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Characterization of Human Blood Tissue Using Bioimpedance Measurements

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Dedication

First of all I would like to dedicate this thesis to my Husband who has been a great source of motivation, inspiration, endless love, support and encouragement at all the stages of my study in the master program.

I dedicate this thesis to my parents whom I thank for their unconditional support during my studies. I am honored to have you as my parents. Thank you for giving me a chance to prove and improve myself through all my walks of life. Thank you as you supported me all the way since the beginning of my studies. Please do not ever change. I love you.

Also I would like to dedicate this thesis to my family. I thank them for believing in me; thank you for allowing me to further my studies. Please do not doubt my dedication and love for you. Hoping that with this research I have proven to you that there is no mountain higher as long as God is on our side.

This thesis is dedicated to all the people who never stop believing in me and who along with god, have been my 'footprints in the sand'

My Husband

My Sons

My Fathers

My Mothers

My brothers

My Sisters

Finally, this thesis is dedicated to all those who believe in the richness of learning.

Enas Sadi Abed Alsalam Qabaja

Declaration:

I certify that this thesis submitted for the degree of master, is the result of my own research, except where otherwise acknowledged, and that this study (or any part of the same) has not been submitted for a higher degree to any other university or institution.

Signed:.....

Enas Sadi Abed Alsalam Qabaja

Date:.....

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Abstract

Bioimpedance approaches are given much attention by many researchers from different fields. Scientists from biological, industrial and medical backgrounds performed much research and development combining state-of art technology, electronics, medical research and bioimpedance approaches.

Bioimpedance techniques have a lot of advantages such that: Low cost of measurements and instrumentation, easiness to be used, the ability to be applied non invasively, enable online monitoring and finally low hazard.

While impedance is a characteristic property of any material, bioimpedance measurements can give valuable information about the tissue under measurement, hence characterizing the tissue or monitoring a physiological event. In some cases bioimpedance techniques can be an important alternative for an invasive measurement. Bioimpedance measurement is done using an external current source. This measurement can be used for characterizing the tissue or for the purpose of obtaining other information such as differentiate between normal and cancerous tissues.

The research part in this work includes two phases; the first phase includes the pilot measurements using wet lab and dry lab. In dry lab the simulated biological tissue based on electronic circuit with known impedance value is used instead of real human tissue in order to test the bioimpedance analyzer and to verify the instrumentation accuracy while 10 blood samples are used in this pilot measurements in the wet lab. The main objectives of this pilot measurements is to verify the experimental procedure that will be used in the second phase which will include larger number of human blood samples.

From the two phases the impedance measurements are obtained. The impedance values are translated to give valuable information about the blood tissue under measurement including

blood Haematocrit HCT, blood intracellular water content ICW, and extracellular water content ECW.

Special program is built using LABVIEW for continuous measurements of bioimpedance, 33 fresh human blood samples are used, for each blood sample the multi-frequency impedance measurements are taken at six frequencies. For data analysis, a special program is built using MATLAB in which the complex impedance as function of frequency, impedance module and phase angles are all fitted into the Cole-Cole model. Cole-Cole parameters are evaluated including characteristic frequency *f*c, and the characteristic parameter of the distribution of the relaxation frequencies α.

The impedance values at zero frequency R_0 and infinite frequency R_∞ are then extrapolated from the Cole-Cole model and used in the estimators to determine the blood Extracellular volume (ECW) ,Intracellular volume (ICW) and Haematocrit (HCT).

Different estimators are used:

1) Xitron first generation Estimator.
2) Xitron second generation Estimator.
3) Surkhi Estimator.

For each of the 33 blood samples the accuracy of those estimators in evaluating blood water content and blood Haematocrit are evaluated and compared to results of blood Haematocrit sample measured using clinical blood analyzer .

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List of Acronyms

Abs	Absolute
BCM	Body Cell Mass
BI	Bio-Impedance
BIA	Bio-Impedance Analysis
BIS	Bio-Impedance Spectroscopy
CBC	Cell Blood Counter
ECF	extra-cellular fluid
ECW	Extra Cellular Water
Fc	Characteristic Frequency
HCT	Haematocrit
HF	High Frequency
HGH	Hebron Governmental Hospital
ICW	Intra Cellular Water
LF	Low Frequency
RPM	Revolution Per Minute
TBK	Total Body potassium(K)
TBW	Total Body Water
TTW	Total Tissue Water

Chapter One: Introduction

1.1 Research Objectives

In this thesis human blood tissue is characterized using electrical bioimpedance spectroscopy measurements. There are two phases of work including dry and wet lab according to working with simulated biological tissues or human blood tissue respectively.

The main objectives of this work are:

1) Perform electrical bioimpedance spectroscopy measurements using wide frequency range on human blood tissue.
2) Evaluate tissue parameters out of the bioimpedance measurements such as :
    - Intracellular water volume
    - Extracellular water volume
    - Blood Haematocrit (HCT)

3) Study and compare the most used ICW and ECW estimators available in literature
4) Study the accuracy of ICW and ECW estimators and try to indicate source of inaccuracies.
5) Develop new ICW and ECW estimators .

1.2 Research Problem and Possible Contribution

Different estimators are used by bioimpedance systems producing company such as Xitron company. Those estimators include Xitron first generation and Xitron second generation that differs from each other in estimating tissue intracellular water content.

This thesis is trying to study those estimators and prove if they give accurate estimating for tissue intracellular and extracellular water contents or not. The biological tissue that is used in this thesis is human blood tissue, so this thesis used the most used estimators in literature which are Xitron first and second generations to estimate human blood intracellular and extracellular water contents along with Haematocrit. Add to that using the developed Surkhi estimator to estimate the mentioned parameters above and try to compare, analyze and discuss the results to indicate the most accurate estimator.

The Cole-Cole parameters are also extrapolated and calculated to find the characteristic values of human blood tissue.

From the above research problem and objectives points; the contribution and outcomes from this work can be guessed to be as follows:

1) Measure the intracellular and extracellular water contents in the tissue based on low and high frequency measurements. Most of common used estimators for ECW water is based on low frequency measurements. There are fewer works dealing with ICW at high

frequency than with ECW. In most cases ICW is obtained by subtracting ECW from total tissue volume.

2) Different ECW and ICW estimators are currently used, many authors have reported inaccuracies in those estimators and errors in their results. In this work different estimators will be compared and analyzed.

3) Develop blood Haematocrit HCT estimator based on high frequency impedance measurements. Currently the used method to estimate blood HCT is using low frequency measurements to evaluate the blood ECW. In this work the blood HCT is evaluated using high frequency measurements to evaluate blood cell content.

4) Different blood samples are used with different medical conditions (different HCT values, different health conditions) and investigated. blood condition Indicators based on bioimpedance measurements are developed.

Chapter Two: State of the Art

2.1 Historical Review

Bioimpedance research has attracted many researchers from different fields of specialization due to the advantages of this approach. Dielectric properties are characteristic property of any material. Bioimpedance measurements can give valuable information about the tissue under measurement, hence characterize the tissue or monitor a physiological event. In some cases bioimpedance measurements become a noninvasive solution. Nowadays bioimpedance techniques are commercially available and it is the basic principle of several medical instrumentation used for different purposes such as monitoring, imaging and diagnostic systems.

One of the first researchers that had worked in this field is G.N. Stewart in the year 1894 when he studied the blood circulation between organs depending on the blood electrical conductivity. After that he detected the growth of the bacteria based on the electrical

conductivity changes of the culture media[1]. Hober in the year 1910 measured the electrical impedance of red blood cell suspensions and concluded that the cells are surrounded by a poorly conducting membrane[2].

The electrical properties of the pleuropneumonia-like organism over the frequency range from 0.5 to 250 MHz is determined by P. Schwan et al. in 1962 [3], in his model and after analyzing the obtained data, the cells displayed frequency dependent capacitance and conductivity values.

The electrical properties of tissues, macromolecular solutions and cell membranes at frequencies from the extra low frequency to microwave range is summarized by P. Schwan in 1980 [4]. In that work, the electrical properties of tissues which are the conductivity, resistivity and the dielectric permittivity relative to free space are reviewed covering the total frequency spectrum up to microwave frequencies.

In 1987, A review of the dielectric properties of various mammalian tissues and biological fluids for the frequency range from 1 Hz to 10 GHz is given by Pething, the properties that are considered are the permittivity and the electrical conductivity[5], in his paper he showed that the dielectric properties of all tissues tend to follow the same dependence on frequency and in the range of frequencies from 100KHz to 100MHz there are large changes in dielectric properties associated with the resistive nature of cell membranes, also he showed that there are dielectric differences between normal and cancerous tissue because the cancerous cells have a higher water content and sodium concentration than the normal one, also the electrochemical properties of their cell membranes are different.

Measuring the extracellular water (ECW), Total Body Water (TBW) and the intracellular water (ICW) by using the bioimpedance spectroscopy is first reported in 1992[6]. In vitro electrical impedance spectroscopy is performed on tissue samples excised from sheep in the year 1995 by Rigaud et. al.. The measured data have been processed to reduce dispersion in measurements and to provide a criteria that is useful for tissue comparison, two electrical

model are proposed for tissue which are the one-circle impedance locus and the two-circle impedance locus[7].

In the year 1996, a parametric model is developed by S. Gabriel et. al. to describe the variation of dielectric properties of tissues as a function of frequency, the experimental spectrum from 10 Hz to 100 GHz is modeled with four dispersion regions[8].

Maasrani et.al. in 1997 tested a technique for continuous measurements of Haematocrit and plasma volume in the arterial line of dialyzed patients in vitro and in vivo, that technique uses impedance measurements at 5 KHz and require a single Haematocrit measurement[9], his study is relied on two assumptions, the first is that the plasma resistivity does not change during dialysis and the second is that the blood resistivity obeys Hanai's model. The Haematocrit measured in vivo by that method is in good agreement with the direct measurements from blood samples, then he used the Haematocrit variation to monitor the changes in the plasma volume.

The body cell mass is predicted in 1997 by Delorenzo et.al. using bioimpedance based on theoretical methods, a technological review is done, in this method the body cell mass (BCM) defined as intracellular water (ICW) is estimated in 73 healthy men and woman by using the total body potassium (TBK) and by bioimpedance spectroscopy (BIS), in other 14 subjects extracellular water (ECW) and total body water (TBW) are measured by using bromide dilution and deuterium oxide dilution, the Cole-Cole model and Hanai Mixture theory are used to obtain the unknown volumes and parameters[10].

In the year 1997, a method is presented in order to monitor the relative variation of extracellular and intracellular fluid volumes using the Cole-Cole extrapolation technique and using the multifrequency impedancemeter, this method is presented by Jaffrin et.al.[11]. Jaffrin found that the extrapolation is necessary to obtain reliable data for the resistance of the intracellular fluid, the extracellular and intracellular resistances can be approached using frequencies of 5KHz and 1MHz, and he found that the use of 100KHz leads to an unacceptable errors.

The clinical achievements in which the applications of bioimpedance techniques are extensively extended and managed to find their place in the routine clinical and industrial world is reviewed by O. Al-Surkhi [12]. In his paper Al-Surkhi tried to review some of the achievements technological in clinical practice with some examples from the medical industry products. First he reviewed the techniques that are used in the assessment of body composition with its two branches which are the Bioimpedance Analysis (BIA) in which it uses a single frequency for measurement and the Bioimpedance Spectroscopy (BIS) in which a range of frequencies are used. Monitoring the body fluid during haemodialysis and how Bioimpedance techniques can provide noninvasive measurements of the Total Body Water (TBW), the distribution of intra (ICW) and extracellular (ECW) fluids and how they changes during haemodialysis are discussed. How the blood Haematocrit can be monitored by using Bioimpedance are also mentioned, then he described the method used in cardiology depending upon Bioimpedance. After that he mentioned the image that is taken by using Bioimpedance approach using a set of electrodes to form an array around the volume conductor of interest to obtain the image after processing. Finally he described how abnormal tissues can be characterized and monitored by using Bioimpedance. In addition the technique of electrical impedance spectroscopy for in-vivo and in situ characterization of different organ tissues in normal state and with the progress of tissue ischemia level is reported by O. Al-Surkhi [13]. In this paper he took in-vivo measurements of different tissues from pigs using electrical impedance spectroscopy analyzer with frequency range between 100KHz to 1MHz with a 4-electrodes system, and in those measurements different degrees of ischemia are obtained in the tissue by the occlusion of the arterial blood supply, he adjusted the experimental data to the Cole-Cole model and the Cole parameters are eventually calculated.

In 2010 Richelle Leanne Gaw thesis entitled “The Effect of Red Blood Cell Orientation on the Electrical Impedance of Pulsatile Blood with Implications for Impedance Cardiography” to obtain the degree of philosophy doctoral studied the impedance cardiography as an application of bioimpedance analysis primarily used in a research setting to determine cardiac output. The cardiac output is calculated from the measured impedance using the parallel conductor theory and a constant value for the resistivity of blood. The resistivity of blood has been shown to be velocity dependent due to changes in the orientation of red blood cells induced by changing

shear forces during flow. The overall goal of his thesis is to study the effect that flow deviations have on the electrical impedance of blood [15].

S. Abdalla et. al. in the year 2010 studied the effects of the blood microstructure on the electrical conduction from two different but correlated properties: Electrical and Mechanical (Viscosity), and derive useful parameters for the evaluation of electrical conduction as a function of the blood viscosity. In their work, they have shown that bioimpedance measurements could provide an important method for the noninvasive investigation of blood internal structure and properties for monitoring physiological change i.e., “static” or “dynamic” human organism properties [16].

Chapter Three: Theory: Principle of Bioimpedance Measurements

3.1 Theoretical Background

The main principle of Bioimpedance measurements is quite simple and depends on how the biological tissues respond to an applied external electrical field.

Biological tissues consists of cells which are the main building blocks of any tissues. Biological cell microstructure can be modeled into three main components which are:

1) Extracellular water (ECW).
2) Intracellular water (ICW).
3) Cell membrane that separates between ECW and ICW.

Figure 3.1 represents a typical biological cell. As shown in figure 3.1, each cell in the body is surrounded by a cell membrane with the known lipid bilayer structure. The cells contain intra-

cellular fluid, abbreviated ICF (also called ICW for intra-cellular water). The cells are surrounded by extra-cellular fluid, ECF (also called ECW).

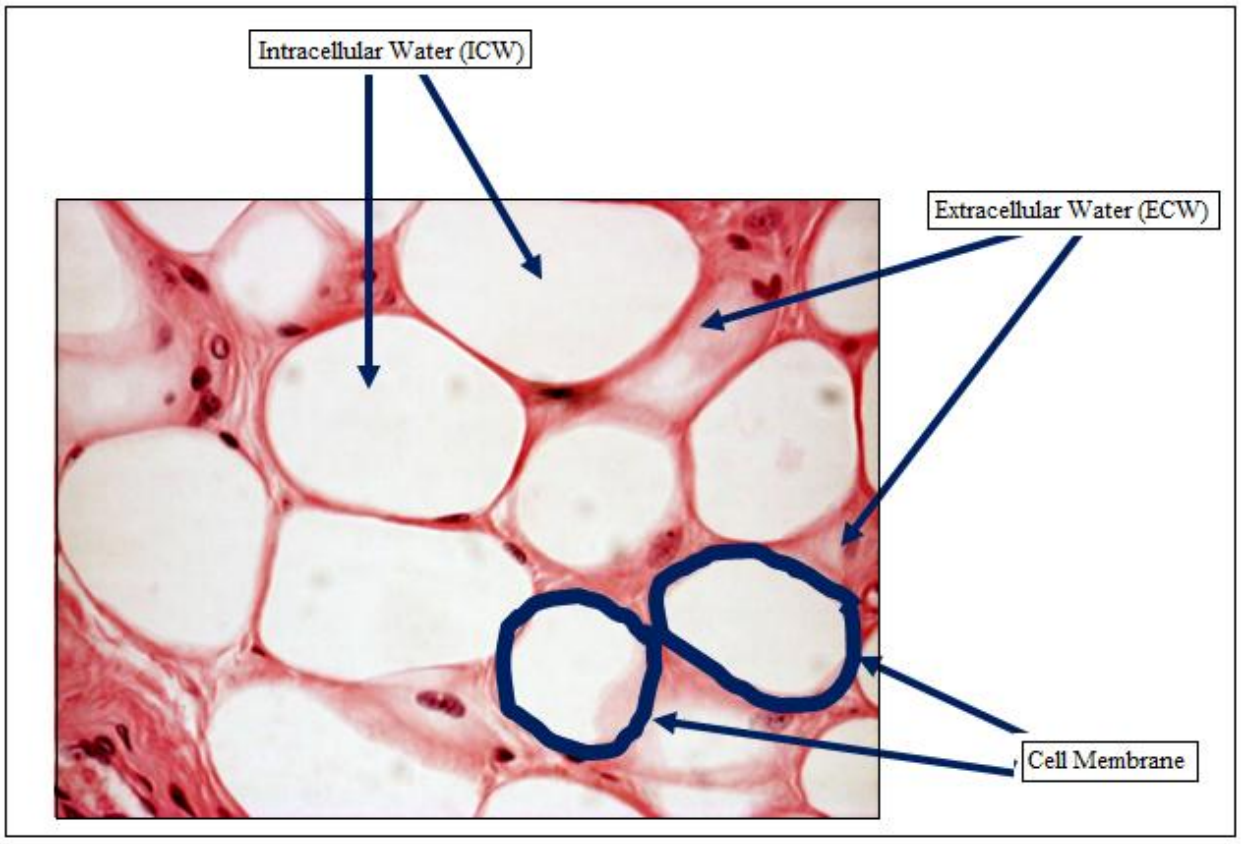

Figure 3.1: Biological Cell Microstructure .

The cell membrane is known to have a lipid Bilayer structure. Lipids also known to have dielectric properties thus the cell membrane microstructure is a non conductive structure acting as an electrical capacitor [1] [10]. Therefore the measured bioimpedance is a function of frequency of the ejected electrical current.

At low frequency LF (approaching to zero Hz), the cell membrane will act as an open circuit capacitor and blocking the electrical current to penetrate the ICW space and the current will pass though only the ECW space. In this case the measured impedance is pure resistive and represents the impedance of the ECW volume.

At high frequency HF(approaching to infinite Hz) the cell membrane will act as a short circuit capacitor allowing the electrical current to penetrate both the ICW and ECW spaces and the measured impedance is also pure resistive and represents the impedance of the ICW and ECW volumes (TBW).

The biological tissue can be modeled with an electrical model. Both the extracellular and intracellular water content are modeled with resistances while the cell membrane is implemented by a capacitor as shown in figure (3.2).

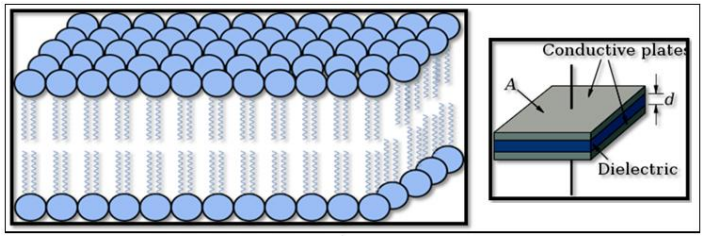

Figure 3.2: Electrical Model of Cell Membrane Microstructure .

Thus the equivalent electrical circuit model of biological tissue can be represented in the following figure [10]:

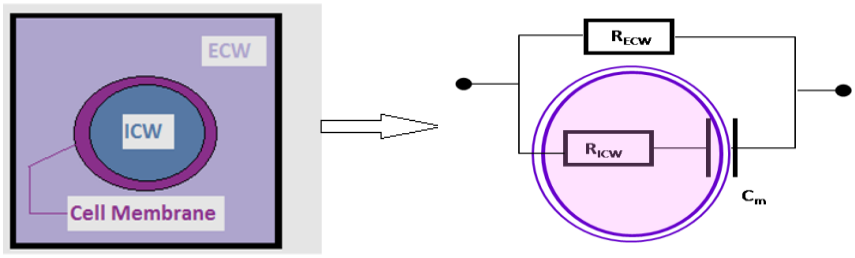

Figure 3.3: Equivalent Circuit model for biological tissue .

Where:
R_{ECW}: Extracellular water content resistance.
R_{ICW}: Intracellular water content resistance.
C_m: Cell membrane capacitance.

3.2 Bioimpedance measurements

Bioimpedance measurements are performed by injecting an electrical current with certain frequency in to the biological cell which has a geometric properties such as length and volume. It has a dielectric properties such as conductivity and permittivity. If electrical current injection occurs, an induced voltage will be obtained and voltage can be measured. As the injected current is known and the induced voltage is measured, the impedance (Z) can be calculated and it is called bioimpedance as the deal here with biological cells. Bioimpedance will be a function of frequency, dielectric properties and geometry, then the expression of biological impedance will be as follows :

$$Z\ (\Omega) = \frac{V\ (volt)}{I\ (amp)} \qquad (Ohm's\ Law) \qquad \textit{Eq(3.1)}$$

In order to understand the principle of the electrical bioimpedance, see figure 3.4.

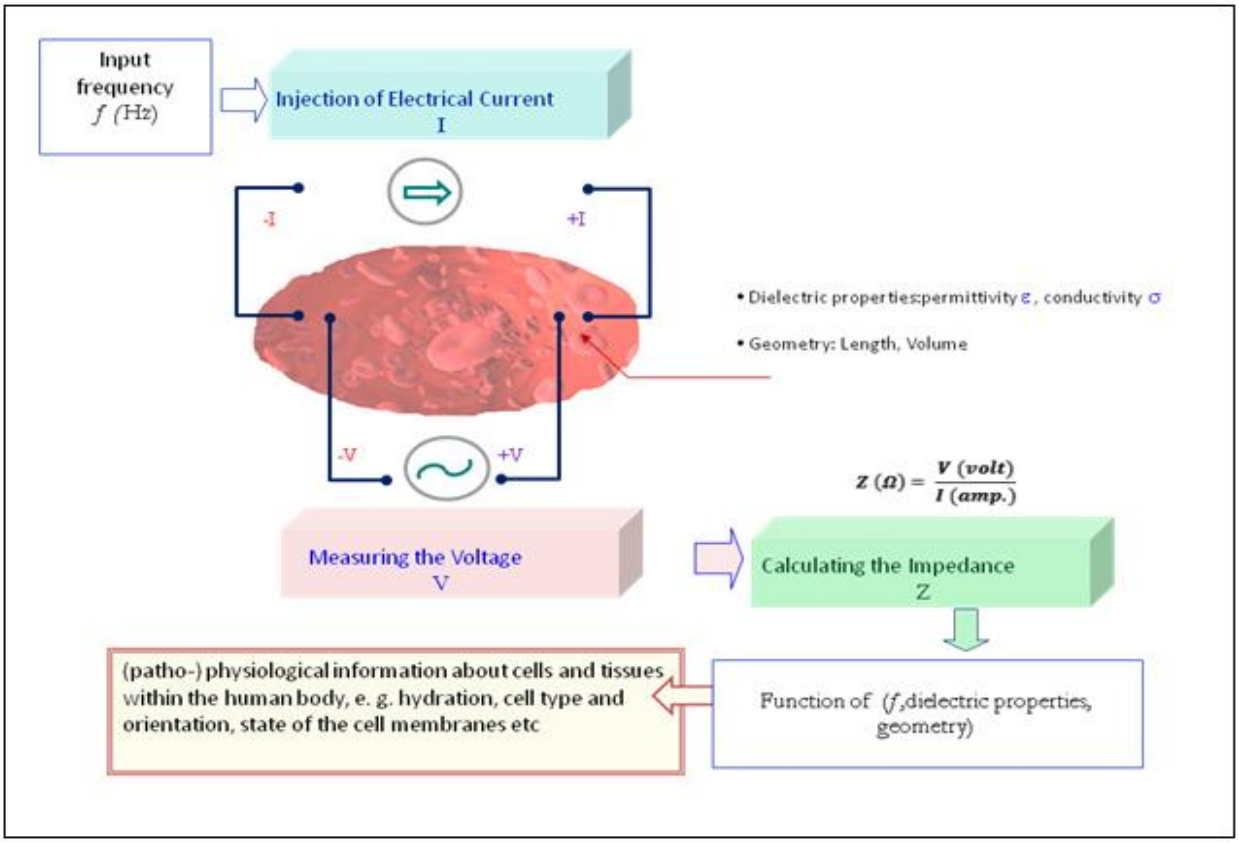

Figure 3.4: Principle of the Electrical Bioimpedance .

