Development Of MATLAB Based Software For The Analysis Of The Impedance Data Obtained From In-House Developed Bio-Impedance Analyzer

A Thesis submitted in partial fulfillment of the Requirements for the degree of

Master of technology

In

Biomedical Engineering

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Rourkela, Odisha, 769008, India

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Under the guidance of

Dr. Kunal Pal

(Project Supervisor)

Department of Biotechnology and Medical Engineering

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Rourkela, Odisha, 769008, India

May 2015

Dedicated to…

Gurudev, My Parents, my brothers, Sister in-laws

And

Kunal Sir

DEPARTMENT OF BIOTECHNOLOGY AND MEDICAL ENGINEERING

NATIONAL INSTITUTE OF TECHNOLOGY, ROURKELA

ROURKELA – 769008, ODISHA, INDIA

Certificate

This is to certify that the work done in thesis "**Development Of MATLAB Based Software For The Analysis Of The Impedance Data Obtained From In-House Developed Bio-Impedance Analyzer"**by **Dibyajyoti Biswal(213BM1003)** in partial fulfillment of the requirements for the award of the degree of Master of Technology in Biomedical Engineering during session 2013-2015 in the Department of Biotechnology and Medical Engineering, National Institute of Technology Rourkela is an authentic work carried out by her 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 or Diploma.

Place: NIT Rourkela **Dr. Kunal Pal**

Date: 24th May2015 Assistant Professor

DEPARTMENT OF BIOTECHNOLOGY AND MEDICAL ENGINEERING

NATIONAL INSTITUTE OF TECHNOLOGY, ROURKELA

ROURKELA – 769008, ODISHA, INDIA

Declaration

I, Dibyajyoti Biswal, declare that this thesis titled, "**Development Of MATLAB Based Software For The Analysis Of The Impedance Data Obtained From In-House Developed Bio-Impedance Analyzer**" and the work presented in it are my own.

I confirm that:

- I certify that the work contained in this thesis is original and has been done by me under the guidance of my supervisor.
- The work has not been submitted to any other Institute for any degree or diploma. • I have followed the guidelines provided by the Institute in preparing the thesis.
- I have confirmed to the norms and guidelines given in the Ethical Code of Conduct of the Institute.
- Whenever I have used materials (data, theoretical analysis, -gures, and text) from other sources, I have given due credit to them by citing them in the text of the thesis and giving their details in the references.

Dibyajyoti Biswal

26th May 2015

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ABSTRACT:-

Biological samples are sensitive to physical changes, mechanical as well as electrical. When any force is applied or temperature of the environment is increased or any torsion given to the system or electricity flows through it then it shows some changes behaviorally which in some distinguishable into eyes and is predictable. But there are two types of observations present which is taken into consideration. One is qualitative and other one is quantitative. Qualitative way is meant just to detect whether changes happening. But when it comes to comparison at that time one needs proof in which case this qualitative study fails. Again as biological samples are very sensitive to changes and different sample have different properties ,so they act differently in different time duration to the changes. Some changes such as due to electrical flow the changes in the behavior of the sample might not be visible but it can be predicted by the proposed method in quantitative approach. This study shows an easy approach to show the changes due to electrical current flow through the system. This quantitative approach also help in finding the stability as well as the utility of the model both in estimated and predicted cases. Here the well-known impulse response, Nyquist plot and Bode plot and the location of poles and zeros were used for detail analysis of the model behavior. Here hydrogel is taken as the dummy model for all the studies. This approach can be proposed in industrial way for samples stability prediction, its utility in required conditions and the productivity of the designed sample.

Key words:- *Nyquist, Bode ,poles, zeros, estimated, predicted*

Chapter 1

1.1 Introduction:-

Hydrogels are defined as 3D polymer matrices which have the ability to accommodate water within its polymeric structure. Under normal circumstances, the water is not released from the hydrogel structure. This is due to the surface active forces acting at the interface of the polymer-water interface. Due to the presence of water within the structure, hydrogels electro-active in nature. Electro-active polymers have found wide applications in biomedical industry. Some of the commonly used applications include development of architectures for developing muscle-like actuator, wound dressings, tissue engineering and control drug delivery system. The various polymers which have been tried in such applications include gelatin, collagen, sodium alginate, guar gum, chitosan, chitin etc. Amongst the various polymers used in biomedical industry, gelatin has received much attention since the inception of this field of study. This is due to the inherent biocompatible nature of the gelatin matrices. Additionally, gelatin has been reported to be non-immunogenic, biodegradable, bioactive. Further gelatin is available commercially at much cheaper prices as compared to the many other polymers of biomedical importance. Gelatin gels are also used because of its ability to provide mechanical strength to the hydrogels. The only problem associated with the gelatin gels is its at higher temperature. Gelatin gels are converted into sols at temperatures > 35^0C. Many scientists have improved the gelatin hydrogels either by cross-linking with multifunctional reagents (Glutaraldehyde(GA),genipin, EDC cross-linker(1-ethyl-3-(3-dimethylaminopropyl))) or by using charged polymers which can form poly-electrolyte complexes (e.g. chitosan). Many studies have reported the variation in the cell proliferation rates of mammalian cells on the gelatin based constructs in gelatin concentration dependent manner. Some literatures have suggested that the electro conductivity of

hydrogels may be improved by incorporating multi-world carbon-nanotubes (MWCNT). Such hydrogels were used for culturing electro-active cells like muscles ,neural cells(1).

