COLE PARAMETERS OF HUMAN BLOOD WITH DIFFERENT ANTICOAGULANTS

by

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ABSTRACT

COLE PARAMETERS OF HUMAN BLOOD WITH DIFFERENT ANTICOAGULANTS

Impedance spectroscopy of blood samples with Acid Citrate Dextrose, Ethylene Diamine Tetra-acetic Acid, Lithium Heparin and Sodium Citrate anticaogulants are performed in the frequency range 20Hz-1MHz, using the two probe method, at room temperature. The measurement cell is a cylindrical-like plastic tube of 1.45 cm diameter and 14 ml volume with two stainless-steel electrodes. Blood samples of 5 ml are drawn from 9 healthy male donors between ages 22 to 28, and centrifuged at 5500 rpm for 8 minutes to constitute different hematocrit values in the range from 29 % to 60 %. Multifrequency impedance measurements are fitted to Cole-Cole diagrams using the Matlab algorithm; Cole parameters R_0, R_∞, f_c and α are then used to model the equivalent electrical circuit of blood. Only high frequency data (100kHz-1MHz) are used in fitting the Cole circle where effects of electrode polarization are negligible. It is later shown that this is acceptable since the characteristic frequency of the blood samples occurs around few MHz. Resistivities of plasma alone are measured as 70 $\pm$ 1.3 Ω.cm : 79.2 $\pm$ 1.3 Ω.cm : 72 $\pm$ 3.4 Ω.cm and 78.3 $\pm$ 1.2 Ω.cm for EDTA, LH, SC and ACD respectively. At 100 % hematocrit when the extracellular conductivity is zero the intracellular resistivities are 200 Ω.cm for EDTA; 214 Ω.cm for LH; 261 Ω.cm for SC and 176 Ω.cm for ACD. The rate of increase in extracellular resistance with Ht is measured higher with LH and EDTA. In the physiological range of hematocrits, Re is the highest with LH and lowest with SC; at h=0.6 LH samples are 34 % higher than SC samples. The characteristic frequency fc changes with the type of anticoagulant; the frequency span at Ht=50 % is from 0.8 MHz (LH) to 1.1 MHz (ACD). The angle of depression is the highest with LH.

Keywords: erythrocyte, hematocrit, electrical impedance of blood, impedance spectroscopy, Cole-Cole plot, Maxwell-Fricke Formula.

ÖZET

FARKLI ANTIKOAGULANLARDAKI KANIN COLE PARAMETRELERI

Farklı antikoagulan içeren, hematokrit değeri % 29 ve % 60 arasında değişen tam kanın ve eritrosit süspansiyonlarının spesifik empedansı, 100 kHz-1 MHz frekans aralığında ölçülmüştür. Ölçüm haznesi 1.45 cm çapında, 14 ml hacimli silindir şeklinde plastik bir tüptür ve içinden geçen elektrodlar aracılığı ile elektrotların bulunduğu noktalar arasındaki voltaj farkı ölçülür. Her bir ölçüm için 5 ml kan kullanılmıştır. Kan örnekleri 22-28 yaş aralığında sağlıklı erkek bireylerden alınmıştır. Farklı yoğunluklarda eritrosit süspansiyonları elde etmek için 5500 rpm'de 8 dakika santrifüj edilmiştir. Çoklu empedans ölçümleri, Matlab algoritması kullanılarak Cole-Cole diyagramlarına fit edilmiştir. Cole parametreleri Re, Ri, f_c and α oda sıcaklığında belirtilen hematokrit değer aralığında kanın elektriksel modellemesi için kullanılmıştır. Re ve Ri, Maxwell-Fricke denklemlerine fit edilmiş, f_c and α parametrelerinin hematokritle değişimleri analiz edilmiştir. Bütün parametreler istatiksel olarak antikoagulanların etkisini karşılaştırmak için kullanılmıştır. Sonuçlar bize kanın elektriksel model parametrelerinin hematocrite bağlı olarak değiştiğini ve antikoagulanların kanın elektriksel empedansına etki ettiğini gösterir. Bu yüzden kanın eletkriksel empedansı çalışılırken, antikogulanın etkisi göz önünde bulundurulmalıdır.

Anahtar Sözcükler: eritrosit, hematokrit, kanın elektriksel empedansı, empedans spektroskopisi, Cole-Cole diyagramı, Maxwell-Fricke Formulü.

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LIST OF ABBREVIATIONS

f	Frequency
fc	Characteristic frequency
h	Hematocrit in decimal form
H	Hematocrit
Im(Z)	The imaginary part of impedance
k_{cell}	Cell constant
Re(Z)	The real part of impedance
Ri	Intracellular fluid resistance
Re	Extracellular fluid resistance
Rm	The cell membrance resistance
R_0	The sample resistance at f=0
R_∞	The sample resistance at f=∞
Z	The sample impedance
α	The spread of relaxation times
ϕ	The depression angle
ρ_i	Intracellular Resistivity
ρ_e	Extracellular Resistivity
ρ_p	Plasma Resistivity
τ	Time constant

1. INTRODUCTION

1.1 Electrical Properties of Tissues

The electrical properties of biological tissues, in other words bioelectricity, have been studied for over the past 150 years, as the instruments for the measurement of electrical resistance and capacitance become avaliable, by researchers from a wide variety of scientific areas. These properties determine the current pathways through the body and are therefore important in many studies such as in the measurement of physiological parameters using impedance techniques, studies of physiological effects of electromagnetic fields, electrocardiography, muscle contraction and nerve transmission [1]. The existence of cell membrane was firstly demonstrated by the dielectric studies on cell suspensions [2].

The bioelectrical impedance technique is the measurement of tissue impedance upon the application electrical current. The techique was used by early investigators and still continues to be important for clinical applications.

Peltier discovered in 1834 that animal bodies behave as polarization cells if exposed to direct currents (DC), they are able to store electricity and release it after the termination of the current. Hermann, in 1872, probably was the first to measure the resistance of tissues exposed to alternating currents (AC) [1]. Du Bois and others (1848-1860) demonstrated that the resistance of animal tissues decreases with increasing frequency [3]. Bernstein proposed, in 1902, that the interior of a living cell was an electrolyte and the cell was seperated from its environment by a membrane only slightly permeable to ions [2]. Cole offered a particularly interesting survey of these early works from 1920s up to 1940s by Höber, Fricke and others [4]. Schanne and P.Ceretti presented a summary of many dielectric studies on tissue [5]. Foster and Schwan, in 1989, published a critical review emphasizing the mechanisms responsiple for the observed electrical properties [1]. Recently, McAdams and Jossinet provided an

historical overview suggesting to highlight the contributions of various research areas such as physics, electrochemistry, electrophysiology and biomedical engineering. They declared that this wide variety of areas leads to misunderstanding and misuse of terminology and concenpts suggested by others, so in their review they explained some of the most important of these terms/concepts [2].

The impedance of biological tissue is related to its water content. Tissue contains intracellular and extracellular water spaces that act as an eletrical conductor. The cell membrane separating these two water spaces behaves as an electrical capacitor [6]. At low frequencies the alternating current passes through the extracellular space since cell membranes are relatively highly resistive, whereas at high frequencies cell membranes become conductive[2, 6]. There are many studies using this technique to estimate water content in tissues and the whole body [7, 8, 9, 10, 11].

1.2 Blood

Blood is the extracellular fluid of the body.It carries nutrition from small intestine to the body, carries oxygen from lungs to the heart, from heart to whole body. It also carries toxic waste products and carbondioxide from tissues to the kidneys and lungs, respectively. It includes the cells destroying foreign materials. In addition, blood carries the hormones from their sites of production in the endocrine glands to their target organs in other locations [12].

Blood tissue consists of a cellular compartment and a fluid medium called the plasma. The blood cells float freely within the plasma medium. The cells are mostly red cells (erythrocytes), with smaller numbers of white cells (leukocytes) and platelets (occasionally called thrombocytes) [13].

1.2.1 Plasma

The plasma occupying 55 % of the blood consists mainly of water (90 %). It helps to dissolve the blood proteins such as fibrinogen, albumins, and globulins. The blood plasma contains high concentrations of Na^+ and Cl^-(87 %). Typical values of ionic concentrations in plasma are given in Table 1.1.

Table 1.1
Ionic concentrations in mEq.per liter in plasma. [12]

Ion	Blood Plasma
Na+	142.0
K+	4.0
Mg+	2.0
Ca++	5.0
Cl-	102.0
HCO3-	26
PO4-	2
Other	6
Protein	17

1.2.2 Red Blood Cells(Erythrocytes)

An erythrocyte is a cell without a nucleus containing protein,haemoglobin and few vorganelles in its cytoplasm surrounded by a cell membrane. It has a disc-like shape with a diameter about 7.2 μm in dried films and about 8.5 μm in suspension. Although red blood cells have the same, constant shape in blood samples, they can be deformed as they pass down the capillaries, but they can revert to their original shape [14].

1.2.3 Cell Membrane

The cell membrane is an asymmetric structure consisting of a lipid bilayer, membrane proteins and cell coat. The total thickness of the cell membrane is typically

about 7.5 nm. The cell membrane contains approximately equal amounts of lipids and proteins, with smaller amount of other substances including water, cholesterol, sugar groups and metal ions. The lipid bilayer is composed of phospholipids which are amphiphatic molecules. These molecules, which have both hydrophylic and hydrophobic properties, can form, in the presence of water, a bimolecular leaflet where the hydrophylic end of the molecule are facing outwards and the hydrophobic hydrocarbon chains point inward to form the membrane interior. The thickness of such a lipid bilayer is 5 nm. The cell membrane is not a rigid structure and can be described by the fluid mosaic model [15, 16, 13].

1.3 Applications of Bioelectrical Impedance Technique for Blood

Blood, as a vital body fluid, has been extensively studied using the electrical impedance techique [17]. The earliest application of blood impedance measurements was the determination of cardiac output. Continuous recordings of the change in conductivity of blood using arterial detectoes was realized by Wiggers and White to determine cardiac output and was improved by Geddes and Hoff by employing specially designed electrodes placed on the surface of an intact artery [9, 17, 18, 19, 20].

The impedance of peripheral systems of the human body was first found by Mann to change with the cardiac cycle, today known as impedance plethysmography. This technique is based on the fact that blood has a lower resistance than most other living tissues. Hence, when the volume of blood is increased in the region of interest, the impedance of the segment will be decreased. It was developed by Kubicek into impedance cardiography for non-invasively monitoring the cardiac output and stroke volume [20, 21, 22].

As blood cells are much less conductive than plasma, Stewart declared that the resistivity of blood increased as the erythrocyte concentration, which is called hematocrit, was increased [23]. Höber revealed that the red blood cell membranes were highly resistive at low frequencies($\approx$ 1kHz) that red blood cell suspensions had a resistivity

exceeding 1000 Ω whereas at high frequencies the resistivity fell to a value around 200 Ω [2, 17] and helped to clarify the reason for the strong frequency dependence of the resistance of blood [1]. Fricke made extensive studies on blood impedance and he was the first who determine the capacity of red blood cell membrane as 0.81 μF cm^{-2} and made the theoretical study on the relation between the impedance and hematocrit of blood [24, 25, 26]. A practical apparatus for determination of the hematocrit value based on the electrical conductivity of blood was introduced by Okada and Schwan [27].

The dielectric relaxation properties of whole blood and hemoglobin have been revealed by Schwan [27]. Whole blood exhibits beta ,gamma ,delta relaxations but no alpha dispersion (see Figure 1.1). For blood with a hematocrit of 40%, the beta dispersion has a total permittivity increment of about 2000 and is centered at about 3 MHz. The gamma and delta dispersions are similar to those found in hemoglobin solutions of similar concentration [2, 28].

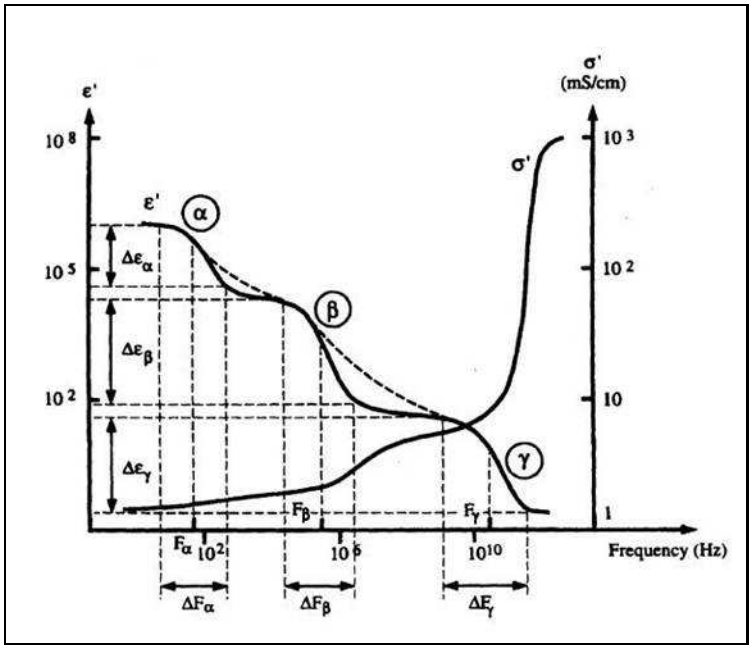

Figure 1.1 α, β and γ dispersions [29].

1.4 Multifrequency Model of Tissues

Early work has been discussed previously that du Bois Reymond suggested the resistance component of animal tissue whereas Bernstein proposed the cell membrane and Höber explained its frequency dependent property [2].

In 1907, Lapicque investigated the use of the three-component model, which is the parallel combination of a resistance and a capacitance,both in series with another resistance, to model the excitation of the nerve membrane.

Following the studies by Philippson, Fricke and Morse [30] developed a theory for the resistance of suspensions of spherical or spheroidal cells. They found that their measurements on suspensions of red blood cells at various frequencies could be accurately fitted to a circuit similar to that shown in Figure 1.2. Re was thought to represent the resistive properties of the suspending medium, Ri those of the interiors of the corpuscles, Cm is the capacitances of the membranes and Rm is the resistance of cell membranes which is negligible. Hence at low frequencies the mebranes have high reactance and do not allow the current passage, so the current flows through the suspending medium and the total impedance is relatively high and equal to Re. On the contrary, at high frequencies the capacitance of the membranes decrease and they become conductive allowing the current flow, so there remains the limiting resistive value of the intracellular fluid.

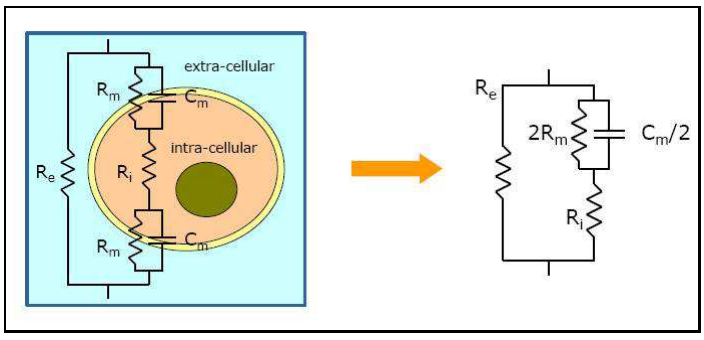

Figure 1.2 Electrical equivalent circuit of biological tissues [29].

