MODELING OF PHYSIOLOGICAL PROPERTIES OF STORED HUMAN BLOOD BY COMPLEX IMPEDANCE MEASUREMENTS

by

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ABSTRACT

MODELING OF PHYSIOLOGICAL PROPERTIES OF STORED HUMAN BLOOD BY COMPLEX IMPEDANCE MEASUREMENTS

In this study, the relationship between physiological properties of human blood, namely Na^+, K^+, Cl^- concentrations, pH, 2,3-DPG and ATP, and its electrical parameters, the Cole-Cole parameters- the resistance of the extracellular fluid (R_e), the resistance of the red cell interior fluid (R_i), phase angle (α), characteristic frequency (F_c) and the capacitance of the cell membranes (C_m)- is investigated.

Measurements are performed on 51 erythrocyte suspension (ES) samples, subject to 42 days of storage at 4^oC, on day 0, 10, 21, 35 and 42. On whole blood (WB) samples (31 samples) under 21 days of storage, same measurements are done on day 0, 10 and 21. Electrical measurements are performed in the frequency range from 100 kHz to 1 MHz at room temperature. Multifrequency complex impedance data are fitted to Cole-Cole diagrams using Least Mean Square algorithm to give Cole-Cole parameters for the equivalent electrical circuit model of blood samples.

Variance analysis (ANOVA test) is used to evaluate differences in blood properties relative to storage time. The relationship between the physiological and the electrical parameters of blood is investigated by regression analysis using SPSS. A multiple regression model is developed for ES and WB separately, where the physiological parameters are expressed in terms of the electrical parameters.

In a case study, the models are tested for 20 donors, and it is seen that the model for WB is appropriate for predicting Na^+, K^+, Cl^-, pH and ATP at all time, for ES the model is only appropriate for the first 35 storage days. The models can not estimate 2,3-DPG at all, at any time.

This study clearly showed that complex impedance measurement technique can be a valuable tool in predicting the viability of stored blood.

Keywords: Na^+-K^+ ion current, Red blood cell, Blood storage lesions, Bioimpedance.

ÖZET

SAKLANAN İNSAN KANININ FİZYOLOJİK ÖZELLİKLERİNİN KOMPLEKS EMPEDANS ÖLÇÜMLERİ İLE MODELLENMESİ

Bu çalışmada, kanın fizyolojik parametreleri- Na^+, K^+, Cl^- konsantrasyonları, pH, 2,3 DPG ve ATP- ile elektriksel parametreleri, Cole-Cole parametreleri- hücredışı sıvı direnci (R_e), hücreiçi sıvı direnci (R_i), faz açısı (α), karakteristic frekans (F_c) ve hücre membran kapasitansı (C_m)- arasındaki ilişki incelenmiştir.

Ölçümler, +4^{o}C'de 42 gün boyunca saklanan 51 eritrosit süspansiyonu (ES) üzerinde 0., 10., 21., 35. ve 42. günlerde gerçekleştirilmiştir. 21 gün saklanan tam kan örneklerinde (31 örnek) ise, 0., 10., ve 21. günlerde ölçümler alınmıştır. Elektriksel empedans ölçümleri, 100 kHz-1 MHz frekans aralığında oda sıcaklığında gerçekleştirilmiştir. Çoklufrekans kompleks empedans ölçümleri, LMS algoritması kullanılarak Cole-Cole diyagramlarına fit edilmiş ve kanın eşdeğer elektriksel devresini modellemek için bu Cole-Cole parametreleri kullanılmıştır.

Parametrelerin zamanla değişimleri, ANOVA test ile incelenmiştir. SPSS'deki regresyon uygulamasıyla, kanın fizyolojik parametreleri ile elektriksel parametreleri arasındaki ilişki incelenmiş, çoklu regresyon analizinin uygulanmasıyla eritrosit süspansiyonu ve tam kan için ayrı ayrı model geliştirilmiştir. Örnek çalışma yapılarak, bu modellerin doğruluğu 20 donor ile test edilmiştir. Tam kan için geliştirilen model, tüm saklama günleri için Na^+, K^+, Cl^-, pH and ATP'ye doğru yaklaşımda bulunurken, eritrosit süspansiyonu için geliştirilen model ancak ilk 35 saklama günü için doğru yaklaşımda bulunmuştur. 2,3-DPG için, hem tam kan hem de eritrosit süspansiyonunda yaklaşımda bulunulamamıştır.

Kan bankasında saklanan kanların kalite tespitinde, biyoempedans tekniğinin kullanılabileceği görülmüştür.

Anahtar Kelimeler: Na^+-K^+ iyon akışı, Kırmızı kan hücresi, Saklanan kan lezyonları, Biyoempedans.

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α	The spread of relaxation times
ε	The relative permittivity
σ_b	The electric conductivity of blood
σ_∞	The tissue conductivity at very high frequencies
τ	The time constant

LIST OF ABBREVIATIONS

ACD	Acid-citrate-dextrose
AS-1	Adsol
AS-3	Nutricel
AS-5	Optisol
ATP	Adenosine triphosphate
BTHC	Butyryl-trihexyl citrate
C_{eff}	The effective capacitance of red blood cell
C_m	The cell membrane capacitance
CPD	Citrate-phosphate-dextrose
CP2D	Citrate-phosphate-dextrose with higher dextrose
CPDA-1	Citrate-phosphate-dextrose with less dextrose, additive adenine
DEHP	Di-2-ethyl-hexyl phthalate
DPG	Diphosphoglycerate
ES	Erythrocyte suspension
ESR	The erythrocyte sedimentation rate
f	Frequency
F_c	Characteristic frequency
Hct	The hematocrit in per cent
Im (Z)	The imaginary part of impedance
k	The conductivity measurement cell constant
PVC	Polyvinyl chloride
RBC	Red blood cell
R_e	The extracellular fluid resistance
Re (Z)	The real part of impedance
R_i	The intracellular fluid resistance
R_m	The cell membrane resistance
R_0	The cell resistance at $f = 0$
R_∞	The cell resistance at $f = \infty$
SAGM	Sodium-adenin-glucose-mannitol
V_b	The volume of blood sample

WB	Whole blood
X_c	The capacitive reactance
Z	The sample impedance
Z_{CPA}	The constant phase angle impedance

1. INTRODUCTION

Red blood cells stored in the blood bank, normally undergo a series of chemical alterations, or storage lesions. The ultimate consequence of these lesions is a decrease in the viability of the red cells following transfusion [1]. Red cell viability of stored blood classically can be defined as the percentage of cells remaining in the circulation of the recipient, 24 hours after transfusion. If red cells are transfused at the time of maximum storage, up to 20-30% of the red cells may be destroyed within 24 hours, the remainder showing a survival close to normal [2].

The chemical alterations mentioned above are mainly changes in levels of Na^+, K^+ and Cl^- concentrations, pH, 2,3-DPG and ATP levels, and they affect the electrical impedance of blood [1]. Red cell viability is generally evaluated by such measurements of pH, adenosine triphosphate levels, 2,3-DPG levels, etc. [3]. These parameters can be used in the assessment of blood quality.

The electrical impedance is determined mainly by the resistance of intracellular and extracellular fluids (R_i) and (R_e), respectively, and the effective capacitance of the cell membranes (C_m). The cell membrane separating the two water spaces acts as an electrical isolator. At low frequencies, the alternating current mainly passes through the extracellular space while at high frequencies, cell membranes become conductive and intracellular conduction also takes place. This allows the impedance technique to compartmentalize the water distribution of tissues.

The bioelectrical impedance technique is commonly utilized to estimate the water content in tissues and in whole body [4]-[9]. When this technique is applied to blood, high correlations between the resistivity or conductivity of blood and the hematocrit have been reported [10]. Recent studies have shown strong correlation between the hematocrit and the plasma resistance R_e, the intracellular resistance R_i, and the cell membrane capacitance C_m; yet the accuracy of impedance method for estimating hematocrit has not been well established [10].

Blood impedance parameters might find clinical applicability since they could be related to certain pathological conditions of blood. For example, C_m of blood cells with high erythrocyte sedimentation rate has been found significantly higher than that of normal blood cells. The impedance parameters are, however, strongly dependent on the hematocrit which varies among individuals, especially among patients. Hence it is necessary to know the dependence of R_e, R_i and C_m on the hematocrit [11].

In Chapter 2 blood physiology and electrical properties of blood are introduced. The quality of blood and the appropriate storage conditions are given in this chapter.

Chapter 3 explains the impedance spectroscopy measurements and the physiological parameter measurements performed on human blood samples, under storage. Cole-Cole representation of complex impedance and the method for conductivity measurement of blood are described here.

Physiological and electrical impedance alterations during storage are explained in Chapter 4, as experimental results.

Chapter 5 describes the biophysical interpretation of electrical impedance changes.

The multiregression models of the physiological parameters for erythrocyte suspensions and whole blood samples are derived in Chapter 6 where the Cole-Cole parameters are proposed as predictors of blood quality.

The study is finally concluded in Chapter 7.

2. BLOOD PHYSIOLOGY AND ELECTRICAL PROPERTIES OF BLOOD

2.1 Introduction

Blood is the body's principal extracellular fluid. Its flow through the tissues permits its numerous transport functions, which ensure nutrition, respiration, physiological regulation, and defense. During its course through the tissue capillaries, blood delivers nutrients from the small intestine and oxygen from the lungs to the cells. It also removes the toxic waste products of cellular metabolism, such as urea and carbon dioxide, from the tissue environment and eliminates them as it circulates through the kidneys and lungs respectively. In addition, blood carries the hormones from their sites of production in the endocrine glands to their target organs in other locations [12].

Two compartments, a cellular compartment and a fluid medium called the plasma, make up blood tissue. The blood cells float freely within this medium. The cells are mostly red cells (erythrocytes), with smaller numbers of white cells (leukocytes) and platelets (occasionally called thrombocytes). Separation into these two compartments is achieved by centrifuging the blood in a small capillary tube. The centrifuged blood separates into a colorless fluid supernatant on the top and a red precipitate on the bottom. The supernatant, amounting to about 55% of the blood volume, is the plasma.