In this study we report the development of gelatin hydrogels by varying the concentration of the gelatin polymer in the range of 10% and 20%. The gelatin polymers were cross-linked with GA so as to improve the thermal stability of the hydrogels. The electrical properties of the hydrogels were studied in depth. Various commonly used equivalent electrical models for the analysis of biological tissues were used to have an understanding of the electrical properties of the hydrogels prepared by varying the concentration of gelatin. The variation in the concentration of gelatin.

Chapter 2

2.1 Literature Review:-

Bio-impedance analysis can be defined as the method to characterize biological samples. Many bio-impedance analyzers consist of applying multi-frequency sinusoidal current of constant amplitude of the sample[1]. So the thing to be kept in mind is the injecting current has constant amplitude over a wide range of frequency. This is required as we have to find out the impedance values at different frequency ranges. So the supply or the input voltage has to be constant with a constant feedback resistor. For V-I analysis the frequency should be constant whereas, the input voltage will be varying with constant feedback resistor. For any sample the V-I analysis would be constant independent of any condition as it describes the characteristic of the sample[2]. Studies suggest that tissue undergoes compression in a variety of physiological , clinical and research experience. In this paper the it has taken 0.25mm diameter platinum electrode and is 19mm diameter as shown before[3]. The electrodes were equispaced by 3mm and the electrode spacing was adopted for better result in Agilent 33120A function generator[3]. In the projected work of mine I kept the distance between two electrodes as the length of the mould and I have used gold plated electrodes of (Height-14.10mm,width-3.03mm)[3].Now coming to the method of bio-impedance measurement there are many for different types of measurement. Those are FFM(fat-free mass), ECW(Extracellular water), ICW(Intracellular water), BCM(Body cell mass) which can be defined as the cell mass other than ECW [4, 5]. The ability of measurement of BCM would set a reference for measurement of consumption of oxygen, requirement of calories, BMR[6, 7].The below figure 2.1 is the pictorial representation of equivalent electrical circuit taken throughout study.

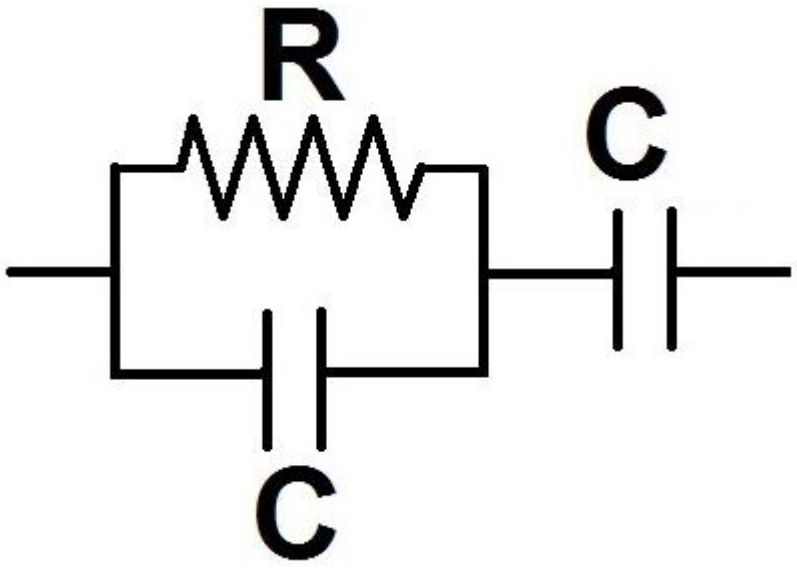

Figure 2.1.Equivalent electrical circuit for (RC)C

According to this paper ICW is closely related to BCM in terms of values. Accordingly, bio-impedance measurements can be used to estimate BCM noninvasively by measuring ICW.

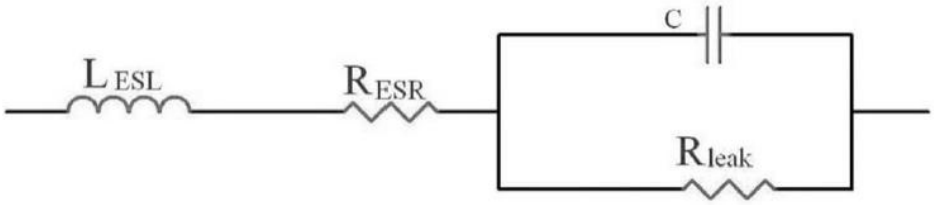

Figure 2.2.Equivalent circuit of water electrolyte

Investigators given the statement that ECW, ICW and TBW can be predicted best using a bio-impedance spectrometer[7-9]. The proposed method by Thomas suggests the fixed single low-frequency at 1kHz used for measuring ECW and frequency of 100kHz for measurement of TBW[10].Third type is at constant frequency of 50kHz[10]. Basic theoretical and analytic bio-impedance principles proposed the studies suggest that the cell membrane behave as capacitance and impedance is purely frequency dependent[10]. It suggests that at zero frequency or lower

frequency the impedance is known to be initial resistance i.e. denoted as R_0 and the impedance value at higher frequency i.e. 1MHz . When ac supply is provided the membrane capacitance charges and discharges the current as per its property. The value of the internal resistance is as showed in the equation-16 below. According to the studies at R_0 and R_∞ the impedance value does not depend on membrane capacitance[10].

$$\frac{1}{R_I} = \frac{1}{R_0} - \frac{1}{R_\infty} \quad (1)$$

As per this study I have taken the models which are with the internal resistance and membrane impedance mainly. In some cases I have taken the consideration of external resistance also. The figure 2.2 shows the circuits taken for electrical modeling as well as analysis of the impedance profile. The study suggests that the water electrolysis cell has structural similarities with the structure given below in figure 2.3[11].Water electrolytic cell consists of the two parallel electrode plates with dielectric material in between. The aim of saying this is that the path of flow of current of such structure also might have followed an equivalent electrical circuit model so as to complete the circuit. The series circuit next to the inductor is called Randle's circuit which is analyzed mainly by frequency response[12].