1.5 Cole-Cole Representation of Blood Impedance

The complex impedance of tissues or other biological materials, (Z=R+jX), give rise to a locus having the form of a minor arc of a circle whose centre lies below the real axis when equivalent resistance, R[Z], and the equivalent series reactance, Im[Z], plotted on the complex impedance plane. This locus, which is called Cole-Cole plot, is shown in Figure (1.3). The complex impedance equation which is also called Cole equation of tissue is given by the following Equation 1.1, where Φ ($(1-\alpha)\pi/2$) is the angle of depression, R_0 is the resistance at zero frequency, R_∞ at infinite and τ is the mean relaxation time constant. The plot gives the Cole parameters R_0, R_∞, characteristic frequency f_c ($=1/2\pi\tau$) and α. α takes values between 0 and 1, when it is equal to 0, Equation 1.1 turns into Debye equation.

$$Z = R_\infty + \frac{(R_0 - R_\infty)}{1 + (jw\tau)^{1-\alpha}} \tag{1.1}$$

$$\alpha = (2/\pi) sin^{-1}(d/r) \tag{1.2}$$

where w is the angular frequency. In the Equation (1.2) d is the distance from the real axis and r is the radius of the depressed circle.

The imaginary part of Z is maximum at f_c that is determined from the intersection of the straight line, obtained by plotting lnw against $ln(\frac{|v|}{|u|})$, with lnw. The phasors u and v are defined in Figure 1.3.

$$|u| = \sqrt{(Im\{Z\})^2 + (R\{Z\} - R_\infty)^2} \tag{1.3}$$

$$|v| = \sqrt{(Im\{Z\})^2 + (R_0 - R\{Z\})^2} \tag{1.4}$$

In Figure 1.2, R_0 is equal to Re and, R_∞ is the parallel combination of Ri and Re. The cell membrane capacitance is seen as a parallel combination of cell membrane resistance (which is negligebly small) and capacitance. However, even within the same tissue, cells will have slightly different shapes and structures and will have different time constants. To account for this effect, capacitive effects of cell membranes are lumped into a constant phase angle impedance Z_{CPA} defined by

$$Z_{CPA} = 1/K(jw)^{1-\alpha} \tag{1.5}$$

where K is the constant magnitude of the capacitance in units of Siemens.seconds$^{1-\alpha}$ and when α is equal to zero, Z_{CPA} becomes purely capacitive.

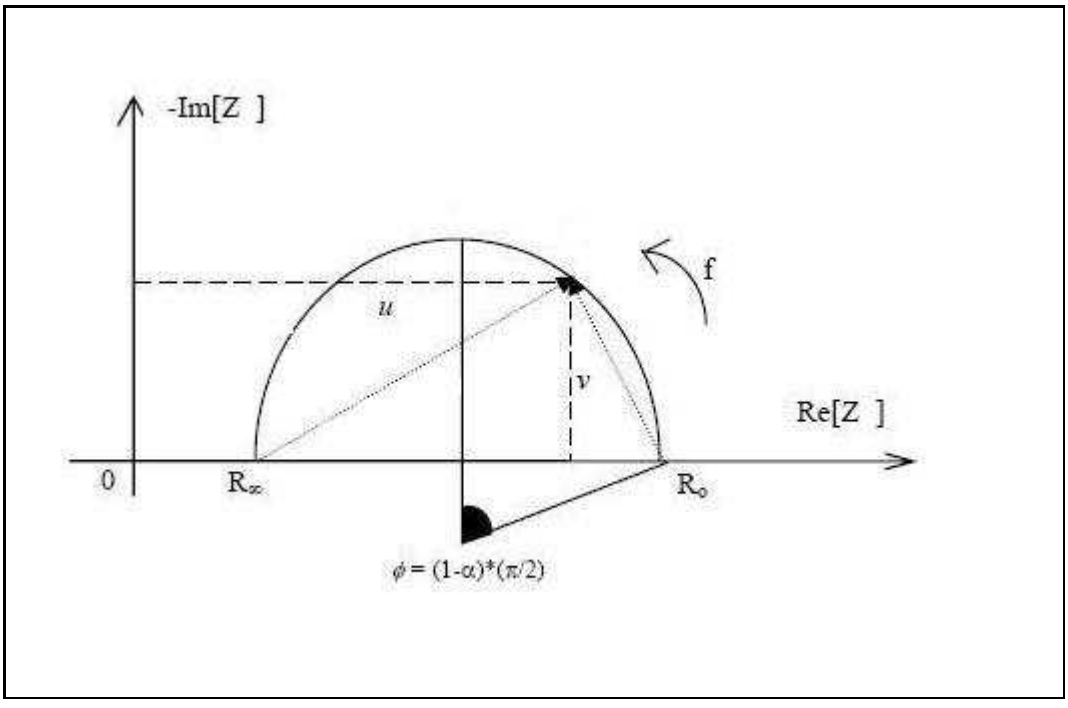

Figure 1.3 The Cole-Cole Plot for impedance of living tissues.

1.6 Objective of the Study

Blood is a vital tissue and plays important role in clinics. There are lots of diseases related to this tissue such as Hepatitis, Aids. Electrical bioimpedance technique is a time-saving and cheap method to study the properties of blood in treatments and diagnosis of diseases. Blood coagulates when it is drawn from vein. Hence, to study the electrical properties of blood in vitro, anticoagulants should immediately be added to the blood. At this point, there occurs a pitfall that the measured impedance may not reflect the intrinsic impedance value of blood. Therefore, the effect of anticoagulant on the blood impedance should be figured out to see the exact electrical properties of blood. There are several types of anticoagulants used in clinics such as CPDA, for blood storage and EDTA, for determining hematocrit. In this study, we used four types of anticoagulants in standardized blood collection tubes: EDTA, LH, SC and ACD all of which have different kinds of uses. The proportions of anticoagulants in the collection tubes are not same; hence it was also the intention to observe the effects of increased amount of anticoagulant.

2. MATERIALS AND METHODS

2.1 Materials

2.1.1 Preparations for Blood Collection

The blood samples are collected at Medical Center of Bogazici University from 14 healthy male donors between ages 22 to 28. Donors who voluntarily participated in the study, are tested to met the standard blood donor criteria. The study is approved by the Ethics Committee of Bogazici University.

2.1.2 Collection and Preparation of Blood Samples

Blood samples are drawn from each donor into the four types of blood collection vacuum tubes with anticoagulants, ACD (Acid Citrate Dextrose), EDTA (Ethylenediaminetetraacetic acid), LH (Lithium Heparin) and SC (Sodium Citrate) by vacutainer system. From each subject, 27 ml of blood are drawn into three 9 ml Vacuette EDTA tubes coated with K3EDTA, 30 ml blood into three 10 ml Becton Dickinson Vacutainer LH tube coated with LH, 25.5 ml blood into three 8.5 ml Becton Dickinson Vacutainer ACD-A tube including 1.5 ml ACD-A solution and 27 ml blood into three SC tubes. The maximum volume of SC vacuum tube in commercial use is 6 ml, which is small for the study because increased number of tubes leads to difficulties during blood donation. So SC tube is prepared by adding SC solution in 1:9 ratios by syringe into CAT coated 10 ml Becton Dickinson tube without any anticoagulant in it. SC solution is prepared by adding 109 mmol SC ($Na_3C_6H_5O_7 2H_20$) to 1000 ml distilled water and is stored in the refrigerator. Abbott Bayer Hemogram-1200 analyzer is used to determine the hematocrit value of the donor. Approximately 1.5 ml blood is drawn into 2 ml EDTA tube and it is placed in the analyzer. After blood is drawn into three tubes, they are put on a mixer for 10 minutes in order to homogenize the anticoagulant

in the blood. The tubes are centrifuged at 5500 rpm for 8 minutes using ElectroMag M615M and transported to blood laboratory (RNA room) at Molecular Biology and Genetics Department of Bogazici University to perform the measurements. ACD-A tubes are made of glass; they are fragile and there is a risk of breaking them during centrifugation. So they are allotted to 15 ml falcon tubes and then centrifuged in Beckman Coulter AllegraTM X-22R Centrifuge at blood laboratory at the same settings. After centrifugation, one of the three tubes is used as the original sample with the hematocrit value measured in the Hemogram-1200. So it is agitated for several times until plasma and red blood cells are evenly distributed in the tube. The reason for centrifugation of this tube is to eliminate any possible difference due to centrifugation. For constitution of erythrocyte suspensions, plasma and red blood cells are separated into separate tubes. By diluting red blood cells with plasma in different volumes using 5000 ml Eppendorf Micropipette, at least three erythrocyte suspensions with hematocrits between 30% and 60% are determined. Hence, for each subject at least four samples with different hematocrits are determined. Erythrocyte suspensions are prepared and measured immediately after being drawn from subjects. All measurements are performed at room temperature.

2.2 Instrumentation

Blood complex measurements are performed by HP 4284A LCR Meter using two-probe configuration. Figure 2.1 shows the two probe configuration of the attachment of the sample to the LCR meter.

The calibration of system to eliminate impedance effects of connecting BNC cables is performed according to the open circuit/short circuit correction procedure of HP 4284A manual. The open circuit and the short circuit configurations are measured and it is seen that the residual capacitance and inductance of the probe are negligibly small.

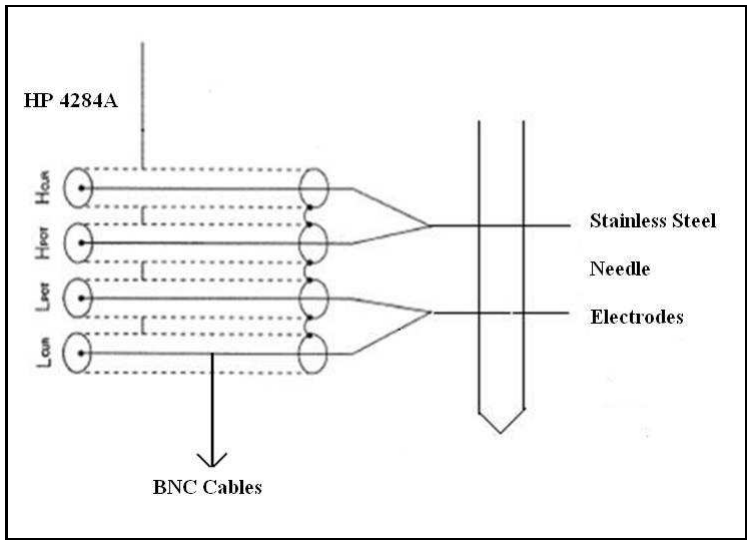

Figure 2.1 The block diagram of the 2-electrode measurement set-up.

2.3 Methods

2.3.1 Electrical Impedance Measurements

The measurement system consists of the HP 4284A LCR Meter, the measurement cell placed vertically in a holder and two connecting BNC cables, of 31.5 cm length each (Figure 2.2).

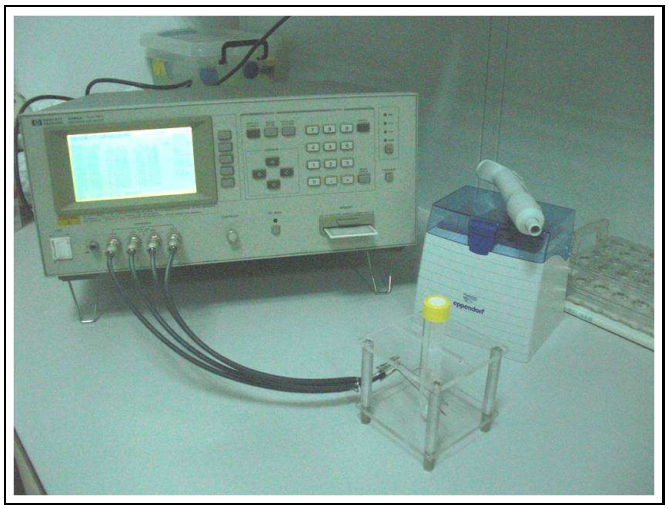

Figure 2.2 The Experimental Set-Up.

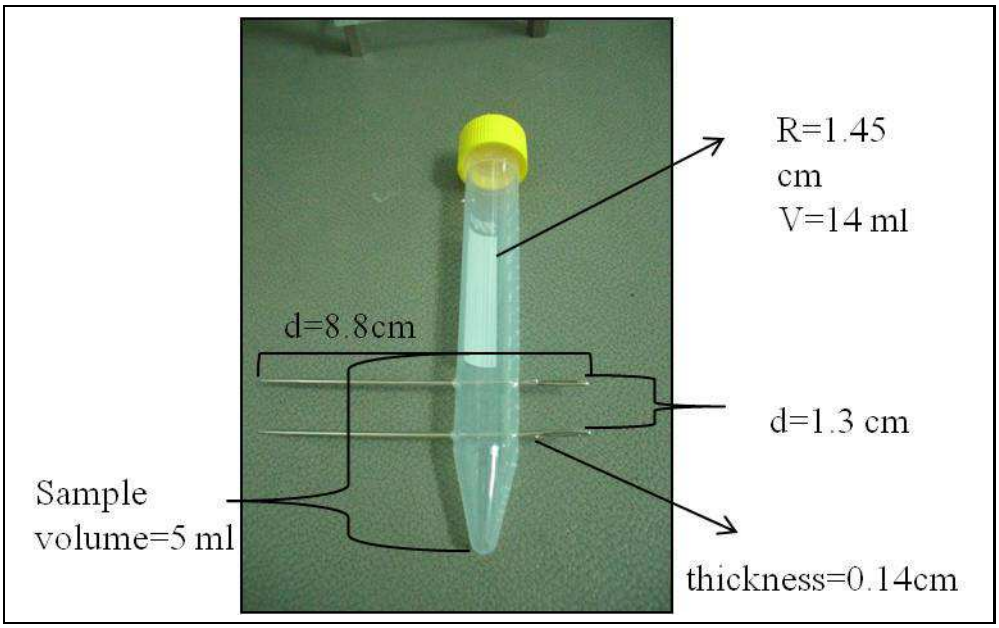

Figure 2.3 The Measurement Cell.

The measurement cell is a cylindrical-like plastic tube of 1.45 cm diameter and standard volume of 14 ml. Two stainless-steel needle electrodes, each measuring 0.14 x 8.8 cm and separated by 1.3 cm, are used to inject the current along the axis of sample chamber and measure voltage across them (Figure 2.3). The needle electrodes are connected to the impedance analyzer HP 4284A through BNC cables.

5 ml of blood is allotted using 5000 ml Eppendorf Micropipette into the measurement cell and the resistance R and the reactance X are measured in the frequency range 20Hz - 1MHz. The measurement cell constant, $k_{cell}=(\sigma R)^{-1}$, is determined by using different concentrations of NaCl solutions; i.e. solutions that are purely resistive. The conductivities of the NaCl solutions are measured by HINOKI Conductivity Meter. The impedance measurements are done at four different frequencies 10 kHz, 100 kHz, 800 kHz and 1 MHz with two different electrode separations, of 1.3 cm and 1.95 cm (Figure 2.4- 2.5). The cell constant for 100 kHz and 1.3 cm electrode separation is found to be 1.14 cm^{-1}.

