about good attachment of the maskant, improper cleaned surface which could result in inexact final dimension. The material may additionally be immersed in basic cleaners or particular de-oxidizing results.

2.5.2 Masking:

In this process the maskant material is applied to the surface of the copper laminated PCB sheet to assure that just required area to be etched.

2.5.3 Etching:

It is the procedure of applying the masking PCB sheet immersed in the ferric chloride (Etchant) solution for removing unmasked part of copper by shaking the solution [25].

$$\mathbf{FeCl_3 + Cu \rightarrow FeCl_2 + CuCl}$$

$$\mathbf{FeCl_3 + CuCl \rightarrow FeCl_2 + CuCl_2}$$

In above reaction firstly iron(III) chloride is convert into copper(I) chloride then copper(II) chloride for the design of PCB chip (Electrode).

2.5.4 Demasking:

It is the combined procedure to clearing the maskant material. It is generally removed with a wash of normal water.

2.6 Impedance analyzer Circuit development:

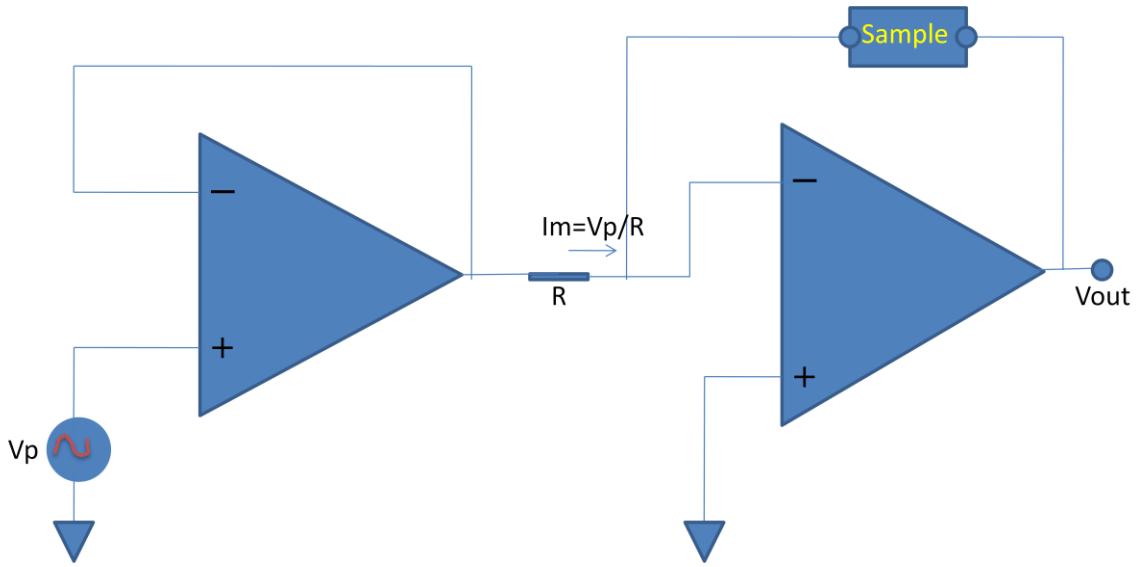


Figure 2.5: Impedance analyzer circuit

The main consideration of circuit development for impedance analyzer is sinusoidal signal wave generator and voltage controlled constant current source, but here we used Function generator for generating sine wave signal. The output of function generator was fed into unity gain feedback voltage buffer working in the non-inverting mode shown in figure 2.5. This voltage buffer reduces the loading affect of signal generator. Impedance analysis turns around the injection of constant current, of variable frequencies into the sample and the subsequent measurement of Vrms of the sample. In the current study, voltage controlled constant current source was developed as shown in figure 2.6. The output of unity gain voltage buffer connected into VCCS, which working on the non-inverting mode [26]. The regulated constant AC current injected into the sample whose impedance was to be analyzed. The combination of unity gain voltage buffer and VCCS provides as a impedance analyzer. Subsequently, the developed impedance analyzer was used for the analysis of electrical behavior of biological sample. The picture of whole developed set up has been shown in figure 2.5.

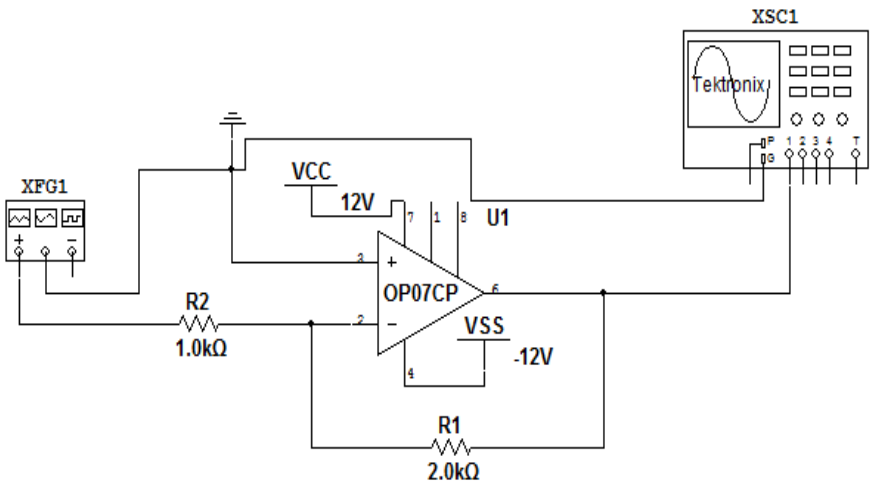


Figure 2.6: Circuit diagram of VCCS using Multisim 11.0 software

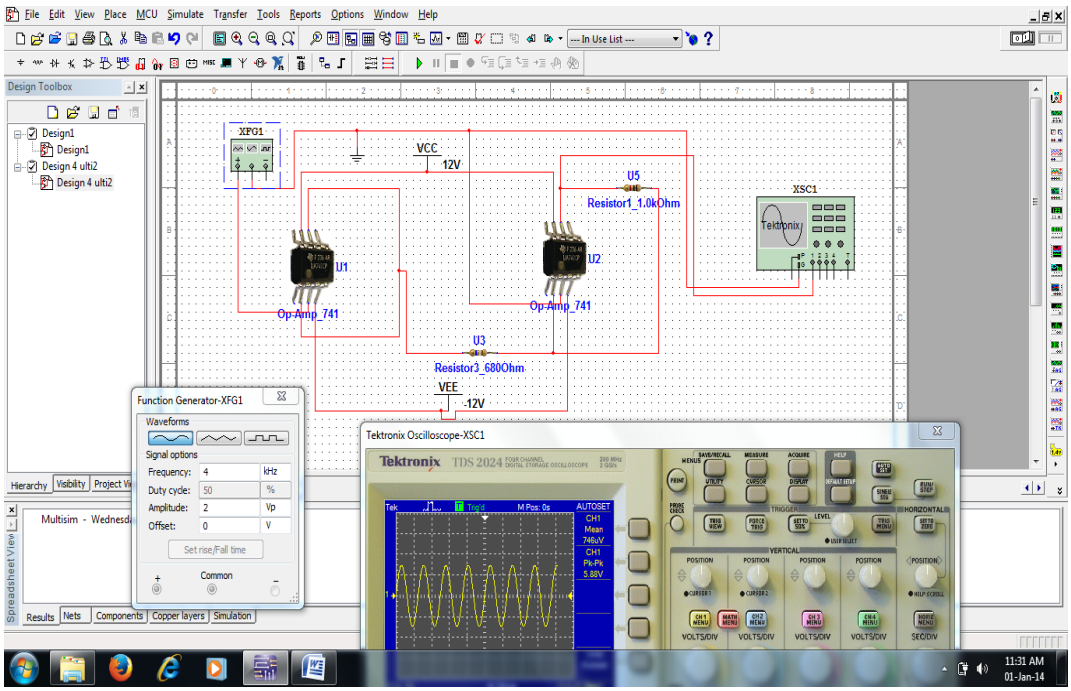


Figure 2.7: Circuit diagram of impedance analyzer using Multisim 11.0 schematic software