2.1.1 Blood Components

2.1.1.1 Blood Plasma. The plasma consists mainly of water (90%), which helps dissolve the blood proteins (e.g., fibrinogen, albumins, and globulins). The blood plasma contains high concentrations of Na^+ and Cl^-. Typical values of ionic concentrations in plasma are given in Table 2.1. In plasma, the ionic concentrations depend on the steady state (state maintained by the metabolism) and are affected by ionic transfers and fluid movements.

Table 2.1
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
HCO_3^-	26.0
PO_4^-	2.0
Other	6.0
Protein	17.0

2.1.1.2 Red Blood Cells. The erythrocyte has no nucleus and contains no intracellular membranes. The erythrocyte normally adopts the shape of a biconcave disk 7 micrometer in diameter. It is very flexible, however, and often squeezes through capillaries much thinner than 7 micrometer. Aged or deformed cells that do not possess this flexibility are trapped in the capilleries of the spleen and ingested by macrophages [13].

Red blood cells (erythrocytes) are produced in the bone marrow under the controlling influence of the renal hormone erythropoietin. After entering the bloodstream, they have a life-span of approximately 120 days before being broken down in the reticuloendothelial system. The red cells contain the iron-containing pigment haemoglobin, whose primary function is to store and transport oxygen.

The haemoglobin molecule is made up of four sub-units, each of which is composed of an iron-containing ring surrounded by a peptide chain. Each sub-unit of haemoglobin can reversibly combine with one molecule of oxygen. Thus each molecule of haemoglobin can combine with a maximum of four molecules of oxygen [14].

Red cells are the most numerous of the cells in blood and normally occupy about 45% of the whole blood volume.

Without the benefit of mitochondria or of ribosomes for synthesizing protein, the erythrocyte survives for more than 4 months in the face of repeated oxidant stress from the high concentrations of O_2 and repeated mechanical stress from many daily passages through capillaries of diameter saller than that of the cell. This cellular longevity seems to depend on a simplified metabolic organization with three main functions: to provide

energy for maintaining cellular volume, to provide reducing power to protect the cell against oxidation and to help control the affinity of haemoglobin for O_2.

The red cell interior conductivity results from K^+, Na^+, Cl^-, Mg^{+2} and HCO_3^- ions. Typical values of ionic concentrations in erythrocytes are given in Table 2.2.

Table 2.2
Some typical values of intracellular ionic concentrations in mEq/liter of erythrocytes [12].

Ion	Erythrocytes
Na^+	19
K^+	136
Mg^+	6
Ca^{++}	0
Cl^-	78
HCO_3^-	18
PO_4^-	4
Protein	36

2.1.1.3 White Blood Cells. They are produced in the bone marrow and lymphatic tissue. Their principal role in the blood is to identify, destroy and remove any foreign material that has entered the body. These cells are therefore important in fighting infection and in developing resistance to infection in response to natural exposure or immunization. White cells occupy less than 1% of the total blood volume.

2.1.1.4 Platelets. Platelets are small fragments of cells (megakaryocytes), which are produced in the bone marrow and contain enzymes and other biologically active substances.

Their function is to respond to any vascular wall damage by gathering together at the site of injury to form an initial temporary platelet plug and releasing their contents into the blood.

2.1.1.5 Cell Membrane. Apart from certain exceptions (e.g.: neurones) the cell size is generally in the range 5-20 microns. The cell membrane is an asymmetric structure comprising a lipid bilayer, membrane proteins molecules and the 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].

The integral membrane proteins also subserve important cellular functions. One has binding sites for several glycolytic enzymes and provides transport facilities for HCO_3^- and Cl^- ions entering and leaving the cell [14]. Other proteins include the Na^+, K^+-ATPase of the sodium pump and a Ca^{+2}, Mg^{+2}-ATPase, which mediates active efflux of calcium from the cell**.**

There are many varied forces producing movement of substances across membranes. They include:

1 *Diffusion*, in which a substance passes from an area where it has high concentration to an area of lower concentration.

2 *Filtration*, where fluid is forced through a membrane under pressure.

3 *Active Transport*, where certain substances are specifically 'pumped' across membranes.

4 *Osmosis*, the process in which freely moving substances, such as water, are drawn across a membrane towards a region where there is a higher concentration of molecules to which the membrane is impermeable. These molecules are termed 'osmotically active' [17]. In body fluids, they include the electrolytes sodium, potassium and chloride, and the proteins.

2.1.2 Total Blood Volume

The volume occupied by both cells and plasma in the vascular system is called the total blood volume.

In an adult, this is approximately 7% of body weight or 70 ml/kg. As children have a higher water content, the blood volume is calculated as the 8% of their body weight.

Table 2.3
The blood volume for different age groups [17].

Age group	Blood volume
Neonates	85-90 ml/kg
Children	80 ml/kg
Adults	70 ml/kg

2.1.3 Main Function of Blood

The most basic physiological function of blood is to ensure a constant supply of oxygen to the tissues and organs of the body in order that life can be sustained. To achieve this, four important steps must take place.

1. Oxygen transfer from the lungs into the blood plasma.
2. Oxygen storage on the haemoglobin molecule in the red blood cells.
3. Oxygen transport to the tissues of the body via the circulation.
4. Oxygen release from the blood to the tissues, where it can be utilized.

2.1.3.1 Oxygen Transfer from Lungs to Plasma. When a breath is taken, the air initially becomes humidified in the upper airway and is then transferred by ventilation to the alveoli of the lungs. The partial pressure of oxygen in the alveoli constitutes the 'driving force' resulting in the transfer, by diffusion, of oxygen into the blood. Gases diffuse from areas of high pressure to those of low pressure. The partial pressure of oxygen in the alveoli is 100 mmHg, but it is only 40 mmHg in the pulmonary capillary returning blood from the tissues. Oxygen therefore rapidly diffuses down its pressure gradient across the alveolar-capillary membrane to dissolve into the plasma of the pulmonary blood.

2.1.3.2 Oxygen Storage in Blood. Oxygen storage in blood depends almost entirely on the presence of haemoglobin within the red blood cells. Haemoglobin has the ability to combine with oxygen to such an extent that its presence increases the oxygen-carrying capacity of blood by 70 times. Without it, the dissolved oxygen in plasma would be totally inadequate to supply the demands of the tissues.

Each gram of haemoglobin can carry up to a maximum of 1.36 ml of oxygen. When it is in this state, it is fully or 100% saturated with oxygen [17].

Once diffusion of oxygen from the alveoli into the plasma of the pulmonary blood has taken place, oxygen at its high partial pressure (98 mmHg/13KPa) rapidly crosses into the red blood cells and binds to the molecules of haemoglobin until they become almost fully saturated. Typically, the haemoglobin in arterial blood is about 97% saturated with oxygen.

The relationship between plasma partial pressures of oxygen and the degree of saturation of haemoglobin is given by the oxygen dissociation curve, shown in Figure 2.1.

This curve embodies the unique ability of haemoglobin to combine with oxygen at high partial pressure in the lung, and then to lose its affinity at lower partial pressures found in the tissues and release its oxygen to them.

Several factors can alter the position of the curve. Some move it to the right, reducing haemoglobin affinity and facilitating oxygen release to the tissues. Others move it to the left, increasing its oxygen affinity and ensuring full saturation of haemoglobin in the pulmonary capillaries.

2.1.3.3 Oxygen Transport to the Tissues. Once haemoglobin becomes saturated with oxygen in the pulmonary circulation, it must then be transported in the blood flow to the systemic circulation and onwards to the tissue capillaries of the body [14].

2.1.3.4 Oxygen Release to the Tissues. The final stage of oxygen delivery involves the release of the stored oxygen in blood to the tissues. Once again, this process is controlled by the tissues themselves and is regulated according to their demand for oxygen.

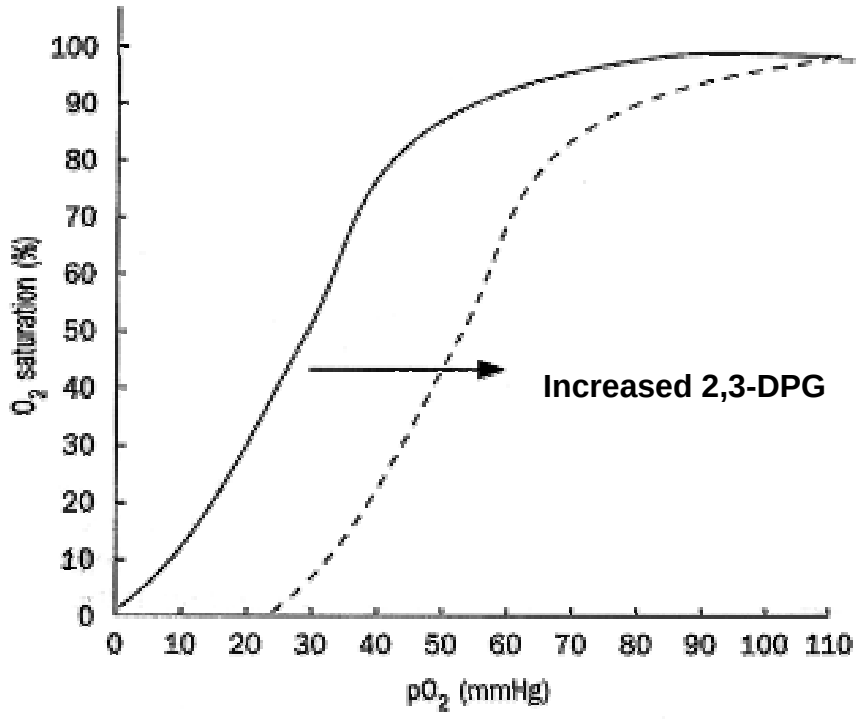


Figure 2.1 The Oxygen dissociation curve [17].