From bioimpedance measurement a physiological information can be gotten about cells and tissues within the human body such as hydration, cell type and orientation, the state of the cell membranes and others.

As mentioned previously, if the current is injected with certain frequency through biological cell, an induced voltage will be obtained and bioimpedance can be calculated from that two parameters. Suppose that the frequency of the injected current is low; then at low frequencies it is assumed that the current flow is limited to the Extracellular Water (ECW) because the cell membranes act as capacitors and as known that at low frequencies the capacitor will be an open circuit so the current will not penetrate through the intracellular water (ICW) and go around the cell through ECW as the current flow through extracellular fluid only and the measured impedance is $Z_0 = Z_{ECW}$ (see figure 3.5).

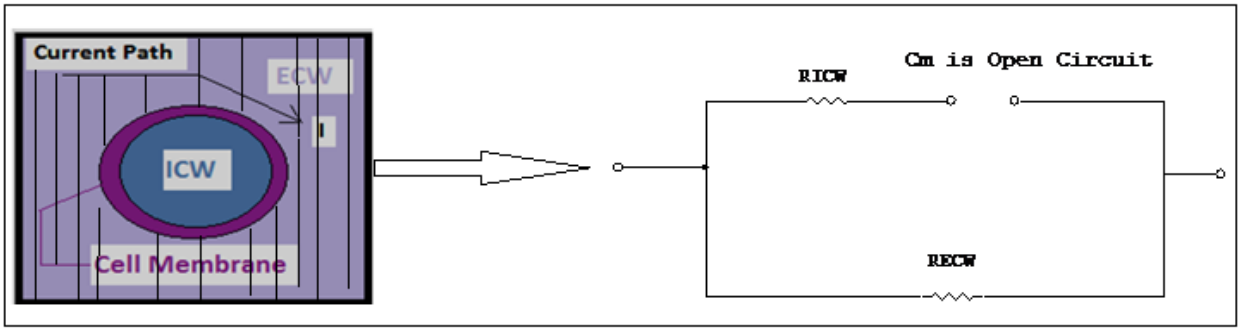

Figure 3.5: Low Frequency Equivalent Circuit.

Hence at low frequencies bioimpedance is regarded as a measure of the ECW. The measured impedance at low frequencies is a Pure Resistive (Real Ro).

$$Z_{\text{Low Frequency}} : \quad R_0 = R_{ECW}$$

Eq(3.2)

At higher frequencies the electric current flows through the Intracellular water (ICW) as well as the ECW because the cell membranes act as capacitors and at high frequencies the capacitor will be a closed circuit so the impedance index is a measure of the volume of the Total Tissue Water (TTW).

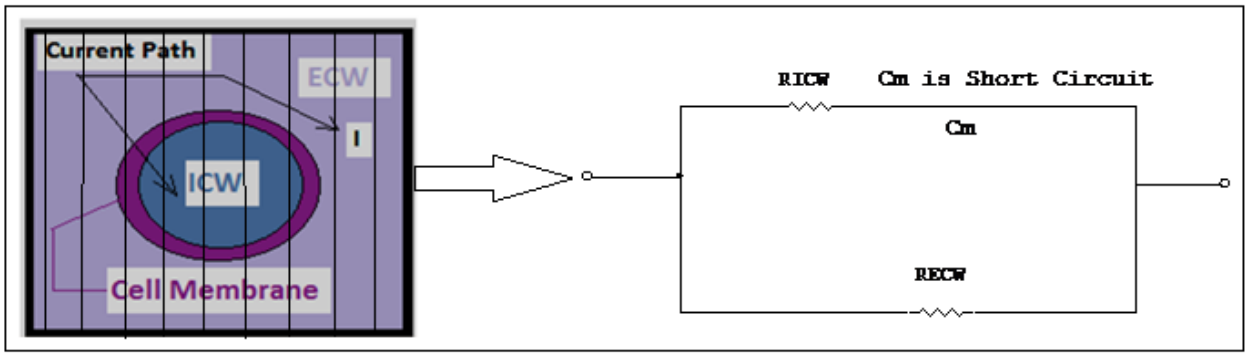

Figure 3.6: High Frequency Equivalent Circuit .

At high frequencies the body is modeled as two resistors in parallel, one representing the resistance of the ECW, the other is the ICW. The resultant impedance is that obtained from the two resistors in parallel and it is a Pure Resistive (Real R∞) as the current flow through the total tissue fluid and the measured $Z_\infty = Z_{ICW} // Z_{ECW}$

$$Z_{\text{High Frequency}}: \quad R_\infty = \frac{(R_{ECW}\, R_{ICW})}{(R_{ECW} + R_{ICW})} \qquad \textit{Eq(3.3)}$$

From eq(3.2) and eq(3.3) one can write :

$$\frac{R_\infty}{R_o} = \frac{R_{ICW}}{R_{ICW} + R_{ECW}} \qquad \textit{Eq(3.4)}$$

At low frequencies, the measured impedance will be only the ECW resistance whereas at high frequencies, the measured impedance will be the resultant impedance from ECW and ICW as they are in parallel. R_{ECW} is known from the first case so R_{ICW} can be computed.

At Frequencies between low and high frequencies, Cell Membrane (Capacitor) will play an important part. Hence bio-impedance will consist of two parts which are Pure & Complex.

$$Z_{\text{Frequencies Between}} = R + jX_c \qquad \textit{Eq(3.5)}$$

Experimentally it is found that the change of impedance as a function of the measurement frequency can be described with a mathematical equation which is the mathematical solution for the electrical model of the biological model that takes the following form according to Cole-Cole model:

$$Z^* = R_\infty + \frac{R_0 - R_\infty}{1 + \left(j\frac{f}{f_0}\right)^{1-\alpha}} \qquad \textit{Eq(3.6)}$$

[11] [14] [17]

$$Z_{\text{Frequencies Between}} = R + jX_c \qquad \textit{Eq(3.7)}$$

Where: Z^*: complex impedance

R_0: limiting impedance at low frequencies

R_∞: limiting impedance at high frequencies

α: shape factor

f_c: characteristic frequency, at which the imaginary part displays the minimum value

Equation(3.6) is referred to Cole Equation or Cole-Cole Equation [11] [14] [17]. It is worth noting that the limiting values at low and high frequencies, R_0 and R_∞, are not measurable in practice, because of other effects showing up when the measured frequency decreases or increases far from f_0.

This idea will be clear when drawing the measured impedances in the complex plane (real by imaginary) (see figure 3.7) [1] [14] [18].

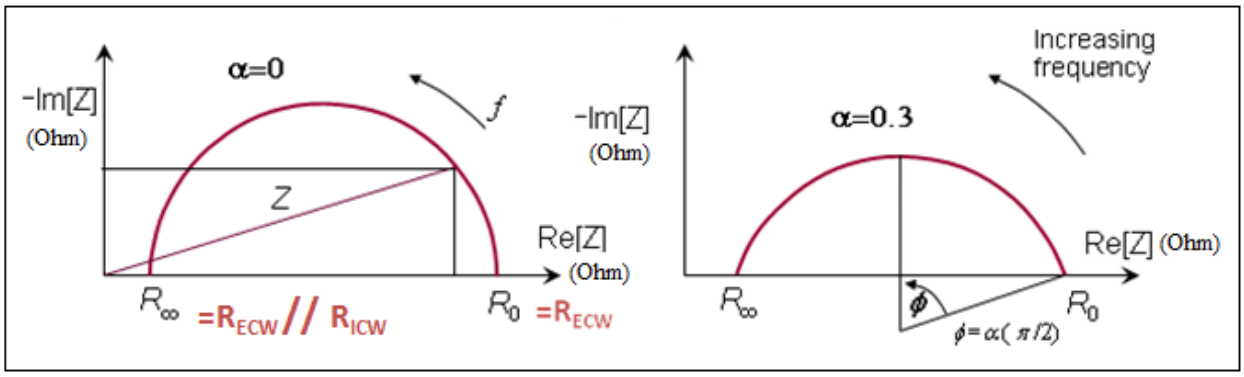

Figure 3.7: Debye (Left) & Cole-Cole (Right) Models .

The measured data isn't fitting to Debye model. There is a depression when drawing the measurements in the complex plane. Debye model is enhanced by Cole-Cole model by adding the shape factor (α) which represents the depression value or the arc center. The ideal capacitor element that is used in the earlier models is replaced by "Constant Phase Element" (CPE) in order to fit the modeled impedance with the actual biological measurements.

CPE cannot be physically represented by ordinary electrical component, but it is described as capacitance that is frequency dependent and at α = 0, CPE will act like ideal capacitor.

The representation of the Cole complex impedance in the Wessel diagram (real Vs imaginary part of Z) as shown in figure 3.7 does not show a semicircle centered in the real axis (Debye Model), instead the center of the arc will be depressed below the real axis depending on the value of α. α in Debye model is set to be zero but it has a value in Cole-Cole model depending upon the depression.

The values of R_0 and R_∞ can be extrapolated by fitting the measurements to Cole-Cole model. R_0 and R_∞ values are the intersection of the semicircle with the x-axis (real (Z)) where R_0 at the right and R_∞ at the left with the direction of increasing the frequency as shown in figure 3.7.

As a result Bio-Impedance is a function of frequency as its value depends upon the frequency of the injected current, you can see figure 3.8 to understand how bioimpedance is a function of frequency.

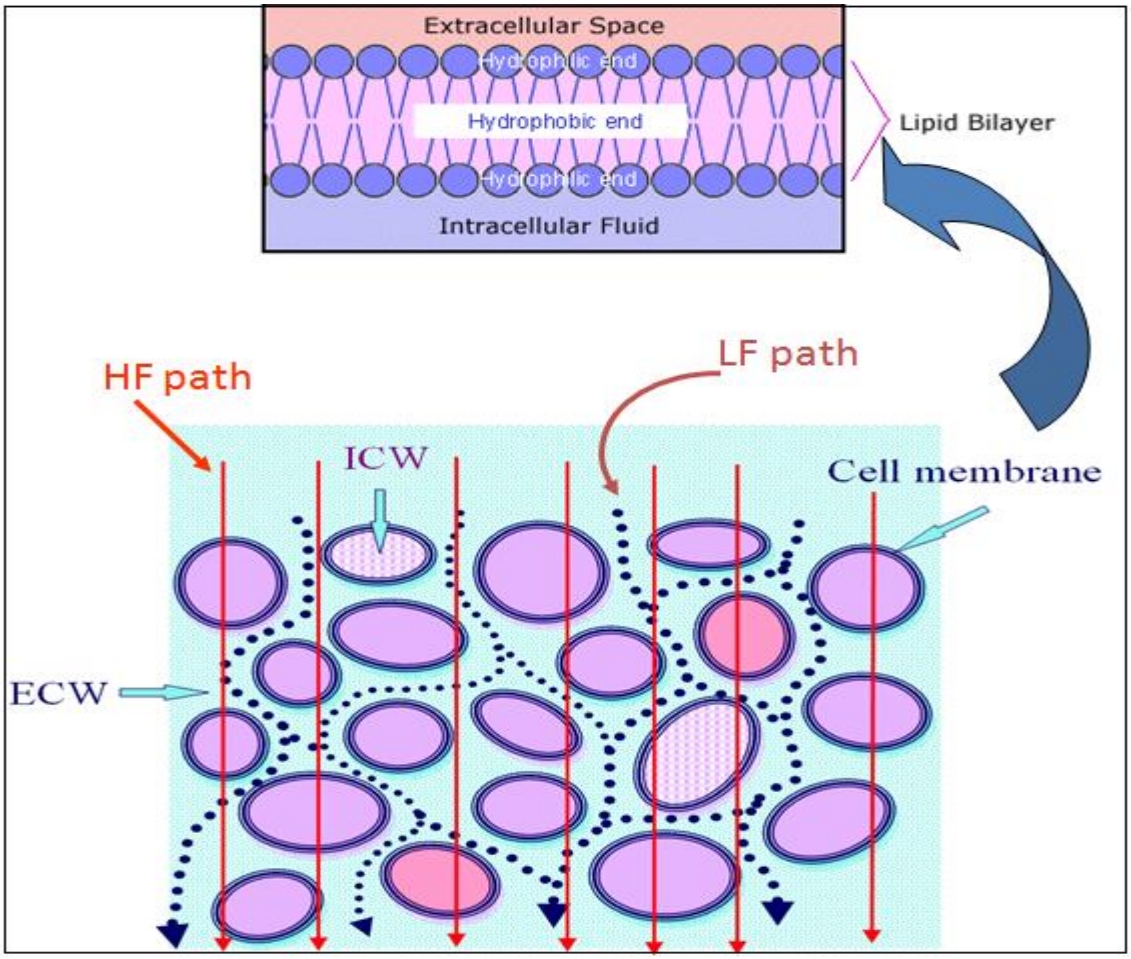

Figure 3.8: Current Path in Low and High Frequency .

The idea of bioimpedance spectroscopy (BIS) is to measure the impedance of biological tissue at a series of frequencies. This is done by sending weak alternating currents through the tissue, while measuring both the impedance and the phase difference for each frequency.

3.3 Hanai Mixture Theory

Blood tissue is not a homogenous material as it is a suspension of low conductivity (cells) in conducting media (plasma) [9] [18]. For zero frequency, the current can't enter the cells and flow in the extracellular fluid only. The extracellular fluid has itself resistivity ρ_{ECF}. The current can't take the direct route through the fluid but must flow around the cells. For this reason the resistivity of the tisse takes a higher value than ρ_{ECF}.

The apparent resistivity problem was solved and analyzed by Hanai in 1968 by the Apparent Resistivity Theory or Hanai Mixture Theory [18]. This theory solves the problem of calculating the resistivity of a conducting fluid mixed with non conducting spheres [18].

According to Hanai results if the non conducting spheres take up the part C of the total volume of the conducting media (see figure 3.9).

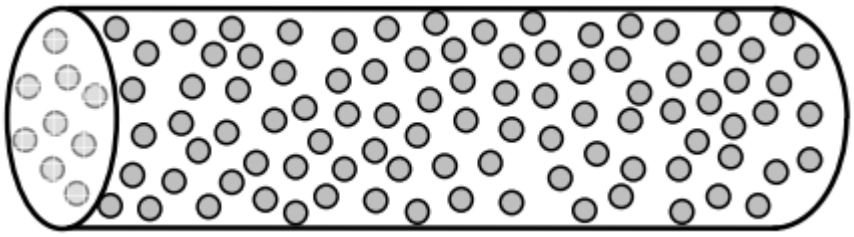

Figure 3.9: Non-Conducting Spheres (Cells) in Conducting Media (Plasma) .

Then:

$$C = \frac{\text{Cell Volume}}{\text{Total Volume}} \qquad \textit{Eq(3.8)}$$

The apparent resistivity is given by Hanai Mixture Theory:

$$\rho = \frac{\rho_{ECF}}{(1-C)^{3/2}} \quad [18] \qquad \textit{Eq(3.9)}$$

Chapter Four: Experimental Methods and Instruments

4.1 Instruments and Tools

The instruments that are used are the following:

1. Multi Frequency Impedance Analyzer –Microtest 6379(Material Lab)
This instrument measures the impedance with angle according to a predetermined frequency. In addition to all other electrical parameters and quantities. The instrument has a software to work with. The number of frequencies and all other parameters related to the measurements can be determined using the software or the instrument screen directly. The graph between the selected parameters can be obtained such as the graph that describes the relationship between time and impedance or between time and phase angle for measurements at the determined frequencies.

2. Local Tissue Impedance Analyzer : multi frequency impedance spectroscopy

3. Tissue simulator : simulators which are models for biological tissues, they act as biological tissues. Those simulators will be used in order to check the validity of the measurements.

4. Clinical Haematocrit (HCT) Analyzer
The Clinical Haematocrit Analyzer will be used for the verification of the obtained results in order to verify the measurements validity.

5. Other Clinical Instruments: cell counter , blood centrifuge , microscope , etc,,,

4.2 Basic Estimators of Blood volumes from bioimpedance data

The most important issue is how to translate the bioimpedance data into volumes estimation. The most used estimators are Xitron first and second generations. This section reviews those two estimators with the developed Surkhi Estimator that are used to make data analysis after bioimpedance measurements are taken.

4.2.1 Data Analysis Approach (Used Estimators)

Biological tissues are not homogenous; in fact they are considered as a suspension of conducting and non conducting materials. The apparent resistivity of suspensions can be calculated by Hanai mixture theory as explained in Chapter Three. The reason for looking at resistivity is the need to do calculations on the volume of a fluid.

To connect the result of bioimpedance measurements with the amounts of body fluid, you must understand what determines the value of the electrical resistance.

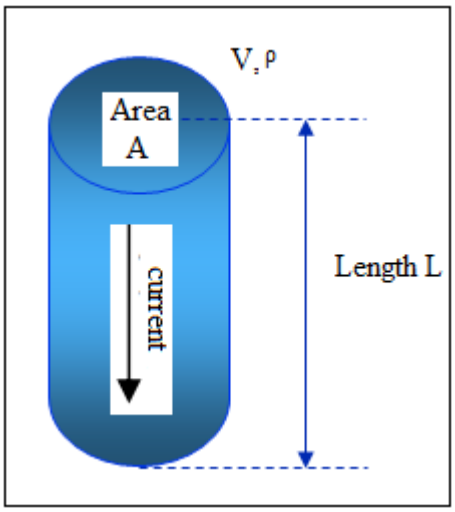

Figure 4.1: Resistance Calculation .

The equation that describes the relation between resistance, resistivity, length and area is:

$$R = \rho.\frac{L}{A} \qquad \textit{Eq(4.1)}$$

Where:

R: is the resistance.

ρ: is the resistivity.

L: is the Length.

A: is the cross-sectional Area.

From equation 4.1, the higher the resistivity, the higher the electrical resistance will be.

Equation 4.1 uses the length and the area. If the length is known, the following relation can be used:

$$Volume = Area \times Length$$

$$V = A \times L \qquad \textit{Eq(4.2)}$$

Rewrite equation 4.1 as follows:

$$R = \rho.\frac{L}{A} = \rho.\frac{L.L}{A.L} = \rho.\frac{L^2}{V} \qquad \textit{Eq(4.3)}$$

Equation 4.3 allows the calculation of the fluid volume in which the current flows:

$$R = \rho.\frac{L^2}{V} \iff V = \rho.\frac{L^2}{R} \qquad \textit{Eq(4.4)}$$

At low frequency ECW (plasma in case of blood tissue) is considered as the conducting material, and ICW which is surrounded by cell membranes is the non conducting material (blood cells in case of blood tissue).

Using Hanai Mixture Theory that gives the apparent resistivity equation and equation (4.4), the volume of the ECW (V_{ECW}) can be calculated by:

$$V_{ECW} = \left[\frac{\rho_{ECW}\, L^2 V_{TTV}{}^{1/2}}{R_0}\right]^{2/3} \qquad [6]\ [18] \qquad \textit{Eq(4.5)}$$

Where:

ρ_{ECW} : is the resistivity of the ECW

L: is the separation distance between the detection electrodes

V_{TTV} : the total tissue volume i.e the volume of the blood chamber

R_0: is the extrapolated impedance at zero frequency

Using the volumetric concentration at high frequency, the volume of the ICW compartment (V_{ICW}) can be calculated by solving :

$$\left(1 + \frac{V_{ICW}}{V_{ECW}}\right)^{5/2} = \frac{R_0}{R_\infty}\left(1 + K_P \frac{V_{ICW}}{V_{ECW}}\right) \qquad [6]\ [18] \qquad \textit{Eq(4.6)}$$

Where

$$K_P = \frac{\rho_{ICW}}{\rho_{ECW}} \quad [18] \qquad \textit{Eq(4.7)}$$

Where:

ρ_{ECW}, ρ_{ICW} are the resistivity of the ECW,ICW in (Ohm.cm) respectively which can be found in literature, and R_∞ is the extrapolated impedance at infinite frequency. Although there is no explicit solution to equation(4.2), a linear relationship may be found for values of ICW/ECW in the range 0.8 to 1.5 and for different values of K_P (3.4 to 3.8). This ranges of values are extracted from literature [6].

The results of equation (4.6) along with the linear approximation are depicted in figure (4.2):

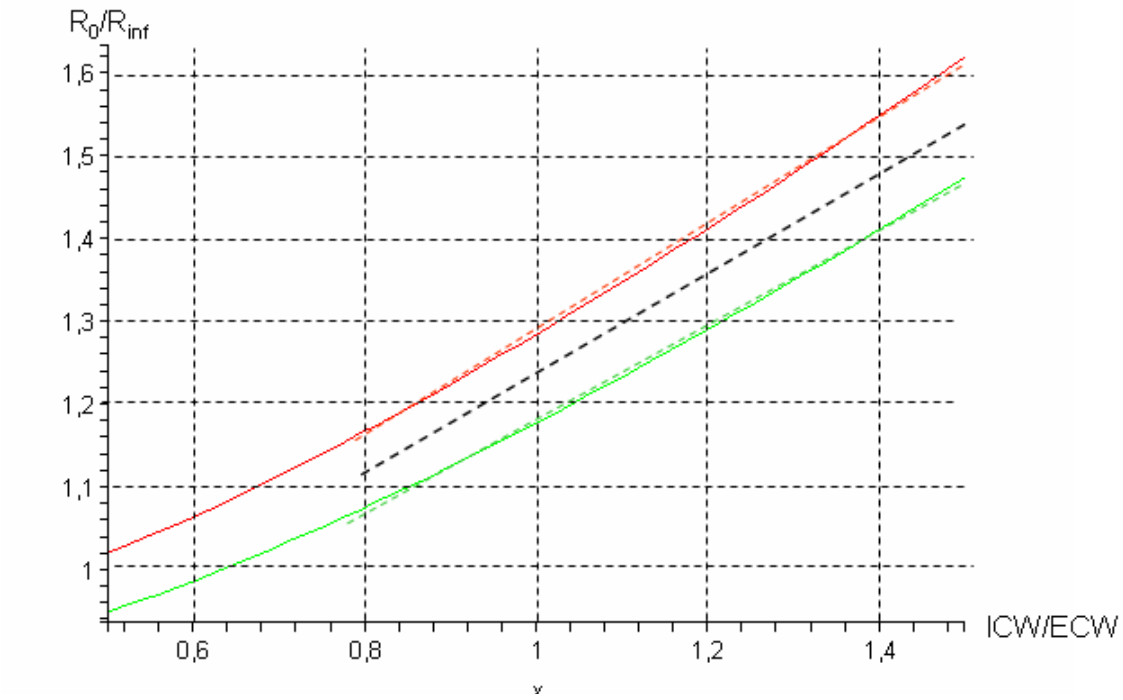

Figure 4.2: Equation (4.6) results with linear approximation .

And the linear relationship obtained (black dotted line in figure 4.1) is the following:

$$\frac{V_{ICW}}{V_{ECW}} = 1.67\frac{R_0}{R_\infty} - 1.067 \qquad \textit{Eq(4.8)}$$

This estimator is reported by many authors [6] [10] and is known to be the first generation estimator used by Xitron since 1990 which is a leading company in producing bioimpedance systems for fluid estimation.