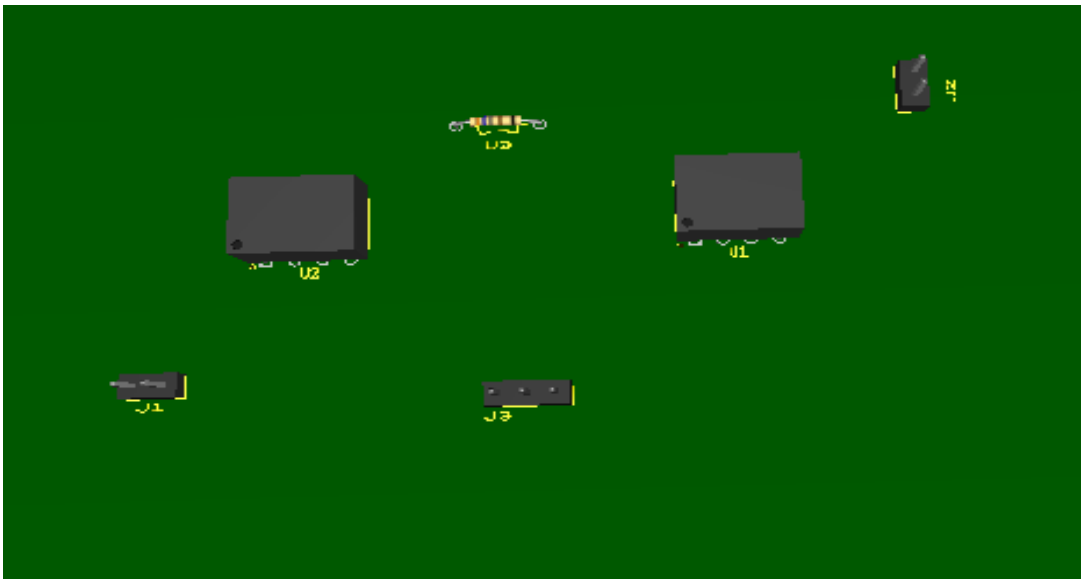


Figure 2.8: 3-D component view of impedance analyzer circuit using Ultiboard schematic software

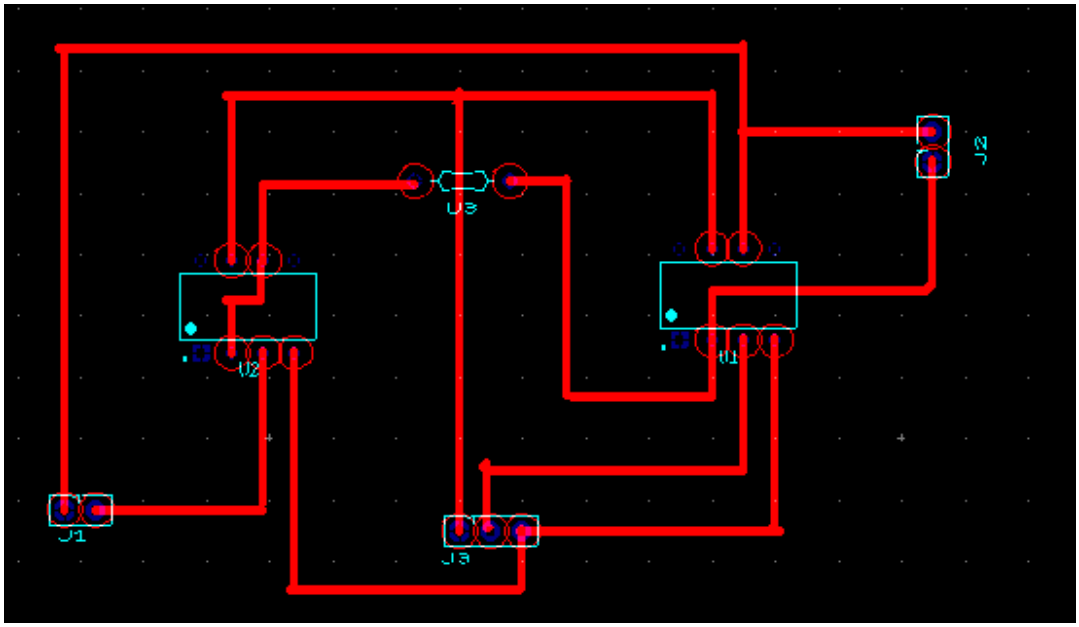


Figure 2.9: Lay-out design of bioimpedance circuit (Top view) using Ultiboard

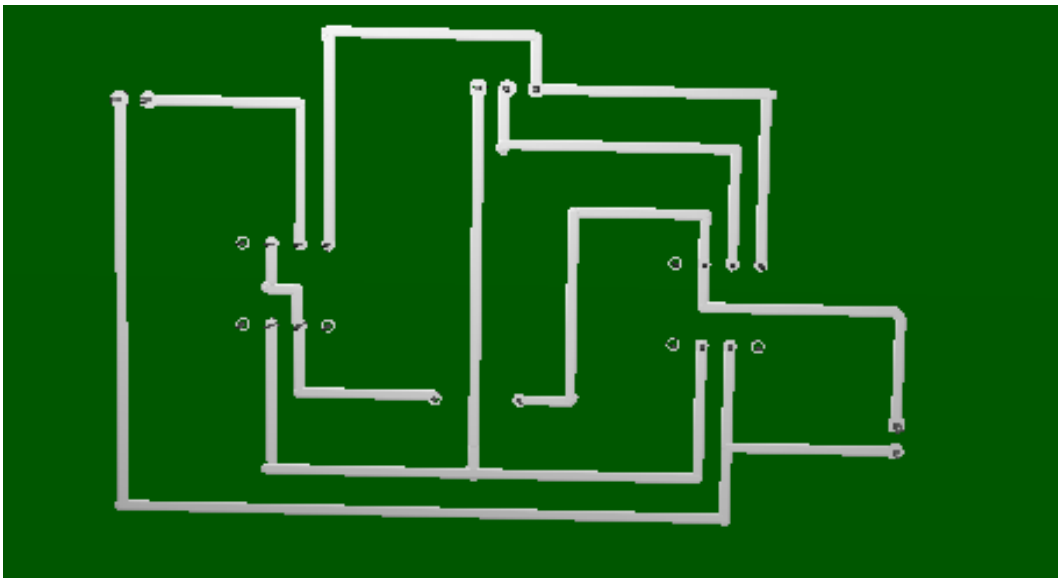


Figure 2.10: Lay-out design of bioimpedance circuit (Bottom view) using Ultiboard

2.7 Sample preparation and its impedance analysis.

2.7.1 Sample preparation of xanthan gum for impedance analysis

The gel was made in the laboratory. The gel was inserted in between the current injection electrode (made up of stainless electrode). The electrode was usually 1 cm apart. Current passed through the sample and corresponding changes in voltage was recorded with the help of oscilloscope with varying frequency. All the measurements were performed with the input voltage of 1V, R=1kΩ and frequency range from 100Hz to 20 kHz.