2.1.3.5 Oxygen Dissociation. The amount of oxygen that the haemoglobin molecule will take up for any given oxygen tension is known as the oxygen affinity of haemoglobin. It is usually expressed as the partial pressure of oxygen required to half saturate the molecule. Because oxygen is continuously being utilized in the cells, the partial pressure of oxygen in the tissues is considerably lower than that in arterial blood entering the capillaries. Oxygen therefore diffuses down its pressure gradient from the capillaries into the tissues, resulting in a fall in the partial pressure of oxygen in the capillary plasma. If one plots a graph of the percentage saturation against the partial pressure of oxygen, a sigmoid curve is obtained [17].

It can be seen from the oxygen dissociation curve that a fall in partial pressure of oxygen in plasma reduces the saturation of haemoglobin. Consequently, haemoglobin releases its stored oxygen into the plasma of the capillaries where it can then diffuse into the tissues.

2.1.3.6 Shifts in the Oxygen Dissociation Curve. In very active tissues, where the demand for oxygen is highest, there are sharp increases in the levels of carbon dioxide and acids derived from metabolism and in local temperature. These changes also affect the

capillary blood and shift the oxygen dissociation curve to the right, reducing the haemoglobin's affinity for oxygen and encouraging its release to the tissues.

When the tissue demand for oxygen returns to normal, the oxygen dissociation curve shifts back to the left, increasing the affinity of haemoglobin for oxygen and reducing the amount released to the tissues.

Another major factor that influences the position of the oxygen dissociation curve is the presence of the red blood cell metabolite 2,3 diphosphoglycerate (2,3DPG). When the concentration of this substance increases in the red cell, the dissociation curve shifts to the right, again facilitating the release of oxygen to the tissues, as shown in Figure 2.1.

Once oxygen extraction by the tissues is completed, desaturated blood with a typical partial pressure of oxygen of about 40 mmHg enters the venous circulation and returns to the heart to complete the cycle again [17].

2.2 The Quality of Red Blood Cells

The quality of the stored red cell is mainly affected by several parameters [18]:

Red cell collection processing,

Component separation,

Red cell containers,

Additive solutions, and

Storage conditions.

2.2.1 Red Cell Collection

Whole blood is obtained from human blood donors by venesection. During donation, blood is collected into a sterile, disposable, plastic pack which contains an anticoagulant-preservative solution [19].

An automated apheresis collection system is essential because there is a significant collection lesion with manual blood collection systems. This system utilizes a system of

metered anticoagulant addition to the whole blood which avoids the osmotic shock of manual collection.

For anticoagulation, citrate is used. The citrate anticoagulates the blood by combining with the blood calcium. Although the first red cell anticoagulant/preservative was acid citrate dextrose-ACD, CPD- citrate-phosphate-dextrose is preferred to ACD because ACD has high osmolality and causes a significant lesion of collection. CPD is less hypertonic and contains additional phosphate [18].

In the standard blood bags, 63 ml anticoagulant for 450 ml + 10% is sufficient. The hematocrit of the whole blood is 40%.

The whole blood can be stored in **ACD**, **CPD** and **CP2D** – 21 days (CP2D higher dextrose), in **CPDA-1** – 35 days (less dextrose, additive adenine) and in **additive solution** – 42 days.

2.2.2 Component Separation

Whole blood may be suitable for transfusion in many clinical situations, such as red cell replacement in acute blood loss. However, the separation of whole blood into its constituent components- red cells, platelets and plasma- is widely practised for use when these specific components only are required.

4 types of red cell preparation are usually used:

Red cells

Washed red cells

Leukocyte-poor red cells

Frozen red cells

When the plasma is removed from whole blood, the red cells can be used as a red cell concentrate or can be formed into a red cell suspension by the addition of an additive diluent solution.

2.2.2.1 Red Cell Concentrate. Red cell concentrate is the simplest red cell component. It is prepared by allowing the blood to seperate under gravity overnight in a refrigerator at a temperature of $+2^{o}C$ to $+6^{o}C$ or by centrifuging the blood pack in a special refrigerated centrifuge. The plasma is then removed by transferring it into a second empty plastic pack, which is supplied connected to the primary whole blood bag to ensure sterility, leaving all the red cells in the original blood collection pack. This process must be generated before the maximum storage time. After process, the hematocrit will be 60-90%. The volume of erytrocyte concentration is 250-300 ml. The red cell concentrate also contains white cells from the donated blood.

2.2.2.2 Red Cell Suspension. Red cell suspension is prepared by removing the plasma into a second empty plastic pack. An additive diluent solution formulated for the best preservation of the red cells is then transferred from a third plastic pack into the original pack.

2.2.2.3 Washed Red Cells. The eytrocyte concentrations can be washed with saline physiology by using centrifuge or continuous-flow cell separator. The washed erythrocytes must be used in 24 hours after the process. There is a risk of bacterial contamination.

2.2.2.4 Leucocyte-depleted (filtered) Red Cells or Whole Blood. White cell transfusions have no proven clinical uses. The removal of the white cells from whole blood requires controlled centrifugation so that the red cells settle to the bottom of the blood pack. The white cells remain in a layer called the 'buffy coat' which forms an interface between the red cells and the plasma. Special filters can be used to remove virtually all the white cells. These filters can be used at the patient bed side during erythrocyte transfusion [20].

2.2.2.5 Frozen Red Cells. Frozen erytrocytes can be stored for years. This process is more expensive than fluid storage. For this process, cryoprotective agent is used.

2.2.2.6 Plasma. Plasma is separated from whole blood by centrifugation or by allowing the red cells to settle under gravity in a blood bank refrigerator. It can also be collected from donors by plasmapheresis.

2.2.2.7 Platelet Concentrates. Platelets separated from plasma obtained from 4-6 donations of whole blood are often pooled to produce a therapeutic dose of platelets for an adult. National guidelines generally require this dose to contain at least $240x10^9$/L platelets [16].

2.2.3 Red Cell Containers

The viability of red cells, after refrigerated storage, is greater when the blood has been stored in certain types of plastic rather than glass containers. The variability in surface characteristics and chemical composition of different plastics, require more rigid control studies than are necessary for glass containers [21].

The blood containers are made of polyvinyl chloride plasticized with di-2-ethyl-hexyl phthalate (DEHP) which makes up 30-40% of the weight of the plastic. DEHP dissolves in the proteins during whole blood storage at a rate of 0.25mg/100 ml/day. In the studies, after 35 days of CPDA-1 whole blood storage DEHP levels of 146ml/l have been observed.

Recently a new plasticizer butyryl-trihexyl citrate (BTHC) is used because it is significantly less toxic. All in vitro parameters are as well maintained with PVC-BTHC as with PVC-DEHP [18].

2.2.4 Additive Solutions

Additional solutions are used to increase the storage time. These solutions are AS-1 (Adsol), AS-3 (nutricel) and AS-5 (optisol). The contents of these solutions are shown in Table 2.4. In these solutions, citrate as an anticoagulant, phosphate as a buffer, dextrose as a source of energy for the metabolism of erythrocytes is used. With the addition of adenine, it is possible to extend storage time to 35 days [22]. Adenine is rapidly taken up by red cells and converted to ATP in the presence of glucose and phosphate. Phosphate is included to stimulate glycolysis. Mannitol is included in additive solutions since it acts as an osmotic stabilizer. Recently SAGM sodium adenine glucose mannitol is used as an additive solution.

Their functions are summarized in Table 2.4.

Table 2.4
The functions of storage solutions [22].

Solutions	Functions
C Sodium citrate	Binds with calcium ions in blood in exchange for the sodium salt so the blood does not clot
P Phosphate	Supports metabolism of the red cells during storage to ensure they release oxygen readily at tissue level
D Dextrose	Maintains the red cell membrane to increase storage life
A Adenine	Provides energy source

2.2.5 Storage Conditions

Red cells and whole blood must always be stored at a temperature between $+1^{o}C$ and $+9^{o}C$. They must never be allowed to freeze.

The upper limit of $+9^{o}C$ is essential to minimize the growth of any bacterial contamination in the unit of blood. The lower limit of $2^{o}C$ is essential because red cells that are allowed to freze become haemolysed. If they are transfused, the presence of cell membrane fragments and free haemoglobin can cause fatal bleeding problem or renal failure.

The solution in the blood bag contains both anticoagulant to stop the blood from clotting and dextrose to feed the red cells during storage. Storage at a temperature between $1^{o}C$ and $9^{o}C$ is essential to make sure the dextrose is not used too quickly [16].

Whole blood and red cells should be issued from the blood bank in a blood transport box or insulated carrier that will keep the temperature under $10^{o}C$ if the room temperature is greater than $25^{o}C$ or there is a possibility that the blood will not be transfused within 30 minutes.

Red cells and whole blood that have been out of the correct storage conditions for more than 30 minutes should never be returned to the refrigerator for later use because of the potential for bacterial contamination and the loss of cell function.

There is no evidence that warming blood is beneficial to the patient when infusion is slow. Cold blood can cause spasm in the vein used for infusion. Blood should only be warmed in a blood warmer [16].

2.3 Electrical Properties of Blood

The dielectric properties of blood were studied by early investigators: the subject continues to be important for practical applications. Cole presented an interesting review of an early work by Höber, Fricke and others [23]. Schanne and P.-Ceretti summarized the results of the many dielectric studies performed on tissue cells [24]. Maxwell (1904) developed an expression for the resistivity of a suspension of spheres of known resistivity in an electrolyte of known resistivity [25]. Fricke (1925), by including a form factor (f), extended the Maxwell expression to allow its application to suspensions of nonspherical particles. Trautman and Newbower have prepared an excellent review of conductivity data from blood, including many references to early work [25]. Some investigators showed that the impedance of blood depends on the components of the suspending medium.

The contents of plasma could contribute to the electrical impedance of blood, especially the capacitance. Plasma can be considered as a NaCl solution since Na^+ and Cl^- ions in the plasma account for the 87 % of the conduction. Changes in plasma chemistry, particularly NaCl and proteins, have significant effects on plasma conductivity. Fibrinogen, as a kind of charged protein, could be easily adsorbed to the interface around the cell surfaces. The absorbed fibrinogen might have a smaller mobility because of its elongated molecular shape and heavy molecular weight, and hence should decrease the conductance or increase the resistance of the interface region. Such an increase in interfacial resistance might contribute to the measured effective resistance [26].