Recently Xitron revealed the second generation of the ICW estimator and it is reported by Matthie et al.2005 as [6]:

$$V_{ICW} = V_{ECW} \times \left\{ \left[\frac{\rho_{TBW} \times R_0}{\rho_{ECW} \times R_\infty} \right]^{2/3} - 1 \right\} \quad [6] \qquad \textbf{Eq(4.9)}$$

Dividing Eq(4.9) by V_{ECW} , Eq(4.10) will be obtained:

$$\frac{V_{ICW}}{V_{ECW}} = \left\{ \left[\frac{\rho_{TBW} \times R_0}{\rho_{ECW} \times R_\infty} \right]^{2/3} - 1 \right\} \qquad \textbf{Eq(4.10)}$$

With:

$$\rho_{TBW} = \rho_{ICW} - (\rho_{ICW} - \rho_{ECW}) \times \left(\frac{R_\infty}{R_0} \right)^{2/3} \qquad \textbf{Eq(4.11)}$$

Dividing equation (4.11) by ρ_{ECW} , the following equation is obtained:

$$\frac{\rho_{TBW}}{\rho_{ECW}} = \frac{\rho_{ICW}}{\rho_{ECW}} - \left(\frac{\rho_{ICW}}{\rho_{ECW}} - \frac{\rho_{ECW}}{\rho_{ECW}} \right) \times \left(\frac{R_\infty}{R_0} \right)^{2/3} \qquad \textbf{Eq(4.12)}$$

Substitute K_P instead of $\frac{\rho_{ICW}}{\rho_{ECW}}$ as indicated before in Eq(4.7), the following equation is obtained:

$$\frac{\rho_{TBW}}{\rho_{ECW}} = K_P - (K_P - 1) \times \left(\frac{R_\infty}{R_0} \right)^{2/3} \qquad \textbf{Eq(4.13)}$$

From the extrapolated values of impedances at low and high frequencies (R_0 and R_∞) respectively which represent the electrical circuit model resistances using Cole-Cole model and by using the computed value of V_{ECW} , it is possible to calculate the value of V_{ICW} using the equations Eq(4.9) and Eq(4.11).

As noticed this section reviewed first and second Xitron generations or estimators.

4.2.2 Data Analysis Approach (Developed Estimator: Surkhi ICW Estimator)

The developed estimator by Dr. Omar Al-Surkhi from Al-Quds University in which the volume of the intracellular volume content is determined.

At HF the capacitive effect of the cell membrane is minimized and both impedances of the ECW and ICW becomes in parallel. Using the conductance expressions for parallel model Surkhi estimator is developed to describe the relation between the measured impedance and the intracellular volume as:

$$\left(1+\frac{V_{ICW}}{V_{ECW}}\right)^{-\frac{1}{2}}=\frac{R_{\infty}}{R_0}\left(1+K_{\rho}{}^{-1}\frac{V_{ICW}}{V_{ECW}}\right) \qquad \textbf{Eq(4.14)}$$

where

$$K_{\rho}=\frac{\rho_{ICW}}{\rho_{ECW}}=\frac{\sigma_{ECW}}{\sigma_{ICW}}$$

Chapter Five: Phase One Measurements and Results (Pilot Measurements)

In this thesis two phases of measurements are performed. The first phase includes the pilot measurements using wet lab and dry lab. In dry lab the simulated biological tissue based on electronic circuit with known impedance values is used instead of real human blood tissue in order to test the bioimpedance analyzer and to verify the instrumentation accuracy while 10 blood samples are used in this pilot measurements in the wet lab. The main objectives of this pilot measurements is to verify the instrumentation accuracy, to decide if we can use the impedance instrument to measure blood tissue impedance, to adjust the experimental parameters such as blood chamber parameters and to decide whether to use multi blood samples with different HCT or generated blood samples from dilution.

From the results of pilot measurements the bigger phase in this thesis which is the second phase known as “research measurements” phase is learned and carried out. In second phase 33 human blood samples are used.

5.1 Dry measurements: Impedance measurements using Microtest impedance analyzer and tissue simulator

This section demonstrates the equipment that are used in performing the dry measurements in phase one.

5.1.1 Microtest impedance analyzer

First part of experiments is done in the Material Lab at the faculty of Engineering at Al-Quds University as all the needed equipment and instruments are located in that site. In this part, the impedance instrument that is used is a Multi Frequency Impedance Analyzer Microtest 6379 .

The specifications of this impedance analyzer are shown in the following table, (see this equipment in figure 5.1).

Table 5.1: Impedance Analyzer Specifications .

Measurement parameters	｜Z｜, ｜Y｜,θ, R, X, G, B, L, C, Q, D, ESR, DCR measurement
Circuit	Series/Parallel
Test Frequency 6379	20Hz ~ 10MHz
Frequency Step Resolution	5 Digits
Frequency Accuracy	±0.01%
Measurement Range	｜Z｜ R X 0.1 mΩ ~ 100MΩ

Figure 5.1: Multi Frequency Impedance Analyzer .

5.1.2 Biological tissue simulator

Before using a biological tissue to be studied by using the above mentioned instrument, a Biological tissue simulator is used –Figure 5.2. This simulator which is a model for biological tissue acts as biological tissue. Those simulators are used in order to check the validity of the measurements. The theoretical values for the impedance module and phase angle for the tissue simulator is shown in table 5.2.

Figure 5.2: Biological Tissue Simulator .

Figure 5.3 shows the schematic circuit of the used biological tissue simulator.

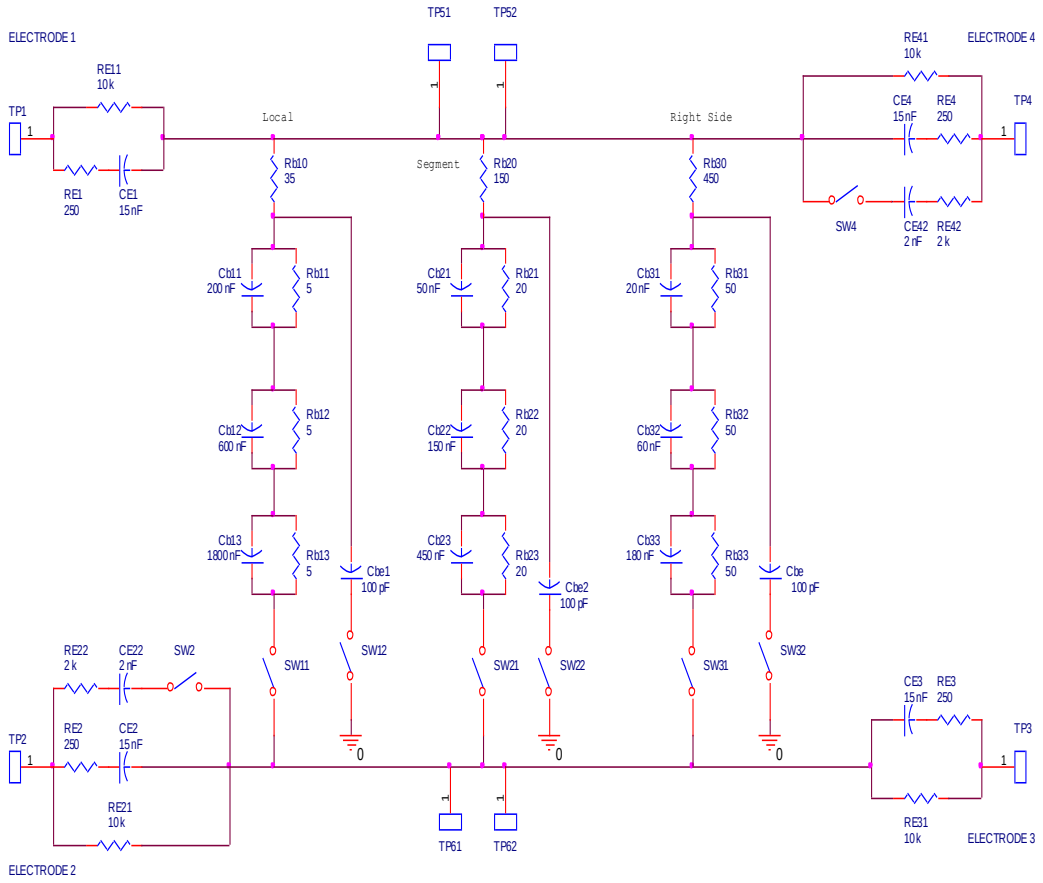

Figure 5.3: Biological Tissue Simulator Schematic Circuit .

Table 5.2 shows the theoretical values for impedance module and phase angle from the tissue simulator.

Table 5.2: Theoretical values for impedance module and phase angle from the tissue simulator

Impedance Module (Ω)Theoretical value		Phase angle (deg) Theoretical value	
10kHz	48.805	10kHz	-3.450
20kHz	47.293	20kHz	-5.522
50kHz	43.697	50kHz	-7.197
100kHz	40.655	100kHz	-7.426
220kHz	37.745	220kHz	-6.065
500kHz	36.049	500kHz	-3.585

5.2 Results from Dry Measurements\Phase One

The measurements setup is shown in figure 5.4.

Figure 5.4: Complete Connection between Multi Frequency Impedance Analyzer Tissue simulator .

The impedance analyzer is attached to the tissue simulator and the impedance module and phase are continuously recorded at 6 frequencies (10,20,50,100,220,500 kHz) with Sampling Rate SR= 3 samples/min. The measured data is shown in tables 5.3 and 5.4. The data is acquired and transferred to the computer and analyzed using MATLAB. The measured impedance is fitted to Cole-Cole model.

Figure 5.4 shows one sample of the measured impedance plotted in the complex plan -Real(Z) Vs Img(Z). The 6 small circles represents the measured impedance data at 6 frequencies and the solid line is the Cole-Cole model. Excellent fitting can be observed for the measured data on the Cole-Cole mathematical model.

Figures 5.5 and 5.6 show the impedance module and the phase angle plotted as function of frequency. The 6 small circles represents the measured data at 6 frequencies and the solid line is the Cole-Cole model.

As you can notice from those figures that there is an excellent fitting to the Cole-Cole mathematical model. But you can see that the last reading at the frequency 500KHz has a bad fitting to Cole-Cole mathematical model, that limits the measurements to be done at the selected range of frequencies (10,20,50,100,220,500 kHz). Fitting needs three readings but here six readings are taken then the readings at the rest of frequencies can be found using Cole-Cole mathematical model.

Tables 5.3 and 5.4 show the first results:

Table 5.3: Measured Impedance module at 6 frequencies .

Reading	10kHz	20kHz	50kHz	100kHz	220kHz	500kHz
1	49.34	47.8	44.26	41.36	38.8	36.77
2	49.34	47.79	44.26	41.36	38.8	36.77
3	49.34	47.79	44.26	41.36	38.8	36.77
4	49.34	47.79	44.26	41.36	38.8	36.77
5	49.35	47.8	44.26	41.36	38.8	36.77
6	49.34	47.79	44.26	41.36	38.8	36.77
7	49.35	47.79	44.26	41.36	38.8	36.77
8	49.35	47.79	44.26	41.36	38.8	36.77
9	49.35	47.8	44.26	41.36	38.8	36.77
10	49.34	47.8	44.26	41.36	38.8	36.77

Table 5.4: Measured Phase module at 6 frequencies .

Reading	10kHz	20kHz	50kHz	100kHz	220kHz	500kHz
1	-3.19	-5.12	-6.86	-6.88	-5.75	-2.71
2	-3.19	-5.12	-6.85	-6.88	-5.75	-2.71
3	-3.19	-5.12	-6.86	-6.88	-5.75	-2.71
4	-3.19	-5.12	-6.86	-6.88	-5.75	-2.71
5	-3.19	-5.12	-6.86	-6.88	-5.75	-2.71
6	-3.19	-5.12	-6.86	-6.88	-5.75	-2.71
7	-3.19	-5.12	-6.86	-6.88	-5.75	-2.7
8	-3.19	-5.12	-6.86	-6.88	-5.75	-2.71
9	-3.19	-5.12	-6.86	-6.88	-5.75	-2.7
10	-3.19	-5.12	-6.86	-6.88	-5.75	-2.71

In order to verify the results, the average of the ten measured impedance and phase angle are found at the six frequencies. Then those values are compared to the theoretical ones by finding the absolute error as shown in tables 5.5 and 5.6:

Table 5.5: Relative Error of the measured Impedance module at 6 frequencies .

Reading	10kHz	20kHz	50kHz	100kHz	220kHz	500kHz
Average Value (Ω)	49.344	47.794	44.26	41.36	38.8	36.77
Theoretical Value (Ω)	48.805	47.293	43.697	40.655	37.745	36.049
Absolute Error	0.539	0.501	0.563	0.705	1.055	0.721

Table 5.6: Relative Error of the measured Phase Angle at 6 frequencies **.**

Reading	10kHz	20kHz	50kHz	100kHz	220kHz	500kHz
Average Value (deg)	-3.19	-5.12	-6.859	-6.88	-5.75	-2.708
Theoretical Value (deg)	-3.45	-5.522	-7.197	-7.426	-6.065	-3.585
Absolute Error	0.26	0.402	0.338	0.546	0.315	0.877

The relative error of the measured impedance and phase angle compared to the theoretical values are very small. This result verify the accuracy of the used instrument.

If one reading from the 10 measured readings is fitted to Cole-Cole model, the following graph will be obtained:

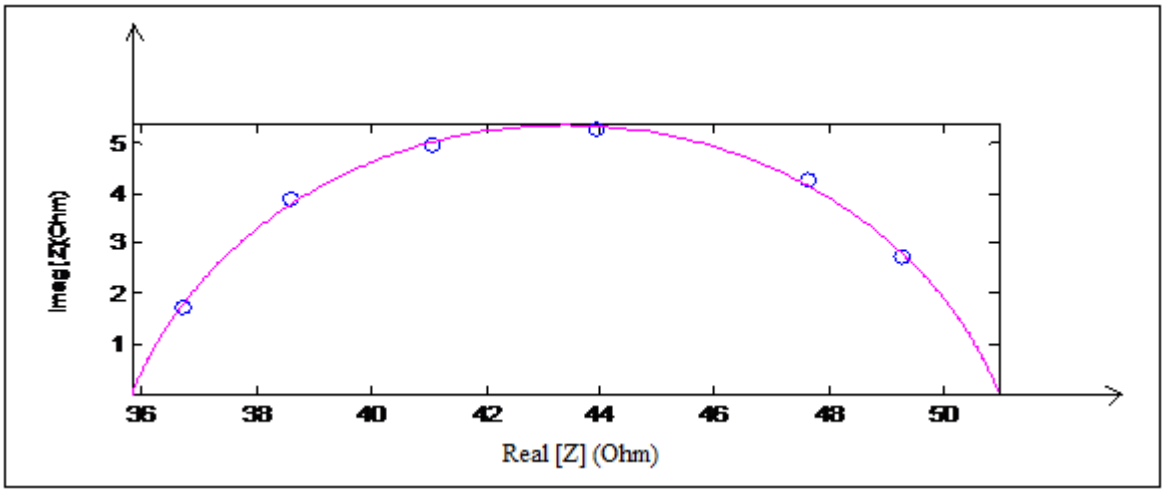

Figure 5.5: One sample of the measured impedance in the complex plane .

The small 6 circles on the curve in figure 5.5 represents the measured data at the six frequencies mentioned previously where the solid line represents Cole-Cole mathematical model. What can be observed from that figure is the best fitting of the measured data to Cole-Cole model.

The same result can be noticed if the impedance module and the phase angle for one reading of the 10 readings are fitted to Cole-Cole model (see figures 5.6 and 5.7).

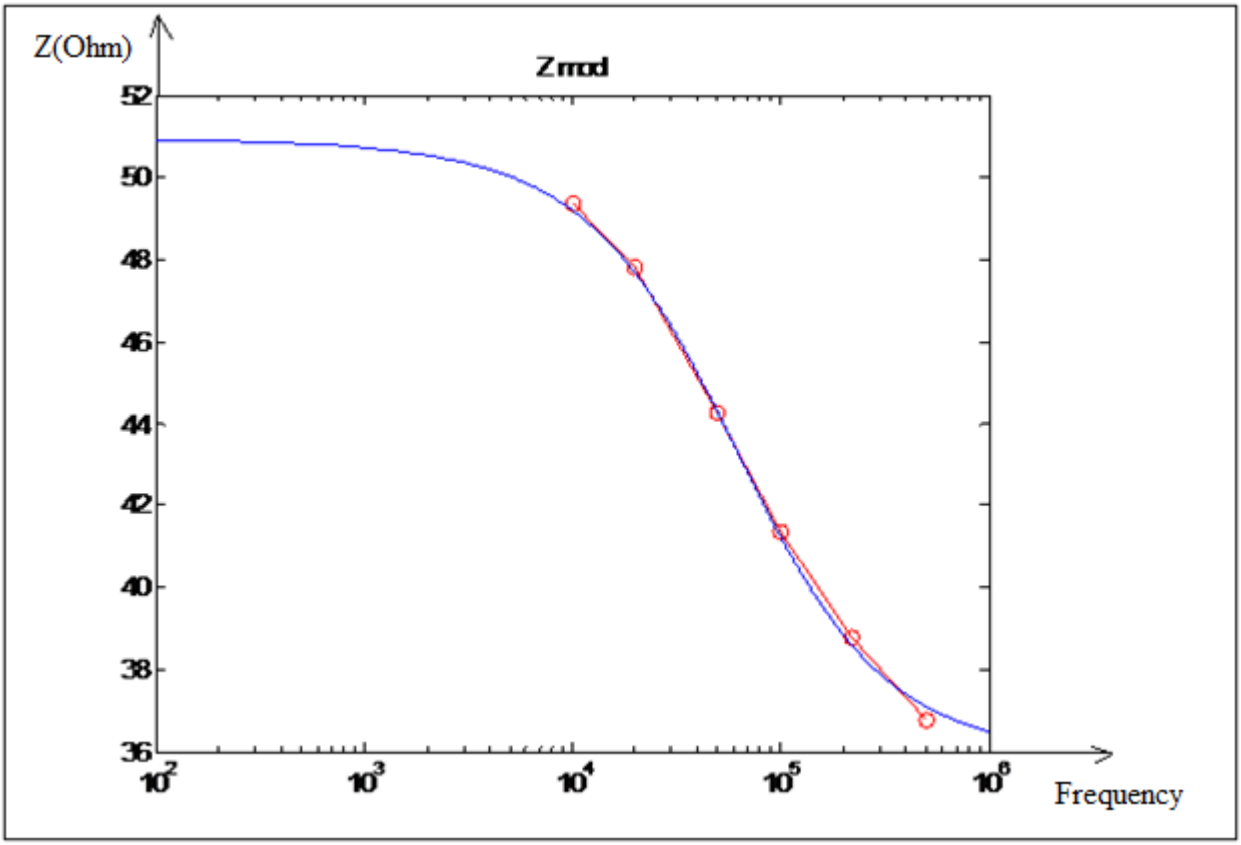

Figure 5.6: Impedance Module as function of frequency .

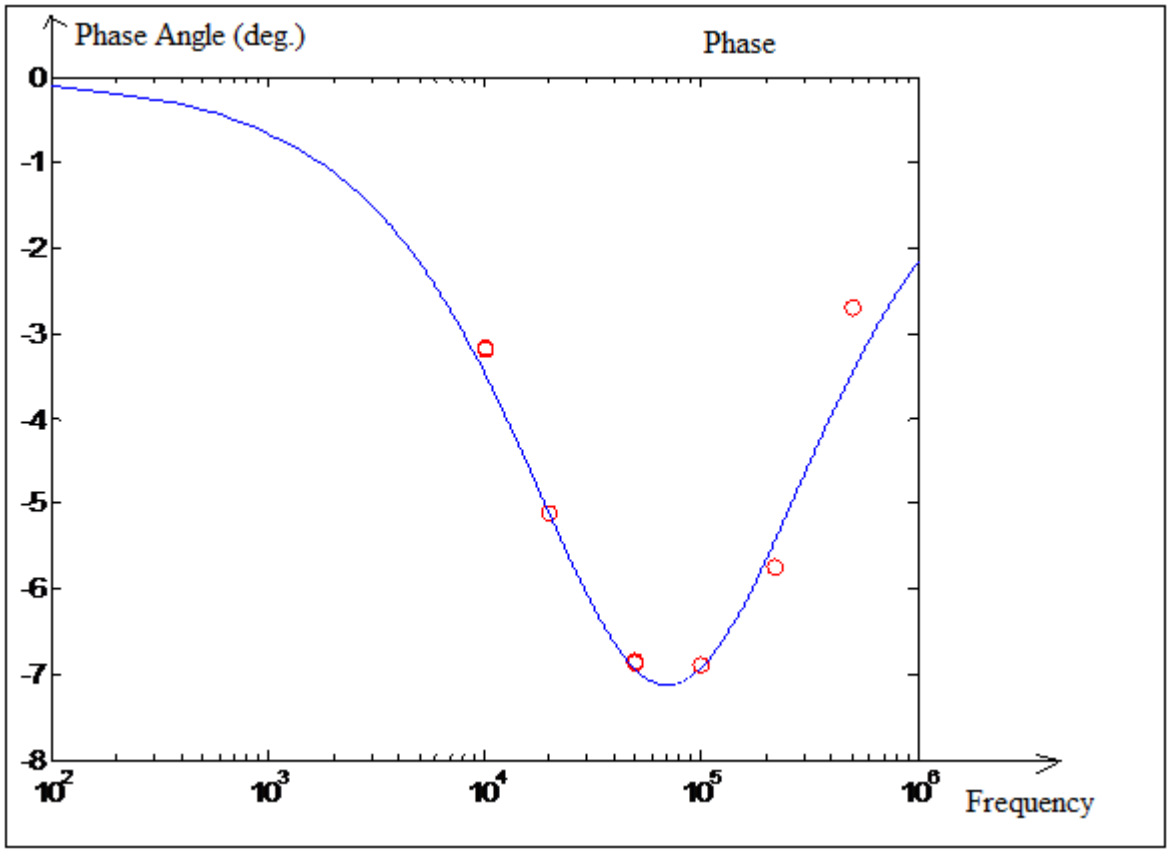

Figure 5.7: Phase Angle as function of frequency .

What can be noticed from figures 5.6 and 5.7 is the excellent fitting of the measured impedance and phase angle to Cole-Cole model. But increasing the frequency above 500KHz has noisy results as the fitting to Cole-Cole model becomes bad. Therefore those six frequencies are chosen to take bioimpedance measurements upon.

5.3 Wet measurements: Impedance measurements using Local Impedance analyzer and blood tissue samples

The second set of measurements in the first phase are wet measurements with 10 blood samples using Local tissue impedance analyzer.

5.3.1 Blood Sample Preparation

A sample of 100 cm^3 of fresh human blood is collected from a healthy subject. The sample is analyzed using conventional clinical cell counter - CBC instrument- to evaluate the sample Haematocrit HCT. The sample is found to have HCT=43.1% or 0.431.

The blood sample is divided into two portions each of 50 cm^3. The first portion is centrifuged using a bench top centrifuge at speed of 3500 RPM for 10 minutes in order to separate plasma from blood cells. Plasma is then collected and used for blood dilution.

5.3.2 Blood Chamber and Impedance Probe

The blood chamber and impedance probes are designed by Dr. Surkhi. The blood chamber and the impedance probes are shown in the figures 5.8 & 5.9.

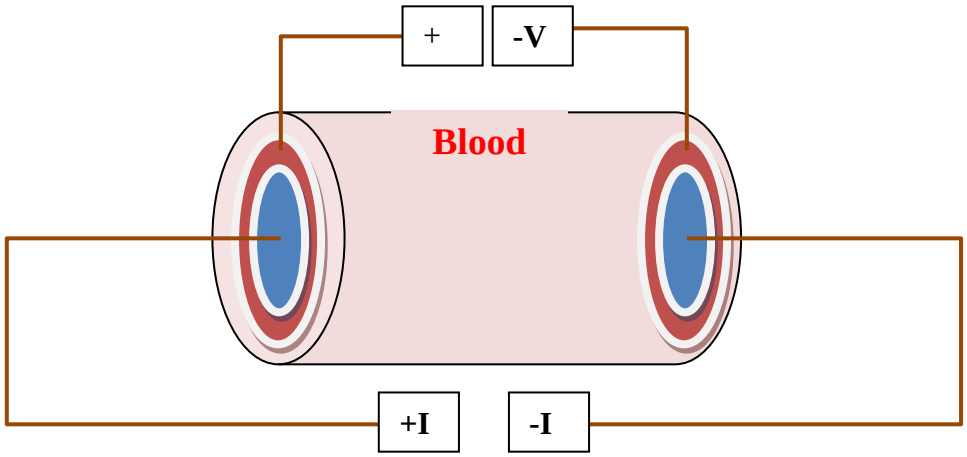

Figure 5.8: The blood chamber and impedance probe .