The Z_C value and Z_L values are found out mainly from the below equations. In these equations 2 and 3, Ldenotes the inductance and C denotes capacitance value.

$$Z_C = \frac{1}{jwC} \quad (2)$$

$$Z_L = jwL \quad (3)$$

Where C is the capacitance and L is the inductance.

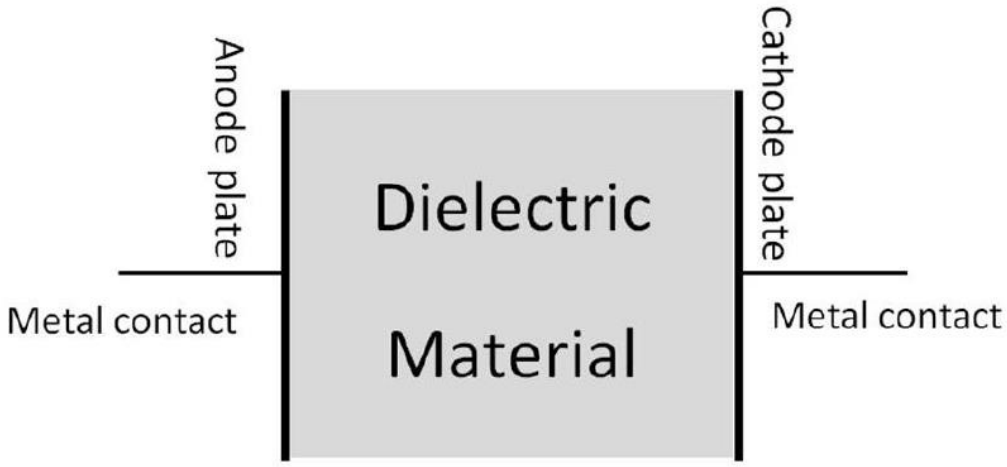

Figure 2.3.Equivalent electrical circuit of water electrolyte[11]

The ohmic resistance of the electrolyte is the main affecting part of impedance of the electroytic cell, as it frequency independent. When the frequency rises, the capacitor begins to lead the electrical current[13]. The requirement of minimizing the electrical impedance of the cell was constructed basically in the light of Ohm's Law[14].This can be written as in Equation 4 where V stands for the potential, Z is the circuit impedance, and I denotes the current flowing through the biological sample.

$$V = ZI \qquad (4)$$

The estimation of every individual piece of the presented proportionate circuit is time variation. Then again, some can be spoken to as elements of the same physical variables. This makes presenting a careful scientific model for the proportional circuit a complex errand. This modeling could help designers to make sensibly precise suspicions when picking or planning force regulation hardware for electrolysis framework. We in this manner accept that numerical/mathematical modeling of the actual transfer function may be an intriguing subject for further research[15]. So from the inferences taken from this study in my work I have tried to implement the equivalent electrical modeling as well as the modeling of the actual transfer

function. Then considering the frequency domain analysis the behavior and property of the biological sample or tissue can be predicted. In case of any critically damped system $\zeta=1$ where ζ is known as the damping co-efficient. If $\zeta \geq 1$ then the roots are real and if $\zeta = \pm 1$ then the roots are equal and if $\zeta < 1$ then roots are non-real and for the last condition $\zeta \leq 1$ then the roots are complex conjugate having damping frequency other than natural frequency[16]. In result and discussion the $\zeta = 1$ which suggests that my system is a critically damped. Accordingly the analysis were done. Transfer function represents a system differential equation wholely, its poles and zeros completely define the profile of system response[17, 18]. Particularly the system poles define the components of homogeneous responses in relation to the decay constant[19].When the roots are real, they are called simple poles and simple zeros[20]. The roots are complex if they occur in pairs of complex conjugates[21]. Bode plot can be drawn from a transfer function and the reverse can be done if we have the bode plot of a system[22]. From the bode plot the stability of the system, systems behavior in different frequency region in terms of phase(in degrees) as well as the magnitude(in dB) which is $20\log_{10}M$ where the value of M is the actual magnitude corresponding to the frequency[23]. The stability of the system in comparison to others can also be predicted from bode plot. From Nyquist plot we can predict the low frequency behavior as well as the high frequency behavior and the bulk resistance to predict more about the properties of the sample[24].

Chapter 3

3.1 Materials and methods:-

3.1.1 Materials:-

Gelatin (Extrapure) was procured from (Himedia. Maharastra, India), GA(25% aqueous solution) procured from (Lobal chemie, State,India),0.1N HCl, 0.3M glycin , ethanol, DMEM which is the media, PBS(phosphate buffer saline), trypsin EDTA(), MTT,DMSO were procured from (Himedia, State, India), falcon tube(Tarson), twelve well plate(Tarson),microtips 1ml and 200ul(Tarson),falsk(Borosil),beakers(Borosil). Double distilled water was used through out study.

Electrical instruments consisted of function generator and CRO (Crystal ray oscilloscope) are from Textronix.Single sided cupper clad as well as resistors were taken from open market from Rourkela, OP07 used were procured from Texas Instruments, Bangalore, India, power supply of 12 V, MATLAB 2010b.