Zhao explained that plasma proteins can make a large contribution to the impedance of blood, the quantity of which seems dependent on the size and shape of the molecules [27]. In Table 2.5, the results of investigations of Zhao are shown.

Table 2.5
Impedance parameters of washed red cells resuspended in various solutions [27].

Solution	R_p (Ωcm)	C_m ($pFcm^{-1}$)
Plasma	389 ± 12.9	928 ± 62.2
SSH	374 ± 6.9	741 ± 35.0
Serum	374 ± 9.6	724 ± 36.1
PBS	289 ± 4.8	592 ± 33.1

Measurement of the cytoplasm conductivity at low frequencies is difficult because of the highly insulating surrounding membrane. The red cell interior (cytoplasm) conductivity results from K^+, Na^+, Cl^-, Mg^{+2} and HCO_3^- ions. If the relative ion and water concentrations in the cytoplasm are known, then the conductivity values give information about the state of these ions and water molecules, for example, whether they are bound or not.

The capacitance of red cells has two components: The cell membrane and the interface between the membrane and the plasma [27], [28]. The membrane electrically acts as a capacitor that is the major origin of the measured capacitance. The membrane is a bipolar lipid layer containing structural and contractile proteins and numerous enzymes and surface antigents. About 50 % of the membrane is protein, 40 % is fats and up to 10 % is carbohydrate. The lipids consist of 60 % phospholipid, 30 % neutral lipids (mainly cholesterol) and 10 % glycolipids. The phospho- and glycolipids are structural with polar groups on the external and internal surfaces and non-polar groups at the centre of the membrane. Carbohydrates occur only on the external surface while proteins are thought to be either peripheral or to be integral, penetrating the lipid bilayer [29].

The contribution of the interface should be influenced by the components in the suspending medium, such as fibrinogen and serum proteins, which can be adsorbed to the interface and hence affect the polarization and the capacitance [30]. The interaction between these molecules will limit the mobility of the lipid molecules and affect the phase transition such that their structural configurations may be quite different from, and more complicated than, those of pure lipid membranes. However, because the major constituents of the biomembrane are lipid molecules, the properties of a biomembrane will more or less reflect those of a lipid bilayer.

2.3.1 Parameters Affecting Blood Conductivity

Conductivity of blood is complex, $\sigma^*(W) = \sigma(\omega) + jW\varepsilon_0\varepsilon(\omega)$, and is a function of many parameters such as; the shape of red blood cells, the volume concentration of red cells (hematocrit), the type of anticoagulant, the temperature and the frequency.

2.3.1.1 The Shape of Red Blood Cells. Blood is electrically anisotropic, because of the influence of the orientation of the erythrocytes. There are only two well defined states of orientation of the erythrocytes: random orientation and parallel orientation (all erythrocytes parallel to each other). Random orientation is attained in stationary blood; parallel orientation in flowing blood.

It is only isotropic when the erythrocytes are homogeneously distributed and in random orientation [31].

2.3.1.2. Hematocrit. Hematocrit is a routine clinical measurement which determines the relative volume occupied by red blood cells in a given blood sample. The hematocrit is determined as the ratio of the total volume of red blood cells to the total volume of blood samples. Recent studies have shown that the hematocrit is highly correlated with plasma resistance, intracellular resistance, and cell membrane capacitance [10].

Hematocrit is one of the most important indices for hemoconcentration and hemodilution. The standard and most widely used techniques for measuring it is to subject the blood to centrifugation so as to achieve packing of the erythrocytes. Another technique is to measure it from the electrical conductivity or resistivity of whole blood. Yamakoshi, Shimazu, Togawa, Fukuoka and Ito measured hematocrit values of blood taken from 16 donors by making use of second technique, and calibrated them against those obtained by the centrifugation method [32]. It was demonstrated that the blood resistivity values by this method were measured within ± 5 percent accuracy.

2.3.1.3 Temperature. The dielectric characteristics of blood cells are insensitive to temperature. The conductivity of blood increases with temperature and the temperature coefficient for inside resistivity of red cells is $-2\ \%\ {}^{0}C^{-1}$. However, it is still useful to stabilize the temperature at body temperature of 36.5 ^{0}C in practical applications, since the temperature dependent plasma resistance might be used to normalize membrane capacitance to eliminate the influence of varying hematocrit [33]. In Table 2.6, the effects of temperature on blood impedance are shown.

Table 2.6
Human blood conductivity and relative permittivity at different temperatures [34].

	Frequency (GHz)	Human Blood 35 ^{0}C	37 ^{0}C
Relative Permittivity	3.0	56.0	53.0
	9.4	47.8	45.0
	24.0	30.2	32.0
Conductivity (mS cm^{-1})	3.0	26.5	27.8
	9.4	103	121
	24.0	344	263

2.3.1.4. Frequency. The electrical properties of a material are determined by the time constants $(\tau = \varepsilon / \sigma)$. The time constant causes the frequency dependence of impedance. The time constants themselves can be calculated by dividing the quotient of imaginary Im (Z) and real Re (Z) parts of impedance by the frequency ω, $\tau = Im(Z) / Re(Z)\omega$.

Impedance at low frequencies is inversely related to the relative volume of extracellular water and thus increased as the red cell volume is increased or the plasma fraction decreased. At high frequencies, the cell membrane becomes conductive and the resistance reflects both the extracellular water (plasma) volume and some intracellular water. In the intermediate frequency range, only a part of the intracellular conductivity is measured.

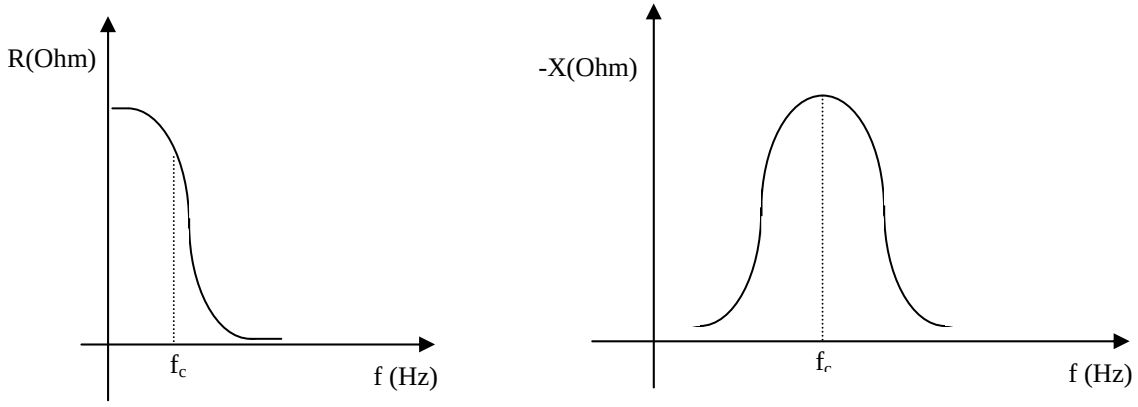


Figure 2.2 Spectra of real and imaginary components of tissue impedance.

2.3.1.5 Anticoagulant. In addition to the plasma proteins` effects on the blood impedance, it was investigated how the blood impedance is influenced by anticoagulants. Nelson and Wilkinson reported a dependence of blood resistivity on the type of anticoagulants [35].

Geddes and Sadler, Hill and Thompson, and Mohapatra and Hill, however, found no such dependence for heparin and EDTA [36]-[38]. Zhao, Jacobson and Ribbe presented that anticoagulants influence not only the resistivity of whole blood but also cell membrane capacitance [33]. The results are shown in Table 2.7. This suggests that the anticoagulants used might have some effect on both the plasma and the cell membranes.

Table 2.7
Human blood parameters for different anticoagulants (h=Hct/100) [33].

Anticoagulants	Sample No	HCT %	R_p (Ωcm)	R_i (Ωcm)	C_m ($pFcm^{-1}$)	R_p/h (Ωcm)	R_ih (Ωcm)	C_m/h ($pFcm^{-1}$)	Plasma (Ωcm)
ACD	1	42.6	147	412	337	346	175	792	65.7
ρ=60.5 Ωcm	2	37.2	128	458	313	346	170	843	65.0
	3	43.5	153	388	339	353	169	780	66.1
	4	41.2	145	417	313	353	172	761	66.6
	5	38.4	136	466	311	355	179	810	66.8
	6	41.4	150	391	315	362	162	762	66.1
	7	36.0	127	478	292	351	172	812	65.2
	8	41.3	148	433	324	357	179	783	65.8
	9	40.1	143	427	316	356	171	788	65.2
	10	39.2	140	441	327	357	173	836	66.2
					AVG	353.7	172.1	796.7	65.9
					STD	4.9	4.7	26.7	0.57
Citrate	1	38.4	126	474	291	329	182	759	62.2
ρ=53.4 Ωcm	2	33.3	113	516	280	340	172	841	62.0
	3	39.3	131	441	301	333	173	766	62.8
	4	36.4	125	523	277	343	191	761	63.4
	5	34.7	118	544	268	339	189	773	63.3
	6	37.8	128	459	269	338	174	711	62.7
	7	31.8	111	516	257	348	164	807	62.1
	8	37.0	126	487	283	341	180	765	62.6
	9	36.9	123	476	279	334	175	757	62.2
	10	35.1	120	564	286	343	198	815	63.0
					AVG	338.7	179.8	775.4	62.6
					STD	5.2	9.7	34.7	0.48
EDTA	1	43.9	146	385	404	332	169	920	62.1
ρ=20.6 Ωcm	2	38.0	127	417	388	333	158	1022	61.1
	3	45.8	156	357	430	341	164	938	62.6
	4	42.2	145	407	385	344	171	914	63.0
	5	39.5	135	414	380	342	164	962	63.1
	6	43.3	149	386	381	344	167	881	62.5
	7	37.5	127	419	384	340	157	1024	62.0
	8	42.8	147	396	413	345	170	966	62.4
	9	42.1	144	386	407	343	163	966	62.1
	10	40.8	142	405	423	348	165	1037	63.0
					AVG	341.2	164.8	963.1	62.4
					STD	4.8	4.5	49.3	0.56

3. BLOOD MEASUREMENTS: MATERIALS AND METHODS

3.1 Introduction

The long-term storage of blood and blood components by the transfusion organization is a routine procedure necessary to meet the various needs of modern medicine [39].