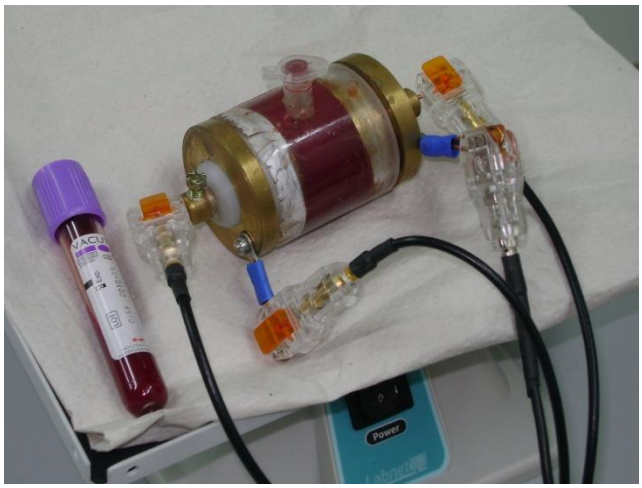

Figure 5.9: The blood chamber and impedance probe .

The blood chamber that is used has a cylindrical shape with the following parameters:

Inner diameter = 2.8cm

Inner cross-section area = 6.157cm^2

Length(distance between the probes) = 2.2cm

Total chamber volume = 13.6cm^3

Four circular shaped impedance probes are designed as shown in figure 5.8. Two of them for current injection (Blue) and the others for the voltage detection (Red).

The blood is injected into the chamber and the impedance is measured via the four probes. Ten Different blood samples are produced with different Haematocrit values. Samples are obtained by removing 1 cm^3 of blood from the chamber and inserting a 1 cm^3 of plasma dilution then the chamber is shaken well, this process is done in order to obtain more than one sample as a small blood sample is obtained at the first time. The Haematocrit for each sample is evaluated using conventional Clinical Cell Counter - CBC instrument. All measurements are performed at temperature of 37°C.

The theoretical value of the blood Haematocrit for each sample is evaluated using the following information : Initial sample HCT, volume of the blood Chamber, removed blood volume and the volume of the added plasma dilution.

The Haematocrit (HCT) is calculated theoretically by using the following criteria:

$$\mathrm{RBC(i)} = \mathrm{HCT(i-1)} \times 12.6\ (\mathrm{cm^3}) \qquad \textit{Eq(5.1)}$$

$$\mathrm{Plasma\ (i)} = 13.6\ (\mathrm{cm^3}) - \mathrm{RBC(i)}\ (\mathrm{cm^3}) \qquad \textit{Eq(5.2)}$$

$$\mathrm{HCT(i)} = \frac{\mathrm{RBC(i)}}{\mathrm{Plasma\ (i)} + \mathrm{RBC(i)}} \qquad \textit{Eq(5.3)}$$

Where:

$\mathrm{i} = 1,2 \ldots 10$: sample number

RBC(i): volume of the Red blood cells in sample (i) (cm^3)

Plasma (i): volume of plasma in sample (i) (cm^3)

HCT(i): Haematocrit of sample(i)

$\mathrm{HCT}(0) = 0.431$

Note that the above criteria for calculating HCT depends upon the values of RBC(i) and Plasma(i). RBC(i) depends upon the value of HCT for previous sample before generating a new one by dilution $\mathrm{HCT}(\mathrm{i}-1)$ and the chamber volume after taking $1\mathrm{cm}^3 = 12.6\ (\mathrm{cm}^3)$. Plasma(i) depends upon the total chamber volume $13.6\ (\mathrm{cm}^3)$ and the value RBC(i). This criteria is derived according to the blood chamber design and the process of generating blood samples by dilution.

5.3.3 Local tissue Impedance Spectroscopy System

A multi-frequency impedance analyzer with four electrode configuration is used. The Performance tests performed on this analyzer gave excellent results in the following range: Module range: $5(\Omega) \leq |Z| \leq 200(\Omega) \pm 2.0\%$:, Phase range: $0.5(\mathrm{deg}) \leq |\phi| \leq 90(\mathrm{deg}) \pm 2.0\%$, Frequency range: 10 kHz-1MHz. The impedance analyzer is attached to the blood chamber and the impedance module and phase are continuously recorded at 6 frequencies (10,20,50,100,220,500 kHz) with Sampling Rate SR= 20 samples/minute. Data is acquired and transferred to the computer using LABVIEW self designed program –Figure 5.10.

As known LABVIEW is a graphical programming environment used by millions of engineers and scientists to develop sophisticated measurement, test, and control systems using intuitive graphical icons and wires that resemble a flowchart. It offers unrivaled integration with thousands of hardware devices and provides hundreds of built-in libraries for advanced analysis and data visualization – all for creating virtual instrumentation.
You can see the experimental setup in figure 5.10.

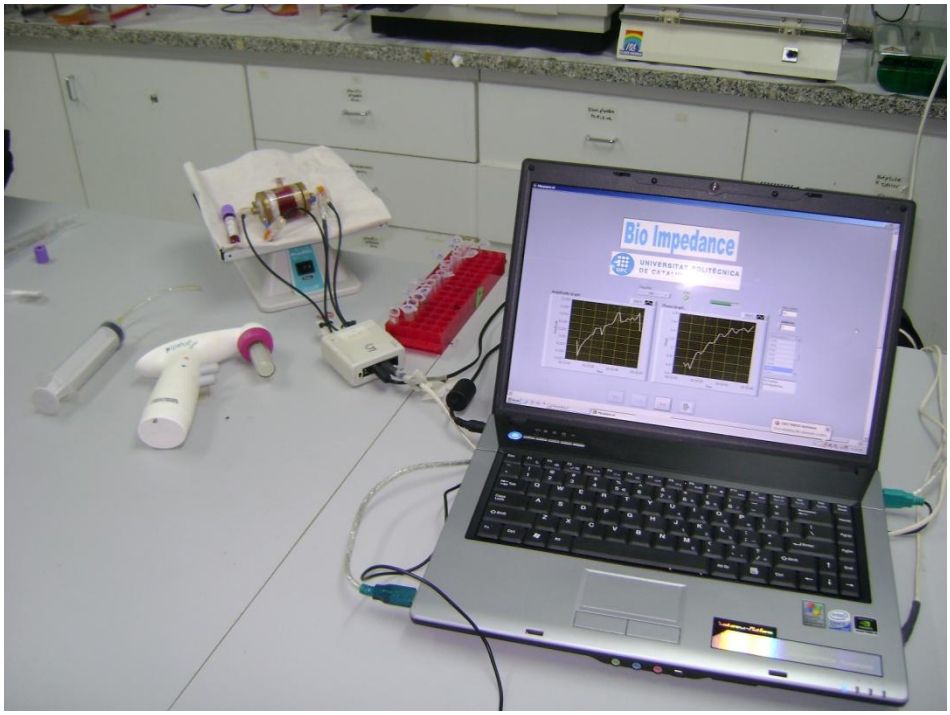

Figure 5.10: The Experimental setup .

5.3.4 Measurement software

Figure 5.11 shows the self-designed LABVIEW program that is designed for acquiring data and transferring it to the computer. By using this program you can determine the value of the used frequency from the frequency list, the wanted maximum points on the curves, the used port. After running this program both Amplitude and phase graph are obtained related to the impedance measurement. The program gives you the ability to save your work, stop working or exit from the program (see figure 5.11).

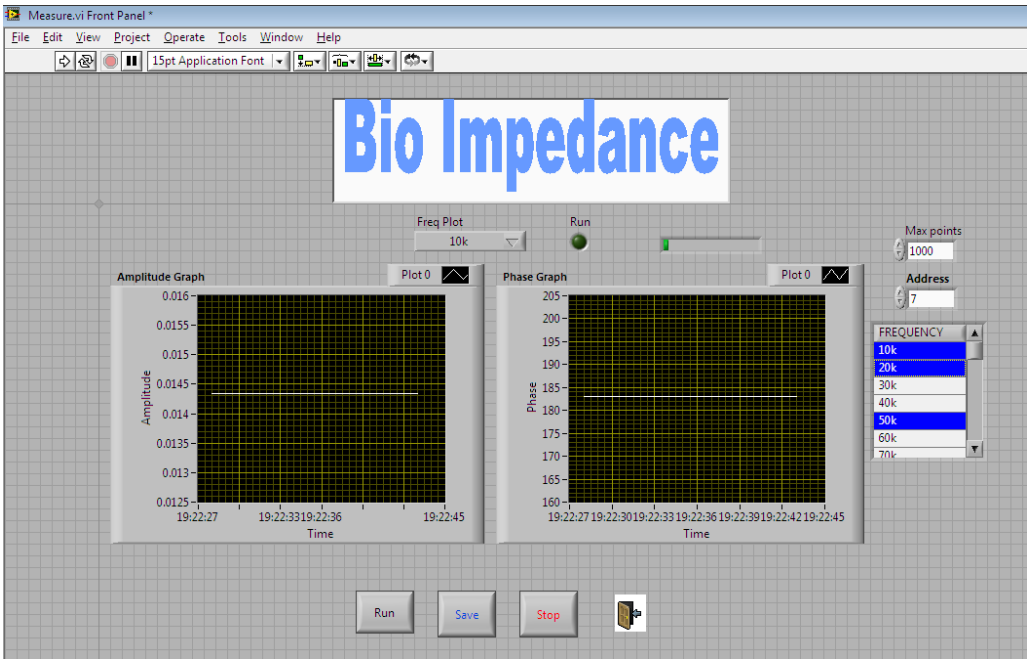

Figure 5.11: Data Acquiring program .

5.4 Results from Wet Measurements\Phase One

In this section, the results from the 10 blood samples that are produced in wet measurements included in phase one are demonstrated. Table 5.7 shows the theoretical and the measured HCT for those 10 blood samples.

Table 5.7: Theoretical & Measured HCT .

Sample	HCT Measured	HCT Theoretical
sample0	0.431	0.431
sample 1	0.410	0.399
sample 2	0.400	0.370
sample 3	0.390	0.343
sample 4	0.309	0.318
sample 5	0.284	0.294
sample 6	0.263	0.273
sample 7	0.236	0.253
sample 8	0.184	0.234
sample 9	0.160	0.217
sample 10	0.133	0.201

If the above results are drawn in order to compare between the theoretical and the measured HCT, the following figure will be obtained:

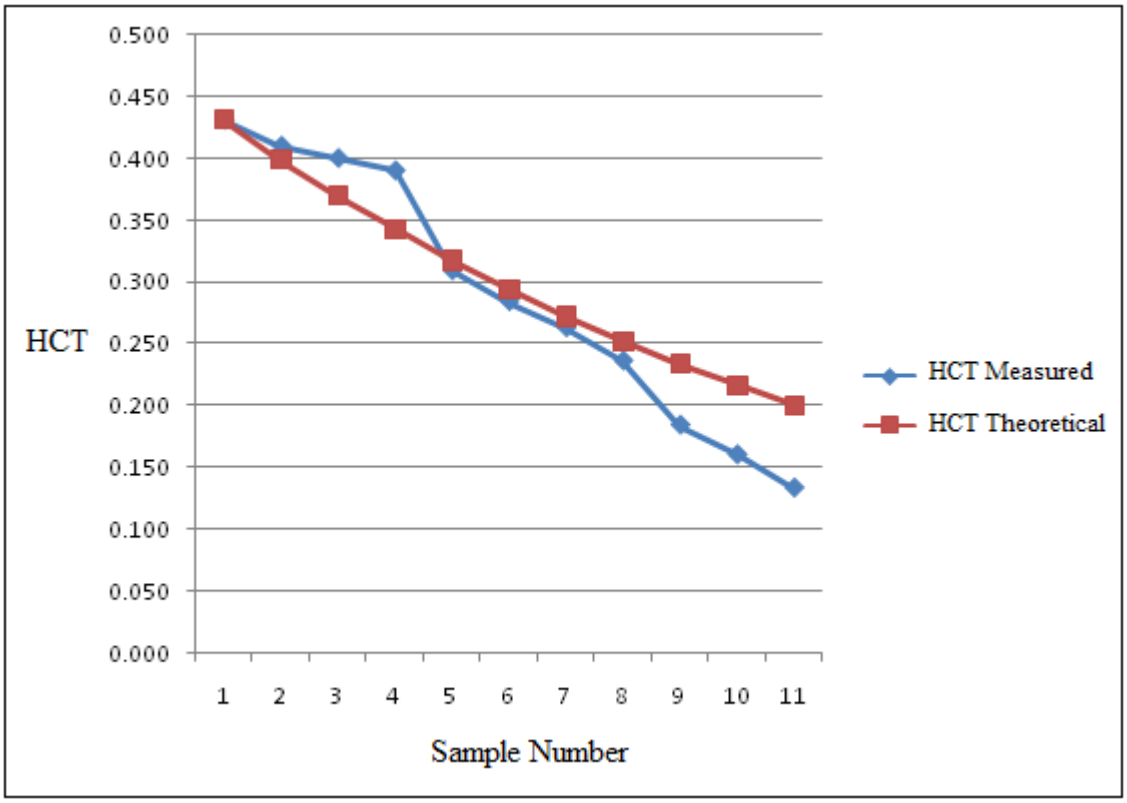

Figure 5.12: Theoretical and Measured HCT for the 10 blood samples .

The theoretical values for HCT are obtained according to the listed criteria above (Eq(5.1), Eq(5.2) and Eq(5.3)) using the total chamber volume, the dilution amount and HCT initial value. The HCT measured values are obtained by measuring HCT for each new sample after dilution is made using the clinical instrument-CBC.

Table (5.7) and figure (5.12) show HCT theoretical and measured values. As noticed the values of HCT drops quickly, thus will affect the values of the measured impedance as will be seen later. When the plasma is added thus means more conducting media is added and the sample becomes a plasma rich sample instead of the blood cells so the plasma increased and hence the value of HCT will be decreased. Thus may result from the volume of the removed blood sample from each sample. For future work a small volume of blood smaller than $1cm^3$ will be removed or the dilution must be made very slow or multi blood samples may be used instead of producing blood samples by dilution..

5.4.1. Measured Data:

For each blood sample, both impedance module and phase are continuously recorded at 6 frequencies which are (10,20,50,100,220,500 kHz). An example of the recorded data is shown in figure 5.13 for blood sample 02.

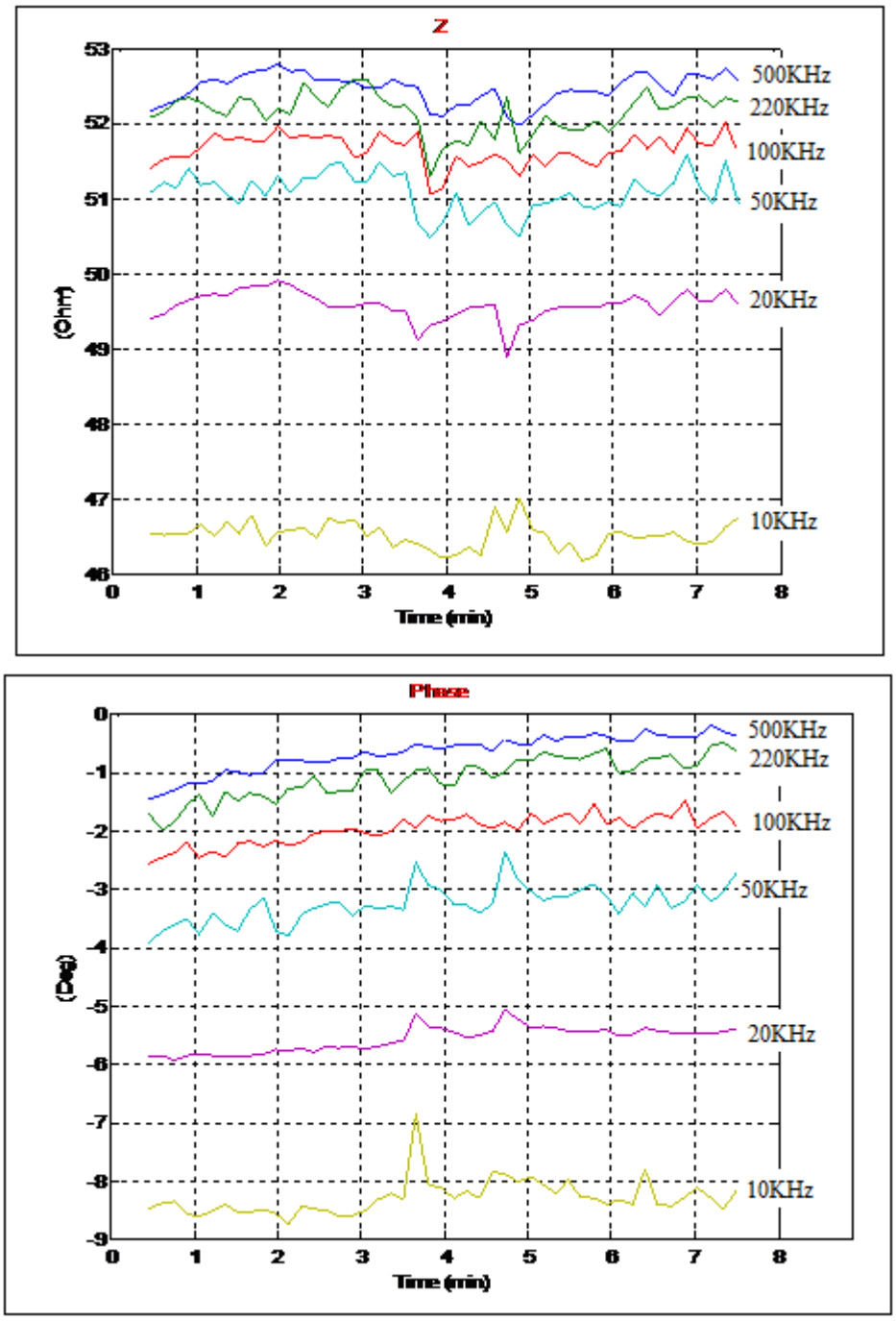

Figure 5.13: Impedance module and Phase angle at 6 frequencies for Sample 02 .

5.4.2 Cole-Cole model fitting

The measured impedance at 6 frequencies (round circle on the curve) is fitted into Cole-Cole mathematical model (Eq(3.6)) (solid line on curve) using a special program developed using MATLAB. For example figure 5.14-a shows all data in sample02 fitted into Cole-Cole model and plotted in the complex plane i.e real part of the impedance vs. the imaginary part. Excellent fit with the model can be noticed.

Figure 5.14-b shows the average impedance data of sample 02 plotted in the complex plane. The limiting values at low and high frequencies, R_0 and R_∞, are not measurable in practice, but they can be extrapolated from the mathematical model.

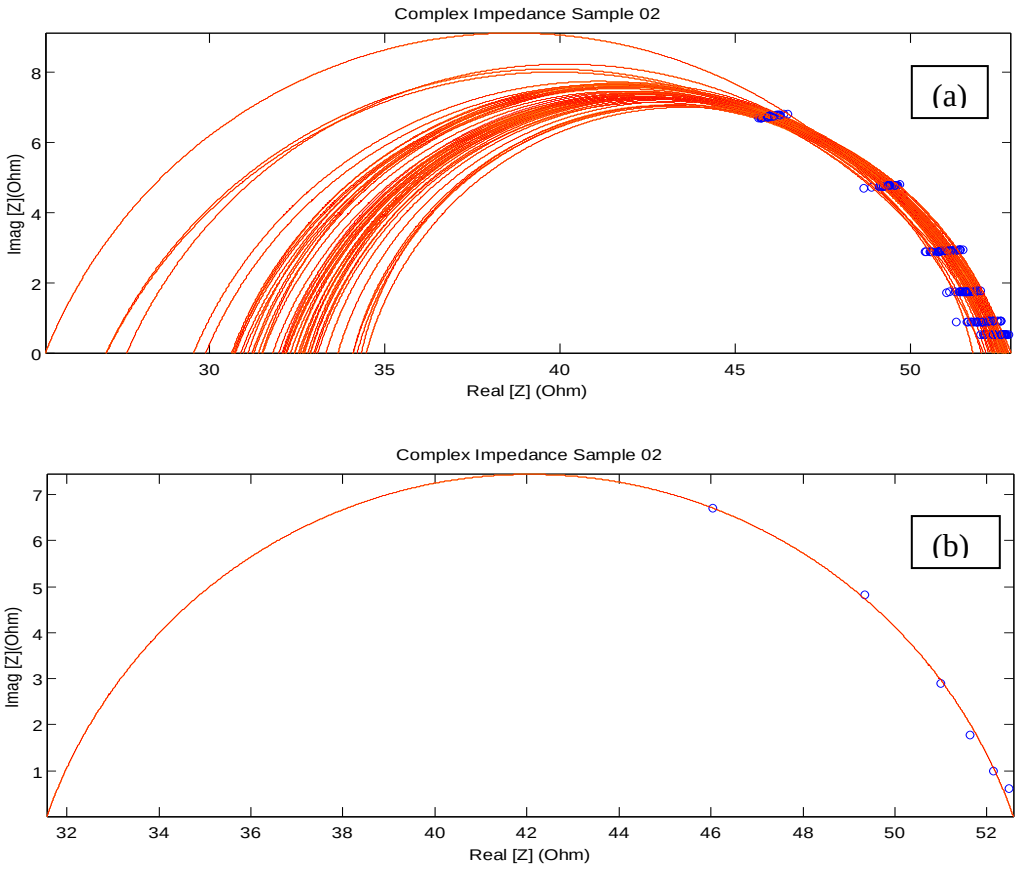

Figure 5.14: Impedance plotted in the complex plane for Sample 02. (a) All measured data (b) Data Average .

The bioimpedance measurement is done for each blood sample for about 8 minutes with 10sample/Minute, so there is nearly 70-80 readings for each blood sample. If the readings average of two blood samples with different HCT are taken and fitted to Cole-Cole model, the effect of α and resistivity can be noticed (see figure 5.15).

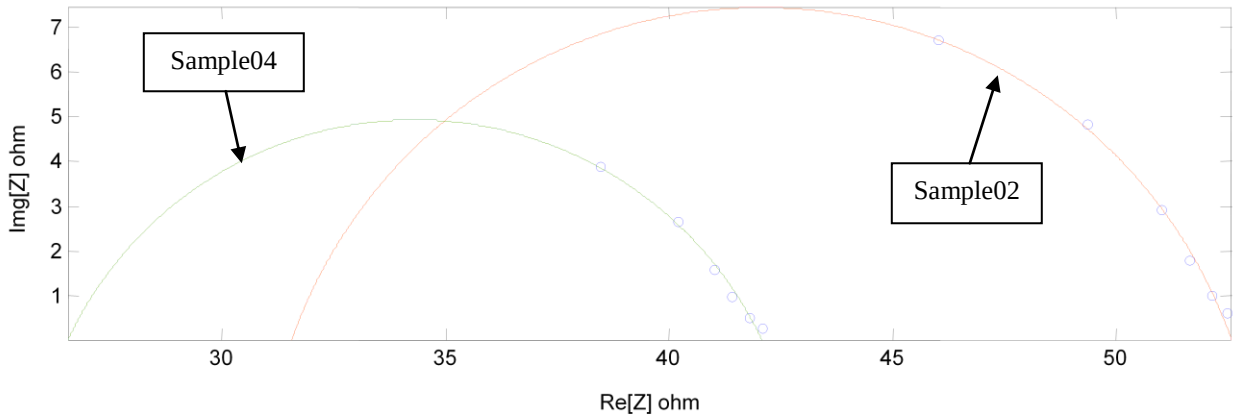

Figure 5.15: Impedance plotted in the complex plane for sample02(HCT=0.4) and sample04(HCT=0.3) .