3.1.2 Methodology:-

3.1.2.1 Preparation of hydrogels:-

Accurately weighed gelatin was dissolved in 60gm of water (50^0C). After complete dissolution of the gelatin, sufficient amount of water (50^0C) was added to make the volume up to 100gm. The gelatin hydrogels were prepared by taking 20gm of the gelatin solution. 0.55ml of GA reagent (0.5 ml of GA + 0.05 HCl) was added to the 20gm of gelatin solution. The mixture was 33mixed thoroughly for 10 seconds at 100rpm using a magnetic stirrer. Subsequently the mixture was transferred to cylindrical mould(height-23.23mm, internal diameter-14.3mm).The mould were kept under room temperature for 5 minutes to allow the gelation of the solution. After 5minutes, the developed hydrogels were retrieved from the mould and were used for further analysis. The composition of the hydrogels have been tabulated in Table 3-1.

Table 3-1.Compositions of the hydrogels taken for study

Hydrogels	Gelatin (%)	Water (%)
G1	10	90
G2	15	85
G3	20	80

3.1.2.2 Electrical properties of the hydrogels:-

The electrical properties of hydrogels were measured using a sinusoidal wave having an amplitude of 2Vpp. The analysis was done in the frequency range of 50Hz-5kHz. The impedance profile, obtained from the test were fitted to various electrical models which are used for the analysis of the biological samples. Then in MATLAB the program has been designed so as to find out the different parameters as well as the plots of the frequency domain. The steps followed after finding out the resistance, capacitance and the homogeneity constants. Then the steps followed are shown in the given flow chart in figure 3.1. The values of the control parameter were produced using the inbuilt control functions. As per the control parameters the results were predicted for the given samples and for the stability conditions. Then the stored values are used for the quantitative analysis of the control parameters for the specialty of the behavior of the circuit in different frequency range.

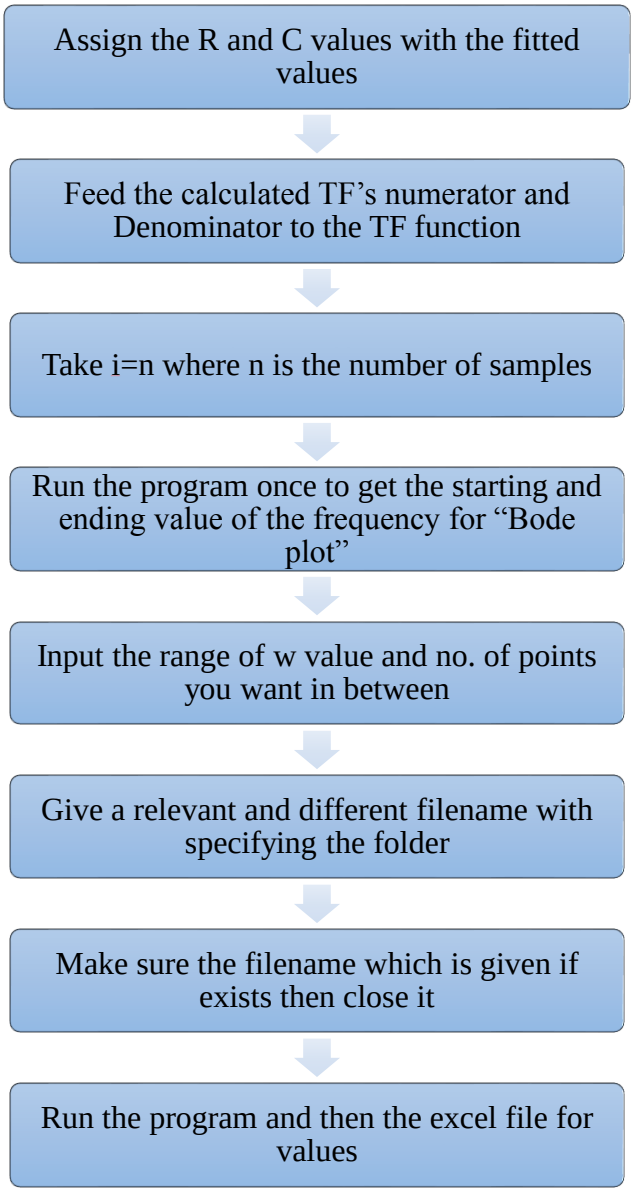

Figure 3.1.Method followed inside designed MATLAB code

Chapter 4

4.1 Results and discussion:-

The impedance profile of the hydrogels has been shown in figure 4.1. A preliminary examination of the profile suggested a decrease in the impedance of the hydrogels with a corresponding increase in the gelatin concentration[25]. This was due to the increase in the amino and the carboxyl groups, present in the gelatin molecules[26]. The impedance profiles of G1,G2and G3 were fitted with (RC)C. The correlation coefficient of the fitted values has been tabulated in table 4-1.