The impedance of sample was calculated by following formula:

$$Z_f = {Vrms}/{Irms} \quad \ldots\ldots\ldots\ldots 2.1$$

$$Irms = {(Vp * 0.707)}/{R} \quad \ldots\ldots\ldots 2.2$$

Where Z_f= Impedance of the sample at frequency, f.

Vrms = Voltage drop in sample at frequency, f.

Irms = Current injected at frequency, f.

Vp = Supply voltage of signal generator

2.7.2 Impedance measurement of salt solution:

Various dilutions of salts solution were made using distilled water. The solution was seeded in the well attached to the current injection electrode. Current passed through the salt solution and corresponding changes in voltage was recorded with the help of oscilloscope with varying frequency. All the measurements were performed with the input voltage of 1V and frequency range from 100Hz to 20 kHz.

The impedance of sample was calculated by above equation 2.1 & 2.2.

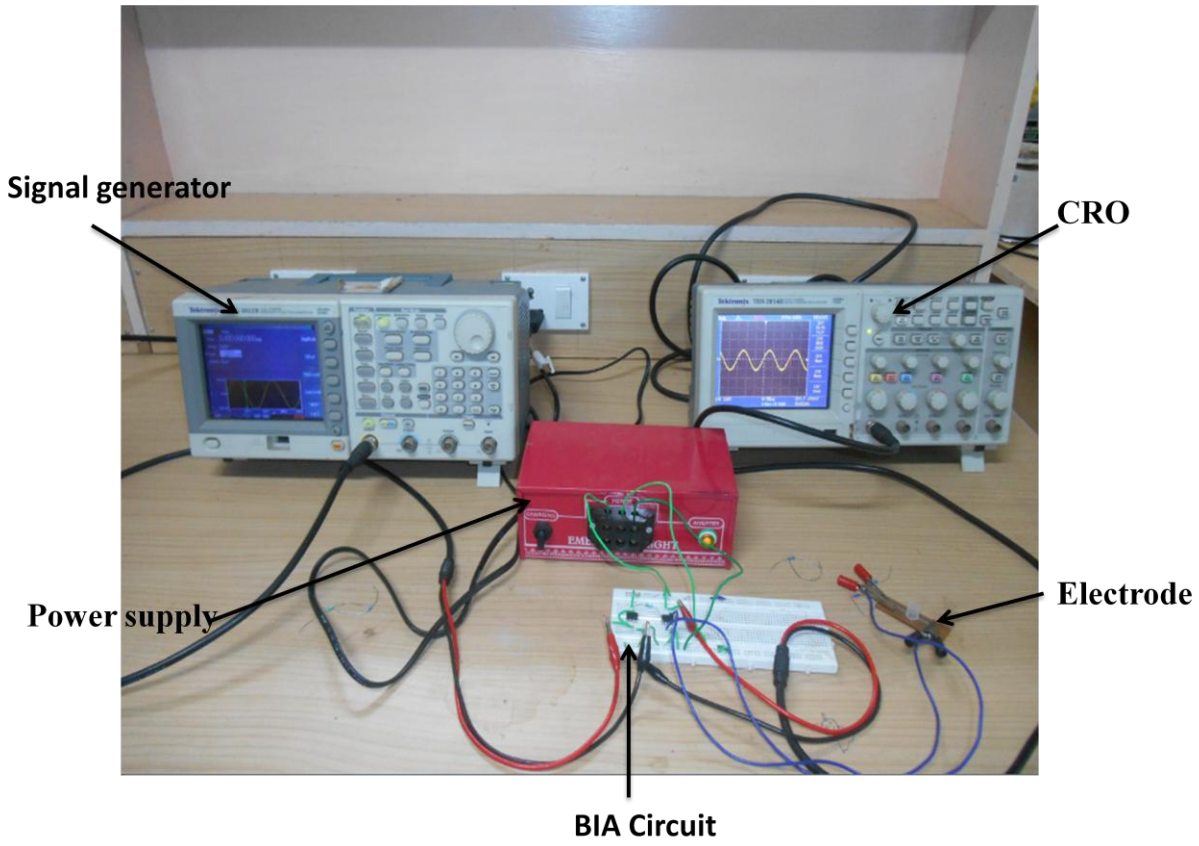


Figure 2.11: Experimental set-up for impedance analysis

2.7.3 Impedance measurement of E.Coli culture solution:

100 ml of distilled water was used to establish a bacterial culture solution. 2.5 gm of Luria Bertani Broth (media) added in distilled water, and mix them properly. After that auto clave was done at pressure-15 psi, temperature- 121°C upto 15-20 minute. 1 ml of mother culture E. Coli culture solution added in the solution when solution reached at room temperature, keep these solution on orbital shaker and continuously check the turbidity. After 18 hours a sufficient turbidity occur in cultured solution. Centrifuge these solution thrice at rpm=5000 for 10 minute. Made various serial dilutions of bacterial culture solution using PBS, and take optical density of all the dilutions at 545nm. The bacterial solutions were seeded in the well attached to the current injection electrode. Current passed through the culture solution and corresponding changes in voltage was recorded with the help of oscilloscope with varying frequency. All the measurements were performed with the input voltage of 1V and frequency range from 100Hz to 20 kHz

The impedance of sample was calculated by equation 2.1 & 2.2.

Table 2.1: Composition of bacterial culture samples

Sample name	Volume(ml)	
	Bacterial culture	Pure PBS
A	0	5
B	1	4
C	2	3
D	3	2
E	4	1
F	5	0

Figure 2.12: Bacterial (E.Coli) culture

2.7.4 Impedance of glucose solution:

Various dilutions of glucose solution were made using distilled water. The solution was added in the well attached to the current injection electrode. Current passed through the glucose solution and corresponding changes in voltage was recorded with the help of oscilloscope with varying frequency. All the measurements were performed with the input voltage of 1V and frequency range from 50 Hz to 20 kHz.

Impedance of sample was calculated using above equation 2.1 & 2.2.