Figure 5.15 shows the average readings for sample02 and sample04 fitted to Cole-Cole model. The two samples differ in HCT values with 0.4 for sample02 and 0.3 for sample04. Sample04 is obtained by adding more conducting media -plasma- to sample03 and sample03 is obtained by adding more conducting media to sample02. Adding more conducting media will decrease the resistivity. Therefore the impedance will be decreased, this result is noticed from figure 5.15. sample04 has less impedances values than sample02, also the depression of the arc and the effect of α can be noticed.

The preliminary data analysis in wet measurements includes:

1. Cole-Cole parameters extrapolation (R0 and R∞).
2. ECW and ICW estimation based on first and second generation Xitron estimators.
3. Blood HCT estimation based on first and second generation Xitron estimators.

Table 5.8 shows Cole-Cole parameters.

Table 5.8: Cole-Cole Parameters .

	Ro	R∞	fc	alfa
Original blood simple	60.52	32.76	7.93E+05	0.17
Sample 1	57.65	32.11	7.79E+05	0.18
Sample 2	52.57	31.56	1.02E+06	0.22
Sample 3	24.23	19.78	4.18E+05	0.03
Sample 4	42.08	26.55	1.33E+06	0.28
Sample 5	20.47	17.90	3.05E+05	0.06
Sample 6	18.63	16.78	2.56E+05	0.10
Sample 7	17.41	15.81	2.15E+05	0.21
Sample 8	16.47	15.16	1.01E+05	0.30
Sample 9				
Sample 10				

Table 5.9 shows high frequency ICW Estimator

Table 5.9: High Frequency ICW Estimator.

Sample	Theoretical $V_{ICW}(Cm^3)$	Measured $V_{ICW}(Cm^3)$	Xitron First Gen. $V_{ICW}(Cm^3)$	Xitron Second Gen. $V_{ICW}(Cm^3)$
Original blood sample	5.86	5.86	9.48	7.65
Sample 1	5.43	5.58	9.33	7.44
Sample 2	5.03	5.44	8.91	6.86
Sample 3	4.66	5.30	6.52	3.68
Sample 4	4.32	4.20	8.60	6.44
Sample 5	4.00	3.86	5.78	2.73
Sample 6	3.71	3.58	5.43	2.29
Sample 7	3.43	3.21	5.33	2.15
Sample 8	3.18	2.50	5.17	1.95
Sample 9	2.95	2.18	4.12	1.04
Sample 10	2.73	1.81	5.38	2.22

5.5 Conclusions from Phase One

Blood Haematocrit is the volumetric percentage of blood cells to the total blood volume. In the case of blood tissue, the volume of blood cells is the V_{ICW} while the volume of plasma is the volume V_{ECW} .

Thus the relation between the HCT and V_{ICW} and V_{ECW} can be written as:

$$HCT = \frac{V_{ICW}}{(V_{ICW} + V_{ECW})} \qquad \textit{Eq(5.4)}$$

Or:

$$HCT = \frac{1}{(1 + V_{ECW}/V_{ICW})} \qquad \textit{Eq(5.5)}$$

Note: Eq(5.5) is obtained by dividing Eq(5.4) by V_{ICW} .

Using the first and second generations for V_{ICW}/V_{ECW} as a relation to Ro and R∞ ,the blood HCT can be evaluated based on those estimators.

The HCT for blood sample is calculated using both the first and second generation of Xitron ICW and ECW estimators. The following table shows those values with the theoretical ones.

Table 5.10: High Frequency ICW Estimator.

Sample	HCT Theoretical	HCT First Gen	HCT Second Gen
Original blood sample	0.431	0.70	0.56
Sample 1	0.399	0.69	0.55
Sample 2	0.370	0.65	0.50
Sample 3	0.343	0.48	0.27
Sample 4	0.318	0.63	0.47
Sample 5	0.294	0.42	0.20
Sample 6	0.273	0.40	0.17
Sample 7	0.253	0.39	0.16
Sample 8	0.234	0.38	0.14
Sample 9	0.217	0.30	0.08
Sample 10	0.201	0.40	0.16

The results for blood sample02 is shown in the following figure:

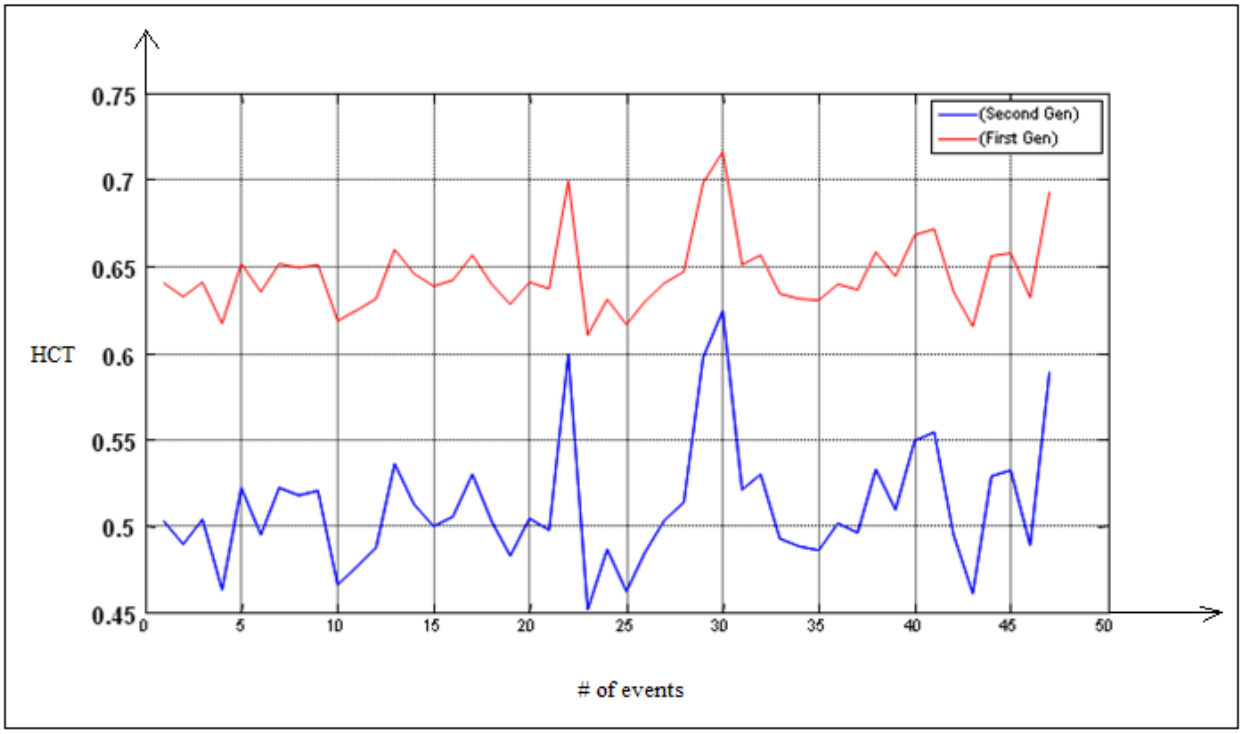

Figure 5.16: Haematocrit Value by First and Second Generation for sample02 .

Referring to Table 5.7 which includes the measured and theoretical HCT values for 10 blood samples obtained by dilution, HCT(sample02)=0.4 (measured) and 0.37(theoretical). From figure (5.15), it is noticed that both Xitron first and second generations give overestimation for the Haematocrit value of sample02, but the overestimation in the first generation is more than that in the second generation, so the second generation gives us more realistic results than the first one and thus the second generation is the development and enhancement of the first one; therefore there is a relationship between the HCT value and the Bioimpedance measurements.

From the pilot measurements some points may be concluded as:

- In dry measurements, no significant differences between the measured impedance of tissue simulator and the theoretical values which indicates excellent accuracy of the instrument.
- In wet measurement, both instruments are able to measure bioimpedance of blood.
- In wet measurements as HCT is reduced below 30%, data are not measured as bioimpedance data and had bad fitting to Cole-Cole model. In general ,the error in estimating HCT value increased as the HCT values decreased so multi blood samples must be used and not diluted samples.
- In wet measurement, the estimated HCT value based on the first and second Xitron estimators had significant errors compared to the measured or the theoretical HCT values.
- The first and second generation of volume estimation give us overestimation of ICW volume values but second generation results are better than first generation as first generation gives more overestimation than second one.
- Blood chamber size is good.
- The used six frequencies are also fine.
- From the results of phase one, we decided to use Local Impedance Analyzer Instrument.

Chapter Six: Phase Two Measurements and Results (Research Measurements)

6.1 Blood Samples

In this phase 33 human fresh blood samples not diluted (multi blood samples) are used. Many parameters will be investigated such as the extracellular and intracellular water and Haematocrit. Samples will be taken and measured by using Local Impedance Analyzer equipment at the material lab (Al-Quds University). The used blood chamber is the same as used in phase one. The frequencies that are used to do measurements are the same as phase one (10,20,50,100,220 and 500 kHz). In this phase three estimators are used to make comparison and data analysis (Xitron first, second generation and Surkhi Estimator).

Before testing the blood sample at Al-Quds University, the same sample is previously investigated using Clinical Blood Analyzers at the Hospital from which the blood is collected. After testing the blood sample at the material lab the same blood sample will be tested again using Clinical Blood Analyzer at the medicine school at Al-Quds university in order to compare the results.

6.2 Phase Two Objectives

The initial research questions that may be asked to introduce the problem statement include:

- How to estimate water content inside cells and outside cells ?
- Is this possible to do using bioimpedance measurements ?
- Can Blood Haematocrit HCT using bioimpedance measurements be estimated ?

From those initial research questions; research objectives can be guessed and introduced and can be summarized in the following points:

1. Perform Electrical Bioimpedance spectroscopy measurements using wide frequency range on biological tissues, mainly blood tissues.
2. Evaluate tissue parameters out of the Bioimpedance measurements such as :
    - Intracellular water volume
    - Extracellular water volume
    - Blood Haematocrit HCT
3. Study and compare the most used ICW and ECW estimators available in literature
4. Study the accuracy of ICW and ECW estimators and try to indicate source of inaccuracies.
5. Develop new ICW and ECW estimators .
6. Study the Cole-Cole parameters of the used blood samples.

After introducing the objectives from this work, the possible contribution and expected outcomes from the work may be predicted as follows:

1. Measure the intracellular and extracellular water content in the tissue based on low and high frequency measurements.

2. Different ECW and ICW estimators are currently used, many authors have reported inaccuracies in these estimators and errors in their results therefore different estimators will be compared, analyzed and corrected .

3. Develop blood Haematocrit HCT estimator based on high frequency impedance measurements.

In order to achieve the desired goals and objectives from this work, let us remember that pilot study and measurements are done to crystallize the idea and find out any directions, measurements or procedure that can be chosen and to check the possibility for application.

From the pilot study and measurements that are done previously, some points are taken in considerations which are:

- In dry measurements, no significant differences between the measured impedance of tissue simulator and the theoretical values which indicates excellent accuracy of the instrument.
- In wet measurement, the estimated HCT value, based on the first and second Xitron estimators, had significant errors compared to the measured or the theoretical HCT values.
- In general, the error in estimating HCT value increases as the HCT value decreases.
- HCT dropped sharply as 1 cm^3 of blood volume is taken from the sample and replaced by plasma to take different HCT reading. Hence a small volume smaller than 1 cm^3 must be taken or multi blood samples may be used.
- The first and second generations of volume estimation give us overestimation of ICW volume values.

From previous points control and limitations of this work is known and may be summarized in the following points:

- In Wet Measurements, the HCT values dropped sharply, so a small volume of blood smaller than 1 cm^3 must be taken in order to take different measurements or multi blood samples may be replaced instead of generating blood samples using dilution.
- When the HCT is reduced in the sample, then the noise is expected to increase and thus will affect the measurements. Measurements had very bad fitting to Cole-Cole model due to reduced amount of cells.

From all previous measurements limitations and controls, guidelines can now be drawn in order to achieve the goals and objectives of this work as follows:

- Indicate source of errors in Xitron estimators
- Develop ICW and ECW estimator
- Increase the frequency range up to MHz
- Work on more blood samples with HCT range between the physiological values 50-25%

6.3 Phase Two Experimental Procedure

This section describes the procedure with which the results are collected.

To obtain blood samples that are well tested, hospital management department has been addressed by the administration of faculty of engineering and the thesis supervisor. Hospital management department addresses Hebron Governmental Hospital (HGH) in order to facilitate the task and obtain the required blood samples. Then blood samples are measured in material lab at Al-Quds University with different steps that are discussed briefly below.

About 33 blood samples are collected from Hebron Governmental Hospital, all blood samples are fresh and well tested. The following steps are done to each one of the collected blood samples:

1. The blood sample is taken from blood bank at HGH. The sample of 40-45 cm^3 is fresh and well tested. Clinical tests include HBSAy, HIV, HCV. Those clinical tests must be negative for the blood sample and those results can be checked from blood bank records.
2. By using the spectroscopy equipment at HGH, all related blood tests are done including HCT.
3. After that the blood sample kept cool to maintain a suitable blood temperature and to be transported from HGH at Hebron to Al-Quds University without damaging the blood cells.
4. After nearly one hour, the blood sample arrived to the Material laboratory in Al-Quds University.
5. Prepare the blood sample for measurements by shaking it for about 10 minutes using the shaker (See figure 6.1).

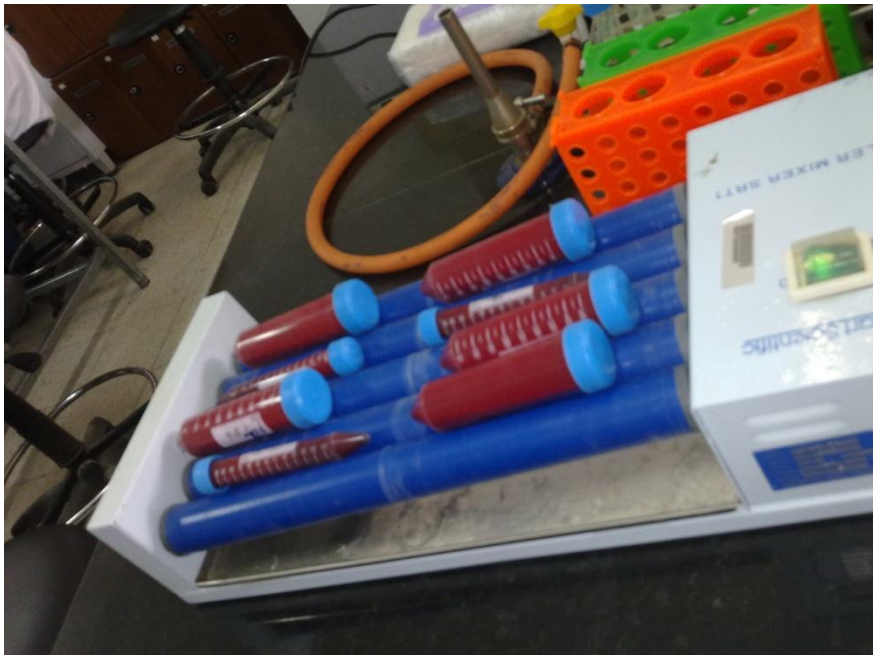

Figure 6.1: Blood Samples on the shaker .

6. Taking into account all the safety requirements during the work with blood sample in the laboratory by wearing lab coat, gloves and taking the considerations to obtain the cleaning of everything used.
7. After 10 minutes, a syringe is used to fill chamber C with blood.

Figure 6.2: A Blood Sample in the chamber .

8. Using the local tissue analyzer, injection and detection electrodes and the labview acquiring program to record the impedance and phase angle result at different frequencies.

Figure 6.3: Connecting chamber with frequency analyzer electrodes .

9. Save the data.
10. Taking the blood sample to medicine college in order to measure its Haematocrit by using the centrifuge equipment at the medicine laboratory.

Figure 6.4: Haematology Analyzer .

By using the capillary tubes, the technician take an amount of the blood sample after shaking it and put it in the centrifuge equipment, indicating RPM and centrifuge time. Usually 1300 rpm and 5 minutes are taken as parameters.

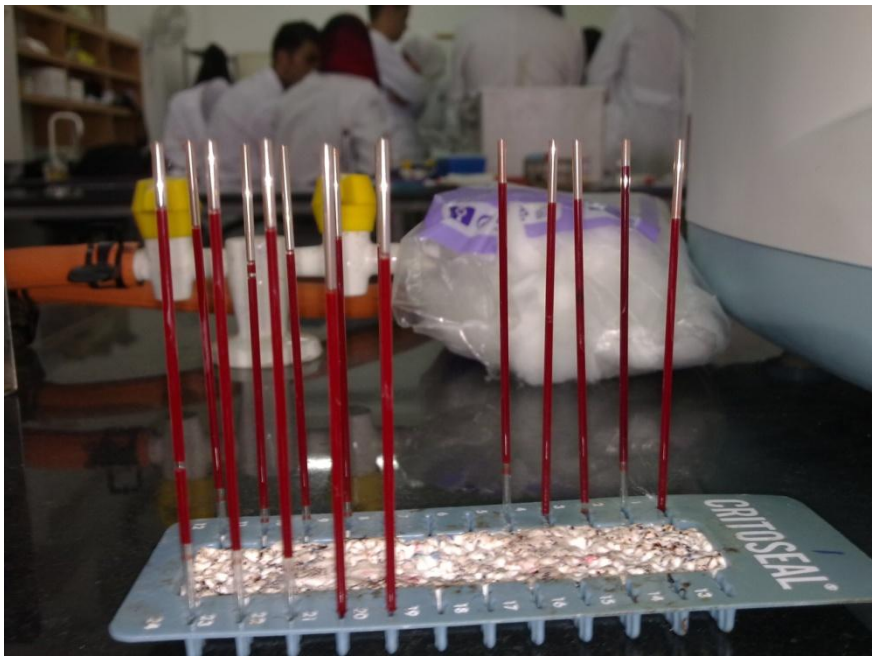

Figure 6.5: Capillary Tubes .

11. Record the Haematocrit value in order to compare it with the one that the hospital equipment had measured and the estimated value that will be estimated.

12. Returning to the data file that is saved, this file include readings of impedance and phase at different frequencies determined by the labview acquiring program.
13. By using matlab program a matlab code is written in order to estimate the Haematocrit value based on Xitron first and second generation therefore compare those values with the values obtained in hospital and university, absolute error and relative error are calculated.
14. The Cole-Cole parameters are extrapolated for each blood sample.
15. Extracellular and intracellular volumes are calculated at low and high frequency based on Xitron first and second generation.

6.4 Phase Two: Results

According to the research measurements procedure mentioned above and by using the equations of the used and developed estimators, this section presents the results that are obtained for Haematocrit, Volumes Estimation and Cole-Cole parameters.

6.4.1 Haematocrit Estimators

The first results obtained are the values of HCT depending upon Xitron first generation, Xitron second generation and the developed Surkhi estimator. This section shows those results.

6.4.1.1. Xitron First Generation:

Table 6.1 shows the results of the first generation estimator used by Xitron impedance systems company for the 33 blood samples. In that table the values of HCT measured in hospital and university are shown, add to that the values of HCT using first generation equation are calculated, then absolute and relative errors are determined.

Table 6.1: First Generation Results .

Sample#	Sample	Hospital HCT	University HCT	First Generation HCT	Abs. Error	1st Gen. Relative Error (%)
1	sample00	46.5	46	68.344±0.009	22.344	-48.574
2	sample05	43.3	42.5	67.700±0.011	25.200	-59.294
3	sample066	43	43.5	67.061±0.012	23.561	-54.163
4	sample06	43.4	43.5	68.470±0.010	24.970	-57.402
5	sample07	47.6	45.5	67.843±0.011	22.343	-49.105
6	sample08	40.7	34	64.859±0.026	30.859	-90.762
7	sample09	47	43	70.614±0.025	27.614	-64.219
8	sample10	42	42	66.969±0.020	24.969	-59.450
9	sample11	45.8	42.5	65.950±0.017	23.450	-55.176
10	sample12	52.9	43	67.246±0.016	24.246	-56.386
11	sample13	34	32	55.970±0.028	23.970	-74.906
12	sample14	42	40	65.731±0.017	25.731	-64.328
13	sample15	40	37.5	64.215±0.025	26.715	-71.240
14	sample16	58	58	75.244±0.051	17.244	-29.731
15	sample17	39	37	64.205±0.032	27.205	-73.527
16	sample18	48	47	68.722±0.027	21.722	-46.217
17	sample19	39	38	62.461±0.016	24.461	-64.371
18	sample20	51	51	70.600±0.035	19.600	-38.431
19	sample21	40	38.5	63.932±0.020	25.432	-66.057
20	sample22	58	57.5	75.796±0.044	18.296	-31.819
21	sample23	31.6	32	58.319±0.077	26.319	-82.247
22	sample24	35.7	35	57.517±0.075	22.517	-64.334
23	sample25	41.5	41	60.132±0.065	19.132	-46.663
24	sample26	42.3	40	61.373±0.059	21.373	-53.433
25	sample27	38.6	40	59.717±0.064	19.717	-49.293
26	sample28	24.9	25	47.347±0.118	22.347	-89.388
27	sample4e	46	45	67.696±0.059	22.696	-50.436
28	sample 1_old	43	39.9	68.610±0.042	28.710	-71.955
29	sample 2_old	40	37	50.462±0.049	13.462	-36.384
30	sample 3_old	38	34.3	65.623±0.056	31.323	-91.321
31	sample 4_old	39	31.8	63.204±0.064	31.404	-98.755
32	sample 5_old	35	29.4	62.266±0.093	32.866	-111.789
33	sample 6_old	33	27.3	59.942±0.127	32.642	-119.568

6.4.1.2. Xitron Second Generation:

Table 6.2 shows the results of the second generation estimator used by Xitron impedance systems company for the 33 blood samples. In that table the values of HCT measured in hospital and university are shown, add to that the values of HCT using second generation equation are calculated, then absolute and relative errors are determined

Table 6.2: Second Generation Results .

Sample#	Sample	Hospital HCT	University HCT	Second Generation HCT	Abs. Error	2nd Gen. Relative Error (%)
1	sample00	46.5	46	54.369±0.012	8.369	-18.200
2	sample05	43.3	42.5	53.48±0.015	10.98	-25.835
3	sample066	43	43.5	52.607±0.016	9.107	-20.936
4	sample06	43.4	43.5	54.542±0.013	11.042	-25.384
5	sample07	47.6	45.5	53.68±0.015	8.18	-17.978
6	sample08	40.7	34	49.597±0.036	15.597	-45.874
7	sample09	47	43	57.502±0.035	14.502	-33.726
8	sample10	42	42	52.481±0.027	10.481	-24.955
9	sample11	45.8	42.5	51.085±0.024	8.585	-20.200
10	sample12	52.9	43	52.861±0.023	9.861	-22.933
11	sample13	34	32	37.642±0.038	5.642	-17.631
12	sample14	42	40	50.787±0.023	10.787	-26.968
13	sample15	40	37.5	48.721±0.035	11.221	-29.923
14	sample16	58	58	63.957±0.070	5.957	-10.271
15	sample17	39	37	48.708±0.045	11.708	-31.643
16	sample18	48	47	54.889±0.037	7.889	-16.785
17	sample19	39	38	46.342±0.022	8.342	-21.953
18	sample20	51	51	57.483±0.048	6.483	-12.712
19	sample21	40	38.5	48.336±0.027	9.836	-25.548
20	sample22	58	57.5	64.733±0.061	7.233	-12.579
21	sample23	31.6	32	40.771±0.105	8.771	-27.409
22	sample24	35.7	35	39.7±0.103	4.7	-13.429
23	sample25	41.5	41	43.201±0.089	2.201	-5.368
24	sample26	42.3	40	44.872±0.081	4.872	-12.180
25	sample27	38.6	40	42.645±0.088	2.645	-6.613
26	sample28	24.9	25	26.326±0.158	1.326	-5.304
27	sample4e	46	45	53.478±0.080	8.478	-18.840
28	sample 1_old	43	39.9	54.735±0.058	14.835	-37.180
29	sample 2_old	40	37	50.462±0.068	13.462	-36.384
30	sample 3_old	38	34.3	50.64±0.077	16.34	-47.638
31	sample 4_old	39	31.8	47.348±0.088	15.548	-48.893
32	sample 5_old	35	29.4	46.079±0.121	16.679	-56.731
33	sample 6_old	33	27.3	42.947±0.166	15.647	-57.315

6.4.1.3. Developed Surkhi Estimator Results:

Table 6.3 shows the accuracy of the developed Surkhi estimator according to this work for the 33 blood samples. In that table the values of HCT measured in hospital and university are shown, add to that the calculated values of HCT using Surkhi Estimator equation (equation 4.14), then absolute and relative errors are determined.