Table 4-1.Shows the values of correlation co-efficient of G1, G2 and G3

Models	**G1**	**G2**	**G3**
(RC)C	0.905308	0.812287	0.762722

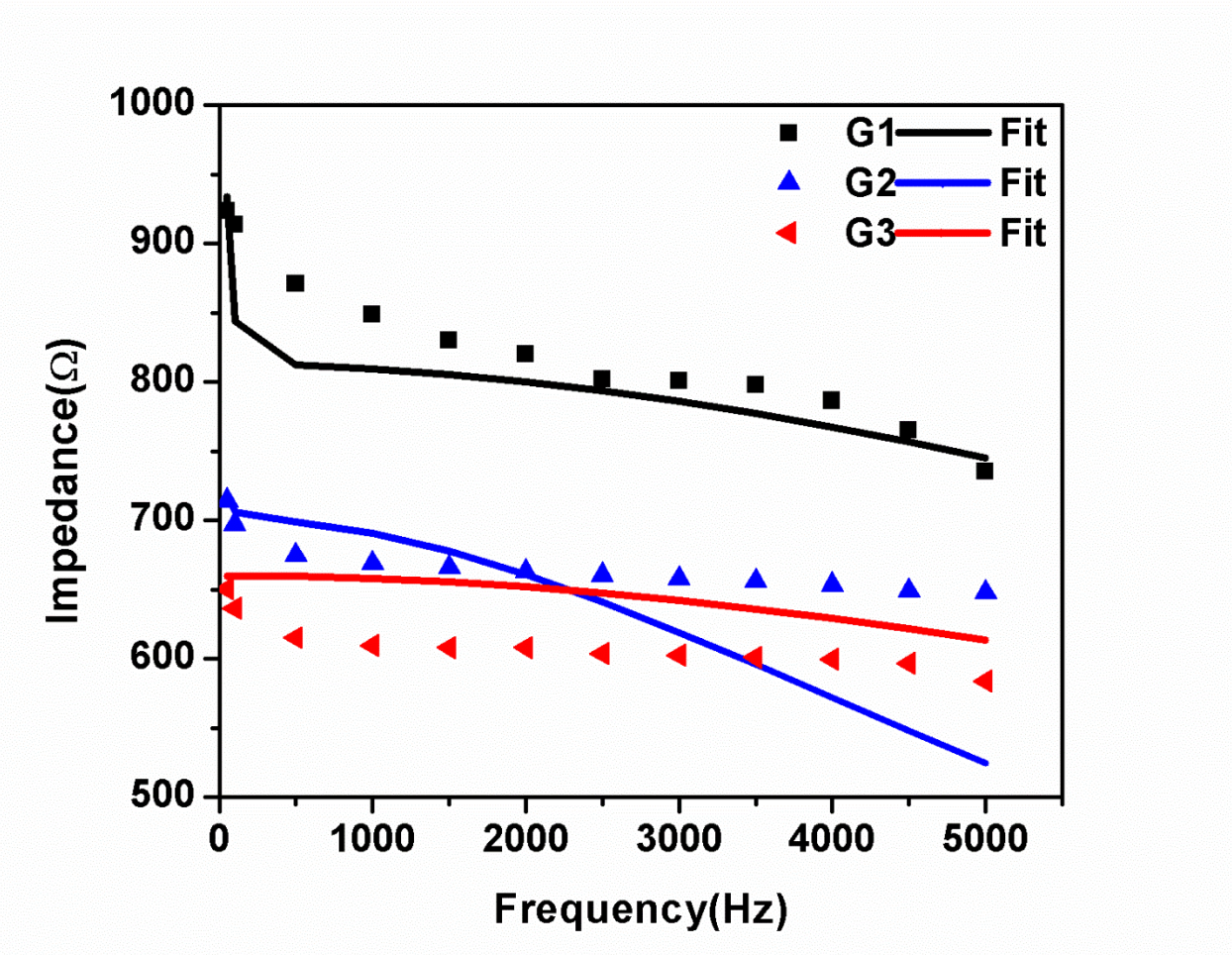

Figure 4.1.Impedance profile of (RC)C

It was observed that the correlation coefficient was lower as the concentration increases. Keeping this point to be noted and analyzing further some conclusion might be drawn for such observation. The model better fits the G1 sample than any other. So there might be one reason that the circuit equation is not fitting properly. But that will be possible if the circuit does not contain any capacitive element. As per the impedance profile of the three samples G1 has the highest impedance than others. The values of the electrical elements of the remaining models were determined. These values showed a gradual decrease in capacitive value. But an interesting

phenomena occurred i.e. the capacitance value of the RC circuit present in the equivalent electrical model used for the analysis of electrical activity. As the capacitance value is showing an increase for G2. So the τ value must be showing the similar pattern. To observe the changes due to the time delay or relaxation time the frequency domain analysis will be required The impulse response profiles obtained using the models have been shown in figure-4.2 and individually in Figure 4.3(a,b,c). A stable system has an impulse response tending to zero at $t \rightarrow \infty$. Hence, it can be said that the equivalent electrical model taken is stable. The impedance showed a decreasing pattern which is reflected here also in the impulse response. The initial value of the impulse response i.e. at t=0 is highest for G1 , then G3 and the lowest is for G2. The time constant of G2 sample is more than other two. So the G2's curve decays in a slower rate than the other two samples. So the G2 curve is less sensitive than that of G1 and G3. The advantage of this point is that with applying input it will show an time delayed output which gives the system the amount of time to act in gentle manner rather than responding suddenly like the other two samples. In impulse response it can be deduced that the sample G2 has an unique characteristic among the three samples of different gelatin concentrations. The impulse response profiles were fitted with the equation 5 which is given below. This equation is the second order impulse response equation meant for un-damped, critically damped or under damped systems. This equation is used here because the plot we are getting in MATLAB is the envelope of the sinusoidal impulse response profile we will actually be getting. It will show that among two poles which pole is more affecting to the response of the profile. This can be known from the fitting of the curve i.e. the value of inverse of σ value. The correlation coefficient of the fitting for all the hydrogels was found to be 1. This suggested that using the second order impulse response equation were in exact match. Hence the developed hydrogels may be explained as a

second-order electrical circuit. This showed the second pole which is nearer to the origin was matching with the value fitted in the curve. To analyze more Nyquist plot and bode plot were taken into consideration.