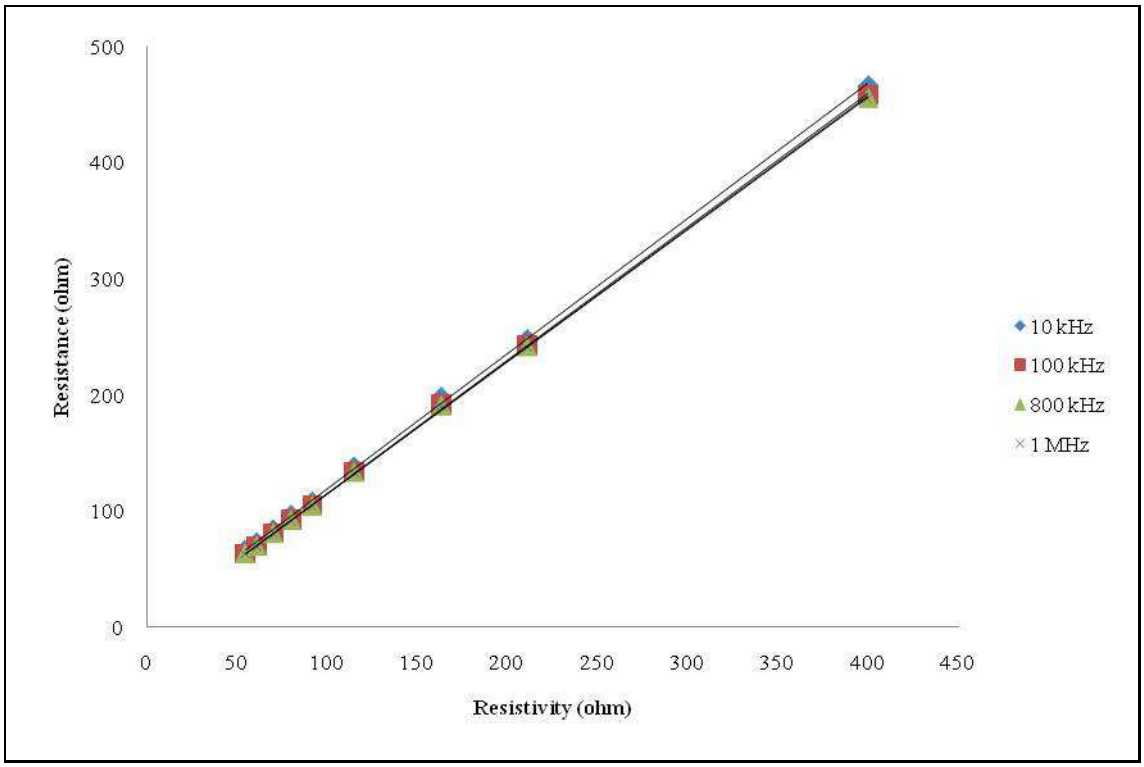

Figure 2.4 Resistances vs resistivities of saline solutions for 1.30 cm electrode separation. R $\geq$ 0.99 for all curves.

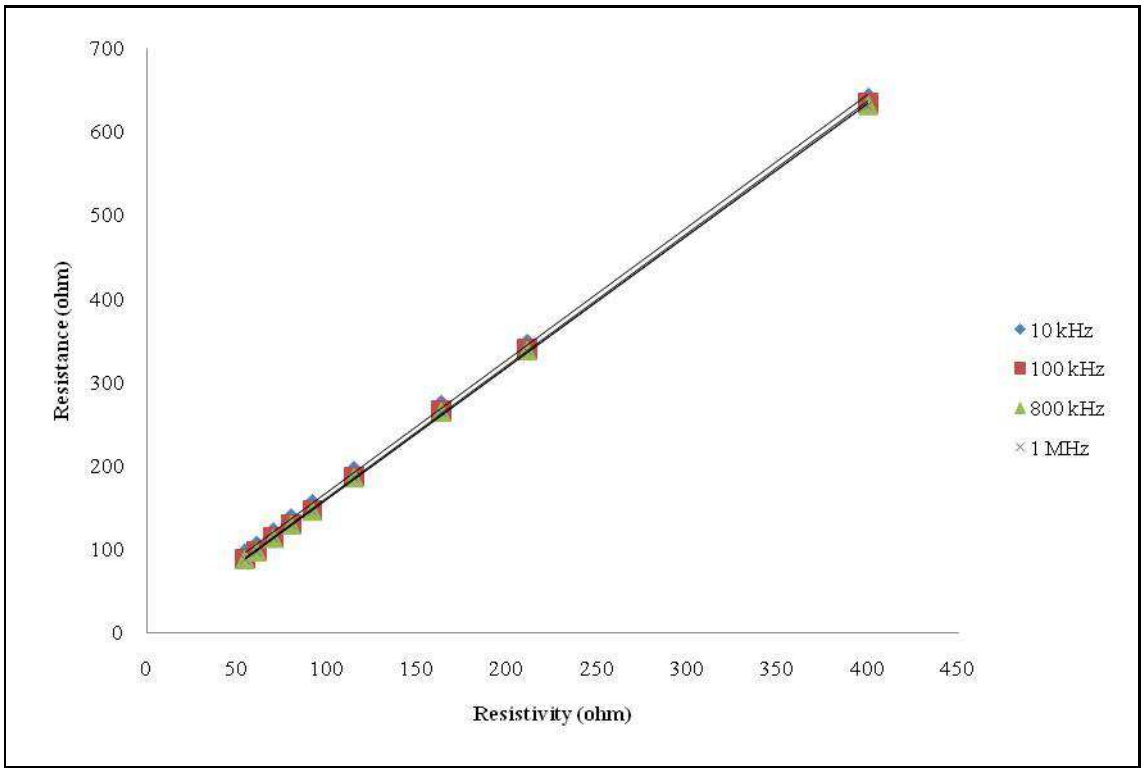

Figure 2.5 Resistances vs resistivities of saline solutions for 1.95 cm electrode separation. R $\geq$ 0.99 for all curves.

2.3.2 Effects of Centrifugation and Pipette Usage on Blood Cells

To obtain erythrocyte suspensions with different hematocrit values, centrifugation and pipette are used. Since red blood cells are too perishable, centrifugation and pipette may deform or even break them and lead to errors in the measurements. To verify this, electrical impedance of whole blood is compared with blood suspensions of the same hematocrit value. From Figure 2.6, we see that the errors contributed in the impedance measurements for Sodium Citrate anticoagulant are around 6 % for donor # 2 and # 9, whereas it is nearly 1 % for donor # 4. On the other hand, the error decreased with inceasing frequency and this observation is taken into account to adjust our data, since the experiments are performed at high frequencies.

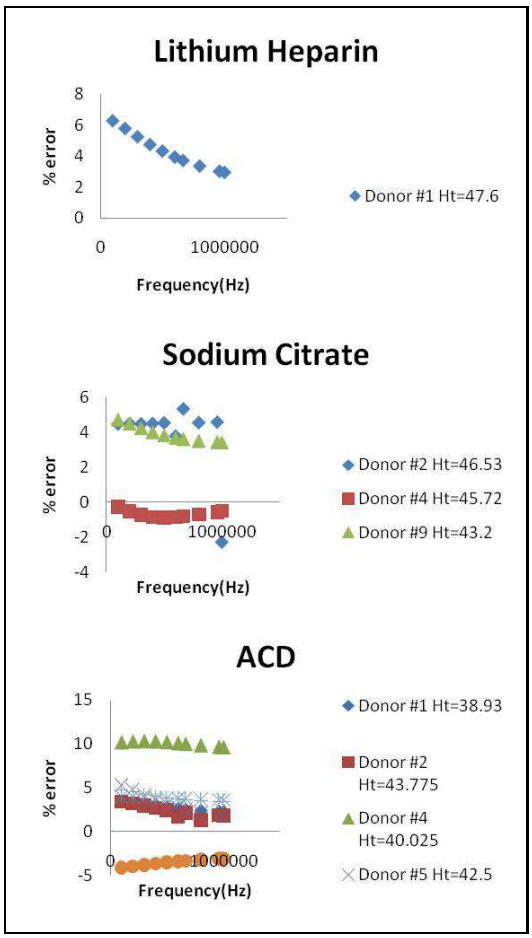

Figure 2.6 The error in the measurements of blood suspensions with respect to whole blood changing with frequency.

3. Electrode Polarization Impedance and Dispersions

3.1 Electrode Polarization Impedance

When studying electrical properties of tissues, electrode polarization impedance on electrolyte conductivities becomes a nuisance [2]. This impedance is found at metal-electrolyte interfaces and affected by several parameters such as electrode material; surface area of electrodes; type of electrolyte and frequency of treatment. It can be represented as a series combination of a resistance (Rp) and a capacitance (Cp):

$$Zp = Rp + \frac{1}{jwCp} \tag{3.1}$$

Both Rp and Cp are frequency dependent and decrease with increasing frequency; and, the phase angle of the polarization (i.e.wRpCp) impedance is almost constant [31]. In Figure 3.1 the sample in contact with electrodes (a), its electrical equivalent circuit(b) and the observed circuit total impedance Z*(c)= R+jwC are seen. The polarization impedance of electrodes Zp=Rp+1/jwCp is seriesly connected with the sample impedance Zs=Rs+1/jwCs.

$$Rp = R_0 f^{-m} \tag{3.2}$$

$$Cp = C_0 f^{-n} \tag{3.3}$$

The measured impedance is the sum of the sample impedance and electrode polarization impedance. The polarization impedance could be eliminated by any of the following methods:

Substitution Method: The electrode impedance characteristics are obtained by using a solution of known conductivity. For example, NaCl solution is used as a reference solution since it is purely resistive: the impedance curve against the frequency

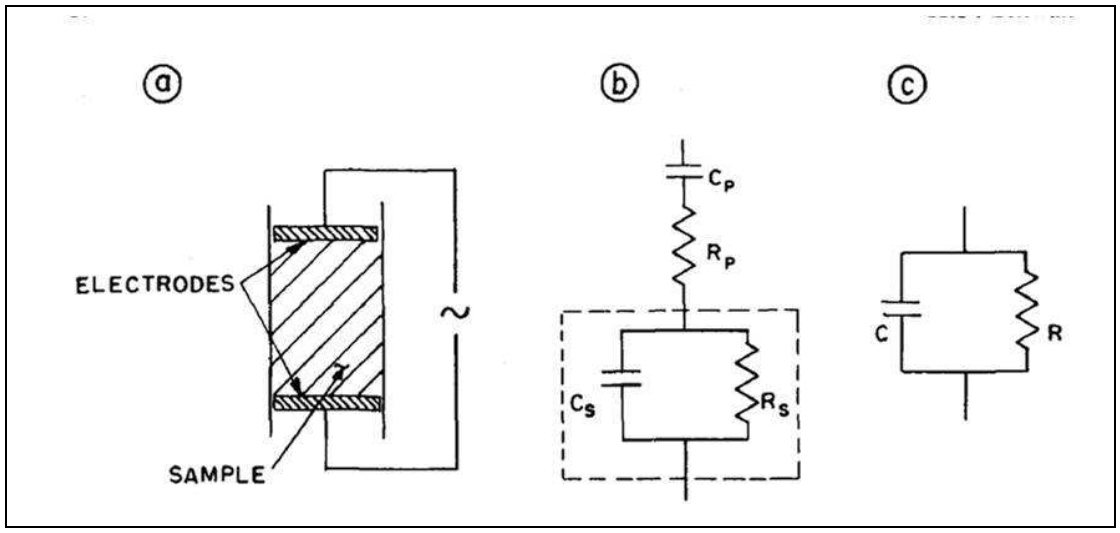

Figure 3.1 Electrode-Tissue Interface Impedance Circuit [31].

is frequency independent. At low frequencies, the impedance curve decreases with increasing frequency up to a constant value. This constant value is the resistance of NaCl solution and when it is subtracted from the measurement value, the resistance of electrode is obtained. Another approach is to use the suspending medium, namely plasma, as reference solution. But, this method is limited to low concentrations of suspension; i.e. the hematocrit value should be less than 10 %.

Electrode-Distance Variation Teachnique: The impedance measurement is performed at two inter-electrode distances. When subtracting the impedance of the smaller volume of blood from the larger one, one should obtain the impedance of the sample. The limitation of this technique is that the polarization impedance becomes large in comparison with the sample impedances at low frequencies and small errors in the determination of the total impedance (Zsample+Zpolarization) cause, therefore, large errors in impedance difference [31].

Polarization impedance is investigated by making use of NaCl solutions of known conductivities. In Figure 3.2, the resistive impedance of NaCl is constant and approximately equal to 69.9 Ω. It is subtracted from low frequency measurements to obtain the electrode polarization impedance. The fitting equation in Figure 3.3 shows R_0=5927.6 and m=0.88 in Equation 3.2.

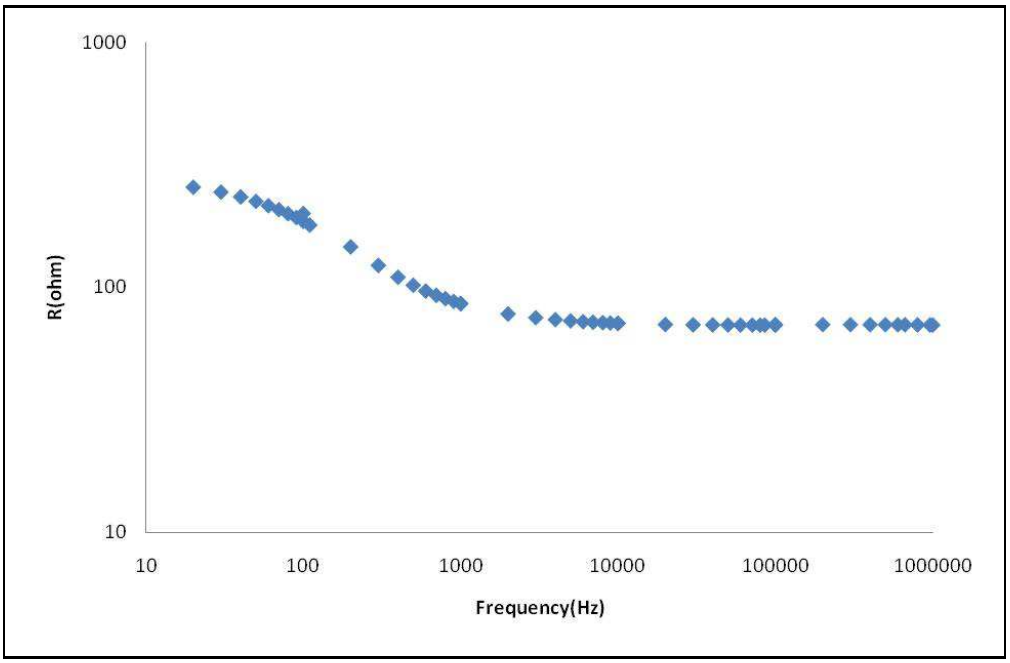

Figure 3.2 The impedance spectroscopy of $NaCl^{-}$ with two electrodes.