2.7.5 Study of the biocompatibility of the impedance analysis platform:

First of all, sterilization of four triplet electrode (3 well electrodes) was done by using iso-prapanol and ethanol under the laminar hood [27]. After 20 minutes of time, the electrodes were washed with phosphate buffer solution (PBS). Minimal essential media was added to the 3 well electrodes to provide nutrients for the growth of HT-29 cell line. 0.25% tripson/EDTA

was used for detaching the cells from the surface of culture flasks. 10^4 cells/well were seeded. Cell seeded electrodes were placed in CO_2 incubator and maintained at 37°C and 5% CO_2. After incubation for 24 hours, electrodes were loaded with a drug namely cis-platin on two sets of triplet electrodes, and the other two sets were kept as control. Two electrodes (loaded with drug and control) were taken out from the incubator and the voltage drop of the cell suspensions were recorded in a range of 50 Hz to 10 KHz (Vp= 1V, R=1kΩ) using impedance analyzer device. Similarly, the other two sets of electrodes (loaded with drug and control) were taken out from incubator and absorbance (O.D) was measured at 595nm using UV spectrophotometer. Impedance was calculated using the above equation 2.1 & 2.2.

Table 2.2: Absorbance of drug and non-drug solution at 595nm

Solution	Absorbance
Drug	0.666
	0.652
	0.605
Non-drug	0.857
	0.823
	0.847

CHAPTER#3

RESULTS AND DISCUSSIONS

Results and discussion:

3.1 Xanthan gum gel:

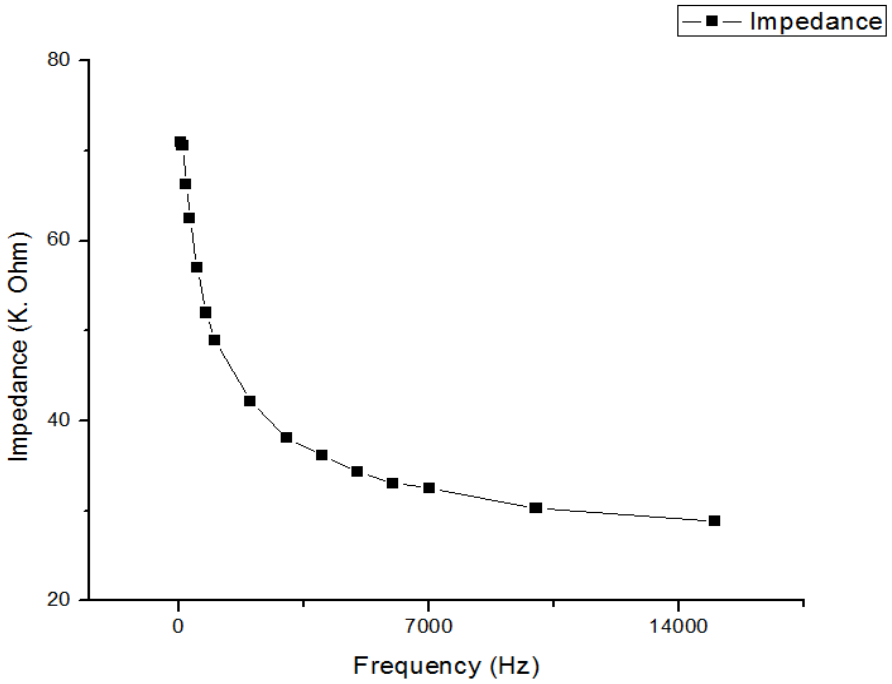


Figure 3.1 Impedance Vs frequency plot of xanthan gum

Impedance analysis of xanthan gum has been done in the frequency range of 50 Hz to 15 KHz (Vp= 1V, R=22 KΩ). The VCCS gives a constant current of 32 µA into the sample during the impedance analysis of the sample. The changes in the impedance of xanthan gum sample as a frequency shown in figure 3.1. The outcomes indicate that the impedance of xanthan gum initially decreased then it goes to constant as the frequency of the current was increased in the frequency range of 50 Hz to 15 KHz. This clearly shows dominance of capacitive component in xanthan gum.

3.2 Impedance analysis of salt solution:

R=150Ω, Vp=1V

Electrode dia: 0.05”, 0.1” & 0.15”

Electrode distance: 0.05”, 0.1” & 0.15”

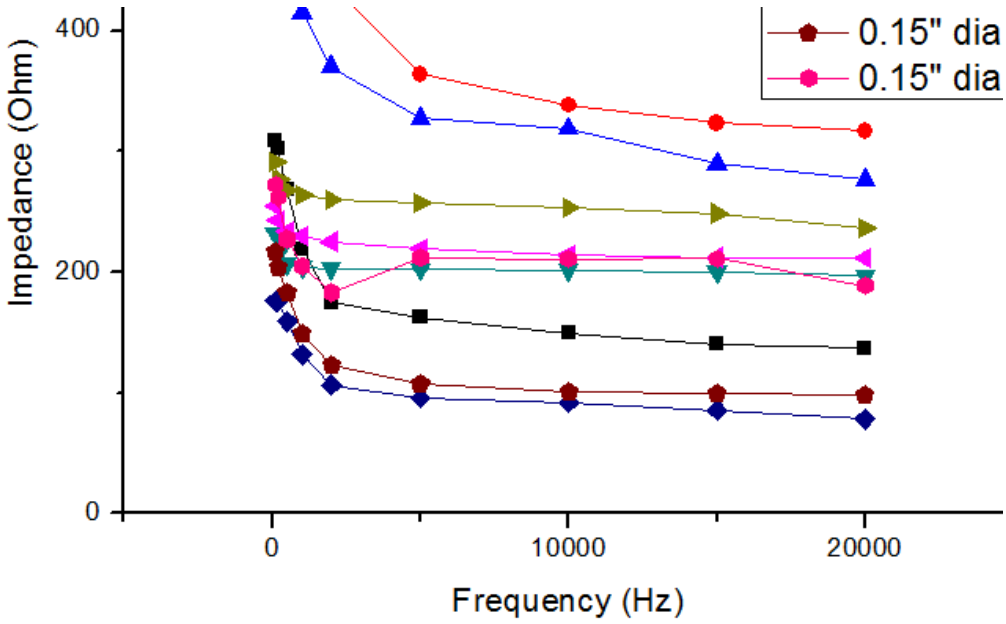


Figure 3.2: Impedance Vs frequency plot of normal saline (NS) at different electrode area and spacing.

In this figure 3.2 the impedance of normal saline depends on the electrode area and spacing. The outcomes indicate that impedance is going to low if electrode area was increased simultaneously this trends also follow in the case of electrode spacing.

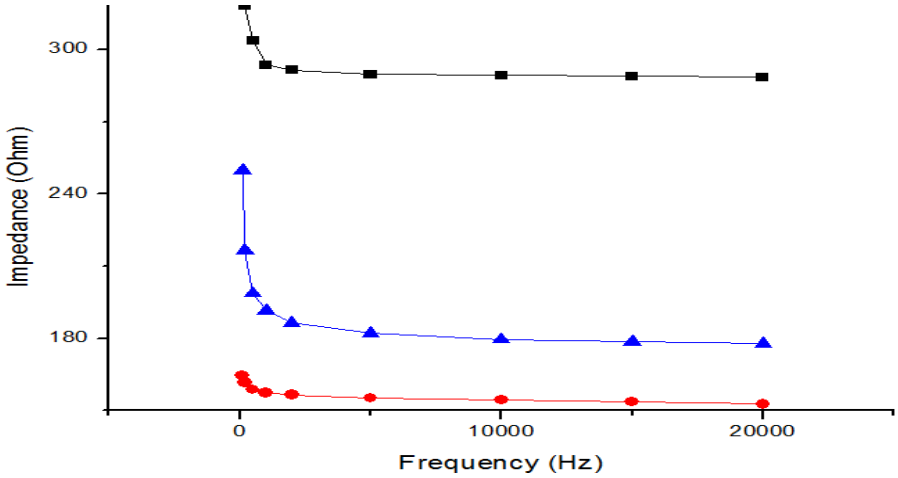


Figure 3.3 Impedance Vs frequency characteristics of NS using optimized electrode

In this figure 3.3 the impedance of normal saline was plotted at the different electrode area and spacing. The outcomes indicate that impedance is going to low if electrode area was increased simultaneously this trends also follow in the case of electrode spacing. The maximum electrode area and minimum spacing of electrode shows low impedance value and it also have more linear region, so we select this electrode for further investigation.