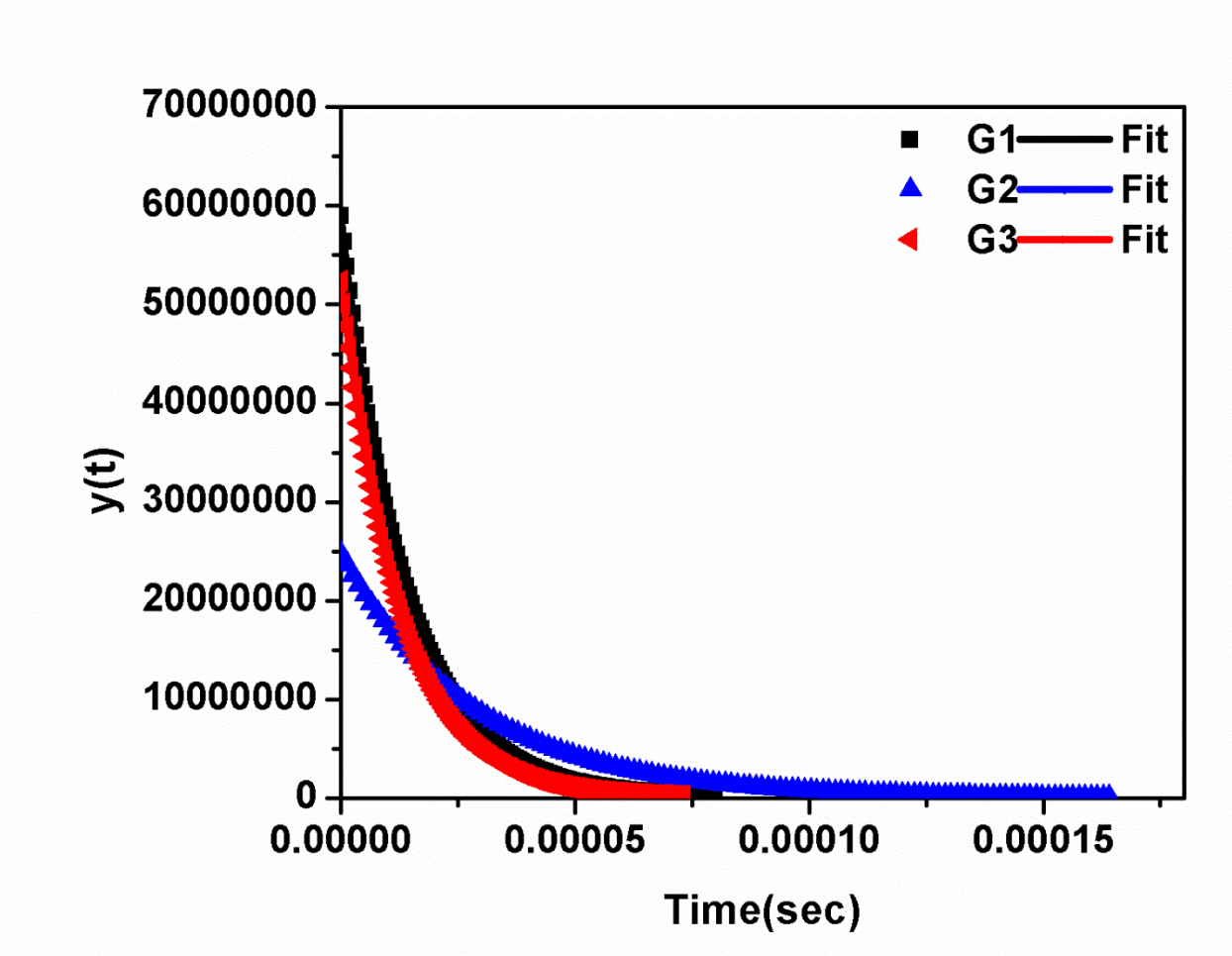

Figure 4.2.Impulse responses of (RC)C

$$y(t) = h(t) = \frac{\sigma^* \omega}{\sigma^2 + \omega^2} \ell^{-\sigma t} \quad (5)$$

$$\tau = \frac{1}{\sigma} \quad (6)$$

Where τ = time constant of the pole nearer to the origin.

Let $M = \frac{\sigma^* \omega}{\sigma^2 + \omega^2}$

Where M=the value of y(t) at time t=0,

Apart from these inferences an increase in the gelatin concentration resulted in a corresponding decrease in the A values. Similar to the A value, B value (amplitude of the impulse response at the highest time) was also in the same order. From this it can be explained that an increase in the gelatin concentration resulted in the increase in the stability of the hydrogels. From here it can be concluded that even from here it can be concluded that even though the electrical stability was high in G2. From here it can be concluded that even though the electrical stability was higher in the hydrogels, which contained higher gelatin proportion, the response time of the equivalent electrical circuit was enhanced.

4.2 Nyquist Plot:-

The nyquist plot of the hydrogels has been shown in figure 4.3(d,e,f). All the hydrogels showed similar nyquist plot. From the plot it was observed that there were two regions i.e.first one is the semicircle and the second one is the straight line going straight. The first semicircle (in the higher frequency region) is associated with the bulk of the material. On the other hand the second straight line (lower frequency range) is associated with the grain boundary. The intercept of the first semicircle with the x-axis at lower frequency range is an indicator of the bulk resistance of the material. Similar to the bulk resistance the grain boundary resistance also followed a same trend. From the grain boundary resistance it can be predicted that the grains of the G2 were of different architecture and did not follow the trend when the gelatin concentration was increased from G1 to G3.

4.3 Bode Plot:-

The bode plot of the hydrogels were similar to each other. The magnitude profile showed decrease in the magnitude response at one frequency. The decrease in the magnitude was same at lower as well as higher frequencies. Corresponding to the decrease in the magnitude values, phase profile showed a distinct peak. The position of the peak(x value) has been tabulated in table 4-2. The natural frequency is also listed in the below table. In both the values it can be noticed that the peak point as well as the natural frequency is lesser for G2. These values were found to be in close approximation of the w_{max} value obtained from the Nyquist plot. The corresponding w_{max} values provide information about the location of the poles. An analysis of the w_{max} suggested that the model used for the prediction of the sample property are stable and hence will provide accurate result. Peak points is x and ω is the natural frequency.