Many advances in the understanding of blood storage have taken place in the past few years. Media have been developed to extend storage time, improve oxygen delivery, and increase plasma yields. However, many of these latter improvements have had negative impact upon red cell storage. The limiting factor in red cell storage is loss of red cell viability following transfusion [40]. When stored RBCs are transfused to a recipient, some of them circulate with a normal lifespan and others are removed within a few hours; the stored cells are alive or dead [41].

Blood donations for clinical use are routinely stored for 21 to 42 days, depending on the composition of the anticoagulant-preservative solution. This time limit has been determined mainly by the survival of the transfused RBCs in the recipient's blood 24 hours after transfusion, which is related to biochemical measures, mainly the cellular ATP level and pH [42]. Since red cells are required to transport oxygen, as well as to survive in the circulation, many groups also measure erythrocyte 2,3-DPG (which influences haemoglobin's affinity for oxygen), as well as cellular ATP when assessing novel red cell storage conditions [43].

The electrical properties of blood have been examined in a number of previous studies [1, 11, 27, 30, 33]. These studies showed that electrical parameters are a potential index for evaluating blood in clinical applications, especially in assessing the quality of stored blood.

Extracellular Na^+, K^+, Cl^- concentrations, pH, 2,3-DPG, ATP and electrical impedances are measured in both erythrocyte suspensions and whole blood samples. The parameters of interest are given in Figure 3.1.

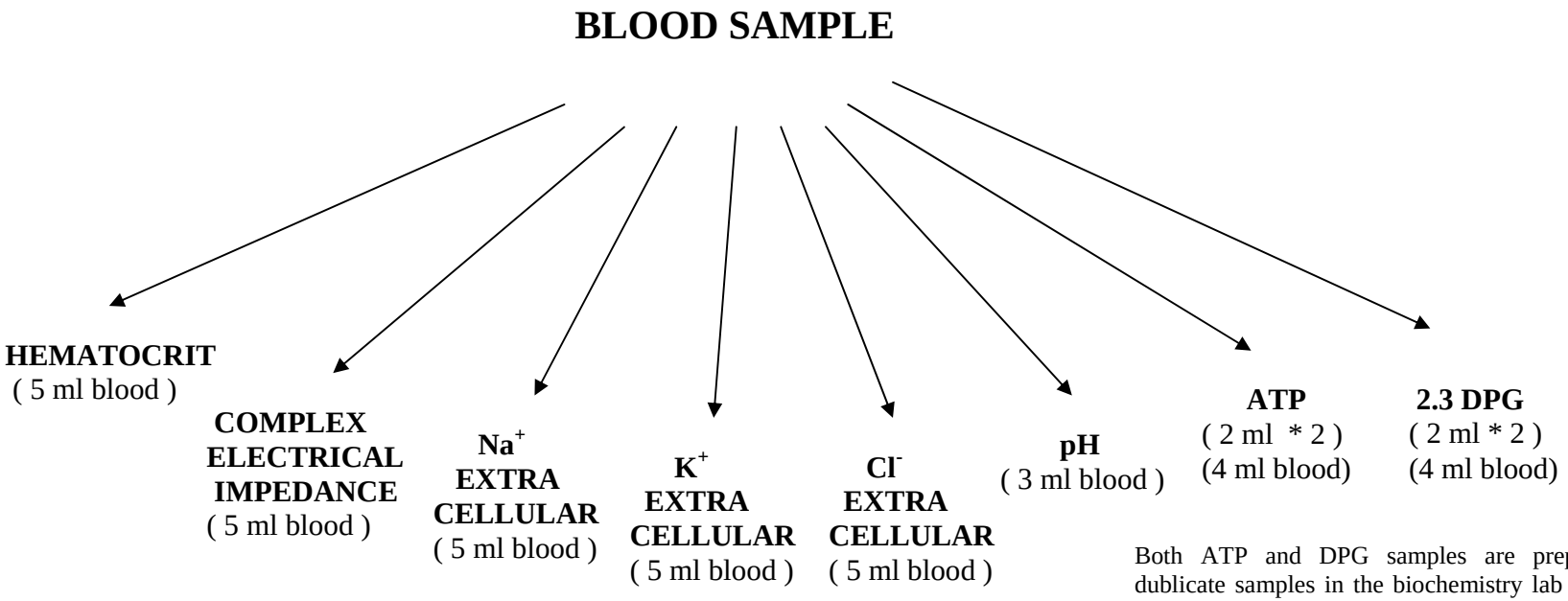


Figure 3.1 Blood measurements.

3.2 Materials

3.2.1 Preparations for Blood Collection

The blood samples are collected at the Blood Bank of Marmara University Hospital (Istanbul-Turkey) from 51 healthy male donors who met the standard blood donor criteria, and gave their consent to participate in the study. The study protocols are approved by the Ethics Committee of Marmara University. Only male donors are selected to keep the hematocrit values in a narrow range. Volunteers are tested to exclude Hepatitis B, Hepatitis C, HIV and syphilis infectivity.

3.2.2 Collection of Blood Samples

Erythrocyte Suspension

Standard units (450 ± 45 mL) of blood are drawn from each donor into the main pediatric blood bag that contained 63 ml of citrate-phosphate-dextrose (CPD). To prepare erythrocyte suspensions, whole blood bag is centrifuged at 3600 G, for 15 minutes. Sodium-adenin-glucose-mannitol (SAGM) is used to extend the lifetime of red blood cells to over 42 days. Approximately 45 ml of erythrocyte suspension is transferred into the first satellite bag for the 0 day measurement. The blood bag system is shown in Figure 3.2 [44].

10^{th}, 21^{st} and 35^{th} day measurements are performed using the 2^{nd}, the 3^{rd} and the 4^{th} satellite bags respectively, with 45 ml of erythrocyte suspension transferred into it, each time. The 42^{nd} day measurement is done with the remaining blood in the main bag. The purpose of using pediatric bags is to have access to erythrocyte suspensions in a closed system avoiding any bacterial contamination. Blood bags are stored at $+4^{o}C$.

All blood bags are labeled accordingly, using a coding system, to avoid any confusion. Blood withdrawal date, donation number, and identity are written on blood bags. For example; for 10^{th} day measurement of 3^{rd} donor on erytrocyte suspensions, the code is ES3-10.

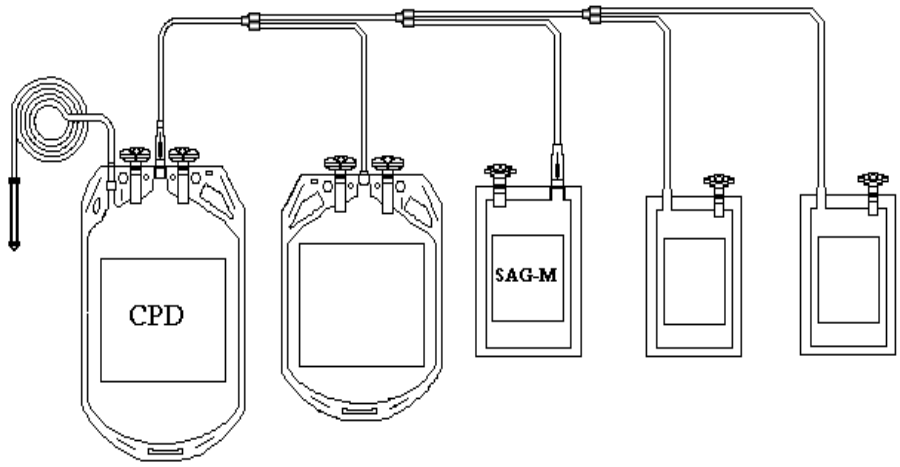


Figure 3.2 Pediatric SAGM blood bag [44].

Whole Blood

From each donor, 450 ml whole blood is drawn into the first largest chamber of the pediatric blood bag that contained 63 ml citrate-phosphate-dextrose (CPD) solution. The pediatric blood bag consists of 4 bags, three of them are filled with approximately 110 ml of whole blood. The blood bag system that is used in this study is shown in the Figure 3.3. For the measurements, the whole blood is used without any preparation.

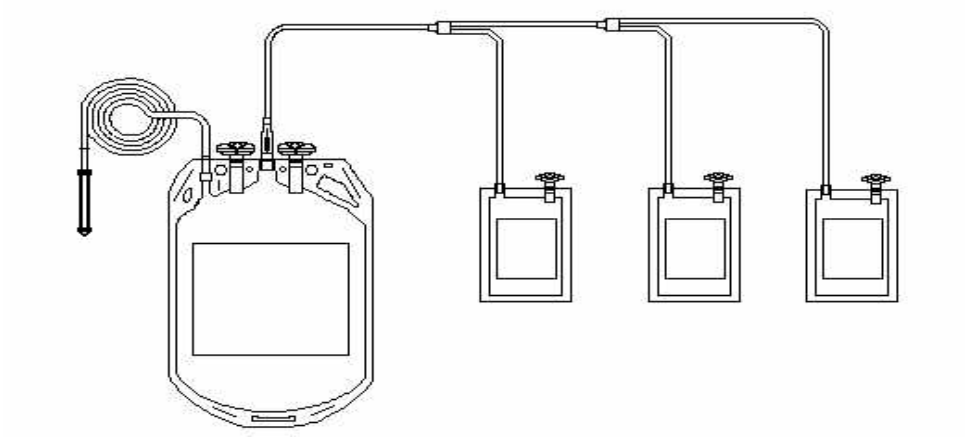


Figure 3.3 Pediatric CPD blood bag [44].