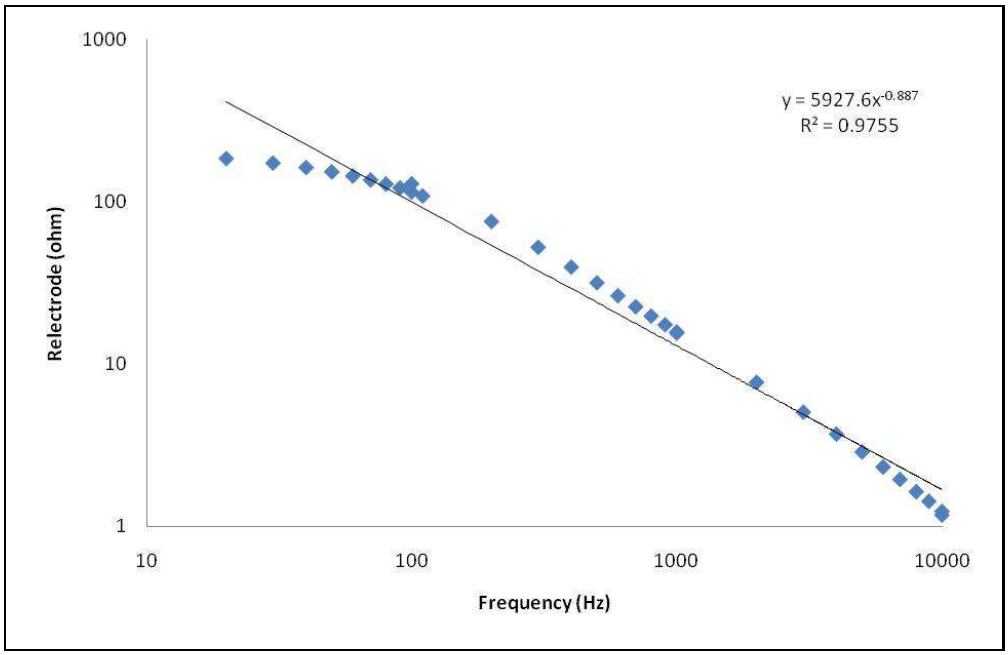

Figure 3.3 The electrode impedance after subtraction in the frequency range 20 Hz - 10 kHz.

3.2 Dispersions

The Debye (Equation 1.1) representing the complex impdednace Z = R + jX, when separated into its real and imaginary components gives Equations 3.4 and 3.5, respectively:

$$R = R_\infty + \frac{(R_0 - R_\infty)[1 + (w\tau)^{1-\alpha} sin(\alpha\pi/2)]}{1 + 2(w\tau)^{1-\alpha} sin(\alpha\pi/2) + (w\tau)^{2(1-\alpha)}} \tag{3.4}$$

$$X = -\frac{(R_0 - R_\infty)(w\tau)^{1-\alpha} cos(\alpha\pi/2)}{1 + 2(w\tau)^{1-\alpha} sin(\alpha\pi/2) + (w\tau)^{2(1-\alpha)}} \tag{3.5}$$

A typical impedance spectroscopy with 2 electrodes is illustrated in Figure 3.4. The measured impedance is the sum of the polarization impedance and blood sample impedance. By fitting in MatLab, Equations 3.4 and 3.5 to the real and imaginary parts of measured impedances respectively in the frequency range from 100 kHz to 1 MHz, where electrode polarization is negligible, the intrinsic impedance of the sample is obtained in the β-dispersion region. β-dispersion curves predicted by Equations 3.4 and 3.5 are consistent with the measurements. Divergence of the measured data from the calculated curves at frequencies below 10 kHz is due to electrode polarization; α-dispersion associated with the interface between the electrodes and blood. The polarization impedance can be obtained by subtracting this intrinsic impedance from the measured one.

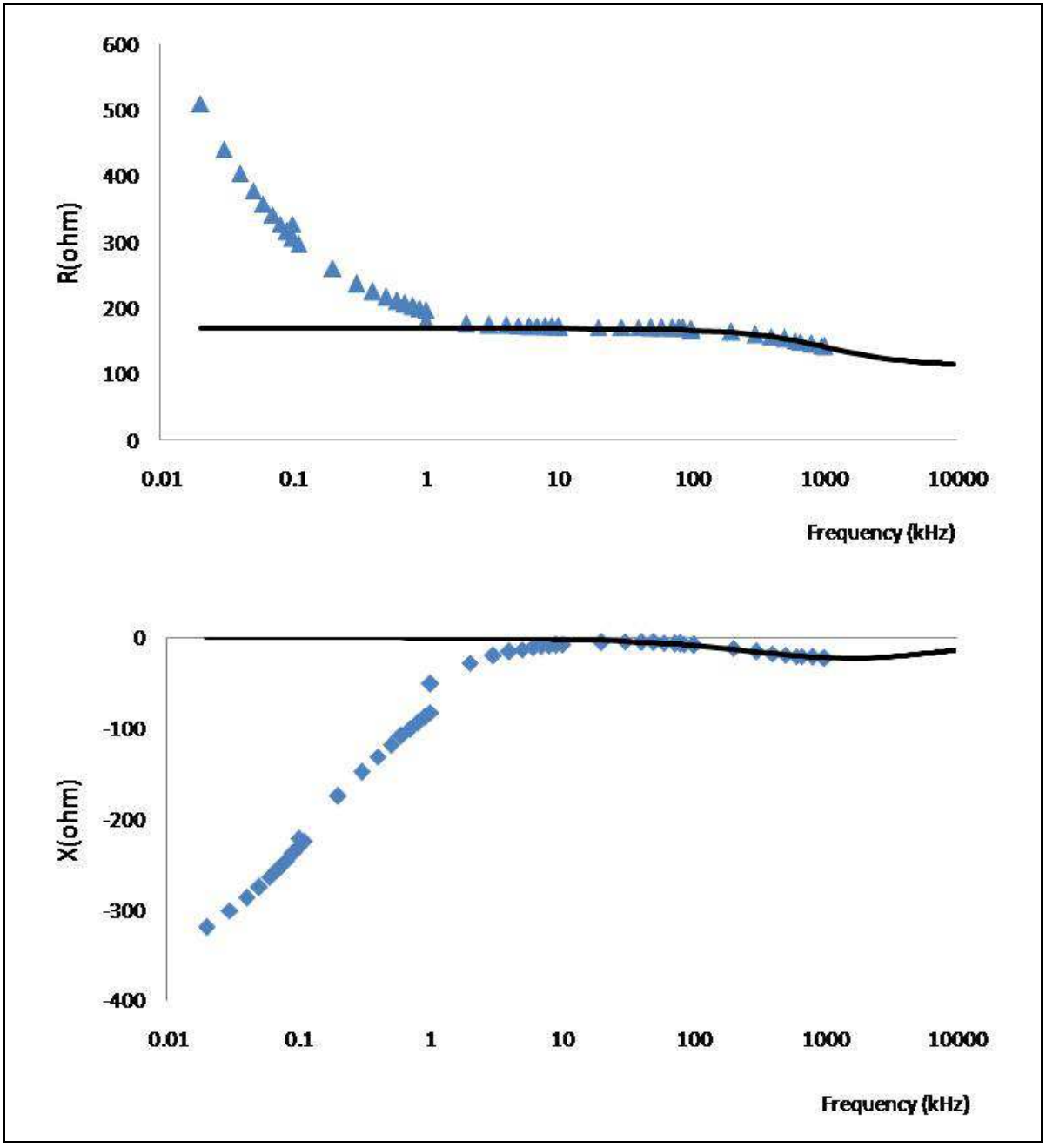

Figure 3.4 Real and Imaginary parts of measured impedance of blood from Donor #1. Solid lines represents Equations 3.4 and 3.5.

4. RESULTS AND DISCUSSION

The complex impedance of measurement of whole blood samples and erythrocyte suspensions, with four anticoagulants (EDTA, LH, SC, ACD), is performed in the frequency range from 100 kHz to 1 MHz where the electrode-tissue polarization interface impedance is negligibly small. In this frequency range, the cell membranes also gradually become conductive so the measured resistances reflect both the intracellular and extracellular properties.

4.1 Cole Diagrams and Cole Parameters

Erythrocytes are diluted with plasma in varying concentrations to obtain different hematocrit values for each donor. Figures 4.1-4.9 illustrate the Cole-Cole Plots of the complex impedance constructed in the frequency range from 100 kHz to 1 MHz. Cole parameters R_0, R_∞, α and f_c are obtained by performing a Matlab code in which the experimental data are fitted into a circle. In Table 4.1, the Cole parameters are listed for each donor.

R_0 and R_∞ increases with increasing hematocrit for each anticoagulant. α, f_c response to hematocrit change for each type of anticoagulant is shown in Figure 4.10 and in Figure 4.11. The alpha parameter remains almost constant with respect to changing hematocrit. The frequency span at H=50 % is from 0.8 MHz (LH) to 1.1 MHz (ACD). f_c gets lower for each anticoagulant as the volume occupied by red blood cells become greater implying a larger effective cell membrane capacitance. The effect is more pronounced with LH.

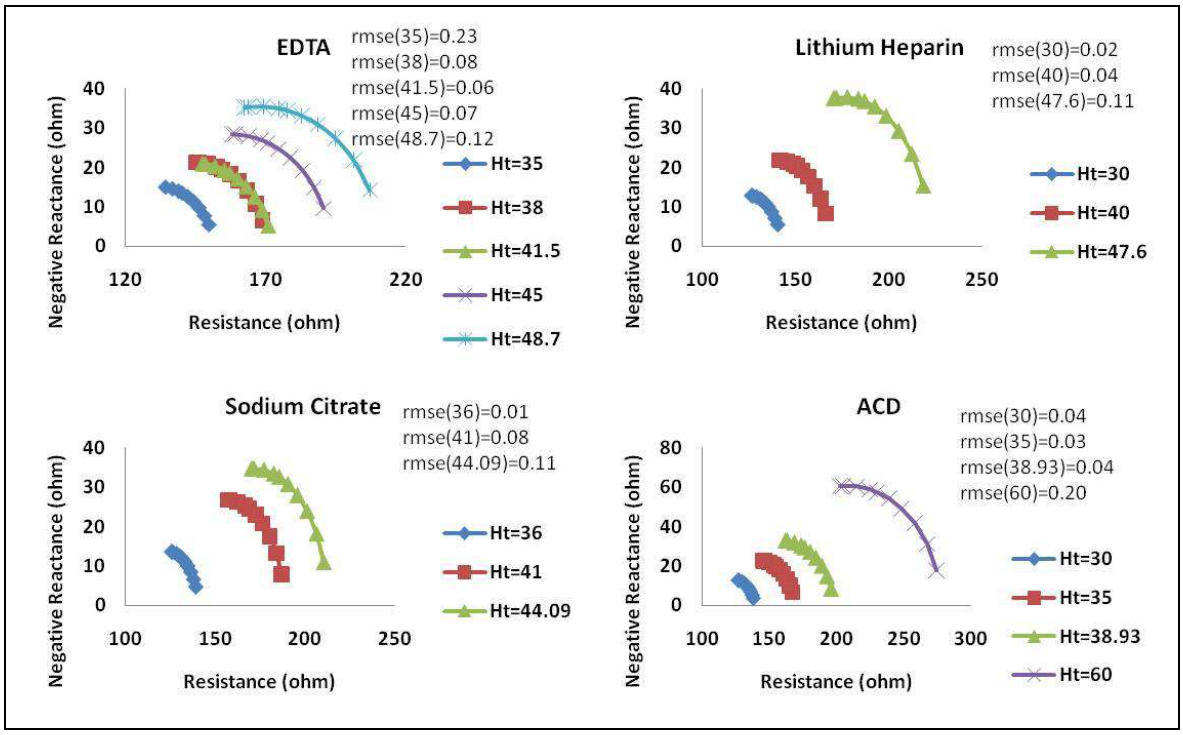

Figure 4.1 The Cole-Cole Plots for donor 1 with different anticoagulants.

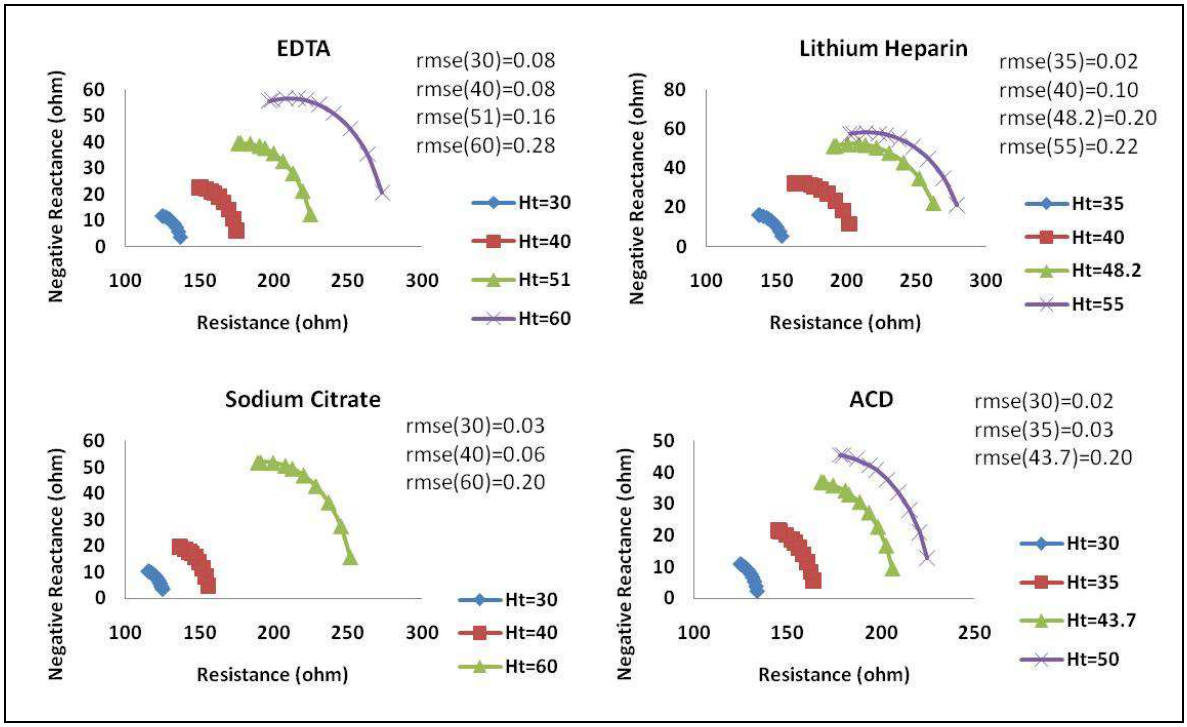

Figure 4.2 The Cole-Cole Plots for donor 2 with different anticoagulants.