Table 4-2.Peaks and natural frequency of bode plot

Samples →	**G1**	**G2**	**G3**
x	3604.435	1607.39	2366.95
ω	72621.64	35360.68	79327.3

The Figure-4.4,4.5 and 4.6 show the MATLAB outputs of the program designed among which the figure 4.4 shows the impulse responses of G1,G2,G3 respectively for model (RC)C. Similarly Figure 4.5 shows the Nyquist plot of the three samples as named and the Figure 4.6 shows the magnitude and phase plot of the bode analysis of the three samples respectively.

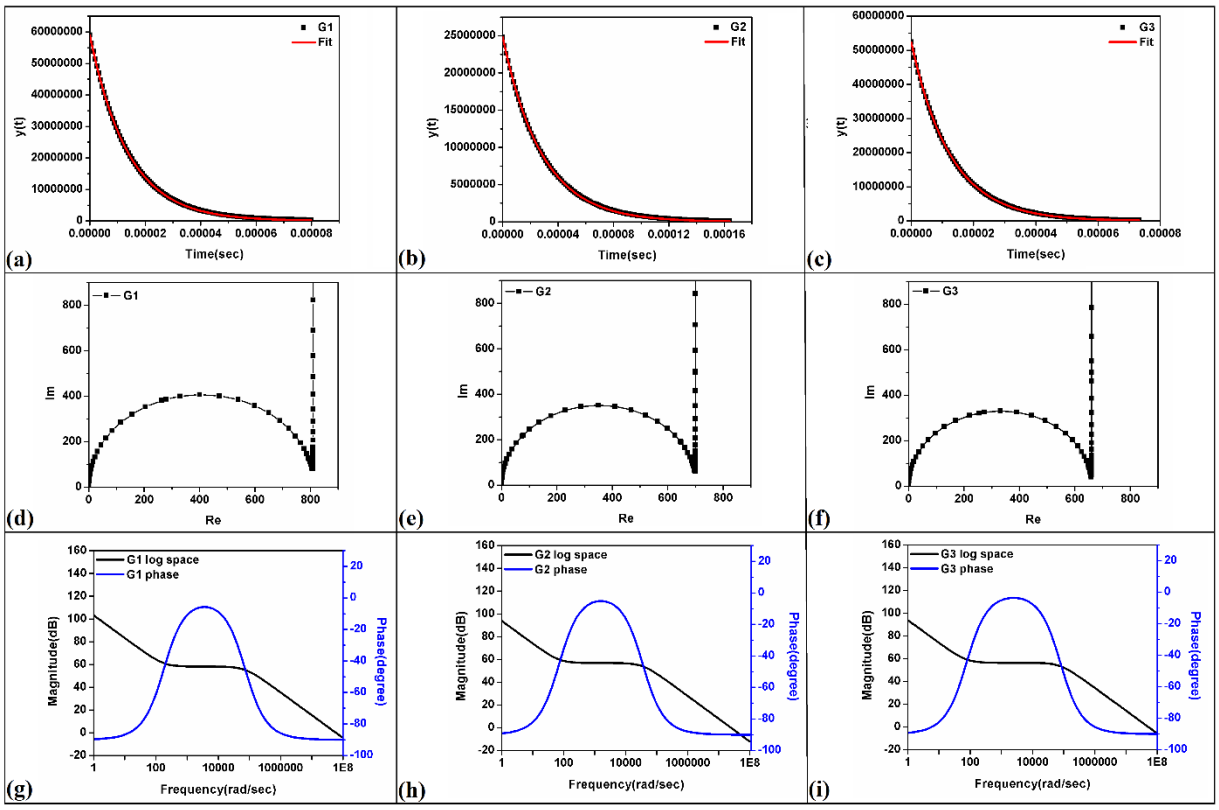

Figure 4.3.Analysis plots (a),(b),(c) are Impulse Responses; (d),(e),(f) are Nyquist plots and (g),(h),(i) are Bode plots of G1,G2,G3 respectively

Moreover the phase margin is assumed to be stable one if it lies in negative phase region. In this case every sample's phase values are nearer to 90^{o} So it can be said that the model is a stable one but there comes the following contradiction which is in the whole process from the results we can conclude that the values show that these are stable but if the model was exactly accurate then it would have fitted more so that correlation coefficient would be more. To describe this the observation in the change of time constant, natural frequency as well as the pole position can be taken. All the three show similar changes. So the problem might be lying in the capacitive element. A biological sample can not behave completely capacitive but the combination of capacitance as well as resistance element. In bio-impedance study CPE(constant phase element) has been introduced which has the same characteristics.