Following the 0^{th} day measurements, the samples are stored in the refrigerator at $+4^{o}C$.

3.3 Methods and Procedures

The blood samples are drawn into the measurement tubes to measure extracellular Na^+, K^+, Cl^-, pH and electrical impedances. Although the hematocrit range is very narrow, each sample's hematocrit is measured for normalization.

For ATP and 2,3-DPG measurements, blood samples are processed in the biochemistry laboratory and then stored at -80^{o}C in the deep freezer. These measurements are always performed after other all measurements are completed.

3.3.1 Electrical Impedance Measurements

The electrical measurements of blood are done in a conductivity cell. The conductivity cell is a uniform cylindrical plastic tube of 0.8 cm diameter, with four stainless steel needle electrodes placed at 1 cm interelectrode distances. The conductance cell is shown in Figure 3.4. The electrodes are connected to the Hewlett Packard 4284A LCR Meter via a unity gain differential preamplifier [45]. The overall gain-phase characteristics of the preamplifier are measured with resistors that are in the physiological range. These measurements are later taken into account for correcting the data from blood samples [46].

The measurement of the complex impedance of blood samples are performed at room temperature in the frequency range from 100 kHz to 1 MHz, using the LCR Meter [10],[33],[47]. From impedance Z, and phase angle α measurements, following the correction for gain and phase characteristics of the amplifier; the resistance R and the reactance X are calculated. Each sample data is fit into the Cole-Cole model; hence the Cole-Cole parameters, intracellular resistance R_i, extracellular resistance R_e, characteristic frequency F_c and phase angle α are obtained, by using a LMS software [48-55].

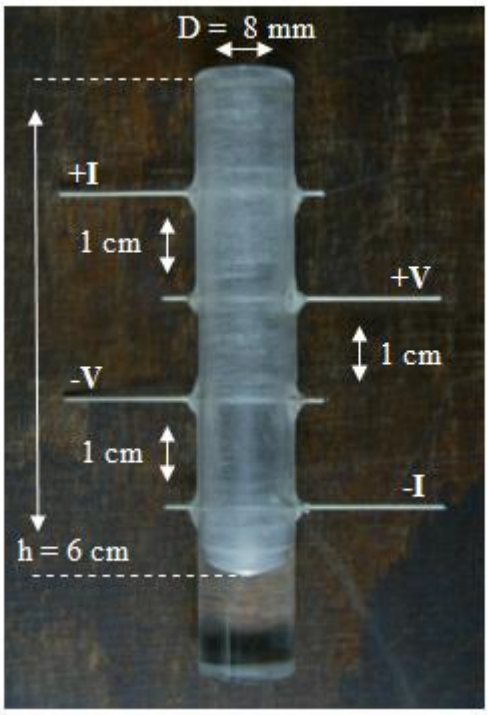


Figure 3.4 The conductivity cell.

The conductivity cell constant, k, in $Z = k/\sigma$, is obtained by using NaCl, that is purely resistive, as reference solution in the conductivity cell. Conductivity cell calibration is further checked by repeating the measurements for saline solutions of known concentrations and conductivities. The conductivities are measured with the HANNA Instruments HI 8733 Conductivity Meter

NaCl solution #1, $\sigma = 20.9$ mS/cm,

NaCl solution #2, $\sigma = 13.95$ mS/cm,

NaCl solution #3, $\sigma = 11.95$ mS/cm,

NaCl solution #4, $\sigma = 9.01$ mS/cm,

NaCl solution #5, $\sigma = 7.13$ mS/cm,

Impedances are measured at 1 kHz, 10 kHz, and 100 kHz.

The plot of resistance vs. resistivities of saline solutions is a straight line and the slope gives the cell constant: k = 1,9 cm^{-1} (Figure 3.5).

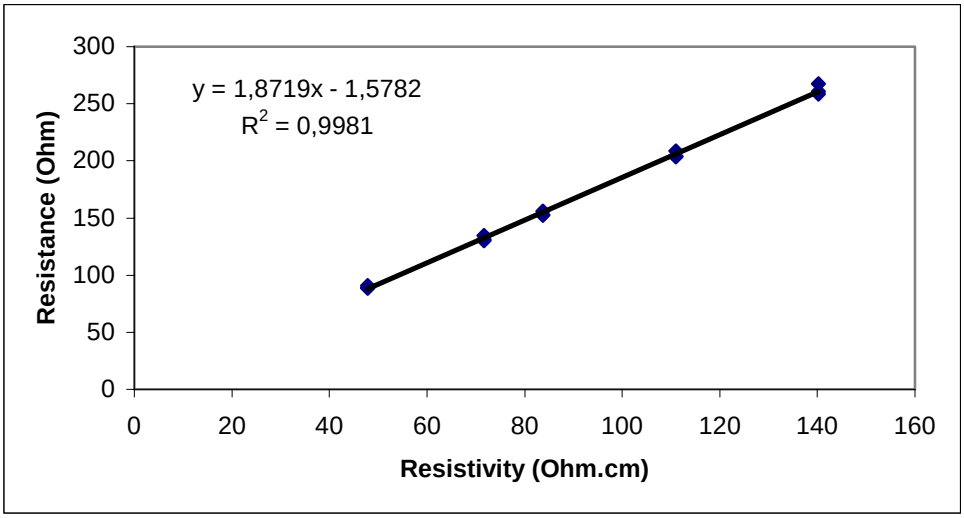


Figure 3.5 Measured resistances vs. resistivities for NaCl solutions.

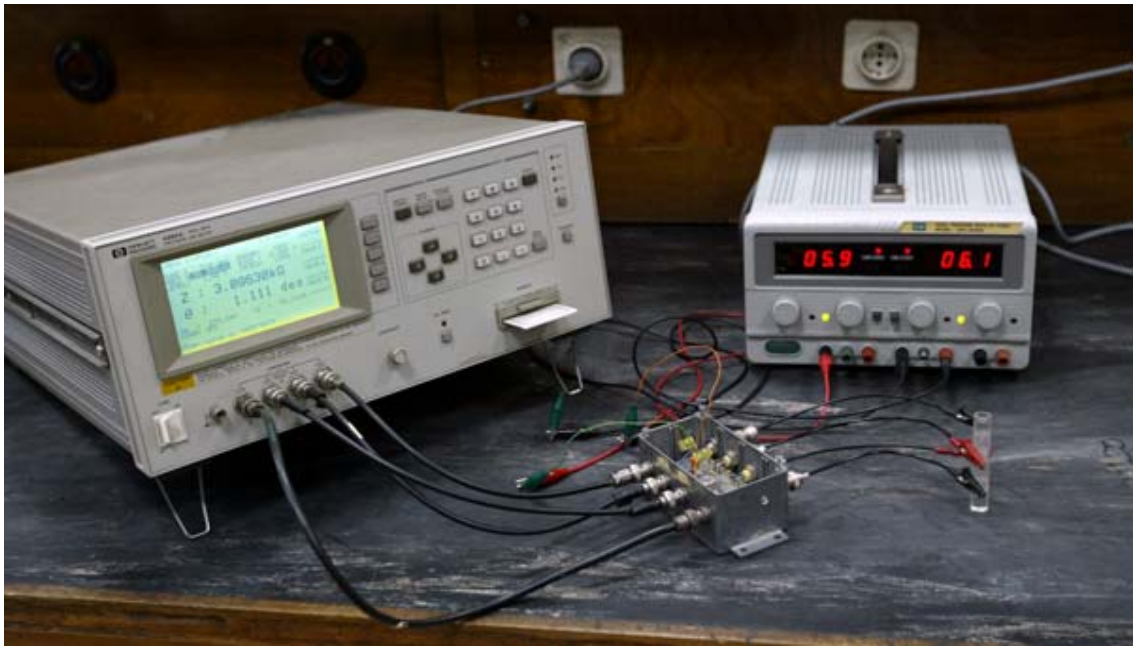


Figure 3.6 The schematic of the experimental set-up.

For testing the Cole-Cole fitting (LMS) software, a single cell electrical circuit is realized with two resistors, R_e = 220 Ohm and R_i = 1 KOhm and a capacitor, C_m = 1.8 nF.

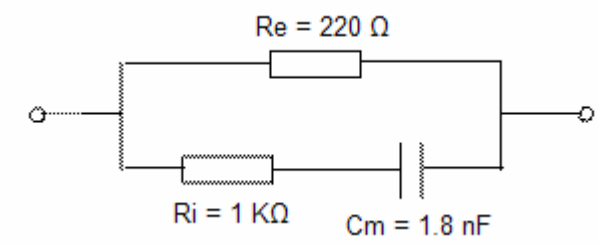


Figure 3.7 The electrical equivalent circuit for a single cell.

The Imaginary vs. Real part of the complex impedance is a circle with the centre on the real axis.

There is little significant difference between the actual values and calculated values by fitting data into the Cole-Cole plot (Table 3.1).

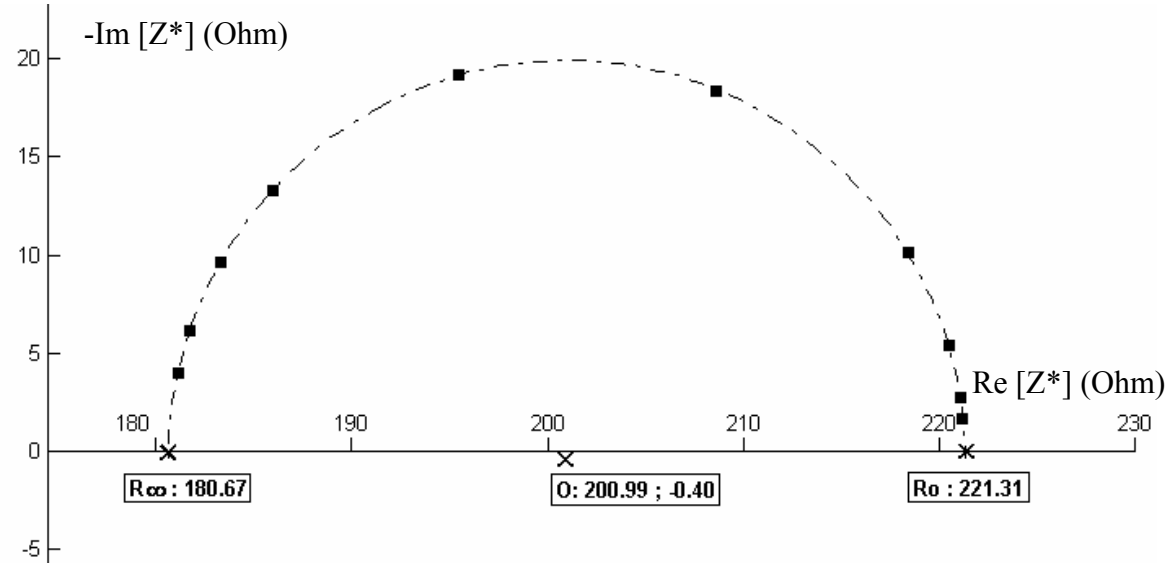


Figure 3.8 The Cole-Cole plot for the electrical circuit.