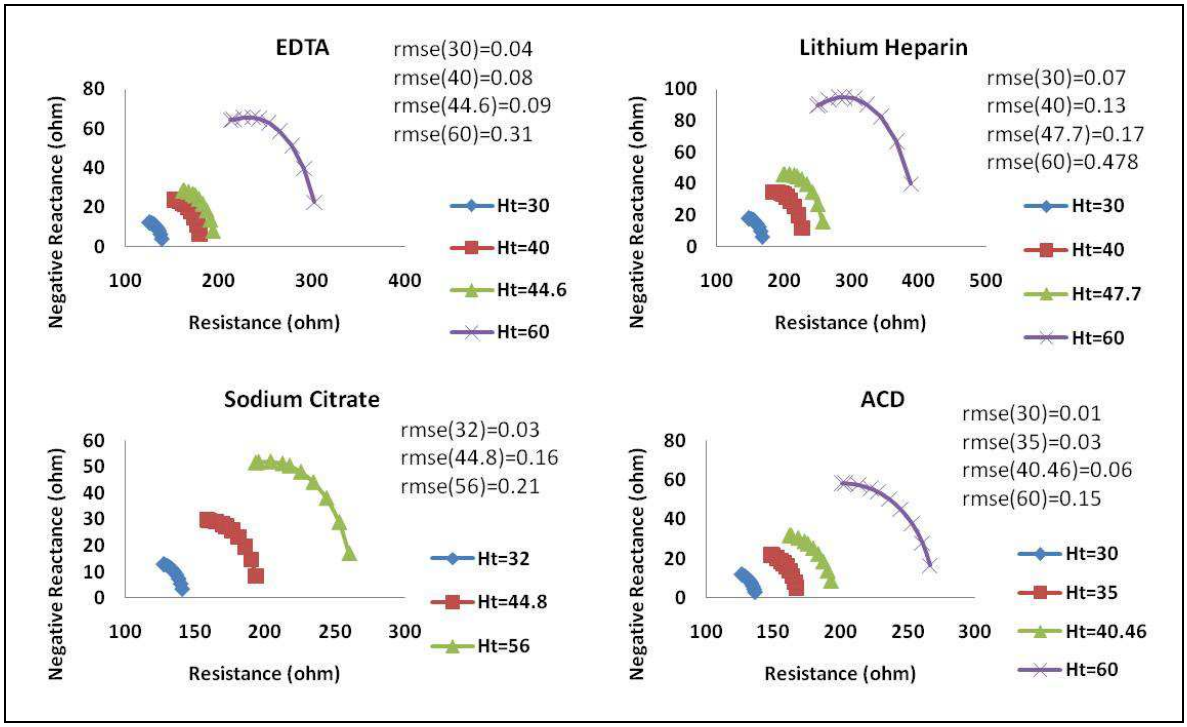

Figure 4.3 The Cole-Cole Plots for donor 3 with different anticoagulants.

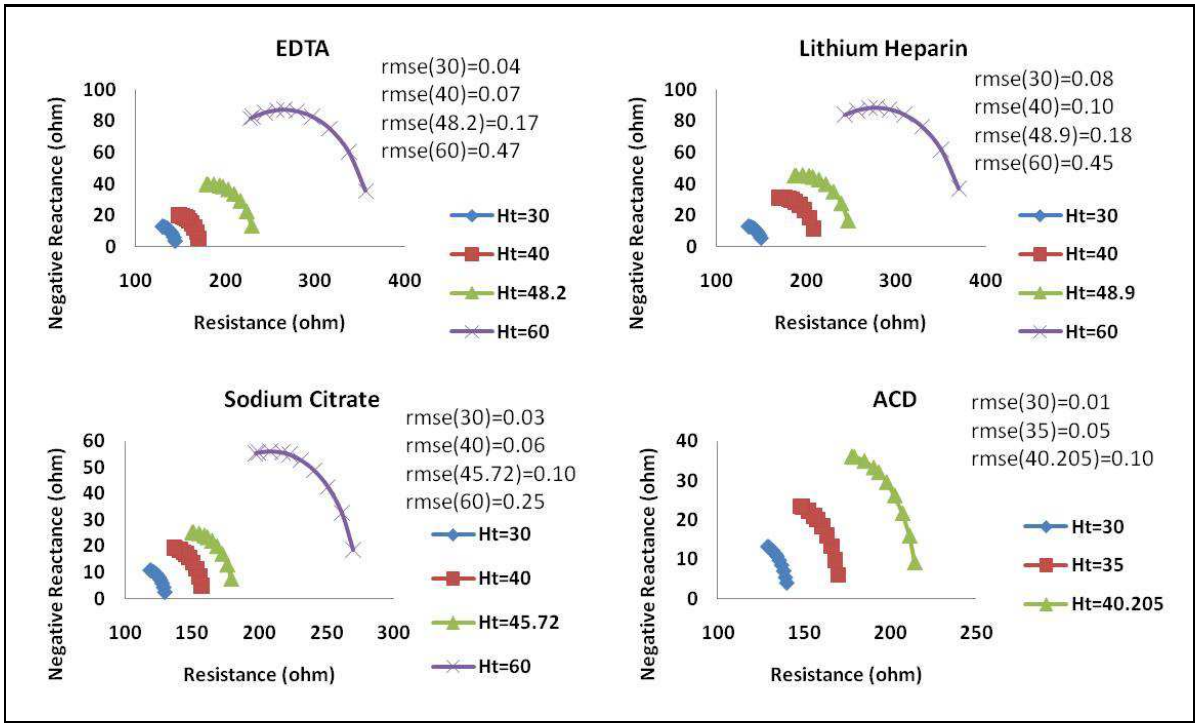

Figure 4.4 The Cole-Cole Plots for donor 4 with different anticoagulants.

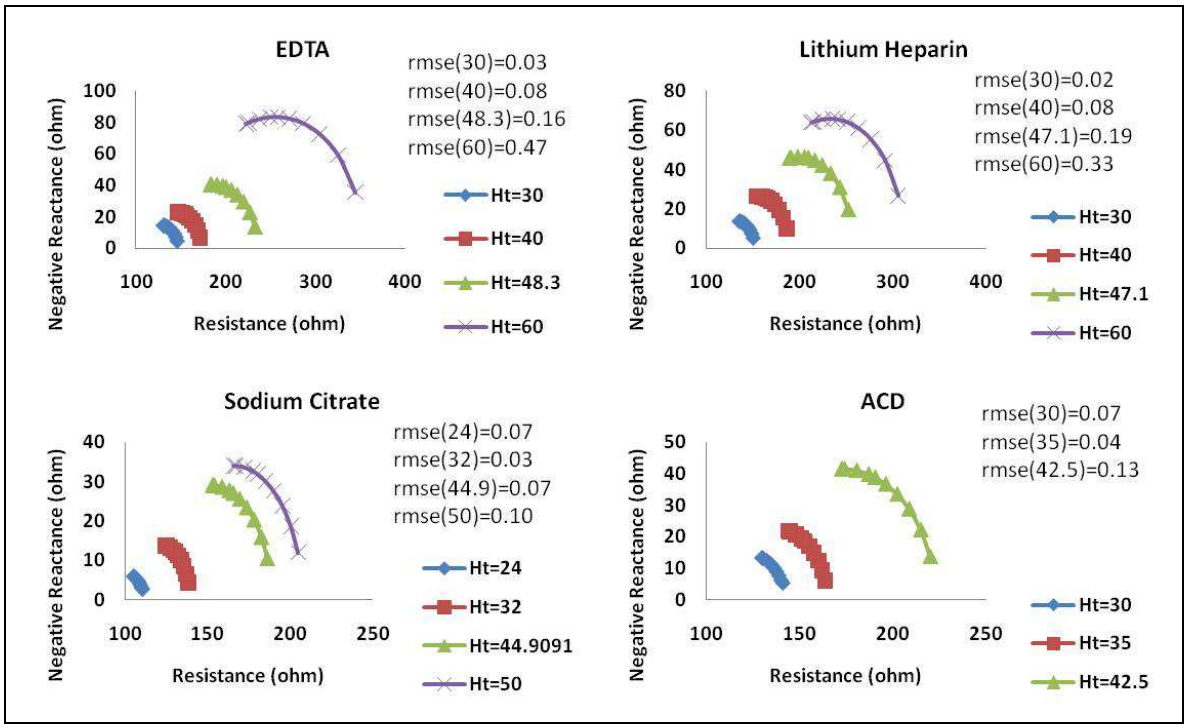

Figure 4.5 The Cole-Cole Plots for donor 5 with different anticoagulants.

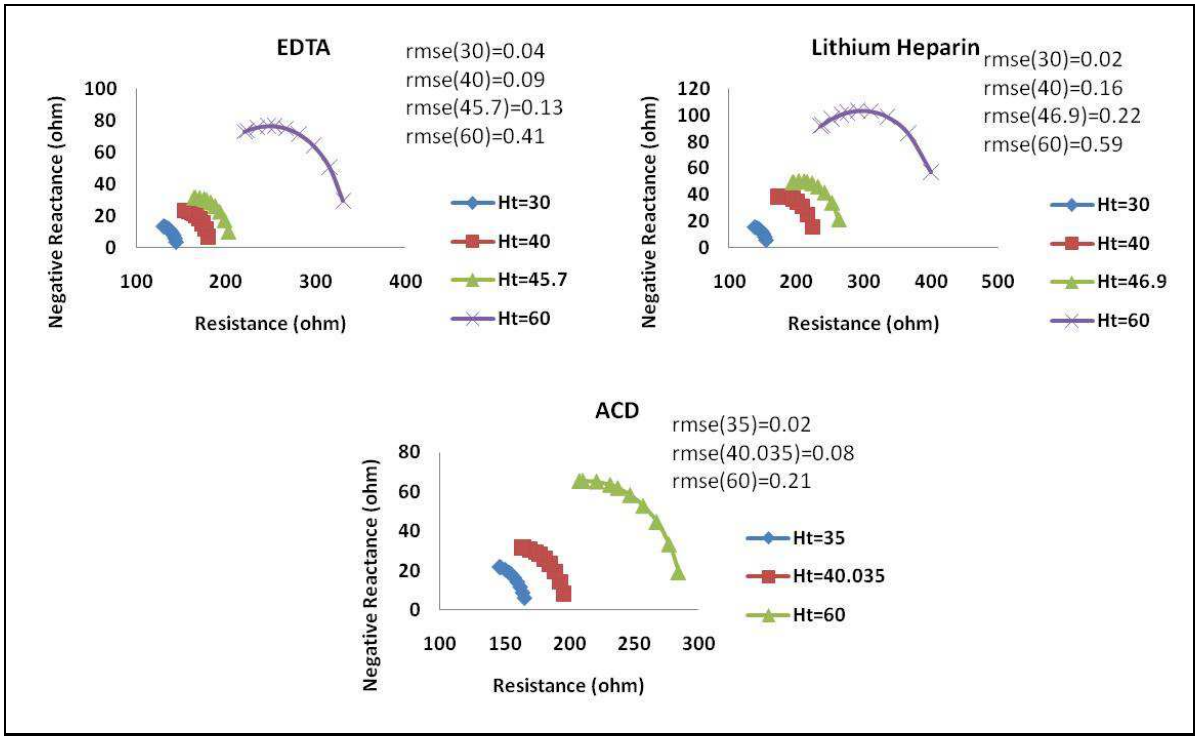

Figure 4.6 The Cole-Cole Plots for donor 6 with different anticoagulants.

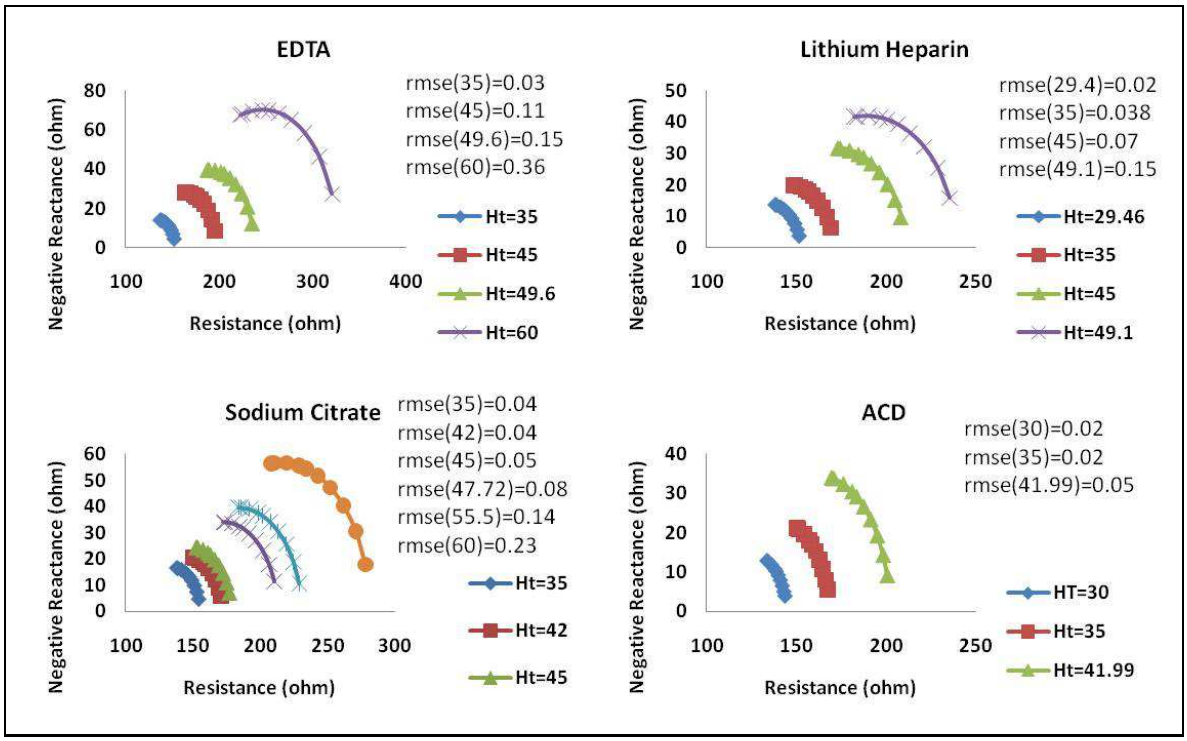

Figure 4.7 The Cole-Cole Plots for donor 7 with different anticoagulants.

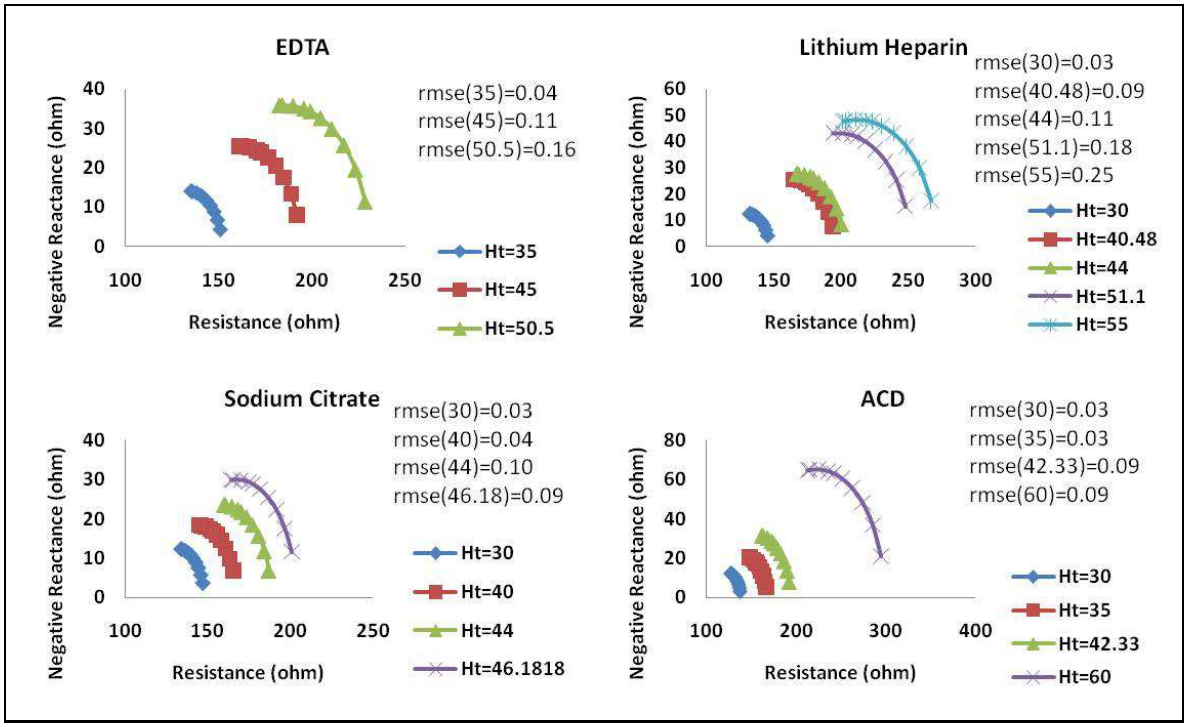

Figure 4.8 The Cole-Cole Plots for donor 8 with different anticoagulants.