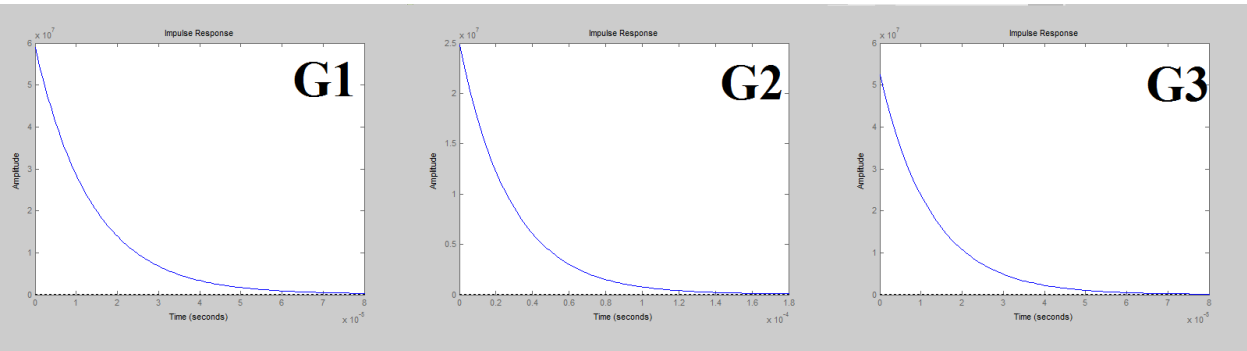

Figure 4.4.Impulse responses of G1,G2,G3 of model (RC)C

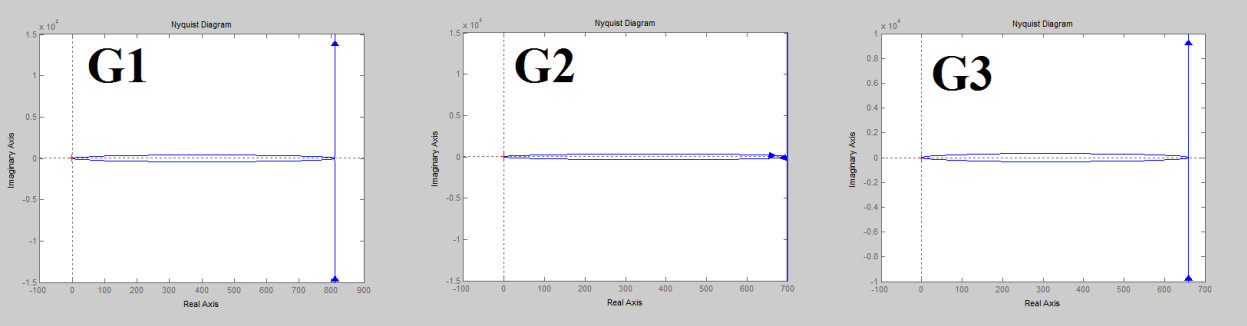

Figure 4.5.Nyquist plots of G1,G2,G3

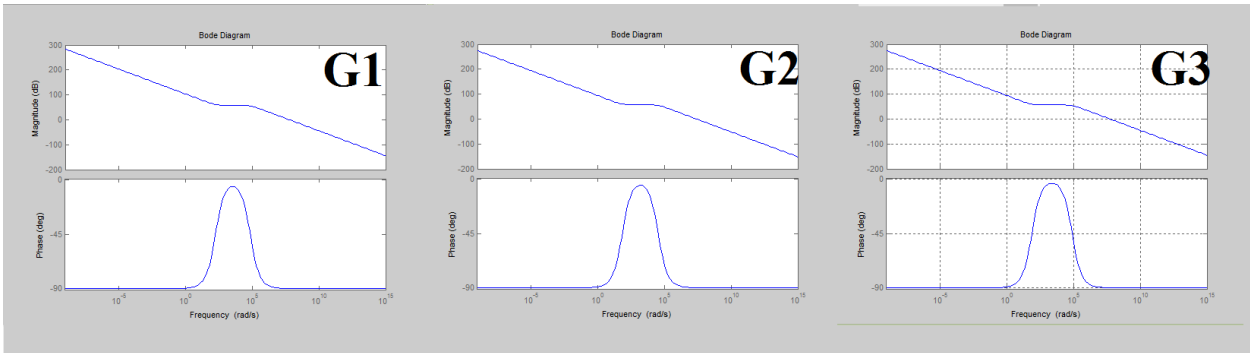

Figure 4.6.Bode plots of G1,G2,G3

Chapter

5

5.1 Conclusion:-

This whole process which is followed here was analyzed thoroughly using different control system plots and different parameters as damping constant, natural frequency, correlation co-efficient. There could be many conclusions as well as inferences taken from this whole process like the method of least square correlation estimation algorithm is the perfect way to estimate the primary parameters of any equivalent electrical circuit. Correlation co-efficient comparison can be one way to predict whether the designed electrical circuit model matches the actual model followed by the soft tissue model taken and if matches then by how many percentage. This only gave the path to analyze more about the significance of CPE than that of completely capacitive element. It showed again from its non-homogeneity constant value's effect on further analysis and analyzing parameters. When the bio-impedance data were collected they were taken by varying the frequency. The plot we got was frequency Vs impedance value. As impedance of biological sample is dependent on time as well as frequency so to know its behavior in different frequency ranges. So the Nyquist and Bode plots and parameters were successful in predicting the changes in the sample behavior of the circuit in different frequency ranges. These also predicted the stability of the sample in terms of control system which can give a clear conclusion in terms of electrical stability while it comes to the use of the samples in biological applications. These analysis were done on different sensors basically till date to know the stability of the sensor in terms of control system. This gives a clear idea on whether the sensor will work properly or yield the required output in desired condition. But this is not highly implemented till now in analysis the behavior and properties of the biological sample in different condition. So it was an attempt to analyze biological sample in this way which lead to many conclusions on the correctness in selecting a sample as well as model for a particular categorized

tissue like fluidic tissues generally should not fir if the external resistance is involved whereas, soft tissues if contain external resistance value then there is no harm. But in our case the tissue showed better fitting when the internal resistance were taken. This observation can be taken as a point to predict the structure of the tissue after processing it more. The programs designed for the above analysis made it a much simpler task as it processed the values by itself and yielded the required parameters as well as plot values which could have been a tedious task if it was done by taking the reading from a machine or the phase values after designing the phasor circuit. Above all the proposed methods in this study could be helpful on the path of bio-impedance analysis.

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