Table 3.1
Actual and calculated values of the equivalent circuit's elements.

	Component values	Measured values	Values from Cole-Cole fit	Error
$\mathbf{R_e}$	220,0 Ω	221,09 Ω	221,31 Ω	0,1 %
$\mathbf{R_i}$	1000,0 Ω	985,50 Ω	983,86 Ω	0,16 %
$\mathbf{C_m}$	1,80 nF	1,73 nF	1,755 nF	1,4 %
α	0,0	0,0	0,0126	1,26%
fc	72,511 kHz	76,284 kHz	75,246 kHz	1,36 %

3.3.2 Physiological Measurements

In physiological measurements, Na^+, K^+ and Cl^- is analyzed automatically on 917 HITACHI chemical analyzer. Avlomni Blood Gas meter (Roche) is used for extracellular pH measurements. All measurements are performed at room temperature.

All 2,3-DPG and ATP measurements are performed after the samplings on the 0^{th}, 10^{th}, 21^{st}, 35^{th} and 42^{nd} day are completed. Prior to 2,3-DPG and ATP measurements, blood samples are processed in the biochemistry laboratory with the 2,3-Diphosphoglycerate and ATP Bioluminescence Assay Kit HS II from Roche Diagnostics and then stored at -80^oC in the freezer, for 3 months, until the measurement day. 2,3-DPG analysis (UV-test at 340 nm) is performed on the PU 8620 PHILIPS spectrophotometer and the ATP analysis (bioluminescence test) is runned on the Berthold luminometer by using the assay kits.

3.3.2.1 Na^+, K^+ and Cl^- Concentration Measurements. For the extracellular Na^+, K^+ and Cl^- measurements, the measurement procedure below is applied and the sample is studied automatically on the 917 HITACHI chemical analyzer.

i). Pipette blood sample into a 5 ml tube,

ii). Centrifuge for 10 min. at 2000 G, and

iii). Perform measurement from the supernatant.

3.3.2.2 2,3-DPG Measurements. To perform 2,3-DPG measurements, blood is pipetted into the heparinized test tubes and deproteinization is carried out immediately. For deproteinization;

- Pipette 5.0 ml perchloric acid and 1.0 ml blood into a 10 ml centrifuge tube and mix.
- Centrifuge mixture.
- Take 4 ml of the clear supernatant and neutralize with 0.5 ml 2.5 M potassium carbonate.
- Keep for at least 30 min. in an ice-bath.
- Centrifuge off in the cold and use 0.1 ml of the supernatant for the assay.

Table 3.2
Assay procedure for 2,3-DPG measurements [56].

	blank	**sample**
Solution 1	2.00 ml	2.00 ml
Solution 2	0.05 ml	0.05 ml
Solution 3	0.05 ml	0.05 ml
Sample (netrulize)	-	0.10 ml
Water	0.10 ml	-
Mix, allow to stand at 20-25^{o}C, read absorbance $\mathbf{A_1}$, after the reaction has stopped.		
Solution 4	0.02 ml	0.02 ml
Solution 5	0.02 ml	0.02 ml
Mix and wait till the end of the reaction (approx. 25 min.). Read absorbance $\mathbf{A_2}$. $\mathbf{(A_1 - A_2)_{sample} - (A_1 - A_2)_{blank} = \Delta A}$		

Solution 1: triethanolamine buffer, $MgCl_2$ and EDTA.
Solution 2: ATP and NADH.
Solution 3: PGM, PGK, GAP-DH, TIM and GDH.
Solution 4: PGM
Solution 5: glycolate-2-phosphate, tricyclohexylammonium salt.

For the reproducibility test of the PHILIPS PU 8620 spectrophotometer that is used for 2,3-DPG measurements, deionized water is measured at 340 nm for five times and the

coefficient of variation is calculated (mean: 0.152, standard deviation: 0.0011 and coefficient of variation: 0.724%). No significant difference ($p<0.05$) is found among the five measurement. The readings are extremely stable.

3.3.2.3 ATP Measurements. For the determination of ATP, the bioluminescence technique is used. This technique depends on the ATP dependancy of the light emitting luciferace catalyzed oxidation of luciferin. The luciferace catalyses the following reaction:

$$\text{ATP} + \text{D-luciferin} + O_2 \longrightarrow \text{oxyluciferin} + PP_i + \text{AMP} + CO_2 + \text{light}$$

The light output is directly proportional to the ATP concentration. The standard protocol of the ATP measurement [57];

- Dilute cells to a concentration of 10^5 to 10^8 cells/ml. If the sample is too dilute, concentrate by centrifugation and discard the supernatant carefully.
- Dilute ATP standard with dilution buffer by serial dilution in the range of 10^{-10} and 10^{-16} M.
- Add to sample or ATP standard the same volume of cell lysis reagent and incubate for 5 min at 15-25^{o}C.
- Transfer the volume of sample prepared above into tube.
- Add luciferase reagent to the samples/standards by automated injection and start measurement after a 1 s delay and integrate for 1 to 10 s.
- Subtract the blank from the raw data and calculate ATP concentrations from a log-log plot of the standard curve data.

For the reproducibility test of the BERTHOLD LB 9506 luminometer that is used for ATP measurements, distilled water is measured five times and the coefficient of variation is calculated (mean: 1116.2, standard deviation: 104.008 and coefficient of variation: 9.318%). No significant difference ($p<0.1$) is found among measured values.

The 0^{th} day measurements are repeated on the 10^{th}, 21^{st}, 35^{th} and 42^{nd} days with erythrocyte suspensions and whole blood samples. Hence, changes of Na^+, K^+, Cl^- concentrations, pH, ATP, 2,3-DPG and electrical impedances during storage are investigated.

4. EXPERIMENTAL RESULTS

4.1 Physiological Alterations During Storage

4.1.1 Introduction

When blood is collected in a standard anticoagulant-preservative solution and stored under blood bank conditions, a number of changes occur. These changes are mainly fall in the ATP, pH and 2,3-DPG levels, loss of membrane lipids and hence reduction in the surface area, formation of spheroechinocytes and increase in membrane permeability [58]-[68].

Red cell ATP has long been used as an indicator of red cell viability. The red blood cell is a highly specialized cell without a nucleus and devoid of protein synthesis. This means that it has to rely on its original contents of enzymes and other proteins for the whole of its limited lifetime. In addition, because it lacks mitochondria, it cannot regenerate adenosine triphosphate (ATP) and ATP falls in storage conditions.

During storage, 2,3-DPG falls causing increasing hemoglobin, oxygen affinity leading to an inability to deliver oxygen. These red blood cells have a left-shifted oxygen dissociation curve (ODC) , greater oxygen affinity, and may supply less oxygen to the tissues [58]-[60].

For stored red cells, a progressive transformation from discocyte to echinocyte to spheroechinocyte occurs. As red cells generate intracellular lactate and accumulate chloride ions, they gradually swell. By lossing of cell membrane lipids, surface area decreases [61],[68].

The permeability of the membranes increases during storage. Since the sodium-potassium ATPase activity is minimal at $4^{o}C$, the leaked Na^{+} and K^{+} ions during bank storage will not be actively pumped back across the red cell membrane. As a result, the sodium level will fall and the potassium level will rise in the suspending medium [66].

These alterations and lesions are given as the storage parameters in Table 4.1.

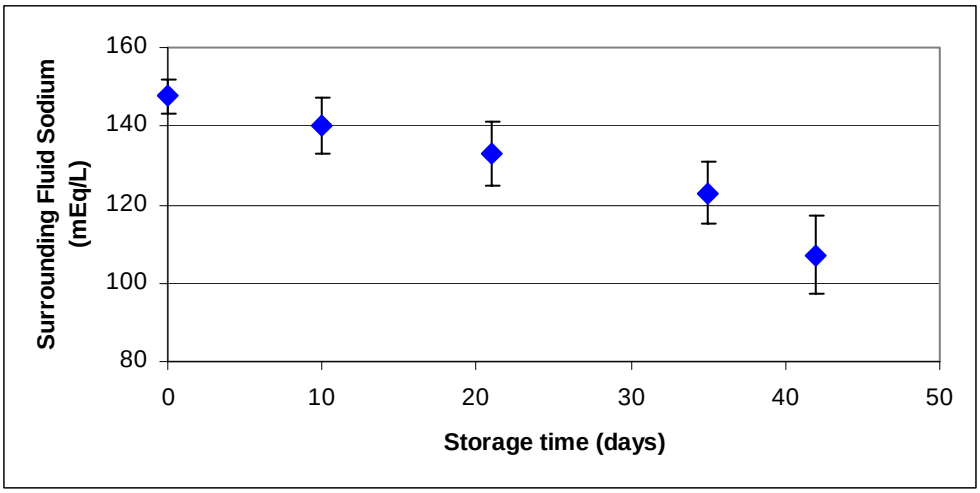
Table 4.1
The parameters affected from storage time.