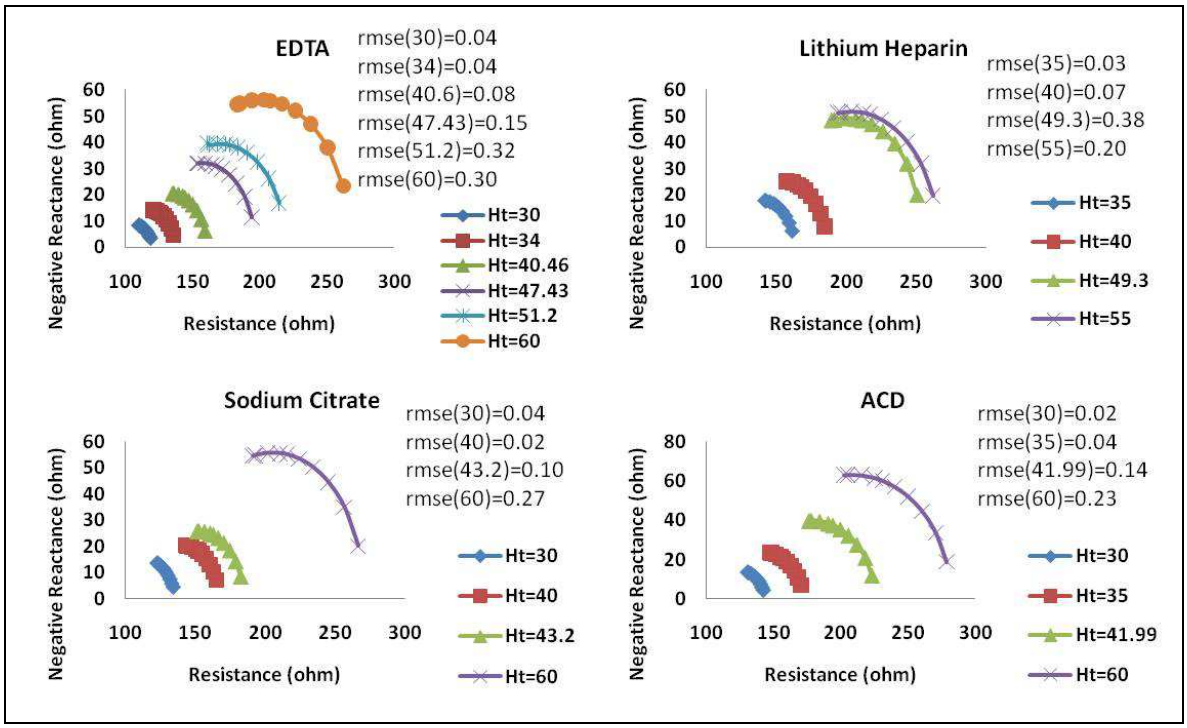

Figure 4.9 The Cole-Cole Plots for donor 9 with different anticoagulants.

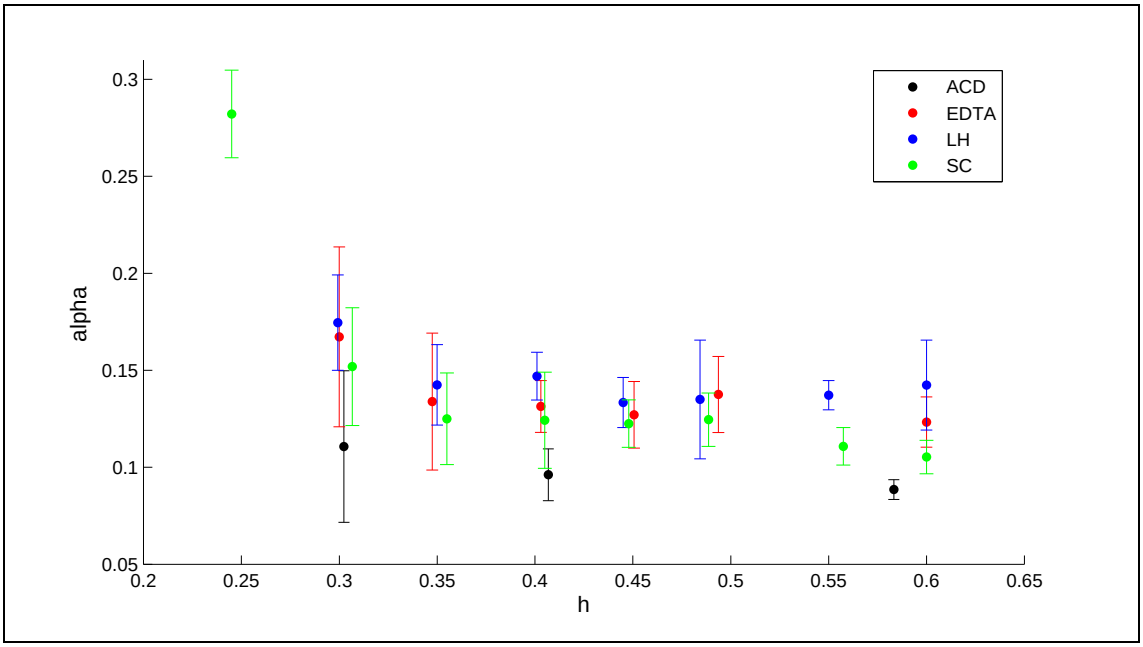

Figure 4.10 α as a function of hematocrit, with different anticoagulants.

Table 4.1
Cole Parameters for all donors with all anticoagulants.

		EDTA				LH				SC				ACD			
	Ht	35.0	38.0	45.0	48.7	30.0	40.0	47.6		36	38.0	44.1		30.0	38.9	40.0	60.0
Donor 1	Ri	532.4	390.9	335.4	293.3	427.1	348.6	303.7		445.5	375.4	343.2		477.6	303.6	361.5	279.8
	Re	151.4	171.5	194.5	213.6	143.4	169.8	225.5		141.3	189.5	214.4		139.3	192.6	169.4	279.5
	α	0.20	0.13	0.1	0.2	0.3	0.2	0.2		0.2	0.1	0.1		0.2	0.2	0.2	0.1
	fc	0.9	1.0	1.0	0.8	1.2	1.0	0.8		1.2	0.9	0.9		1.4	1.2	1.2	0.8
	Ht	30.0	40.0	51.0	60.0	35.0	40.0	48.2	55	30.0	40.0	46.53	60.0	30.0	40.0	43.7	50
Donor 2	Ri	503.2	394.8	319.0	290.4	421.2	322.2	283.2	290.0	447.6	383.9	346.3	285.7	545.6	362.1	302.7	271.8
	Re	138.7	177.2	229.0	281.3	155.9	206.8	272.0	287.0	126.5	157.3	183.5	256.4	134.1	165.3	208.9	228.3
	α	0.1	0.1	0.1	0.1	0.2	0.2	0.2	0.1	0.3	0.1	0.1	0.1	0.1	0.1	0.1	0.1
	fc	1.2	1.0	0.8	0.7	1.2	0.9	0.7	0.7	1.6	1.1	1.0	0.8	1.5	1.4	1.0	1.0
	Ht	30.0	40.0	44.6	60.0	30.0	40.0	47.7	60	32	44.8	56		30.0	35.0	40.4	60
Donor 3	Ri	518.9	402.4	378.1	306.9	455.7	395.3	349.2	301.1	478.7	341.4	298.8		509.1	384.6	322.6	280.4
	Re	140.8	182.4	196.6	310.7	170.1	231.1	263.9	405.3	142.1	196.3	266.6		137.2	168.6	195.1	270.9
	α	0.2	0.1	0.1	0.1	0.2	0.1	0.1	0.1	0.1	0.1	0.1		0.1	0.1	0.1	0.1
	fc	1.0	1.0	0.9	0.7	0.9	0.8	0.8	0.6	1.2	1.0	0.8		1.5	1.4	1.2	0.9
	Ht	30.0	40.0	48.2	60.0	30.0	40	48.9	60	30	40	45.7	60	30.0	40	40.2	
Donor 4	Ri	497.7	412.3	328.3	277.9	494.3	366.7	308.9	300.1	506.3	386.5	366.0	298.3	467.5	370.1	358.1	
	Re	145.6	172.4	234.1	370.9	152.6	213.3	311.5	386.1	130.5	158.6	181.4	276.1	141.3	171.6	216.7	
	α	0.1	0.1	0.1	0.1	0.3	0.2	0.1	0.1	0.1	0.1	0.1	0.1	0.2	0.1	0.1	
	fc	1.1	1.0	0.8	0.6	1.2	0.8	0.7	0.6	1.2	1.1	0.9	0.7	1.6	1.2	1.0	
	Ht	30.0	40.0	48.3	60.0	30.0	40.1	47.1	60	24	32	44.9	50	30.0	40	42.5	
Donor 5	Ri	442.2	378.2	329.9	264.4	484.3	342.2	303.5	287.9	535.4	432.5	308.2	312.2	392.8	354.3	279.3	
	Re	147.5	173.8	237.6	359.9	153.3	191.0	261.4	317.3	112.3	140.1	190.3	209.3	143.7	165.7	224.9	
	α	0.2	0.1	0.1	0.1	0.2	0.2	0.2	0.1	0.3	0.2	0.2	0.2	0.3	0.1	0.1	
	fc	1.2	1.0	0.8	0.6	1.1	0.9	0.7	0.6	2.1	1.2	0.9	0.9	2.0	1.3	0.9	
	Ht	30.0	40.0	45.7	60.0	30.0	40	46.9	50	35	40.0	60					
Donor 6	Ri	483.8	407.0	344.6	288.1	437.9	298.1	297.5	249.1	355.3.5	340.2	272.9					
	Re	145.6	182.6	206.6	342.94	157.5	231.3	272.9	432.9	167.1	197.9	290.2					
	α	0.1	0.1	0.1	0.1	0.2	0.2	0.2	0.2	0.2	0.1	0.1					
	fc	1.2	0.9	0.8	0.6	1.2	0.7	0.7	0.4	1.4	1.1	0.8					
	Ht	35.0	45.0	49.6	60.0	29.4	35	45.0	49.1	35	42	47.7	60	30	40	41.9	
Donor 7	Ri	532.8	391.7	362.7	317.7	523.0	431.5	368.3	322.6	462.7	430.0	352.2	319.5	502.3	398.7	326.7	
	Re	153.4	198.7	239.6	331.9	152.9	171.4	211.7	241.7	155.7	172.7	214.6	284.2	144.9	168.7	203.2	
	α	0.2	0.1	0.1	0.1	0.2	0.2	0.1	0.1	0.1	0.1	0.2	0.1	0.2	0.2	0.1	
	fc	1.1	0.9	0.8	0.6	1.3	1.1	1.0	0.8	1.2	1.2	0.9	0.8	1.8	1.5	1.2	
	Ht	35.0	45.0	50.5		30.0	40.8	44	51.1	30.0	40.0	44.0	46.2	30.0	40.0	42.3	60.0
Donor 8	Ri	484.3	401.3	365.3		548.1	416.9	400.3	343.3	573.2	440.2	442.8	364.6	538.3	416.2	333.0	296.0
	Re	153.1	195.3	233.2		147.6	197.3	203.8	254.1	148.3	168.5	189.5	205.8	138.9	168.4	194.4	301.4
	α	0.1	0.1	0.1		0.2	0.1	0.1	0.1	0.1	0.2	0.1	0.2	0.1	0.1	0.1	0.1
	fc	1.1	0.9	0.8		1.1	0.9	0.9	0.8	1.1	1.1	0.9	0.8	1.3	1.3	1.3	0.8
	Ht	30.0	40.6	47.4	60.0	30.0	40.0	49.3	55.0	30.0	40.0	43.2	60	30.0	40.0	41.9	60
Donor 9	Ri	432.3	348.1	291.2	250.5	421.8	374.9	348.2	291.8	437.6	370.9.5	360.9	283.7	441.5	352.5	318.3	267.8
	Re	94.5	161.7	199.1	272.6	164.3	187.7	256.8	270.3	136.9	168.3	185.7	273.9	143.8	172.7	227.2	284.4
	α	0.3	0.1	0.1	0.1	0.2	0.1	0.2	0.1	0.3	0.2	0.1	0.1	0.2	0.1	0.1	0.1
	fc	1.6	0.9	0.8	0.6	1.1	1.0	0.7	0.7	1.4	1.1	0.9	0.7	1.7	1.2	0.9	0.8

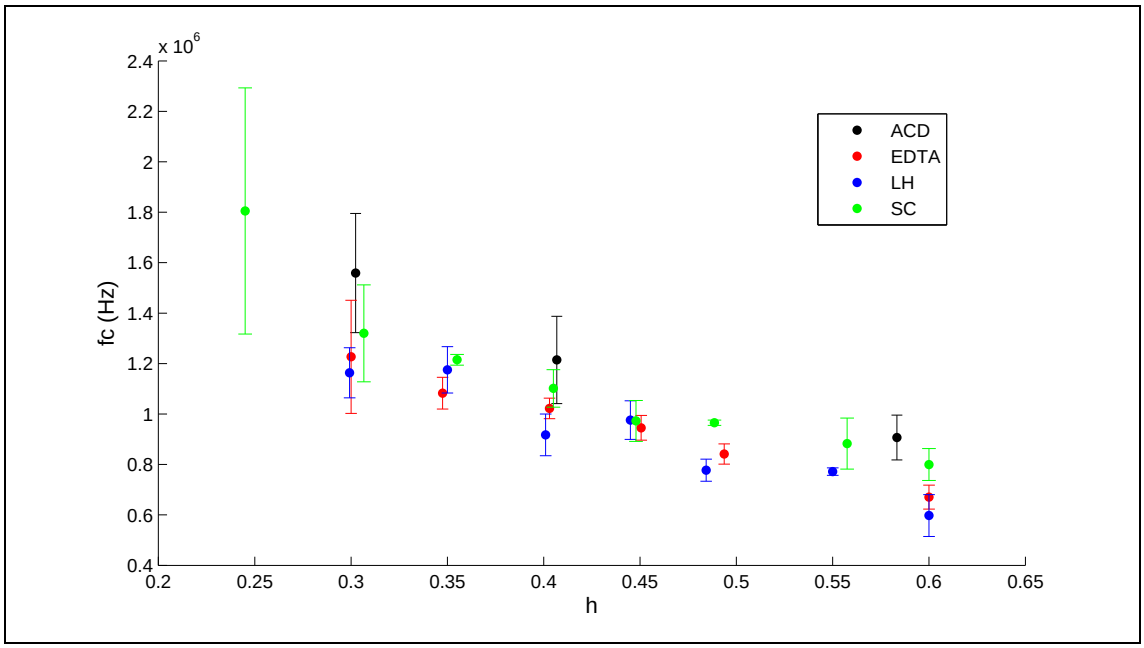

Figure 4.11 f_c as a function of hematocrit, with different anticoagulants.