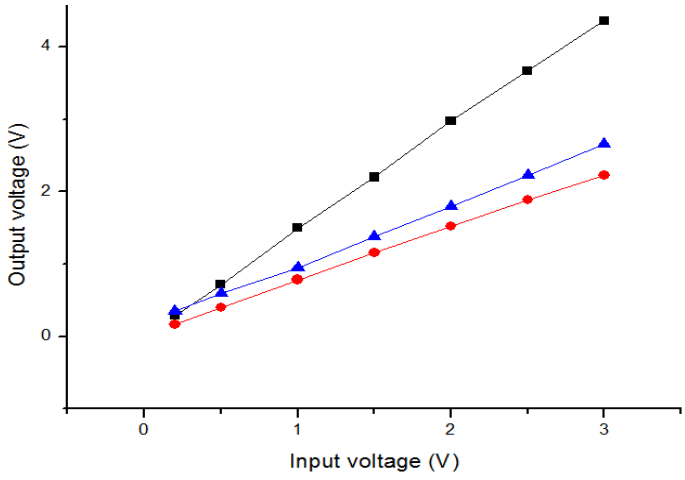


Figure 3.4: Input-Output voltage characteristics of salt solution

This figure 3.4 shows the output-input characteristics of normal saline at the different electrode area and spacing. The outcomes indicate that minimum electrode area and spacing have high value of slope.

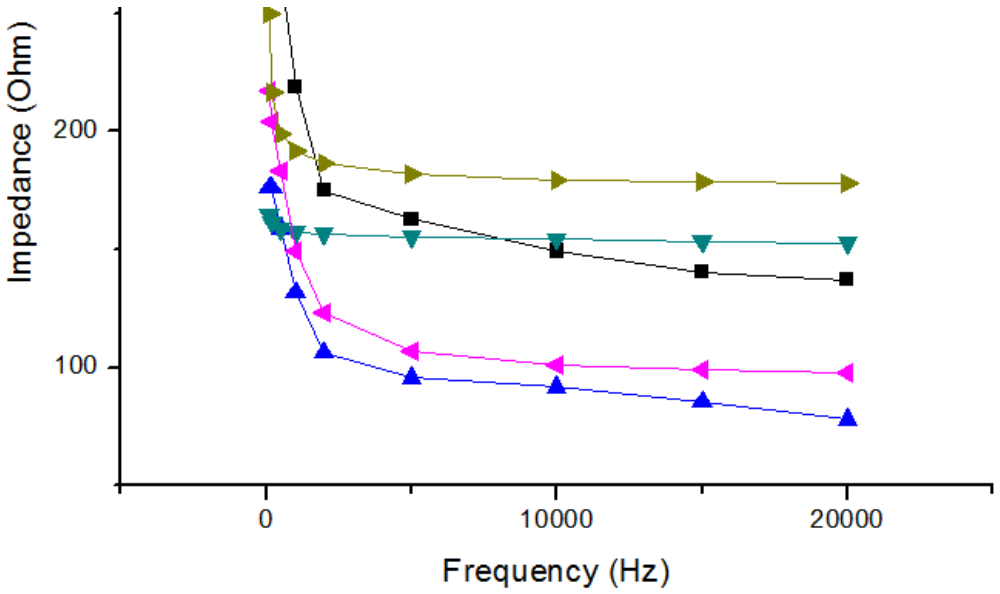


Figure 3.5 Frequency Vs impedance characteristics of NS at different diameter, spacing and voltage

In this figure 3.5 the impedance of normal saline was plotted at the different electrode area, spacing and input voltage. The outcomes indicate that impedance is going to low if electrode area was increased simultaneously this trends also follow in the case of electrode spacing. The maximum electrode area and minimum spacing at low input voltage shows more linear region, so we select this electrode for further investigation.

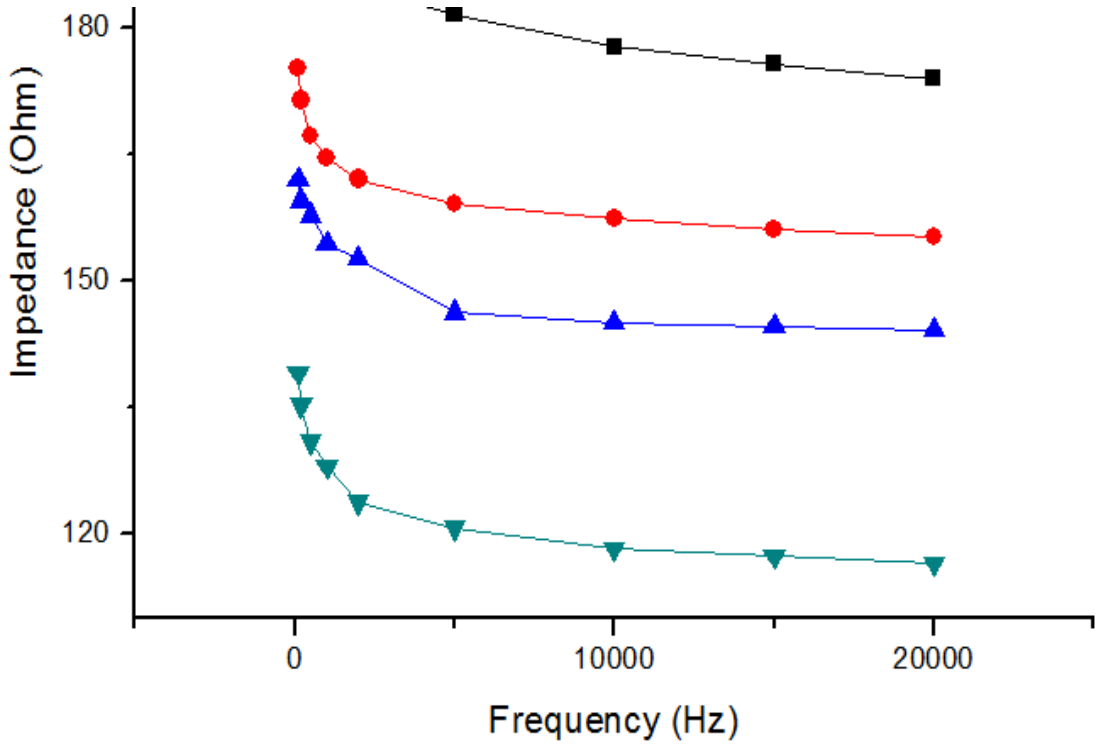


Figure 3.6: Frequency Vs impedance characteristics of salt solution using optimized electrode