Table 6.3: Developed Surkhi Estimator Results .

Sample#	Sample	Hospital HCT	University HCT	Surkhi HCT	Abs. Error	Surkhi Estimator Relative Error (%)
1	sample00	46.5	46	48.511±0.012	2.511	-5.459
2	sample05	43.3	42.5	47.59±0.015	5.090	-11.976
3	sample066	43	43.5	46.701±0.016	3.201	-7.359
4	sample06	43.4	43.5	48.689±0.014	5.189	-11.929
5	sample07	47.6	45.5	47.802±0.015	2.302	-5.059
6	sample08	40.7	34	43.637±0.036	9.637	-28.344
7	sample09	47	43	51.76±0.036	8.760	-20.372
8	sample10	42	42	46.572±0.028	4.572	-10.886
9	sample11	45.8	42.5	45.147±0.024	2.647	-6.228
10	sample12	52.9	43	46.961±0.022	3.961	-9.212
11	sample13	34	32	31.8±0.036	-0.200	0.625
12	sample14	42	40	44.844±0.023	4.844	-12.110
13	sample15	40	37.5	42.751±0.035	5.251	-14.003
14	sample16	58	58	58.577±0.073	0.577	-0.995
15	sample17	39	37	42.738±0.046	5.738	-15.508
16	sample18	48	47	49.048±0.037	2.048	-4.357
17	sample19	39	38	40.361±0.022	2.361	-6.213
18	sample20	51	51	51.74±0.049	0.740	-1.451
19	sample21	40	38.5	42.363±0.027	3.863	-10.034
20	sample22	58	57.5	59.408±0.063	1.908	-3.318
21	sample23	31.6	32	34.848±0.107	2.848	-8.900
22	sample24	35.7	35	33.801±0.104	-1.199	3.426
23	sample25	41.5	41	37.239±0.091	-3.761	9.173
24	sample26	42.3	40	38.896±0.083	-1.104	2.760
25	sample27	38.6	40	36.69±0.090	-3.310	8.275
26	sample28	24.9	25	21.066±0.156	-3.934	15.736
27	sample4e	46	45	47.594±0.081	2.594	-5.764
28	sample 1_old	43	39.9	48.889±0.060	8.989	-22.529
29	sample 2_old	40	37	44.514±0.070	7.514	-20.308
30	sample 3_old	38	34.3	44.694±0.079	10.394	-30.303
31	sample 4_old	39	31.8	41.369±0.090	9.569	-30.091
32	sample 5_old	35	29.4	40.098±0.119	10.698	-36.388
33	sample 6_old	33	27.3	36.988±0.161	9.688	-35.487

6.4.1.4. Comparison between Used and Developed Estimators:

Now let us make a compartment between the most used estimators which are Xitron First and Second Generation and the developed Surkhi Estimator for determining HCT values.

The following figure shows the relationship between time and HCT for sample15:

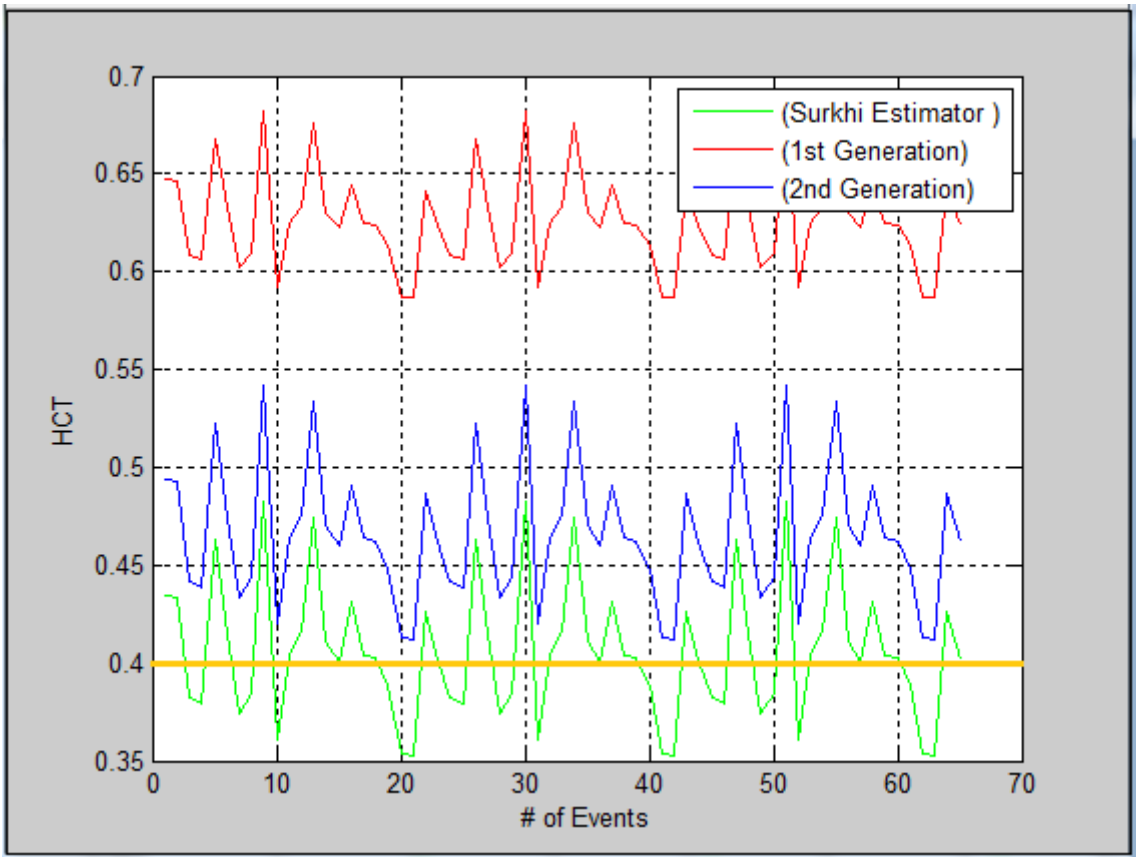

Figure 6.6: Haematocrit Value by First and Second Xitron Generation and Surkhi Estimator for sample15.

As you can notice from figure (6.6) Xitron first and second generation gives overestimation for HCT value which is indicated by the solid gold line (HCT=0.4). First generation gives more overestimation than second generation whereas the developed Surkhi estimator gives the best and the most nearest value from the theoretical value.

Also you can notice that difference from figure (6.7) below.

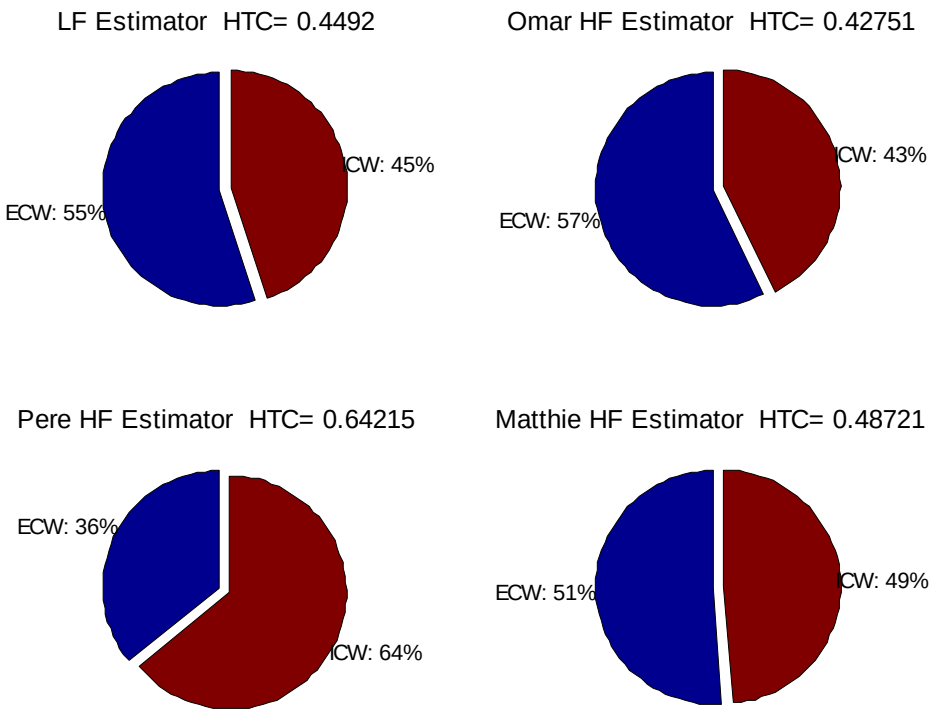

Figure 6.7: Comparison between Used and Developed Estimator in determining HCT value for sample15.

From the tables of determining HCT value by Xitron first, second generation and the developed Surkhi estimator, you can notice that the theoretical value for HCT of sample15 is 40 (Hospital value) and 37.5 (University value). From the above figures you can notice the difference between the accuracies of the used and developed estimators.

6.4.2. ECW, ICW Volumes Estimation:

This section presents the theoretical volumes. The volumes are estimated based upon Xitron first, second generations and the developed Surkhi Estimator.

6.4.2.1. Theoretical Volumes:

The theoretical volumes are found depending upon the volume of the used chamber and the values of HCT for each sample in order to compare the theoretical volumes with the estimated ones using Xitron first, second generations and the developed estimator. Table 6.4 shows those theoretical volumes.

Eq(5.4) describes the relationship between the HCT value and the volumes of Intracellular and Extracellular content, so the theoretical volumes can be determined according to the HCT value and the used chamber volume as shown in the following equations:

$$\mathrm{HCT} = \frac{\mathrm{V_{ICW}}}{\mathrm{Total\,Volume}} \qquad \textit{Eq(6.1)}$$

Where Total Volume = 13.6 cm^3.

$$\mathrm{V_{ICW}} = \mathrm{Total\,Volume} \times \mathrm{HCT} \qquad \textit{Eq(6.2)}$$

$$V_{ECW} = Total\ Volume - \mathrm{V_{ICW}} \qquad \textit{Eq(6.3)}$$

So using those equations, the theoretical volumes for Intracellular and Extracellular content can be determined.

Table 6.4: Theoretical Volumes .

Sample #	Sample	Hospital HCT	University HCT	HCT Mean*	Blood Volume	Theoretical V_{ICW}	Theoretical V_{ECW}
1	sample00	46.5	46	46.25	13.6	6.29	7.31
2	sample05	43.3	42.5	43.25	13.6	5.882	7.718
3	sample066	43	43.5	43.25	13.6	5.882	7.718
4	sample06	43.4	43.5	43.45	13.6	5.9092	7.691
5	sample07	47.6	45.5	46.55	13.6	6.3308	7.269
6	sample08	40.7	34	37.35	13.6	5.0796	8.520
7	sample09	47	43	45	13.6	6.12	7.480
8	sample10	42	42	42	13.6	5.712	7.888
9	sample11	45.8	42.5	44.15	13.6	6.0044	7.596
10	sample12	52.9	43	47.95	13.6	6.5212	7.079
11	sample13	34	32	33	13.6	4.488	9.112
12	sample14	42	40	41	13.6	5.576	8.024
13	sample15	40	37.5	38.75	13.6	5.27	8.330
14	sample16	58	58	58	13.6	7.888	5.712
15	sample17	39	37	38	13.6	5.168	8.432
16	sample18	48	47	47.5	13.6	6.46	7.140
17	sample19	39	38	38.5	13.6	5.236	8.364
18	sample20	51	51	51	13.6	6.936	6.664
19	sample21	40	38.5	39.25	13.6	5.338	8.262
20	sample22	58	57.5	57.75	13.6	7.854	5.746
21	sample23	31.6	32	31.8	13.6	4.3248	9.275
22	sample24	35.7	35	35.35	13.6	4.8076	8.792
23	sample25	41.5	41	41.25	13.6	5.61	7.990
24	sample26	42.3	40	41.15	13.6	5.5964	8.004
25	sample27	38.6	40	39.3	13.6	5.3448	8.255
26	sample28	24.9	25	24.95	13.6	3.3932	10.207
27	sample4e	46	45	45.5	13.6	6.188	7.412
28	sample 1_old	43	39.9	41.45	13.6	5.6372	7.963
29	sample 2_old	40	37	38.5	13.6	5.236	8.364
30	sample 3_old	38	34.3	36.15	13.6	4.9164	8.684
31	sample 4_old	39	31.8	35.4	13.6	4.8144	8.786
32	sample 5_old	35	29.4	32.2	13.6	4.3792	9.221
33	sample 6_old	33	27.3	30.15	13.6	4.1004	9.500

* HCT Mean: The average value of University and Hospital HCT.

6.4.2.2. First Generation Volumes Estimation:

Table 6.5 shows the results related to the volumes estimation depending upon Xitron first generation. Note that the volumes at low frequency are the same for all estimators but the difference will be in estimating the volumes at high frequencies, so each estimator gives a value for the intracellular volume content and so in calculating the extracellular volume.

Table 6.5: Xitron First Generation Volumes Estimation .

Sample#	Sample	HCT Mean*	V_{ICWLF}	V_{ECWLF}	V_{ICWHF}	V_{ECWHF}
1	sample00	46.25	8.010	5.590	9.295	4.305
2	sample05	43.25	7.256	6.344	9.207	4.393
3	sample066	43.25	7.429	6.171	9.12	4.480
4	sample06	43.45	7.110	6.490	9.312	4.288
5	sample07	46.55	7.110	6.490	9.227	4.373
6	sample08	37.35	6.220	7.380	8.821	4.779
7	sample09	45	7.087	6.513	9.604	3.996
8	sample10	42	6.863	6.737	9.108	4.492
9	sample11	44.15	7.007	6.593	8.969	4.631
10	sample12	47.95	7.098	6.502	9.146	4.454
11	sample13	33	5.395	8.205	7.612	5.988
12	sample14	41	6.775	6.825	8.939	4.661
13	sample15	38.75	6.109	7.491	8.733	4.867
14	sample16	58	8.646	4.954	10.233	3.367
15	sample17	38	6.496	7.104	8.732	4.868
16	sample18	47.5	7.656	5.944	9.346	4.254
17	sample19	38.5	6.342	7.258	8.495	5.105
18	sample20	51	8.034	5.566	9.602	3.998
19	sample21	39.25	6.457	7.143	8.695	4.905
20	sample22	57.75	8.653	4.947	10.308	3.292
21	sample23	31.8	6.278	7.322	7.931	5.669
22	sample24	35.35	6.396	7.204	7.822	5.778
23	sample25	41.25	6.869	6.731	8.178	5.422
24	sample26	41.15	6.952	6.648	8.347	5.253
25	sample27	39.3	6.690	6.910	8.122	5.478
26	sample28	24.95	4.896	8.704	6.439	7.161
27	sample4e	45.5	7.256	6.344	9.207	4.393
28	sample 1_old	41.45	5.740	7.860	9.331	4.269
29	sample 2_old	38.5	5.242	8.358	8.907	4.693
30	sample 3_old	36.15	4.597	9.003	8.925	4.675
31	sample 4_old	35.4	3.904	9.696	8.596	5.004
32	sample 5_old	32.2	3.665	9.935	8.468	5.132
33	sample 6_old	30.15	3.107	10.493	8.152	5.448

6.4.2.3. Second Generation Volumes Estimation:

Table 6.6 shows the results related to the volumes estimation depending upon Xitron second generation. As stated previously the difference from generation to another will be in estimating the volumes at high frequencies not at low frequencies.

Table 6.6: Xitron Second Generation Volumes Estimation .

Sample#	Sample	HCT Mean*	V_{ICWLF}	V_{ECWLF}	V_{ICWHF}	V_{ECWHF}
1	sample00	46.25	8.010	5.590	7.394	6.206
2	sample05	43.25	7.256	6.344	7.273	6.327
3	sample066	43.25	7.429	6.171	7.155	6.445
4	sample06	43.45	7.110	6.490	7.418	6.182
5	sample07	46.55	7.110	6.490	7.301	6.299
6	sample08	37.35	6.220	7.380	6.745	6.855
7	sample09	45	7.087	6.513	7.820	5.780
8	sample10	42	6.863	6.737	7.137	6.463
9	sample11	44.15	7.007	6.593	6.948	6.652
10	sample12	47.95	7.098	6.502	7.189	6.411
11	sample13	33	5.395	8.205	5.119	8.481
12	sample14	41	6.775	6.825	6.907	6.693
13	sample15	38.75	6.109	7.491	6.626	6.974
14	sample16	58	8.646	4.954	8.698	4.902
15	sample17	38	6.496	7.104	6.624	6.976
16	sample18	47.5	7.656	5.944	7.465	6.135
17	sample19	38.5	6.342	7.258	6.303	7.297
18	sample20	51	8.034	5.566	7.818	5.782
19	sample21	39.25	6.457	7.143	6.574	7.026
20	sample22	57.75	8.653	4.947	8.804	4.796
21	sample23	31.8	6.278	7.322	5.545	8.055
22	sample24	35.35	6.396	7.204	5.399	8.201
23	sample25	41.25	6.869	6.731	5.875	7.725
24	sample26	41.15	6.952	6.648	6.103	7.497
25	sample27	39.3	6.690	6.910	5.800	7.800
26	sample28	24.95	4.896	8.704	3.580	10.020
27	sample4e	45.5	7.256	6.344	7.273	6.327
28	sample 1_old	41.45	5.740	7.860	7.444	6.156
29	sample 2_old	38.5	5.242	8.358	6.863	6.737
30	sample 3_old	36.15	4.597	9.003	6.887	6.713
31	sample 4_old	35.4	3.904	9.696	6.439	7.161
32	sample 5_old	32.2	3.665	9.935	6.267	7.333
33	sample 6_old	30.15	3.107	10.493	5.841	7.759

6.4.2.4. Developed Surkhi Estimator Volumes Estimation:

Table 6.7 shows the results related to the volumes estimation depending upon the developed Surkhi Estimator.

Table 6.7: Developed Surkhi Estimator Volumes Estimation .

Sample#	Sample	HCT Mean*	V_{ICWLF}	V_{ECWLF}	V_{ICWHF}	V_{ECWHF}
1	sample00	46.25	8.010	5.590	6.597	7.003
2	sample05	43.25	7.256	6.344	6.473	7.127
3	sample066	43.25	7.429	6.171	6.351	7.249
4	sample06	43.45	7.110	6.490	6.622	6.978
5	sample07	46.55	7.110	6.490	6.501	7.099
6	sample08	37.35	6.220	7.380	5.935	7.665
7	sample09	45	7.087	6.513	7.039	6.561
8	sample10	42	6.863	6.737	6.334	7.266
9	sample11	44.15	7.007	6.593	6.140	7.460
10	sample12	47.95	7.098	6.502	6.387	7.213
11	sample13	33	5.395	8.205	4.325	9.275
12	sample14	41	6.775	6.825	6.099	7.501
13	sample15	38.75	6.109	7.491	5.814	7.786
14	sample16	58	8.646	4.954	7.966	5.634
15	sample17	38	6.496	7.104	5.812	7.788
16	sample18	47.5	7.656	5.944	6.670	6.930
17	sample19	38.5	6.342	7.258	5.489	8.111
18	sample20	51	8.034	5.566	7.037	6.563
19	sample21	39.25	6.457	7.143	5.761	7.839
20	sample22	57.75	8.653	4.947	8.079	5.521
21	sample23	31.8	6.278	7.322	4.739	8.861
22	sample24	35.35	6.396	7.204	4.597	9.003
23	sample25	41.25	6.869	6.731	5.064	8.536
24	sample26	41.15	6.952	6.648	5.290	8.310
25	sample27	39.3	6.690	6.910	4.990	8.610
26	sample28	24.95	4.896	8.704	2.865	10.735
27	sample4e	45.5	7.256	6.344	6.473	7.127
28	sample 1_old	41.45	5.740	7.860	6.649	6.951
29	sample 2_old	38.5	5.242	8.358	6.054	7.546
30	sample 3_old	36.15	4.597	9.003	6.078	7.522
31	sample 4_old	35.4	3.904	9.696	5.626	7.974
32	sample 5_old	32.2	3.665	9.935	5.453	8.147
33	sample 6_old	30.15	3.107	10.493	5.030	8.570

6.4.2.5. Comparison between Used and Developed Volumes Estimators:

As you can notice from the tables that present the volumes estimation, the developed Surkhi Estimator gives the best results for volumes estimation. If sample15 is taken as an example and the rational value (V_{ICW}/V_{ECW}) is found, the following figure will be obtained.

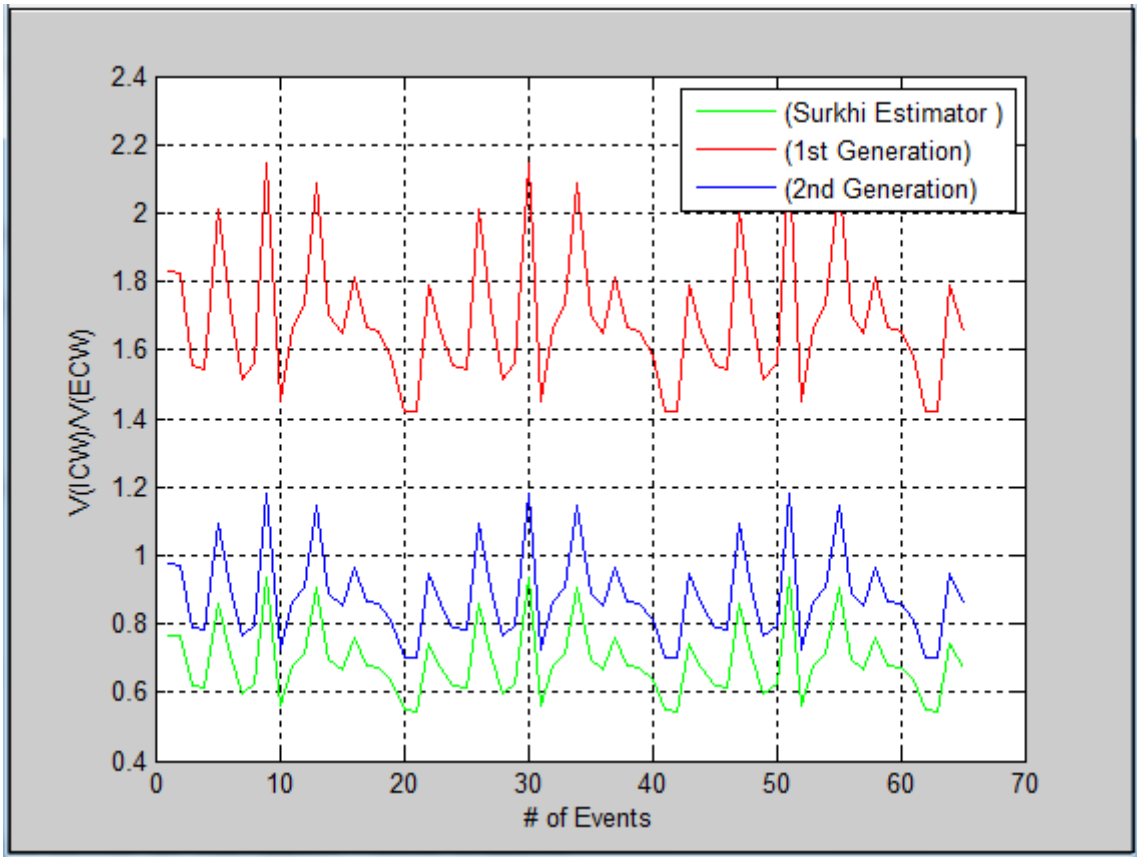

Figure 6.8: Comparison between Used and Developed Estimator in determining volumes for sample15.