4.2 Hematocrit Dependence with Different Anticoagulants

The relationship between intracellular and extracellular resistivities and hematocrit can also be formulated by using the Maxwell-Fricke equation [25]:

$$Re = \rho_p(1 + \xi h)/(1 - h) \tag{4.1}$$

where ρ_p is the resistivity of plasma, ξ= f -1, with f=form factor and h is hematocrit in decimal form. In Zhao's paper [32] Equation 4.1 expressed in the form:

$$Re = a/(1 - h) + b \tag{4.2}$$

where a and b are constants and ρ_p= a + b, ξ= -b/(a + b) and f= 1 +ξ= a/(a + b).

Since (1-h) represents the volume fraction of plasma in the blood, Ri can be obtained by substituting h for 1 - h in Equation 4.2:

$$Ri = c/h + d \tag{4.3}$$

where c and d are constants and c= $\rho_i(1 + \xi)$, d= $-\rho_i\xi$, and f= c/(c + d).

For h=0 in Equation 4.2, Re= ρ_p= a + b since no red blood cells are present. For h=1 in Equation 4.3, ρ_i= (interior resistivity)= c + d.

In Figures 4.12-4.19, Ri and Re values for each anticoagulant are fitted to the corresponding Maxwell-Fricke formula, the coefficients of the fitting equations with the R-square values are listed in Table 4.2. For EDTA 38 , for LH 36 and for SC and ACD 34 data points are considered. It is clearly seen that when hematocrit increases, extracellular resistance increases whereas intracellular resistance is reduced.

Equations in Table 4.2 show that, the rate of increase in extracellular resistance with hematocrit is largest with LH and EDTA. In the physiological range of hematocrit, Re is the highest with LH and the lowest with SC; at h=0.6 Re of LH samples are 34 % higher than SC samples. Zhao has found out that, Re with SH is 20 % higher than SC for 60 % hematocrit.

Table 4.2
Maxwell-Fricke Equations for different types of anticoagulants. R is the correlation coefficient.

		Present Study T=Room Temperature	R	Zhao (1993) $T = 37^0C$	R
Re	EDTA	175.4/(1-h)-117.6	0.996	131.7/(1-h)-78.8	0.994
	LH	203.9/(1-h)-144.8	0.949	-	-
	SC	133.5/(1-h)-54.8	0.994	112.1/(1-h)-52.9	0.998
	ACD	135.2/(1-h)-46.95	0.992	122.6/(1-h)-62.8	0.997
Ri	EDTA	130.9/h+69.2	0.923	155.3/h+7.6	0.985
	LH	111.7/h+101.8	0.921	-	-
	SC	177.9/h+83.2	0.846	158.8/h-1.6	0.97
	ACD	129.1/h+47.3	0.919	156.3/h+7.3	0.986

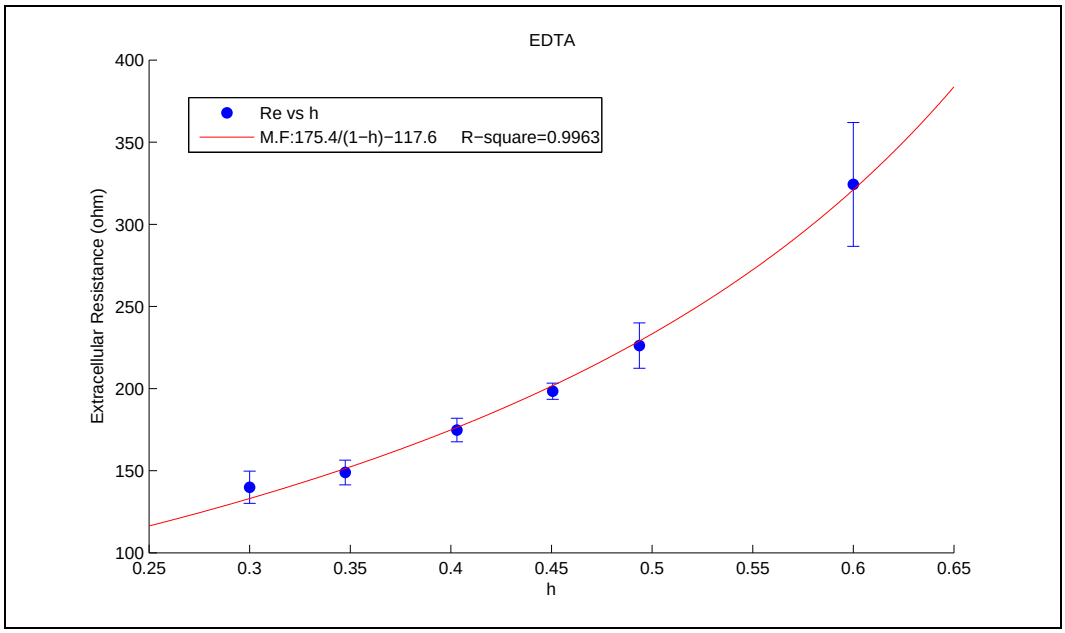

Figure 4.12 Extracellular resistance of blood with EDTA anticoagulant, as a function of hematocrit.

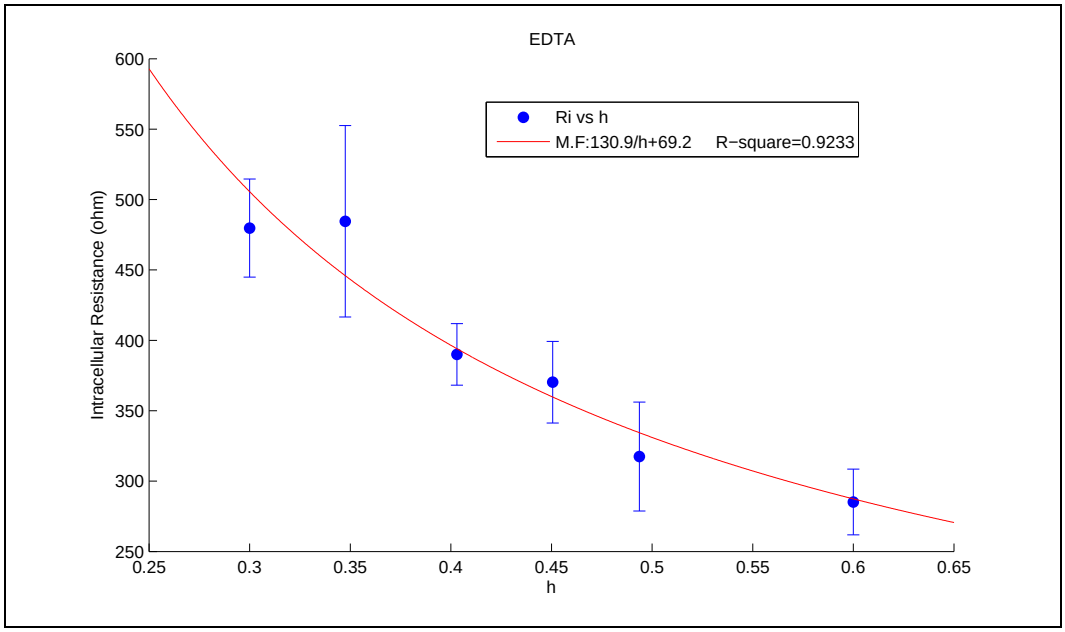

Figure 4.13 Intracellular resistance of blood with EDTA anticoagulant, as a function of hematocrit.

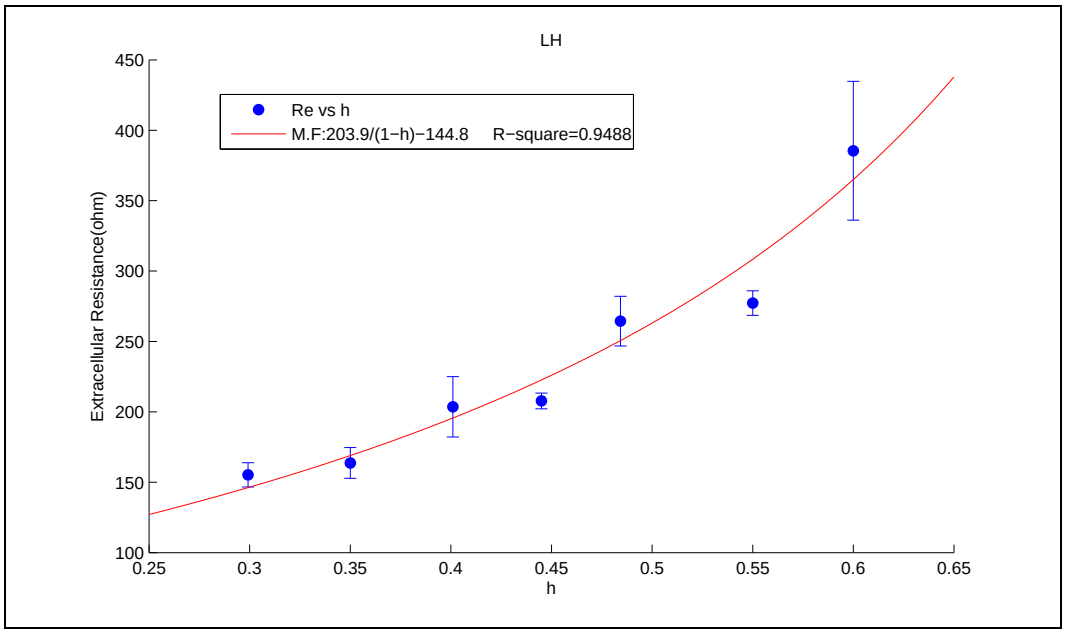

Figure 4.14 Extracellular resistance of blood with LH anticoagulant, as a function of hematocrit.

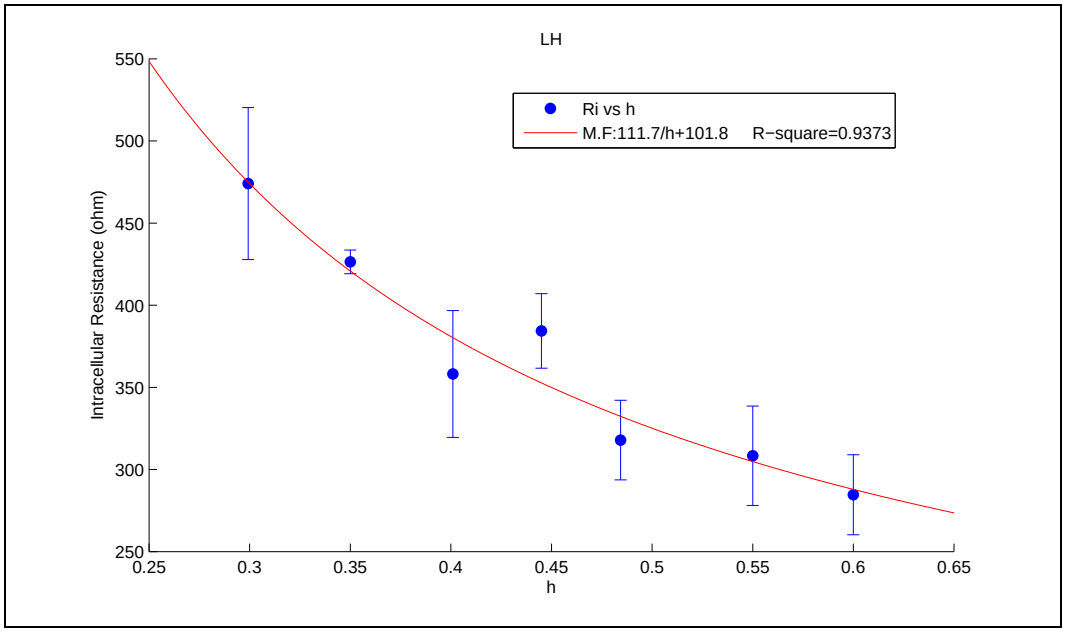

Figure 4.15 Intracellular resistance of blood with LH anticoagulant, as a function of hematocrit.

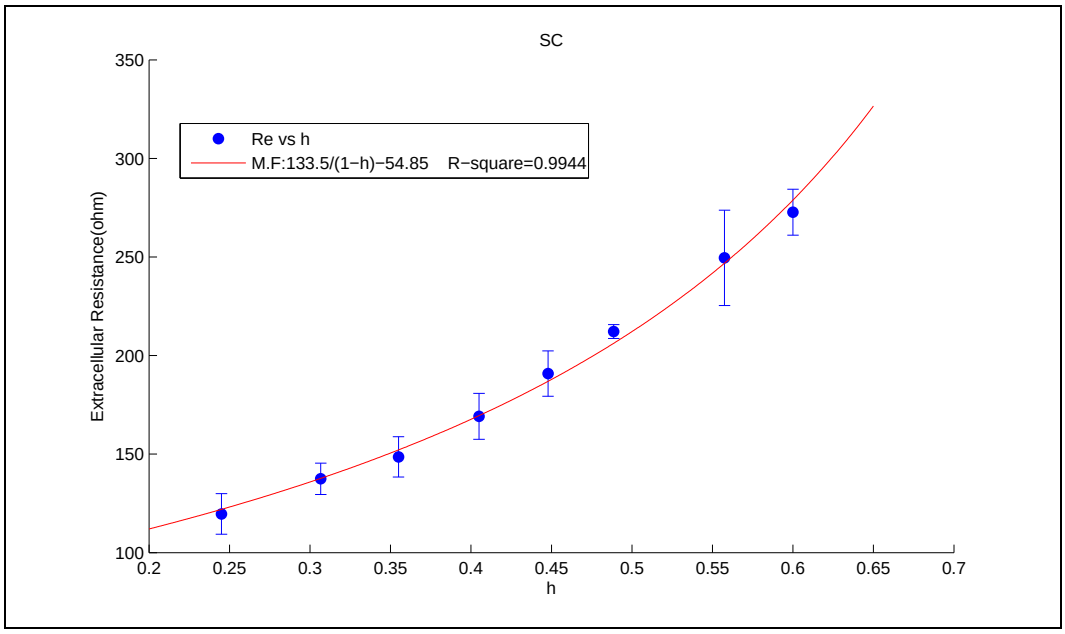

Figure 4.16 Extracellular resistance of blood with SC anticoagulant, as a function of hematocrit.

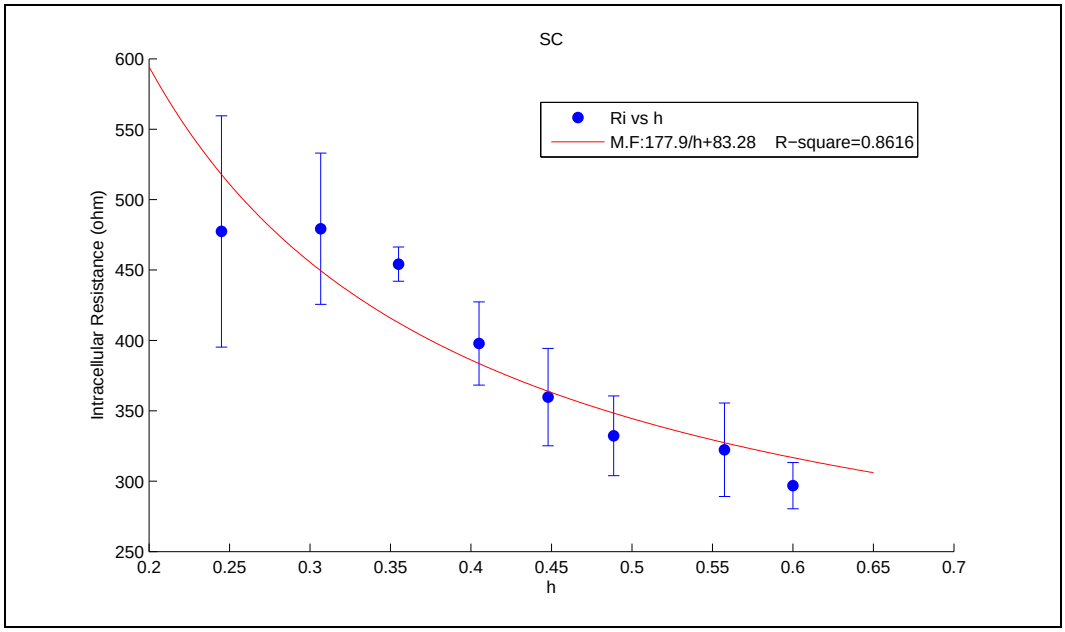

Figure 4.17 Intracellular resistance of blood with SC anticoagulant, as a function of hematocrit.