Impedance analysis of different dilution of salt (NaCl) solution in distilled water has been carried out in the frequency range of 50 Hz to 20 KHz. Similarly, voltage drop in different dilution was recorded in the input voltage range (Vp=0.2 V to 3V). The changes in the impedance of solution as a frequency shown in figure 3.6. The outcomes indicate that the impedance of salt solution initially decreased then it goes to constant as the frequency of the current was increased in the frequency range of 50 Hz to 20 KHz. This clearly shows dominance of capacitive component in salt solution. From the figure, it is obvious that impedance is decreased when the concentration of salt in distilled water increases with the various electrode area and electrode diameter. In this experiment, we observed that a significant value of impedance is found for different concentration of salt solution entire the frequency range. This experiment was repeated two times and similar trends found. From the figure, it is evident that

with the increase of NaCl in distilled water, the impedance was increased and similar trend follows in all the salt solution related experiment.

3.3 Impedance analysis of bacterial (E.Coli) culture solution:

Optical density:

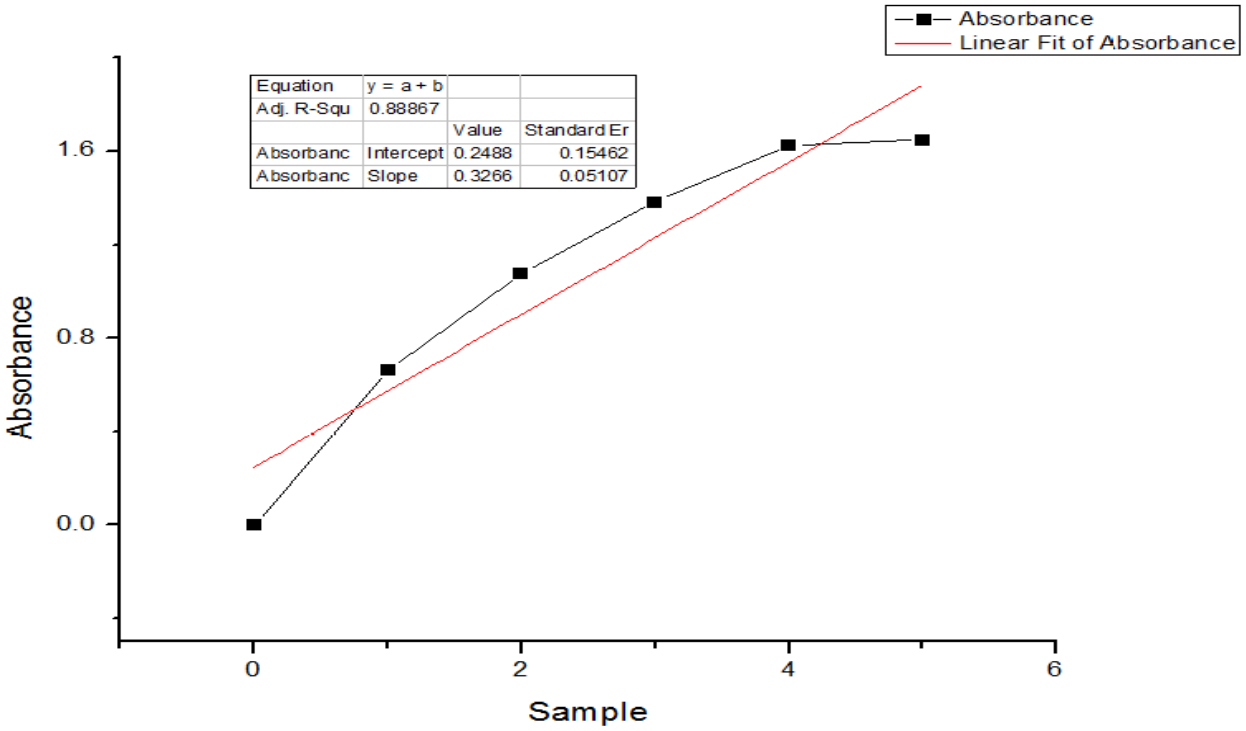


Figure 3.7: Absorbance characteristics of bacterial culture solution of different dilution

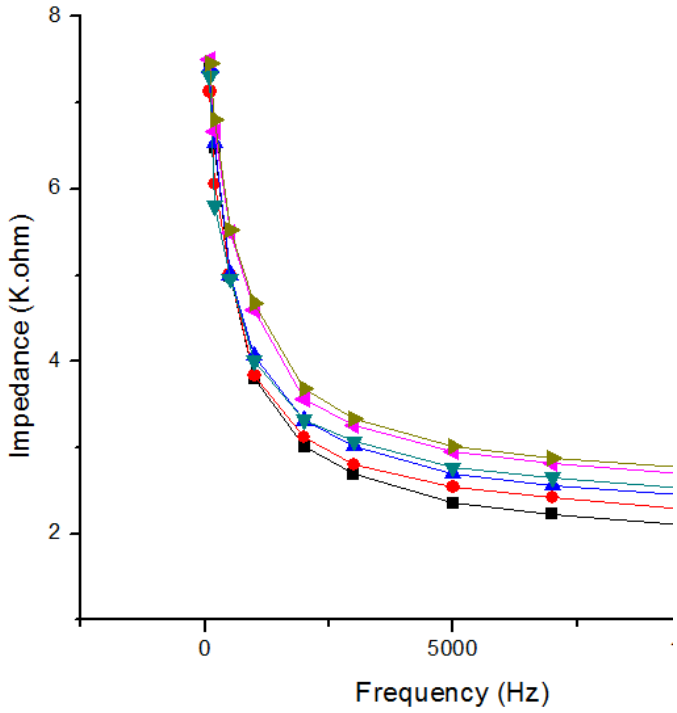


Figure 3.8: Frequency Vs impedance characteristics of bacterial culture solution

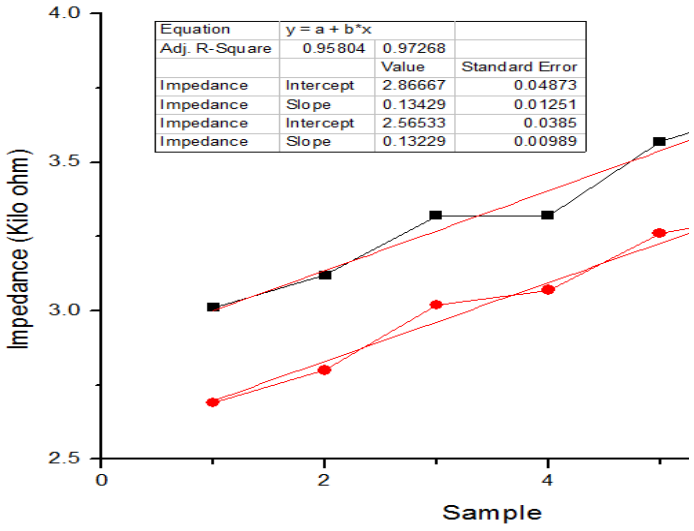


Figure 3.9 Impedance characteristics plot of bacterial culture solution at frequency f=2 & 3 KHz