Figure 6.8 gives the volumes estimation as a rational relation between Intracellular and Extracellular volume content. Referring to table 6.4 which includes the theoretical volumes, the value of the rational quantity (V_{ICW}/V_{ECW}) for sample15 is (5.27/8.33=0.633) and the Surkhi estimator gives the best estimation for that value . As a result the developed Surkhi estimator gives the best estimation for Intracellular and Extracellular volume content.

6.4.3. Cole-Cole Parameters

A common way to describe the behavior of tissue impedance as a function of frequency is to fit the measured data to a mathematical model. Cole-Cole Model is developed by Cole and Cole [1941] and is the most common used model as described in chapter three and Eq(3.6). Table 6.8 shows the obtained results for Cole-Cole parameters according to the Cole-Cole model.

Table 6.8: Cole-Cole Parameters .

Sample#	Sample	Ro (Ohm)	Rinf (Ohm)	Alfa	fc (KHz)
1	sample00	99.113	55.927	0.138	491.000
2	sample05	78.647	46.686	0.134	625.000
3	sample066	75.786	42.671	0.166	737.000
4	sample06	80.307	45.665	0.145	728.000
5	sample07	76.827	43.060	0.164	721.000
6	sample08	62.924	38.269	0.176	954.000
7	sample09	75.244	36.875	0.165	877.000
8	sample10	72.127	41.967	0.149	624.000
9	sample11	74.610	44.574	0.143	509.000
10	sample12	76.086	44.726	0.135	527.000
11	sample13	54.153	37.619	0.119	1342.000
12	sample14	71.505	46.003	0.093	802.000
13	sample15	61.790	39.693	0.136	780.000
14	sample16	115.139	55.365	0.067	515.000
15	sample17	67.264	40.369	0.146	597.000
16	sample18	87.913	49.463	0.112	519.000
17	sample19	64.979	43.145	0.090	478.000
18	sample20	96.770	50.800	0.112	518.000
19	sample21	66.503	45.164	0.081	620.000
20	sample22	115.257	52.642	0.096	506.000
21	sample23	62.998	44.340	0.143	676.000
22	sample24	64.597	47.137	0.079	712.000
23	sample25	72.581	47.149	0.102	800.000
24	sample26	74.066	49.758	0.075	650.000
25	sample27	70.147	48.132	0.084	631.000
26	sample28	49.519	42.157	0.041	490.000
27	sample4e	78.647	46.686	0.134	625.000
28	sample 1_old	58.797	34.195	0.167	673.000
29	sample 2_old	52.672	27.650	0.259	1220.000
30	sample 3_old	47.439	28.672	0.252	1160.000
31	sample 4_old	41.976	29.162	0.238	928.000
32	sample 5_old	40.449	28.319	0.266	1000.000
33	sample 6_old	37.574	24.828	0.350	1280.000

Chapter Seven: Discussion of Results

This chapter discusses and analyzes the obtained results that are presented in chapter five. First the results related to Haematocrit values are discussed and analyzed, then the results related to the volumes estimation are also be analyzed and the inaccuracies of the estimators will be indicated.

7.1 HCT Error (Absolute and Relative Error)

This section introduces the errors between the actual values of the Haematocrit and the estimated values based upon Xitron first, second generations and the developed Surkhi estimator.

7.1.1. Difference between HCT Hospital & University:

HCT Hospital is measured at HGH at the moment of collecting the blood sample. After collecting the blood sample, the sample is taken to the university for experimental works. The travel time plus the time of experiments are about three hours. In order to validate that the blood sample isn’t damaged or the HCT didn’t change significantly during this time

due to clotting, the HCT is measured again after taking bioimpedance measurements in the medical school at Al-Quds University.

The average of the differences between Hospital HCT values and University HCT values is found, the difference is very small and with an average of 2.124 as shown in table 7.1.

Table 7.1: Difference between HCT Hospital & University .

Sample#	Sample	Hospital HCT	University HCT	Difference
1	sample00	46.5	46	0.5
2	sample05	43.3	42.5	0.8
3	sample066	43	43.5	-0.5
4	sample06	43.4	43.5	-0.1
5	sample07	47.6	45.5	2.1
6	sample08	40.7	34	6.7
7	sample09	47	43	4
8	sample10	42	42	0
9	sample11	45.8	42.5	3.3
10	sample12	52.9	43	9.9
11	sample13	34	32	2
12	sample14	42	40	2
13	sample15	40	37.5	2.5
14	sample16	58	58	0
15	sample17	39	37	2
16	sample18	48	47	1
17	sample19	39	38	1
18	sample20	51	51	0
19	sample21	40	38.5	1.5
20	sample22	58	57.5	0.5
21	sample23	31.6	32	-0.4
22	sample24	35.7	35	0.7
23	sample25	41.5	41	0.5
24	sample26	42.3	40	2.3
25	sample27	38.6	40	-1.4
26	sample28	24.9	25	-0.1
27	sample4e	46	45	1
28	sample 1_old	43	39.9	3.1
29	sample 2_old	40	37	3
30	sample 3_old	38	34.3	3.7
31	sample 4_old	39	31.8	7.2
32	sample 5_old	35	29.4	5.6
33	sample 6_old	33	27.3	5.7
Difference Average				2.124

The following figure shows the differences between HCT Hospital and University:

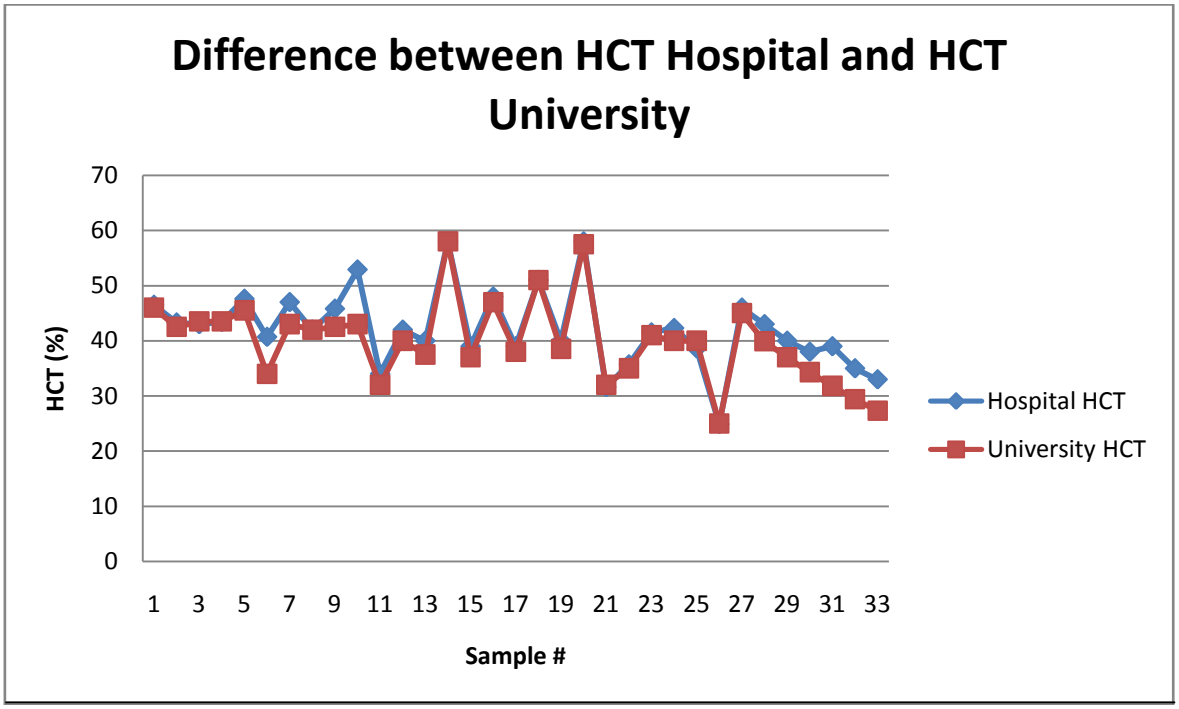

Figure 7.1: Difference between HCT Hospital and HCT University.

As shown in figure 7.1 there is no significant difference between the two measured values of HCT.

7.1.2. Accuracies of Estimators- HCT Error analysis (Absolute & Relative Error):

This section indicates the inaccuracies of the used and developed estimators by comparing the actual values of HCT measured in hospital and university with the estimated values using the estimators.

7.1.2.1. HCT absolute & relative error related to Hospital HCT:

Table 7.2 shows HCT absolute & relative error related to Hospital HCT by taking the mean values for all 33 samples.

Table 7.2: HCT absolute & relative error related to Hospital HCT .

	1st Generation			2nd Generation			Surkhi Estimator		
HCT Hospital	First Gen. HCT	Absolute Error	Relative Error %	Second Gen. HCT	Absolute Error	Relative Error (%)	Surkhi HCT	Absolute Error	Relative Error (%)
42.115	64.368	22.253	55.022	49.457	7.017	18.228	43.748	3.094	3.919

7.1.2.2. HCT absolute & relative error related to University HCT:

Table 7.3 shows HCT absolute & relative error related to University HCT by taking the mean values for all 33 samples.

Table 7.3: HCT absolute & relative error related to University HCT .

	1st Generation			2nd Generation			Surkhi Estimator		
HCT University	First Gen. HCT	Absolute Error	Relative Error (%)	Second Gen. HCT	Absolute Error	Relative Error (%)	Surkhi HCT	Absolute Error	Relative Error (%)
39.991	64.368	24.377	64.264	49.457	9.108	25.313	43.748	4.576	10.139

7.1.2.3. HCT absolute & relative error related to the mean of Hospital and University HCT:

Table 7.4 shows HCT absolute & relative error related to the mean value of Hospital HCT and University HCT by taking the mean values for all 33 samples.

Table 7.4: HCT absolute & relative error related to mean of Hospital & University HCT .

	1st Generation			2nd Generation			Surkhi Estimator		
HCT Mean*	First Gen. HCT	Absolute Error	Relative Error (%)	Second Gen. HCT	Absolute Error	Relative Error (%)	Surkhi HCT	Absolute Error	Relative Error (%)
41.064	64.368	23.304	59.288	49.457	8.543	21.502	43.748	3.684	6.792

7.2 Volumes Error (Absolute & Relative Error)

7.2.1. Low Frequency Extracellular Volume (Absolute & Relative Error):

Table 7.5 shows the low frequency extracellular volume absolute and relative error (Xitron First, Second generation and Surkhi Estimator) by taking the mean values for all 33 samples.

Table 7.5: Low frequency extracellular volume absolute and relative error .

HCT Mean*	Theoretical V_{ECW} (cm^3)	Estimated V_{ECWLF} (cm^3)	Absolute Error (cm^3)	Relative Error (%)
41.064	8.015	7.135	1.058	11.340

7.2.2. Low Frequency Intracellular Volume (Absolute & Relative Error):

Table 7.6 shows the low frequency intracellular volume absolute and relative error (Xitron first, second generations and Surkhi Estimator) by taking the mean values for all 33 samples.

Table 7.6: Low frequency intracellular volume absolute and relative error .

HCT Mean*	Theoretical V_{ICW} (cm^3)	Estimated V_{ICWLF} (cm^3)	Absolute Error (cm^3)	Relative Error (%)
41.064	5.585	6.465	1.058	83.215

7.2.3. High Frequency Extracellular Volume Absolute & Relative Error Related to Xitron First Generation:

Table 7.7 shows the High Frequency Extracellular Volume Absolute & Relative Error Related to Xitron First Generation by taking the mean values for all 33 samples.

Table 7.7: High Frequency Extracellular Volume Absolute & Relative Error Related to Xitron First Generation .

HCT Mean*	Theoretical V_{ECW} (cm^3)	Estimated V_{ECWHF} (cm^3)	Absolute Error (cm^3)	Relative Error (%)
41.064	8.015	4.784	3.231	40.31

7.2.4. High Frequency Intracellular Volume Absolute & Relative Error Related to Xitron First Generation:

Table 7.8 shows the High Frequency Intracellular Volume Absolute & Relative Error Related to Xitron First Generation by taking the mean values for all 33 samples.

Table 7.8: High Frequency Intracellular Volume Absolute & Relative Error Related to Xitron First Generation .

HCT Mean*	Theoretical V_{ICW} (cm^3)	Estimated V_{ICWHF} (cm^3)	Absolute Error (cm^3)	Relative Error (%)
41.064	5.585	8.816	3.231	57.85

7.2.5. High Frequency Extracellular Volume Absolute & Relative Error Related to Xitron Second Generation:

Table 7.9 shows the High Frequency Extracellular Volume Absolute & Relative Error Related to Xitron Second Generation by taking the mean values for all 33 samples.

Table 7.9: High Frequency Extracellular Volume Absolute & Relative Error Related to Xitron Second Generation .

HCT Mean*	Theoretical V_{ECW} (cm^3)	Estimated V_{ECWHF} (cm^3)	Absolute Error (cm^3)	Relative Error (%)
41.064	8.015	6.854	1.161	14.49

7.2.6. High Frequency Intracellular Volume Absolute & Relative Error Related to Xitron Second Generation:

Table 7.10 shows the High Frequency Intracellular Volume Absolute & Relative Error Related to Xitron Second Generation by taking the mean values for all 33 samples.

Table 7.10: High Frequency Intracellular Volume Absolute & Relative Error Related to Xitron Second Generation .

HCT Mean*	Theoretical V_{ICW} (cm^3)	Estimated V_{ICWHF} (cm^3)	Absolute Error (cm^3)	Relative Error (%)
41.064	5.585	6.746	1.161	20.79

7.2.7. High Frequency Extracellular Volume Absolute & Relative Error Related to Surkhi Estimator:

Table 7.11 shows the High Frequency Extracellular Volume Absolute & Relative Error Related to Surkhi Estimator by taking the mean values for all 33 samples.

Table 7.11: High Frequency Extracellular Volume Absolute & Relative Error Related to Surkhi Estimator .

HCT Mean*	Theoretical V_{ECW} (cm^3)	Estimated V_{ECWHF} (cm^3)	Absolute Error (cm^3)	Relative Error (%)
41.064	8.015	7.650	0.365	4.55

7.2.8. High Frequency Intracellular Volume Absolute & Relative Error Related to Surkhi Estimator:

Table 7.12 shows the High Frequency Intracellular Volume Absolute & Relative Error Related to Surkhi Estimator by taking the mean values for all 33 samples.

Table 7.12: High Frequency Intracellular Volume Absolute & Relative Error Related to Surkhi Estimator .

HCT Mean*	Theoretical V_{ICW} (cm^3)	Estimated V_{ICWHF} (cm^3)	Absolute Error (cm^3)	Relative Error (%)
41.064	5.585	5.950	0.365	6.54

7.2.9. A Summary of Volumes Absolute and Relative Errors:

Table 7.13 shows a summary of the volumes absolute and relative error related to the used estimators.

Table 7.13-A: Volumes Absolute & Relative Errors Related to Xitron First Generation .

Theoretical V_{ICW}(cm^3)	**Measured V_{ICWLF}(cm^3)**	**1st Gen V_{ICWHF} (cm^3)**	**Absolute Error (cm^3)**	**Relative Error (%)**
5.585	6.465	8.816	3.231	57.85

Table 7.13-B: Volumes Absolute & Relative Errors Related to Xitron Second Generation .

Theoretical V_{ICW}(cm^3)	**Measured V_{ICWLF}(cm^3)**	**2nd Gen V_{ICWHF} (cm^3)**	**Absolute Error (cm^3)**	**Relative Error (%)**
5.585	6.465	6.746	1.161	20.79

Table 7.13-C: Volumes Absolute & Relative Errors Related to Surkhi Estimator .

Theoretical $V_{ICW}(cm^3)$	**Measured $V_{ICWLF}(cm^3)$**	**Surkhi $V_{ICW\ HF}(cm^3)$**	**Absolute Error (cm^3)**	**Relative Error (%)**
5.585	6.465	5.950	0.365	6.54

As can be noticed from table 7.13, Surkhi Estimator gives the smallest absolute and relative errors compared to Xitron first and second generations. Therefore Surkhi Estimator has the nearest volume values from the theoretical ones.

Chapter Eight: Conclusions, Limitations and Future Work

8.1 Conclusions

The following points can be noticed from the tables listed in chapter seven as conclusion:

- There is no great difference between HCT Hospital and University readings (ΔHCT= 2.124); so the blood samples are carefully protected.

- Surkhi Estimator gives us the best results for HCT values with relative error between 3% and 10%.

- Xitron First Generation has the highest error in determining HCT values with relative error of 55%.

- Low Frequency Volumes Estimation are the same for all generations.

- High Frequency Volumes Estimation differ from generation to other.

- Xitron First & Second Genertaion Volumes Estimation are overestimated compared to the intracellular volumes with relative error of 58% for first generation and 21% for second generation.

- Xitron First Generation is overestimated than Xitron Second Generation.

- Surkhi Estimator gives us the best results for volumes estimation with relative error between 4% and 6.8%.

- The characteristic frequency of the samples occurs between 478 KHz and 1342KHz (mean=910KHz). This result fits with the results in literature. Ulgen & Sezdi in 1998 (937 KHz) [16], Gaw in 2010 (700 KHz to 1 MHz) [15].

In this research, electrical impedance spectroscopy of blood is performed at six frequencies which are: 10, 20, 50, 100, 220 and 500KHz using the two probe method in order to determine the Cole-Cole parameters, namely the resistances R_∞ and R_0, the characteristic frequency (f_c) and the parameter α ($0 < \alpha \leq 1$). Only those six frequencies are used in fitting the Cole circle, where the effects of electrode polarization are negligible. Fitting needs three readings but six readings are taken then the rest of readings are found using Cole-Cole model. As human blood tissue is used in this research the used frequency can't be below 1KHz in order not to conflict with the human body systems such as heart rate for an example. From the results you notice that go above 500KHz make the results noisy so those six frequencies are chosen to take measurements then fitting using Cole-Cole model was used.

All the measurements are done at room temperature (25°C) where as the blood samples are well preserved and tested.

It is shown that this is acceptable since the characteristic frequency of samples occurs between 478 KHz and 1342KHz depending upon the value of the sample HCT. This result matches what is founded by Richelle Leanne Gaw in his thesis in the year 2010 as he

mentioned that the characteristic frequency (f_c) of human blood is in the order of 700 KHz to 1 MHz [15].

The characteristic frequency (fc) gets lower for each anticoagulant as the volume occupied by red blood cells become greater implying a larger effective cell membrane capacitance.

In previous results of Dellimore and Gosling (1975) (a time constant) between 40 and 50 second is reported. Whereas Yamakoshi and Tanaka (1994) reported that the measured frequency is 50 KHz [15].

Bernard Rigaudt, Leila Hamzaouit, Mohamed Ridha Frikhaf, Nicolas Chauveaut and Jean-Pierre Moruccit (1995) had worked upon many tissues such as Muscle, Liver, Lung and Spleen and found that the characteristic frequency (fc) is 27KHz for Muscle, 72 KHz for Liver, 140 KHz for Lung and 373 KHz for Spleen.

DeLorenzo (1997) had worked with Total Body Potassium (TBK) and diluted samples for men and women and his study reported the following results: TBK(Men: 57.02±8.39KHz, Women: 80.14±17.22KHz, Combined: 60.19±12.83). Dilution, Men: 61.96±5.95KHz [10].

The characteristic frequency of stationary blood is reported to be 937 KHz by Ulgen & Sezdi (1998) [16].

Grimnes & Martinsen 2000 stated in their study that the characteristic frequency increase after sedimentation begins[15].

In previous results of Ward and Stroud 2001, the characteristic frequency is reported as 50 KHz.

Richelle Leanne Gaw (2010) reported that the characteristic frequency of bovine blood in the order of 1500 KHz and for stationary blood as 1948 KHz. Also he had investigated the relationship between the characteristic frequency and sedimentation and reported that 15 minutes after sedimentation begins, the characteristic frequency of blood is 1.2 MHz. 90 –

270 minutes after sedimentation begins, the characteristic frequency increased to 1.5 MHz [15].

Resistivity of blood is complex and is a function of many parameters such as: the shape of red blood cells, the volume concentration of red cells (Haematocrit), the type of anticoagulant, the temperature and the frequency.

Because each sample has a different Haematocrit values either normalization is necessary or else the results should be displayed as a function of Haematocrit (h).

At 100% Haematocrit the extracellular conductivity is zero.

As mentioned before that the value of the parameter α is between $(0 < \alpha \leq 1)$ and according to the measurements that are done during this work, the value of α is between 0.041 and 0.349 .

The value of R_0 in this work varies between 37.574 Ohm and 115.257 Ohm depending upon the value of the sample HCT.

The value of R_∞ in this work varies between 24.828 Ohm and 55.927 Ohm depending upon the value of the sample HCT.

8.2 Limitations

The blood samples are collected from Hebron Governmental Hospital at Hebron city and must be transported from Hebron to Jerusalem without damaging human blood tissue. The transportation time of human blood samples from Hebron to Jerusalem and the experimental time was very long, so we can't work with more than one sample a day to make sure the blood samples do not damage.

By using Microtest Impedance Analyzer instrument, raw data are not easily accessible, no driver available and it is hard to connect to labview. Local Impedance Analyzer is more friendly to be used.

8.3 However its worth to mention?

All this research work including the experimental and lab work, data analysis and instrumentation are performed at the labs of engineering school (Material Engineering Lab) plus medicine school at Al-Quds University.

8.4 Recommendation for Future Work

As shown and concluded from this thesis results, Surkhi Estimator gives the best results for determining volumes and Haematocrit values with the small error compared to the theoretical values. Therefore I recommend to use this estimator by bioimpedance systems producing companies. For an example Surkhi Estimator can be used to monitor babies growth or to differentiate between normal and cancerous tissues as cancerous tissues have more water content than normal tissues. Add to that Surkhi Estimator can be used in designing Haemodialysis machines to monitor the fluid volumes.

Also I recommend the people who are interested in this subject to complete this work depending upon the following points:

- Testing blood samples on a wide range of frequencies and using more than the six used frequencies in this work and try to work on a higher range of frequencies.

- Evaluate tissue parameters out of the Bioimpedance measurements such as Normal or pathological conditions of the membrane.

- Investigate different blood samples with different medical conditions (different HCT values, different health conditions) in correlation with bioimpedance.

- Study the correlations between Cole-Cole parameters and blood HCT value.

- Investigate animal blood.

- Working on other biological tissue such as spleen, liver and others.

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Appendices

Appendix A: The Outgoing Books

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Al-Quds University
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التاريخ:2012/01/15

حضرة الدكتور نعيم صبرة مدير عام مستشفيات الضفة الغربية المحترم

تحية طيبة وبعد،،،

تهديكم كلية الهندسة في جامعة القدس أجمل التحيات ونرجو من حضرتكم الإيعاز إلى مستشفى الخليل الحكومي للتعاون مع طالبة الدراسات العليا إيناس قباجة (هندسة طبية) التي تود إجراء مجموعة من القياسات ضمن بحثها لرسالة الماجستير على بعض العينات لدى مختبر الدم التابع لمستشفى الخليل الحكومي.