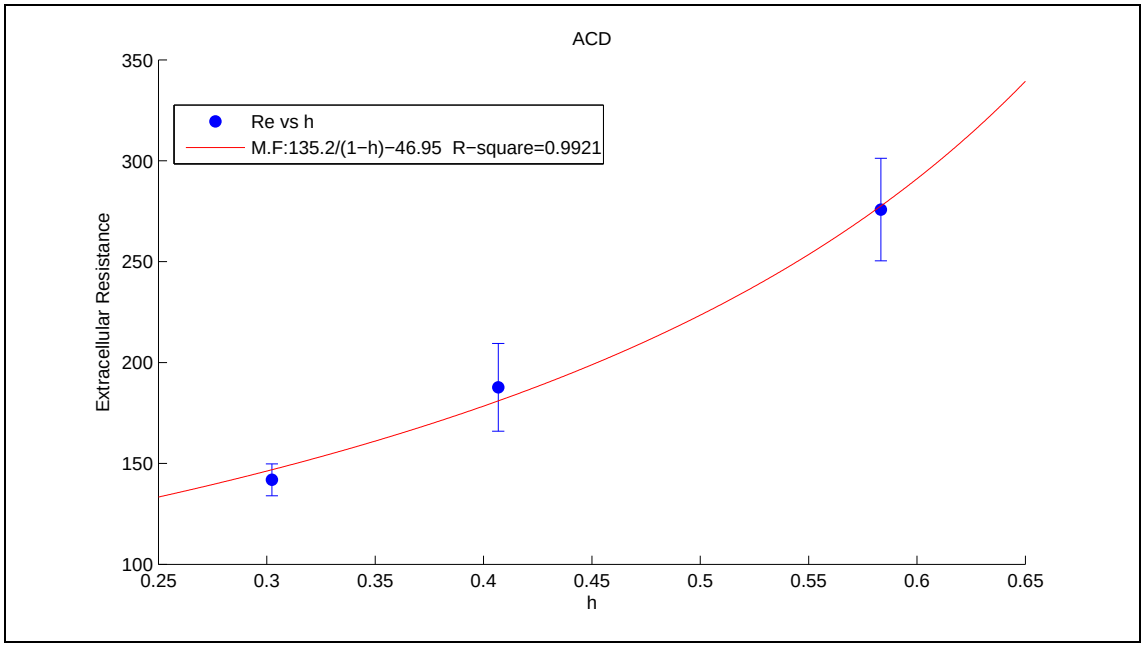

Figure 4.18 Extracellular resistance of blood with ACD anticoagulant, as a function of hematocrit.

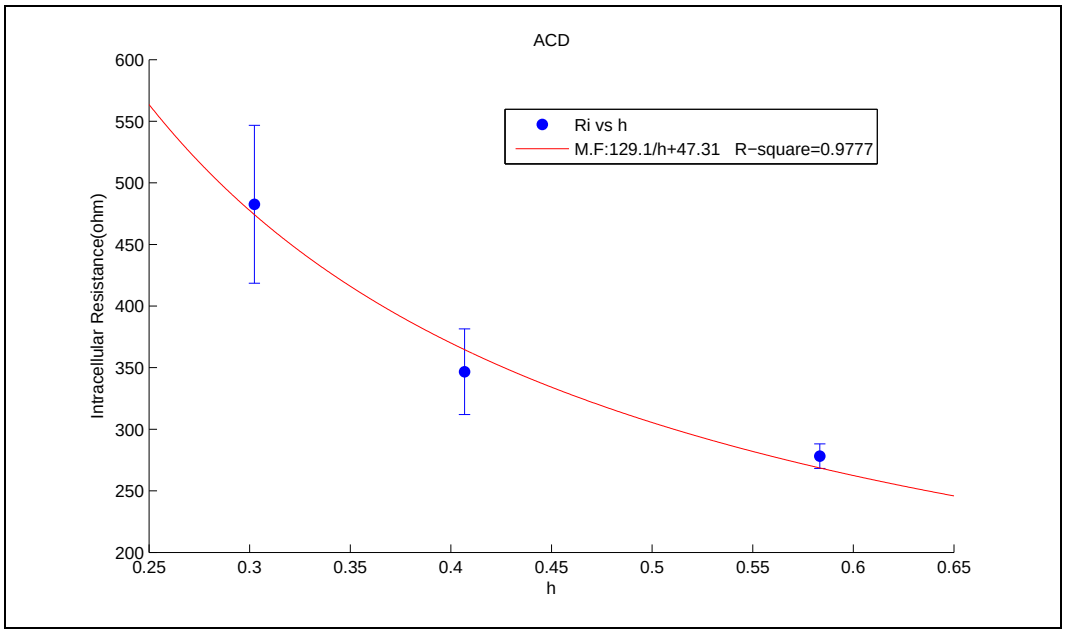

Figure 4.19 Intracellular resistance of blood with ACD anticoagulant, as a function of hematocrit.

4.3 Effect of Increasing the Amount of Anticoagulant

The amount of anticoagulant in standard SC tubes is 1 ml. Hence, when blood is drawn into the tube (10 ml capacity), the hematocrit of whole blood changes. In erythrocyte suspensions that are prepared by making use of different concentrations of plasma and red blood cells, plasma contains an amount of anticoagulant which cannot be disregarded.

In this specific experiment, the impedance of 5 ml blood with SC anticoagulant is measured at 100 kHz by gradually decreasing the volume of plasma and replacing it by the same amount of anticoagulant, such that the hematocrit remained constant. The experiment is performed with whole blood from donor # 2 (H=48,3 %) and donor #3 (H=48,0 %). In Figure 4.20, the increase in the volume of SC decreases the resistivity of blood for both of the donors. Hence the amount of anticoagulant should be taken into consideration when studying the impedance of blood.

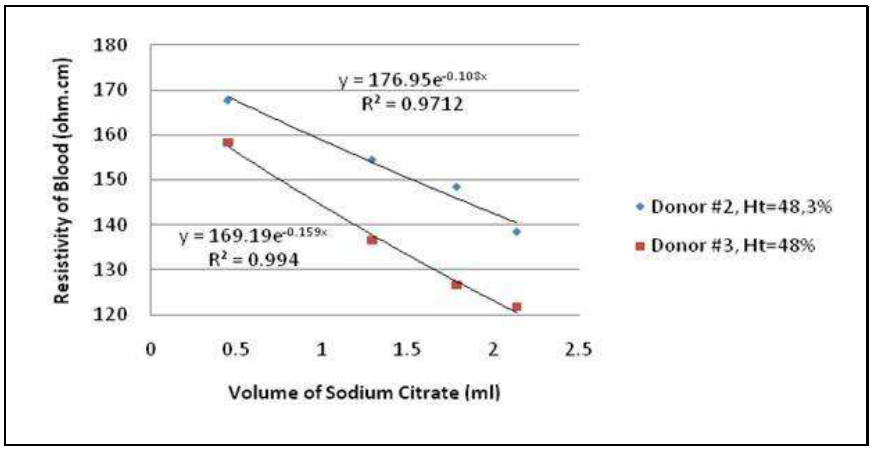

Figure 4.20 The change in the resistivity of blood with respect to volume change of Sodium Citrate.

In the Figure 4.20, it is clearly seen that the quantity of anticoagulant affects the electrical properties. Increasing the amount of Sodium Citrate in the blood increases its conductivity. When extrapolating to zero anticoagulant blood resistivities are 177 Ω.cm and 169 Ω.cm respectively. Corresponding changes in resistivity of blood with 1 ml of SC anticoagulant are calculated as 10.2 % and 10.8 %.

Extracellular resistance values obtained in this study are compared with some of the previous studies such as Geddes and Sadler (1973), Mohapatra and Hill (1975) and Zhao (1993) are tabulated in Table 4.3 [32]. Re value is calculated by inserting the hematocrit values to the Maxwell-Fricke Equations given in Table 4.2.

Table 4.3
Re at various hematocrits from different researchers. RT=Room Temperature.

	Present Study (RT)				Zhao(1993) T=37^{0}C				T=37^{0}C				
H(%)	EDTA	LH	SC	ACD	EDTA	SH	SC	ACD	Kubicek et al(100 kHz)	Geddes and Sadler(24 kHz)	Hill and Thompson (100 kHz)	Mohapatra and Hill(100 kHz)	Sanberg et al(100 kHz)
10	77	82	93	103	68	71	72	73	89	66	51	45	65
20	102	110	112	122	86	91	87	90	102	83	72	69	82
30	133	146	136	146	109	117	107	112	121	103	93	94	103
40	175	195	168	178	141	151	134	142	148	128	114	118	131
50	233	263	212	223	185	199	171	182	186	160	135	142	170
60	321	365	279	291	250	271	227	244	241	199	156	166	228

By applying the Maxwell-Fricke equation to Re vs h and Ri vs h data, the intracellular fluid resistivity, (ρ_i), and the plasma resistivity, (ρ_p), are calculated and shown in Table 4.4. In this study, both ρ_i and ρ_p are smaller than Zhao's findings. A decrease in temperature increases the resistivity, so it is expected that the resistivity values is higher in the present study compared to Zhao's results since the temperature is significantly lower than in Zhao's experiment. Pauly and Schwan (1977) determined ρ_i value as 193 at 25 ^{0}C using heparin as anticoagulant which is close to 213.5 for Lithium Heparin found in this study [33]. Cha [3] et al determined ρ_p as 47.1, in EDTA tube, at room temperature. The volume concentration of anticoagulants vary between the studies. In the present study, EDTA and LH are coated to the tube, and the volume concentrations of SC and ACD is 1:11 and 1.5:10, respectively. In Zhao, 1.50, 0.12, 0.02 and 2.00 ml per 10 ml sample for ACD, EDTA, SH and SC, respectively. Also the difference in the hematocrit values of the data points and the number of data points may affect the parameters.

Amounts of anticoagulants in the vacutainer tubes influence the extracellular medium. The fact that each type of anticoagulant has a different resistivity must also be taken into consideration. In Zhao's paper (1993) the resistivities were reported as

60.5 Ω.cm for ACD, 20.6 Ω.cm for EDTA and 53.4 Ωcm for SC, at 37^0C. In this study, resistivities at 100 kHz, measured at room temperature, are 67.9 Ω.cm for ACD and 52 Ω.cm for SC.

Table 4.4
Estimating ρ_p and ρ_i with Maxwell-Fricke Equation. RT=Room Temperature

		EDTA	LH	SC	ACD
Present study T=RT	ρ_p(ohm.cm)	58	59	79	88
	ρ_i(ohm.cm)	200	214	261	176
Zhao(1993) T=37^0C	ρ_p(ohm.cm)	53	-	59	60
	ρ_i(ohm.cm)	163	-	157	153

In Table 4.5, plasma resistivity values directly measured by conductivity meter and calculated by fitting Maxwell-Fricke equations are seen. According to t-test, in all anticoagulants, there is no significant difference between the calculated and directly measured resistivities of plasma. Hematocrit value, the interaction of red blood cells with plasma and anticoagulant can affect the calculated plasma resistivity. The amount of SC and ACD is higher than EDTA and LH, and therefore their effects are expected to be higher.

Table 4.5
Plasma resistivities (Ω.cm)

	EDTA		LH		SC		ACD	
	ρ_p(cal)	ρ_p(meas)	ρ_p(cal)	ρ_p(meas)	ρ_p(cal)	ρ_p(meas)	ρ_p(cal)	ρ_p(meas)
Donor#1	77	71	65	81	-41	72	102	-
Donor#2	86	69	72	78	76	66	72	77
Donor#3	74	-	78	-	70	73	103	80
Donor#4	64	69	97	-	70	71	7	77
Donor#5	65	69	97	-	70	71	27	79
Donor#7	49	72	86	79	82	74	62	79
Donor#8	63	71	81	80	89	78	84	79
Donor#9	65	69	81	78	85	71	110	77

Statistically, multicomparison analysis is performed on normalized values of Cole parameters. Since Re is almost linearly, and Ri is inversely proportional to hematocrit, we used normalized values; Re/h and Ri.h, respectively. When the anticoagulants are

compared with respect to normalized intracellular resistance, it is found that ACD is significantly different from EDTA and SC; and there is no significant difference between LH and other anticoagulants. Additionally, EDTA and SC are not significantly different from each other.

Multicomparison analysis based on the extracellular fluid showed that EDTA is significantly different from SC and LH is significantly different from all other anticoagulants. When the analysis is performed for R_∞, no significant difference is observed between anticoagulants. For the comparison based on f_c, one can see that all anticoagulants differ from each other, with the only exception that EDTA and LH are not significantly different from each other. The mean values of Re/h are 464±50.3 Ω, 530±63.4 Ω, 437±30.1 Ω, 465±34.8 Ω and Ri.h are 159±16.9 Ω, 153±7.3 Ω, 159±19.1 Ω, 146±16.1 Ω for EDTA, LH, SC and ACD respectively. The statistical results based on the multicompare analysis are displayed in Table 4.6.

Table 4.6
Comparison of Cole-Cole parameters for any two anticoagulants. ∗: p< 0.005, NS: no significance.

	Re/h				Ri*h				fc*h				α			
	EDTA	LH	SC	ACD	EDTA	LH	SC	ACD	EDTA	LH	SC	ACD	EDTA	LH	SC	ACD
EDTA	...	*	*	NS		NS	NS	*	...	NS	*	*	...	NS	NS	*
LH	*	...	*	*	NS	...	NS	NS	NS	...	*	*	NS	...	NS	*
SC	*	*	...	NS	NS	NS	...	*	*	*	...	*	NS	NS	...	*
ACD	NS	*	NS	...	*	NS	*	...	*	*	*	...	*	*	*	...

5. CONCLUSION

It has been shown that, hematocrit value of whole blood can be measured by the multifrequency impedance measurement technique with different anticoagulants. Although anticoagulants change the hematocrit dependence of extracellular fluid and intracellular fluid, due to their chemical composition and different volume concentrations, the common response is that the extracellular fluid is linearly proportional with hematocrit and intracellular resistance is inversely proportional to hematocrit.

Cole fittings of the whole blood samples and hematocrit suspensions are consistent with the theoretical prediction that their centers are below the real axis. Since Re and Ri is determined from R_0 (=Re) and R_∞ (=Re//Ri), Cole parameters R_0 and R_∞ also changes with hematocrit. The characteristic frequency f_c decreases with increasing hematocrit, the effect is more pronounced with Lithium Heparin. The angle of depression α is almost constant with respect to increasing hematocrit and the highest in LH.

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