Impedance analysis of different concentration of bacterial (E.Coli) culture solution in phosphate buffer solution (PBS) has been done in the frequency range of 50 Hz to 10 KHz. Similarly, absorbance (O.D) was measured at 595nm using UV spectrophotometer. The changes in the impedance of as a frequency shown in figure 3.9. The outcomes indicate that the impedance of glucose initially decreased then it goes to constant as the frequency of the current was increased in the frequency range of 50 Hz to 10 KHz. This clearly shows dominance of capacitive component in glucose solution. From the figure, it is obvious that impedance is increased when concentration of bacterial culture solution in PBS increases. In this experiment, we observed that a significant value of impedance is found for different concentration of bacterial culture solution entire the frequency range. This experiment was repeated two times with similar trends. From the figure, it is evident that with the increase of bacterial culture solution in PBS, the impedance was increased and similar trend follows in repeated experiment. The trend line (R-square value) for impedance characteristics of E.Coli cultured solution have better in comparison to O.D value of these solution.

3.4 Impedance analysis of glucose:

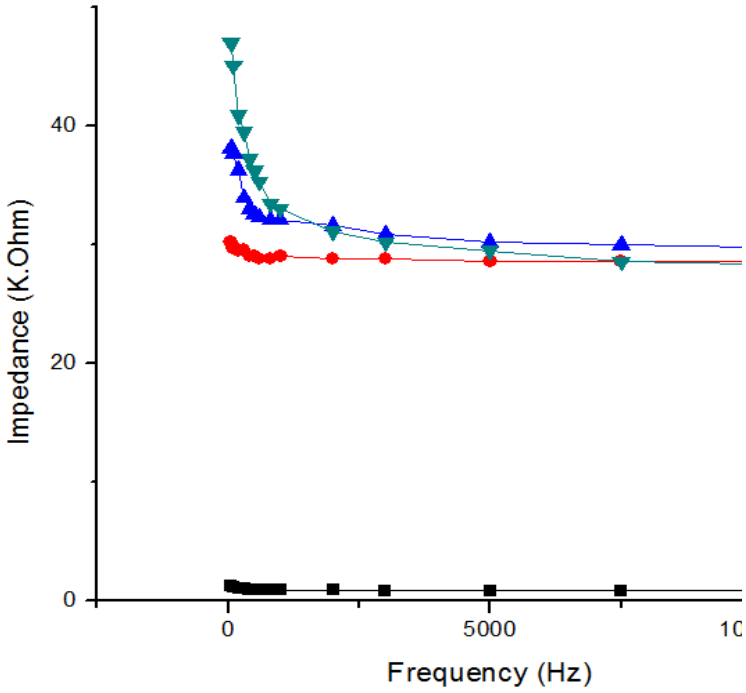


Figure 3.10: Frequency Vs impedance characteristics of glucose solution

Impedance analysis of different concentration of glucose has been done in the frequency range of 50 Hz to 10 KHz. The changes in the impedance of glucose sample as a frequency shown in figure 3.10. The outcomes indicate that the impedance of glucose initially decreased then it goes to constant as the frequency of the current was increased in the frequency range of 50 Hz to 15 KHz. This clearly shows dominance of capacitive component in glucose solution. From the figure.. it is obvious that impedance is increased when concentration of glucose in distilled water increases. In this experiment, we observed that a significant value of impedance is found for different concentration of glucose solution entire the frequency range.

3.5 Biocompatibility test on the impedance analysis platform:

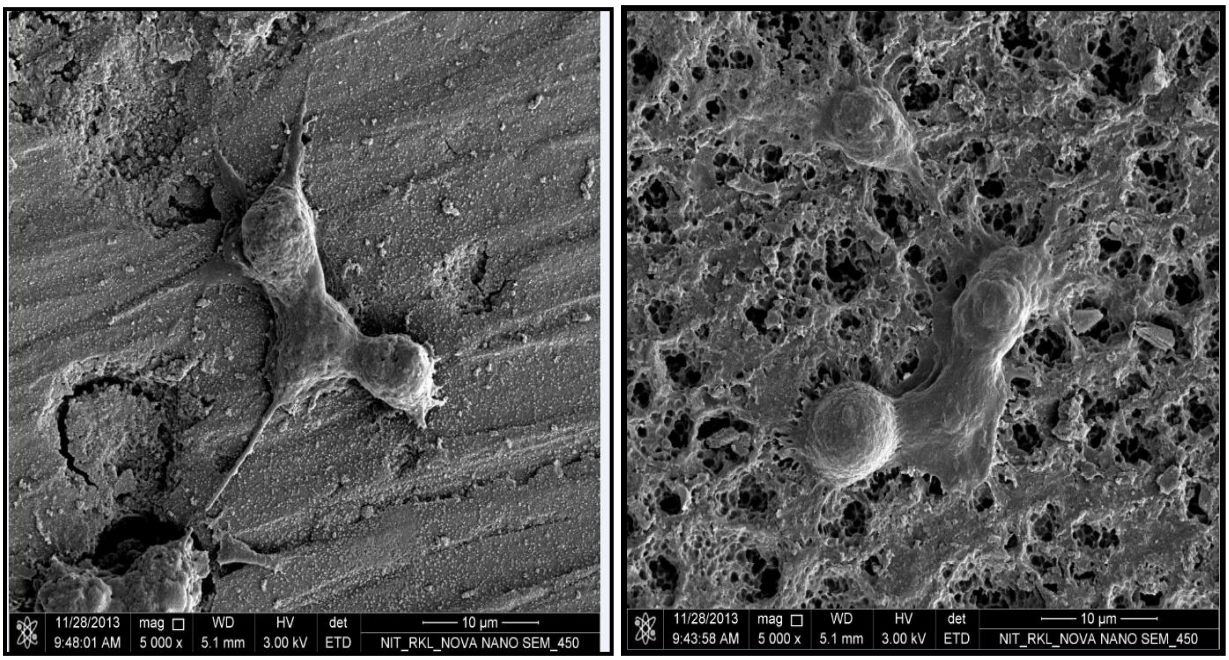


Figure 3.11: FESEM micrograph of HeLa cell cultured on PCB substrate

Cell attachment and spreading is depicted in the figure 3.14 surface of PCB substrate has favored the attachment of seeded cells for 2 days of culture. The cells were aggregated and appear spherical in shape. The cells were found to be well spreaded on the surface of PCB. From these results, it is evident that the surface of PCB has encouraged the attachment and spreading of HeLa cells.