وشكرا جزيلا لحسن تعاونكم،،،

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15 -01- 2012

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Appendix B: MATLAB Codes

1. Matlab M-File for Volumes and Haematocrit Estimation

```
F=[10000 20000 50000 100000 220000 500000];

file=input('Enter file name: ','s');

dat=load(file);

daat=mean(dat(2:length(dat),:));

c1=1;
c2=1;
L=1;

zd=daat(1,8:13);
pd=daat(1,26:31);

z=zd';
ph=pd';
f=F';

dib=1;
 % z module
 % phase
 % frequencies

r(:,1)=(z(:,1).*cos(ph(:,1)*pi/180));% resistance
img(:,1)=(z(:,1).*sin(ph(:,1)*pi/180));    % reactance

 [ro,ri,alf,fc,ecm2]=colez(r,img,f,dib);

% Function for Cole-Cole Parameters & Impedancies
% Outputs:
% ro=resistence zero
% ri=resistence infinity
```

```
% alf=angle alfa
% fc=central frequency
% ecm=error quadratice media

% Inputs:
% r=real part of the impedance vector
% i= imaginary part of the frequency vector
% f= frequencies vector
% dib= 0 for no depression, 1 for depression of the curve module



% Calculation of the center y for the radius of the circle frequencies
% approximation
img=abs(img);
 n=length(f);
for ii=1:4
    for jj=1:4
    m(ii,jj)=mean(r.^(ii-1).* img.^(jj-1));
    end
end
sigmx2=m(3,1)-m(2,1)^2;
sigmxy=m(2,2)-m(1,2)*m(2,1);
sigmy2=m(1,3)-m(1,2)^2;
t1=(-m(4,1)+m(2,1)*m(3,1)-m(2,3)+m(2,1)*m(1,3));
t2=(-m(1,4)+m(1,2)*m(1,3)-m(3,2)+m(1,2)*m(3,1));
t3=sigmx2*sigmy2-sigmxy^2;
a=-0.5*(sigmy2*t1-sigmxy*t2)/t3;
b=-0.5*(sigmx2*t2-sigmxy*t1)/t3;
% b=-abs(b);
radius=mean(sqrt((r-a).^2+(img-b).^2));

% Calculation of  ro,ri,alf
ro=a+(radius^2-b^2)^0.5;
ri=a-(radius^2-b^2)^0.5;
alf=1-(2/pi)*asin((ro-ri)/(2*radius));

% Calculation of fc
u=((r.^2-2*ri.*r+img.^2)+(ri^2)).^0.5;
v=((r.^2-2*ro.*r+img.^2)+(ro^2)).^0.5;
ww=log10(abs(u./v));
```

```
p=log10(2*pi*f);
h=polyfit(p,ww,1);
fc=((1/(2*pi))*10^(-h(2)/h(1)));

% Calculation of the error quadratic media

ye=(-abs(b)+(radius^2.-(r-a).^2).^0.5);
er=(abs(ye)-abs(img))./radius;
ecm=(((sum(er.^2))^0.5)/n)*100;
er2=(abs(ye)-abs(img))./abs(img);
ecm2=(((sum(er2.^2))^0.5)/n)*100;
ecm3=(sum(abs(er)))*100/n;

if dib==1,

    axis(axis);
    dd=ro/ri;
    dd=dd/150;
    xx=[ri:dd:ro];
    c=1-(sin((pi/2)*(1-alf)))^2;
    a=(ro+ri)/2;
    b=((ro-ri)/2)*(c/(1-c))^0.5;
    radius=(((ro-a)^2)+b^2)^0.5;
    yy=(-abs(b)+(radius^2.-(xx-a).^2).^0.5);
    figure(1)
    plot(xx,abs(yy),'r');
    drawnow;
    axis image
    hold on;
    %plot(r,img,'o')
end;
%ro
%ri
%alf
%fc
%ecm2

%TTV =(0.25/pi)*(c2*c2-0.25*(c2-c1)^2)
%K=L^2*47*(TTV)^-0.5;
%ECW_cm3=K*(ro)^(-2/3)
```

```
Recavg=ro;
Roavg=ro;
Rinfavg=ri;
Ricavg=(ri*ro)/(ro-ri);

ff=[100:100:1000000];
dr=ro-ri;
 zf=dr./(1+(i*ff./fc).^(1-alf))+ri;

 figure(2)
semilogx(f,z,'-ro')
title ('Z mod')

semilogx(ff,abs(zf))

figure(3)

 semilogx(f,ph,'ro')

title('Phase')

semilogx(ff, 57.2957795*phase(zf))

AAAavg=[Roavg,Rinfavg,Recavg,Ricavg]



clear ro ri daat
```

```
% 'Select the time of intrest between  ',[ 0 length(dat) ]

%j=input (' Time: ')

daat=dat(2:length(dat),:);

%clean artifacts from data
for j=1:37
for i=1:length(daat)
    if (daat(i,j))> 1.1*(median(daat(:,j)))
     daat(i,j)= median(daat(:,j));

    end
end
end

for j=1:37
for i=1:length(daat)
    if (daat(i,j))< 0.9*(median(daat(:,j)))
     daat(i,j)= median(daat(:,j));

    end
end
end

% step 2: plot all data

for aaa=1:length(daat)

c1=1;
c2=1;
L=1;

zd=daat(aaa,8:13);
pd=daat(aaa,26:31);
```

```
z=zd';
ph=pd';
f=F';
dib=1;
 % z module
 % phase
 % frequencies

r(:,1)=(z(:,1).*cos(ph(:,1)*pi/180));% resistance
img(:,1)=(z(:,1).*sin(ph(:,1)*pi/180));    % reactance


 [ro,ri,alf,fc,ecm2]=colez(r,img,f,dib);

% Function for Cole-Cole Parameters & Impedancies
% Outputs:
% ro=resistence zero
% ri=resistence infinity
% alf=angle alfa
% fc=central frequency
% ecm=error quadratice media

% Inputs:
% r=real part of the impedance vector
% i= imaginary part of the frequency vector
% f= frequencies vector
% dib= 0 for no depression, 1 for depression of the curve module


% Calculation of the center y for the radius of the circle frequencies
% approximation
img=abs(img);
 n=length(f);
for ii=1:4
    for jj=1:4
    m(ii,jj)=mean(r.^(ii-1).* img.^(jj-1));
    end
end
sigmx2=m(3,1)-m(2,1)^2;
sigmxy=m(2,2)-m(1,2)*m(2,1);
```

```
sigmy2=m(1,3)-m(1,2)^2;
t1=(-m(4,1)+m(2,1)*m(3,1)-m(2,3)+m(2,1)*m(1,3));
t2=(-m(1,4)+m(1,2)*m(1,3)-m(3,2)+m(1,2)*m(3,1));
t3=sigmx2*sigmy2-sigmxy^2;
a=-0.5*(sigmy2*t1-sigmxy*t2)/t3;
b=-0.5*(sigmx2*t2-sigmxy*t1)/t3;
% b=-abs(b);
radius=mean(sqrt((r-a).^2+(img-b).^2));

% Calculation of ro,ri,alf
ro=a+(radius^2-b^2)^0.5;
ri=a-(radius^2-b^2)^0.5;
alf=1-(2/pi)*asin((ro-ri)/(2*radius));

% Calculation of fc
u=((r.^2-2*ri.*r+img.^2)+(ri^2)).^0.5;
v=((r.^2-2*ro.*r+img.^2)+(ro^2)).^0.5;
ww=log10(abs(u./v));
p=log10(2*pi*f);
h=polyfit(p,ww,1);
fc=((1/(2*pi))*10^(-h(2)/h(1)));

% Calculation of the error quadratic media

ye=(-abs(b)+(radius^2.-(r-a).^2).^0.5);
er=(abs(ye)-abs(img))./radius;
ecm=(((sum(er.^2))^0.5)/n)*100;
er2=(abs(ye)-abs(img))./abs(img);
ecm2=(((sum(er2.^2))^0.5)/n)*100;
ecm3=(sum(abs(er)))*100/n;

if dib==1,

    axis(axis);
    dd=ro/ri;
    dd=dd/150;
    xx=[ri:dd:ro];
    c=1-(sin((pi/2)*(1-alf)))^2;
    a=(ro+ri)/2;
    b=((ro-ri)/2)*(c/(1-c))^0.5;
```

```
    radius=(((ro-a)^2)+b^2)^0.5;
    yy=(-abs(b)+(radius^2.-(xx-a).^2).^0.5);
    figure(4)
    plot(xx,abs(yy),'r');
    drawnow;
    axis image
    hold on;
    %plot(r,img,'o')
end;
%ro
%ri
%alf
%fc
%ecm2

%TTV =(0.25/pi)*(c2*c2-0.25*(c2-c1)^2)
%K=L^2*47*(TTV)^-0.5;
%ECW_cm3=K*(ro)^(-2/3)


Rec=ro;
Ro=ro;
Rinf=ri;
Ric=(ri*ro)/(ro-ri);


AAA(aaa,:)=[Ro,Rinf,Rec,Ric];
end


%clear i
%for j=1:4
%for i=1:length(AAA)
 %   if (AAA(i,j))> 1.1*(median(AAA(:,j)))
  %     AAA(i,j)= median(AAA(:,j));

   % end
%end
%end
```

```
VicVecOmar=1.19*(AAA(:,1)./AAA(:,2))-1.18;
VicVecPere=2.22*(AAA(:,1)./AAA(:,2))-1.8;
VicVecMath=1.47*(AAA(:,1)./AAA(:,2))-1.43;

HTCOmar=1./(1+(1./VicVecOmar));
HTCPere=1./(1+(1./VicVecPere));
HTCMath=1./(1+(1./VicVecMath));
HTCOmar=abs(HTCOmar);
HTCPere=abs(HTCPere);
HTCMath=abs(HTCMath);

%  Standard Deviation
HTCPere_sd=std(HTCPere);
HTCMath_sd=std(HTCMath)
HTCOmar_sd=std(HTCOmar);



figure(6)
plot(VicVecOmar,'g')
hold on
plot(VicVecPere,'r')
plot(VicVecMath,'b')
xlabel('time')
ylabel('Vic/Vec')
legend('(Omar )','(Pere)','(Matthie)',1)
grid on

figure(7)
plot(HTCOmar,'g')
hold on
plot(HTCPere,'r')
plot(HTCMath,'b')
xlabel('time')
ylabel('HTC')
```

```
legend('(Omar )','(Pere)','(Matthie)',1)
grid on


% omar estimators based on LF and HF
% LF
resplasma= 70.53;

VECLF=((resplasma*2.21^2*13.6^0.5)/AAAavg(1))^(2/3);
VICLF=13.6-VECLF;
HTCLF=VICLF/13.6;

%HF
VicVecOmar=1.19*(AAAavg(1)/AAAavg(2))-1.18;
VicVecPere=2.22*(AAAavg(1)/AAAavg(2))-1.8;
VicVecMath=1.47*(AAAavg(1)/AAAavg(2))-1.43;

HTCHFomar=1/(1+(1/VicVecOmar));
HTCHFpere=1/(1+(1/VicVecPere));
HTCHFmath=1/(1+(1/VicVecMath));



VICHFomar=HTCHFomar*13.6;
VECHFomar=13.6-VICHFomar;

VICHFpere=HTCHFpere*13.6;
VECHFpere=13.6-VICHFpere;

VICHFmath=HTCHFmath*13.6;
VECHFmath=13.6-VICHFmath;



Aresult=[mean(HTCOmar) mean(HTCPere) mean(HTCMath)]
```

```
%.....................................................
% plot using charts
figure (10)

subplot(2,2,1)

x = [VECLF VICLF ];
explode = [0 1 ];
h=pie(x,explode);
colormap JET



textObjs = findobj(h,'Type','text');
oldStr = get(textObjs,{'String'});
val = get(textObjs,{'Extent'});
oldExt = cat(1,val{:});

Names = {'ECW: ';'ICW: '};
newStr = strcat(Names,oldStr);
set(textObjs,{'String'},newStr,'FontSize',8)



val1 = get(textObjs, {'Extent'});
newExt = cat(1, val1{:});
offset = sign(oldExt(:,1)).*(newExt(:,3)-oldExt(:,3))/2;
pos = get(textObjs, {'Position'});
textPos =  cat(1, pos{:});
textPos(:,1) = textPos(:,1)+offset;
set(textObjs,{'Position'},num2cell(textPos,[3,2]))

s='LF Estimator  HTC= ';
 st=[s num2str(HTCLF)]
title(st,'FontSize',10)

%*********************************************
subplot(2,2,2)
```

```
x = [VECHFomar VICHFomar ];
explode = [0 1 ];
h=pie(x,explode);
colormap JET


textObjs = findobj(h,'Type','text');
oldStr = get(textObjs,{'String'});
val = get(textObjs,{'Extent'});
oldExt = cat(1,val{:});

Names = {'ECW: ';'ICW: '};
newStr = strcat(Names,oldStr);
set(textObjs,{'String'},newStr,'FontSize',8)



val1 = get(textObjs, {'Extent'});
newExt = cat(1, val1{:});
offset = sign(oldExt(:,1)).*(newExt(:,3)-oldExt(:,3))/2;
pos = get(textObjs, {'Position'});
textPos =  cat(1, pos{:});
textPos(:,1) = textPos(:,1)+offset;
set(textObjs,{'Position'},num2cell(textPos,[3,2]))

s='Omar HF Estimator  HTC= ';
 st=[s num2str(HTCHFomar)]
title(st,'FontSize',10)

%*****************************
subplot(2,2,3)

x = [VECHFpere VICHFpere ];
explode = [0 1 ];
h=pie(x,explode);
colormap JET
```

```
textObjs = findobj(h,'Type','text');
oldStr = get(textObjs,{'String'});
val = get(textObjs,{'Extent'});
oldExt = cat(1,val{:});

Names = {'ECW: ';'ICW: '};
newStr = strcat(Names,oldStr);
set(textObjs,{'String'},newStr,'FontSize',8)



val1 = get(textObjs, {'Extent'});
newExt = cat(1, val1{:});
offset = sign(oldExt(:,1)).*(newExt(:,3)-oldExt(:,3))/2;
pos = get(textObjs, {'Position'});
textPos =  cat(1, pos{:});
textPos(:,1) = textPos(:,1)+offset;
set(textObjs,{'Position'},num2cell(textPos,[3,2]))

s='Pere HF Estimator  HTC= ';
 st=[s num2str(HTCHFpere)]
title(st,'FontSize',10)
%*******************************************
subplot(2,2,4)

x = [VECHFmath VICHFmath ];
explode = [0 1 ];
h=pie(x,explode);
colormap JET



textObjs = findobj(h,'Type','text');
oldStr = get(textObjs,{'String'});
val = get(textObjs,{'Extent'});
oldExt = cat(1,val{:});

Names = {'ECW: ';'ICW: '};
newStr = strcat(Names,oldStr);
set(textObjs,{'String'},newStr,'FontSize',8)
```

```
val1 = get(textObjs, {'Extent'});
newExt = cat(1, val1{:});
offset = sign(oldExt(:,1)).*(newExt(:,3)-oldExt(:,3))/2;
pos = get(textObjs, {'Position'});
textPos =  cat(1, pos{:});
textPos(:,1) = textPos(:,1)+offset;
set(textObjs,{'Position'},num2cell(textPos,[3,2]))

s='Matthie HF Estimator  HTC= ';
 st=[s num2str(HTCHFmath)]
title(st,'FontSize',10)
```

2. Matlab M-file for Finding Cole-Cole Parameters

```
% This program finds the Cole-Cole parameters for the Z file.
% input file: sample***.CAL
% output : Cole data in the data table called Cole in the order of
%cole=[roo rii fcc alff];

F=[10000 20000 50000 100000 220000 500000];

file=input('Enter file name: ','s');

dat=load(file);



daat=(dat(2:length(dat),:));

for hh=1:length(daat)

zd=daat(hh,8:13);
pd=daat(hh,26:31);

z=zd';
ph=pd';
f=F';



dib=1;

r(:,1)=(z(:,1).*cos(ph(:,1)*pi/180));% resistance
img(:,1)=(z(:,1).*sin(ph(:,1)*pi/180));    % reactance


 [ro,ri,alf,fc,ecm2]=colez(r,img,f,dib);



% Function for Cole-Cole Parameters & Impedancies
% Outputs:
```

```
% ro=resistence zero
% ri=resistence infinity
% alf=angle alfa
% fc=central frequency
% ecm=error quadratice media

% Inputs:
% r=real part of the impedance vector
% i= imaginary part of the frequency vector
% f= frequencies vector
% dib= 0 for no depression, 1 for depression of the curve module



% Calculation of the center y for the radius of the circle frequencies
% approximation
img=abs(img);
 n=length(f);
for ii=1:4
    for jj=1:4
    m(ii,jj)=mean(r.^(ii-1).* img.^(jj-1));
    end
end
sigmx2=m(3,1)-m(2,1)^2;
sigmxy=m(2,2)-m(1,2)*m(2,1);
sigmy2=m(1,3)-m(1,2)^2;
t1=(-m(4,1)+m(2,1)*m(3,1)-m(2,3)+m(2,1)*m(1,3));
t2=(-m(1,4)+m(1,2)*m(1,3)-m(3,2)+m(1,2)*m(3,1));
t3=sigmx2*sigmy2-sigmxy^2;
a=-0.5*(sigmy2*t1-sigmxy*t2)/t3;
b=-0.5*(sigmx2*t2-sigmxy*t1)/t3;
% b=-abs(b);
radius=mean(sqrt((r-a).^2+(img-b).^2));

% Calculation of ro,ri,alf

ro=a+(radius^2-b^2)^0.5;
ri=a-(radius^2-b^2)^0.5;
alf=1-(2/pi)*asin((ro-ri)/(2*radius));
```

```
% Calculation of fc
u=((r.^2-2*ri.*r+img.^2)+(ri^2)).^0.5;
v=((r.^2-2*ro.*r+img.^2)+(ro^2)).^0.5;
ww=log10(abs(u./v));
p=log10(2*pi*f);
h=polyfit(p,ww,1);
fc=((1/(2*pi))*10^(-h(2)/h(1)));



    roo(hh,1)=ro;
    rii(hh,1)=ri;
    fcc(hh,1)=fc;
    alff(hh,1)=alf;

    cole=[roo rii fcc alff];

% Calculation of the error quadratic media

ye=(-abs(b)+(radius^2.-(r-a).^2).^0.5);
er=(abs(ye)-abs(img))./radius;
ecm=(((sum(er.^2))^0.5)/n)*100;
er2=(abs(ye)-abs(img))./abs(img);
ecm2=(((sum(er2.^2))^0.5)/n)*100;
ecm3=(sum(abs(er)))*100/n;

if dib==1,

    axis(axis);
    dd=ro/ri;
    dd=dd/150;
    xx=[ri:dd:ro];
    c=1-(sin((pi/2)*(1-alf)))^2;
    a=(ro+ri)/2;
    b=((ro-ri)/2)*(c/(1-c))^0.5;
    radius=(((ro-a)^2)+b^2)^0.5;
    yy=(-abs(b)+(radius^2.-(xx-a).^2).^0.5);
    figure(1)
    plot(xx,abs(yy),'r');
    drawnow;
    axis image
```

```
    hold on;

end;
end;
```

وصف أنسجة الدم باستخدام القياسات الأومية الحيوية

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الملخص

القياسات الأومية الحيوية ليست من الحقول البحثية الجديده، إذ إن العمل في هذا النوع من البحوث بدأ في نهاية العام 1980 لكن منذ القرن الماضي اهتم العديد من الباحثين ومن مختلف المجالات في هذا النوع من البحوث حيث أن العلماء من مختلف الخلفيات البيولوجيه والصناعية والطبية قد قدموا الكثير من البحث والتطوير حيث قاموا بالجمع ما بين التكنولوجيا الحديثة والالكترونيات والبحوث الطبية والقياسات الأومية الحيوية.

هذه التقنية لها الكثير من المزايا والإيجابيات مقارنه مع غيرها من التقنيات حيث أنها تمتاز بالتكلفة المنخفضه للقياسات و الأجهزة، وسهولة الاستخدام، والقدرة على التطبيق غير الجراحي، وتسمح بالمراقبة المباشرة، بالاضافة الى قلة المخاطر المرتبطة بهذا النوع من القياسات.

بينما تعتبر المقاومة من الخصائص المهمه لأي ماده فإن القياسات الأومية الحيويه تستطيع إعطاء معلومات قيمة عن النسيج تحت القياس، وبالتالي فإنه بالإمكان تشخيص النسيج أو مراقبة الحدوث الفسيولوجية. في بعض الأحيان تعتبر القياسات الأومية الحيوية بديلاً مهماً للقياسات التي لا تحتاج الى جراحة. ويتم عمل القياسات الأومية الحيوية باستخدام مصدر تيار خارجي ثم يتم استخدام هذه القياسات لتحديد صفات الأنسجة تحت القياس أو الحصول على معلومات أخرى باستخدام القياسات الأومية.

يشتمل العمل في هذه الرسالة على مرحلتين تبعاً للعمل باستخدام طريقة محاكاة الأنسجة البيولوجية أو العمل على الأنسجة البيولوجية البشريه وتحديداً الأنسجة الدموية، وبالتالي يمكن تقسيم العمل بحيث يشتمل على المختبر الجاف الذي منه نحصل على القياسات الأولى والمختبر الرطب والذي فيه يتم تجميع القياسات التجريبية والقياسات البحثية على التوالي .

من المرحلتين المذكورتين أعلاه يتم الحصول على القياسات الاومية ثم يتم ترجمة هذه القيم والقياسات من أجل الحصول على المعلومات القيمه عن النسيج تحت القياس أو لوصف الأنسجة البيولوجية ونحن نتحدث هنا عن أنسجة الدم تحديدا كما ذكرنا سابقا، وبالتالي يتم ترجمة هذه المعلومات إلى معلومات عن قيمة نسبة خلايا الدم الحمراء في محتوى الدم، والمحتوى المائي داخل خلايا الدم وخارجه.

تم بناء برنامج خاص باستخدام برنامج اللاب فيو لأخذ قياسات أومية بشكل متواصل، وتم استخدام 33 عينة دم طازجة. لكل عينة دم تم أخذ القياسات الأومية على ستة ترددات وهي الترددات الأكثر استخداماً في مثل هذه القياسات حيث تم ذكرها في المواقع ذات العلاقة. من أجل تحليل المعلومات والبيانات تم بناء كود خاص باستخدام برنامج الماتلاب، بحيث تم من خلاله احتواء القياسات التي تشمل المقاومة والزاوية وكيف أن المقارمة هنا هي اقتران له علاقة بالتردد ياستخدام نموذج كول – كول. ومن ثم تم استخراج المتغيرات التي يتضمنها نموذج كول – كول والتي تشمل المقاومة الإبتدائية والمقاومة النهائية والتردد المميز وتردد الاسترخاء ألفا.

بعد ذلك تم استقراء قيم كل من المقاومة الابتدائية والمقاومة النهائية بالاعتماد على نموذج كول – كول، ثم تم استخدام المقدرات الأكثر شيوعاً من أجل تحديد الحجم خارج الخلوي والحجم بين الخلايا وفي النهاية يتم تحديد قيم نسبة خلايا الدم الحمراء من حجم الدم كله بالاعتماد على قيم الحجوم التي يتم الحصول عليها.

تم في هذا العمل المقارنة بين العديد من المقدرات الموجودة والتي تشمل مقدر الجيل الأول لشركة شترون ومقدر الجيل الثاني بالإضافة إلى مقدر جديد تم استحداثه من قبل الدكتور عمر السرخي مشرف الرسالة. لكل عينة دم من

العينات ال 33 السابقة الذكر تم تقييم دقة هذه المقدرات في تقييم المحتوى المائي وتحديد نسبة خلايا الدم الحمراء من حجم الدم ومقارنة هذه النسبة مع النسبة الأصلية التي تم قياسها قبل وبعد العمل على عينات الدم باستخدام جهاز تحليل الدم في كل من مستشفى الخليل الحكومي ومختبرات كلية الطب في جامعة القدس.