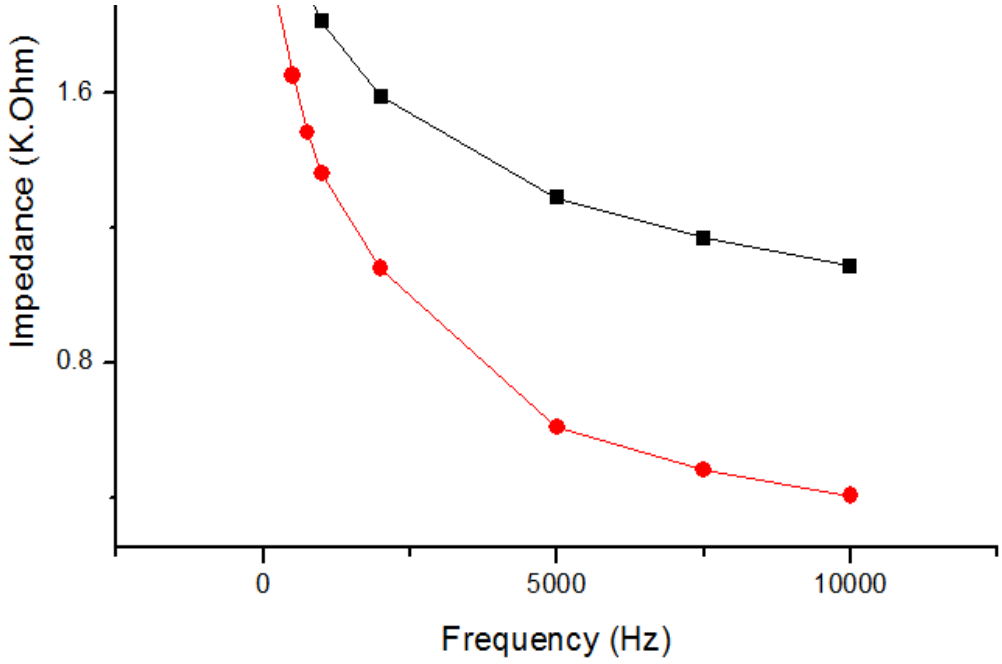


Figure 3.12 Impedance response of anti-cancer drug with frequency

Table 3.1 Calculation of different function of sample

Function	Non-drug	Drug	Non-drug	Drug	Non-drug	Drug	MTT(Non-drug)	MTT(Drug)
Frequency	200 Hz	200 Hz	1 KHz	1 KHz	5 KHz	5 KHz	W.L=595nm	W.L=595nm
Mean	1.486667	1.333333	1.27	0.954333	0.903667	0.427	0.842333	0.641
STDEV	0.023094	0.109697	0.045826	0.114177	0.044095	0.041905	0.017474	0.031953
R.P.I	1	0.896861	1	0.751444	1	0.472519	1	0.760982
RSTDEV	0.015534	0.073787	0.036083	0.089903	0.048795	0.046372	0.020745	0.037934

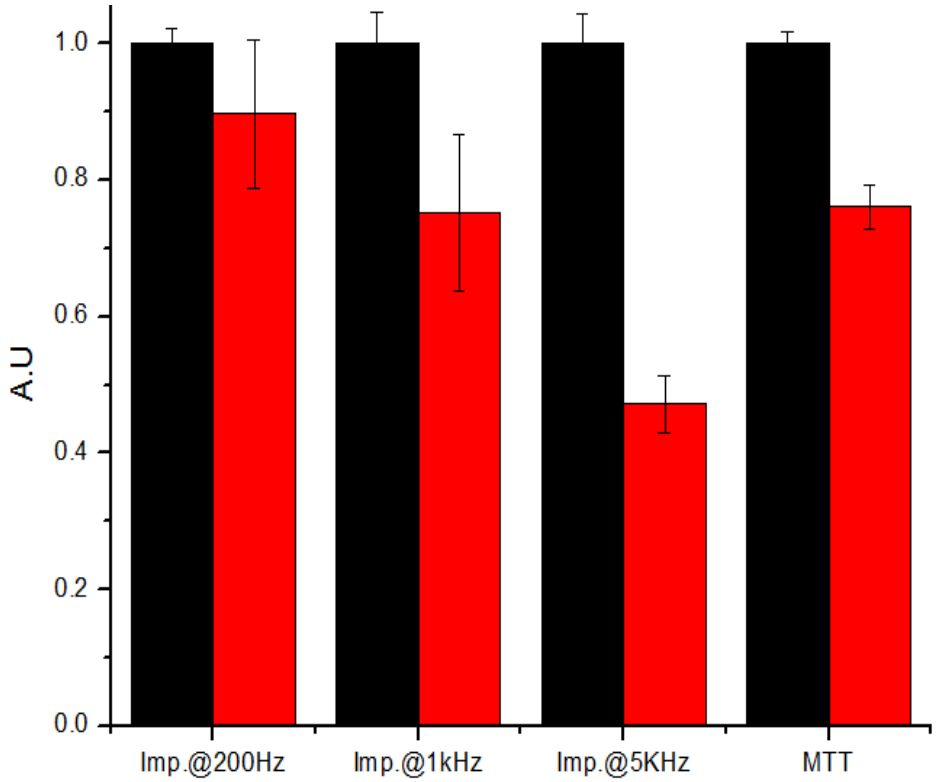


Figure 3.13 R.P.I bar graph of HT-29 cultured cell line

Two electrodes (loaded with drug and control) were taken out from the incubator and the voltage drop of the cell suspensions were recorded in a range of 50 Hz to 10 KHz (Vp= 1V, R=1kΩ) using lab on chip impedance analyzer device. Similarly, the other two sets of electrodes (loaded with drug and control) were taken out from incubator and absorbance (O.D) was measured at 595nm using UV spectrophotometer. Impedance analysis of both loaded and unloaded drug has been done in the frequency range of 50 Hz to 10 KHz (Vp= 1V, R=1KΩ). The VCCS gives a constant current of 0.707mA into the sample during the impedance analysis of the sample. The changes in the impedance of both loaded and unloaded drug as a frequency shown in figure 3.13. The outcomes indicate that the impedance of both (loaded with drug and control) initially decreased then it goes to constant as the frequency of the current was increased in the frequency range of 50 Hz to 10 KHz. This clearly shows

dominance of capacitive component in both (loaded with drug and control). From the figure, it is evident that control (without drug) has more impedance value in comparison to loaded drug with frequency. Mean, standard deviation, and R.P.I (relative proliferation index) were calculated using a standard formula and plot the bar graph which is shown in figure 3.13.

CHAPTER#4

CONCLUSION AND FUTURE WORK

Conclusion:

A suitable device with low cost was successfully developed for measuring bioimpedance of biological samples. The sensitivity of the developed device efficiently works on the range of 50 Hz to 20 KHz. The electrode spacing and area were optimized to improve its efficacy in measuring the bioimpedance. Further, the device was actively used to measure impedance of various biological samples such as xanthan gum, NaCl solution, bacterial culture (E. coli) and cellular responses (HT-29 cell line). All the samples were shown capacitive dominance nature. Screening of anticancer drugs was also performed by using HT 29 cell line. Overall, the present study focuses on measuring impedance values of various samples by the minimal cost. Furthermore, portable nature of the developed device makes it a special among the instruments used for measuring bioimpedance.

Future work:

A simple and portable instrument which can measure the impedance of biological samples is of paramount importance. However, the sensitivity of the device can be improved by digitalization which will reduce the noise during measuring the impedance. The operating range of the device can also be improved by digitalization. Further, the data can be monitored on real time basis by interfacing the device with software such as MATLAB to get accurate data.

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