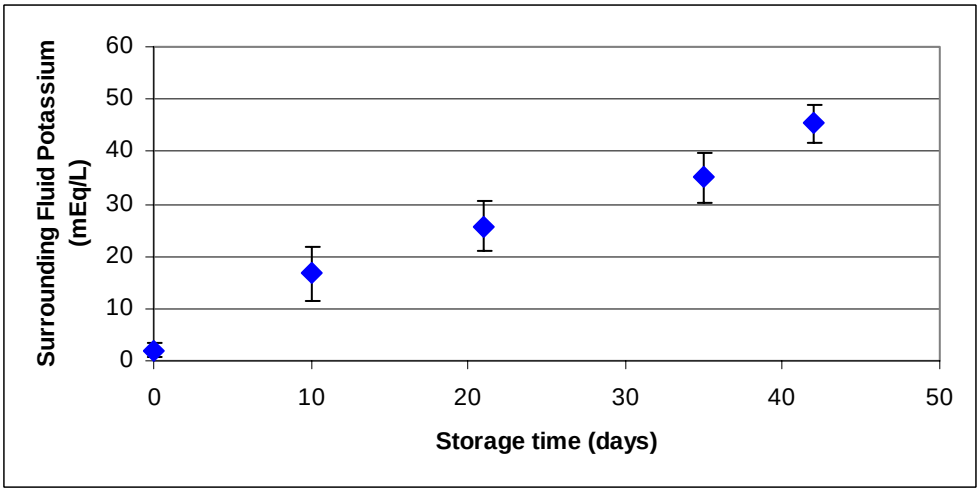
	PARAMETERS
STORAGE TIME ↑	Intracellular Na^+ ↑ Plasma Na^+ ↓
	Intracellular K^+ ↓ Plasma K^+ ↑
	Calcium permeability, Ca^{2+} ↑
	Red Cell Radius, q ↑
	Red Cell Volume, V_c ↑
	Form Factor ↑
	Membrane Surface Area, A_c ↓
	Membrane Lipids ↓
	Internal Viscosity ↑
	Red Cell Filterability ↓
	Extracellular pH ↓
	2,3-DPG ↓
	Red Cell ATP ↓
	Red Cell Viability ↓

↓: decrease, ↑: increase.

4.1.2 Alterations During Storage

4.1.2.1 Erythrocyte Suspensions (ES). Physiological alterations during storage are investigated using the raw data. Storage of red blood cells resulted in a rise in the extracellular (surrounding fluid: SAGM + CPD + residual plasma) K^+ and a fall in the Na^+, Cl^-, pH, 2,3-DPG and ATP (Figure 4.1).





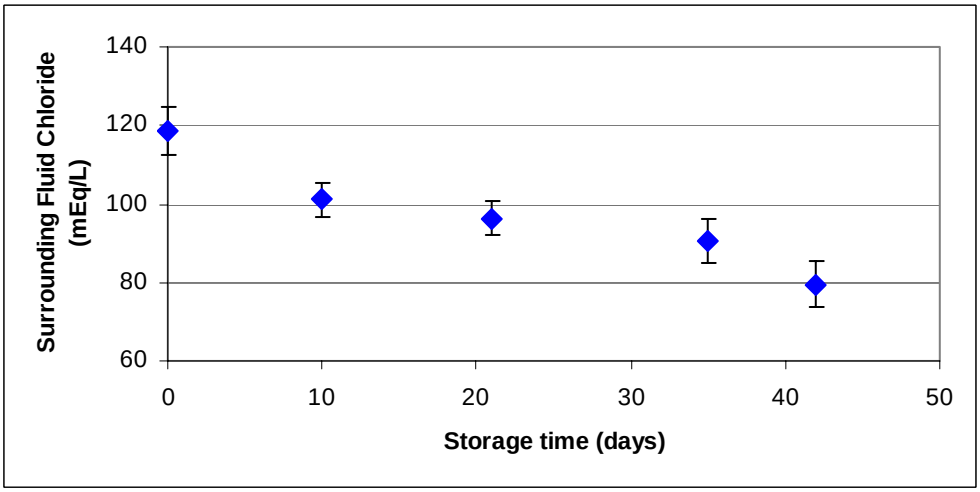
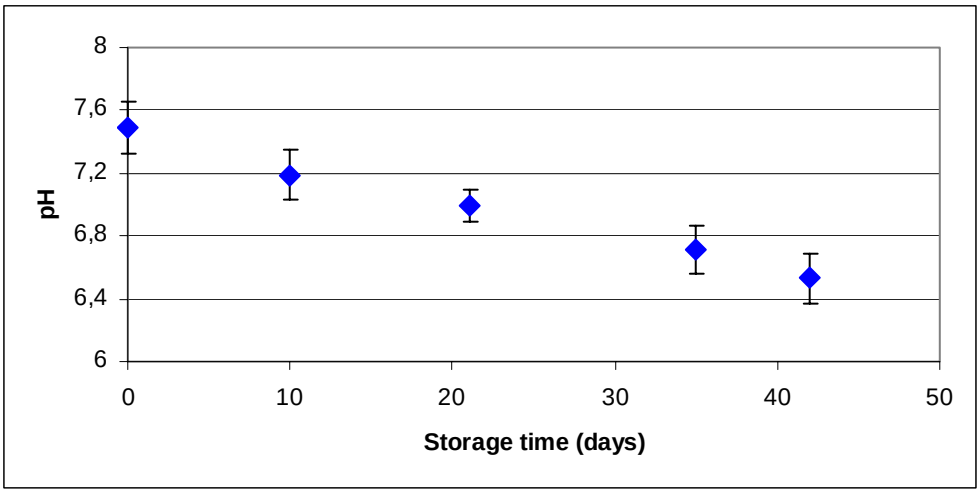
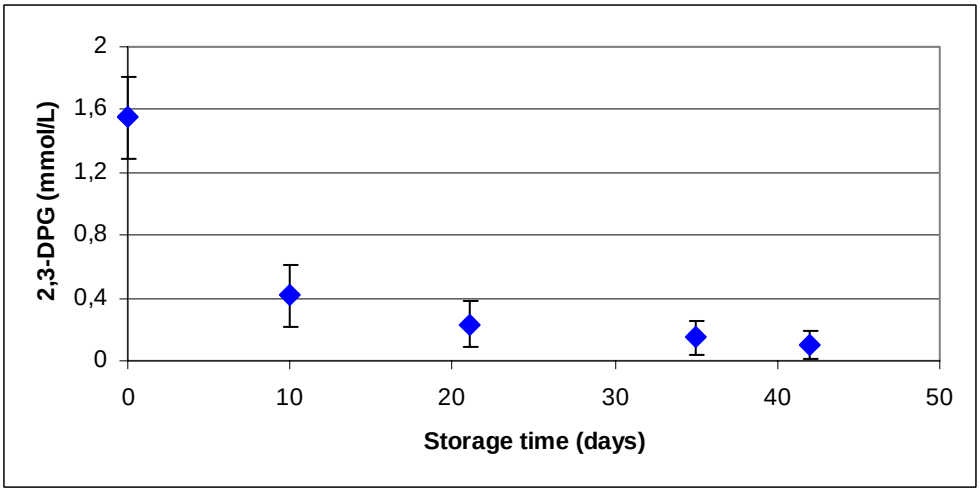


Figure 4.1 Raw physiological data for ES under storage.





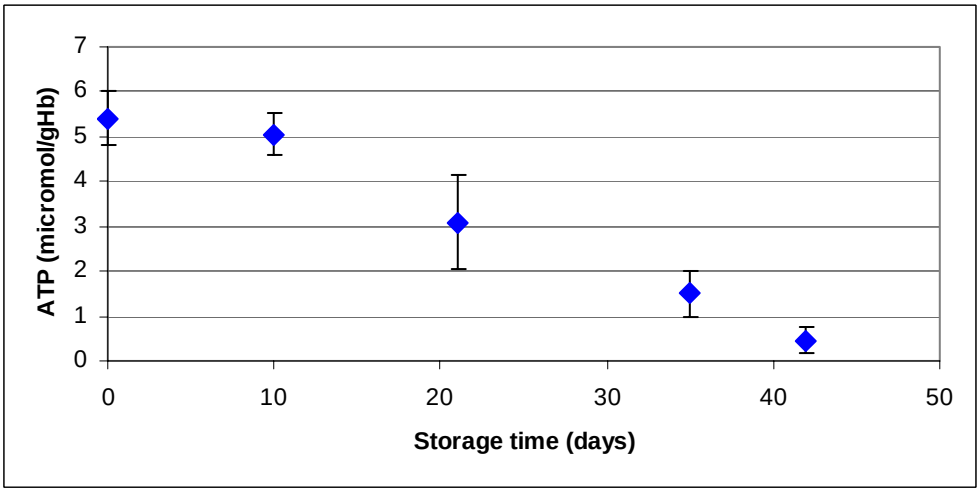


Figure 4.1 continued.

The descriptive statistics in SPSS displays univariate summary statistics for several variables in a single table (Table 4.2).

Table 4.2
Descriptive statistics of raw physiological data for ES.

	Mean	*Std. deviation*	*Skewness*		*Kurtosis*	
			Statistic	*Std.Error*	*Statistic*	*Std.Error*
Extracellular Na^+ (mEq/L)	130,192	16,032	-,598	,153	-,218	,304
Extracellular K^+ (mEq/L)	24,995	15,479	-,137	,153	-1,136	,304
Extracellular Cl^- (mEq/L)	97,228	13,921	,292	,153	-,361	,304
pH	6,981	,372	,138	,153	-,668	,304
2.3-DPG (mmol/L)	,491	,609	1,972	,153	3,935	,304
ATP (micromol/gHb)	9,667	6,042	,095	,183	-1,538	,364

For comparison among the donor samples with different total ion concentration, it is necessary to normalize the ion concentrations. For normalization of the Na^+, K^+ and Cl^- concentrations, total ion concentration on the 0^{th} day, $t = [Na^+]+[K^+]+[Cl^-]$, is taken as reference. Therefore, $[Na^+]_n$ (%) = 100 $[Na^+]_{meas.}/t$, $[K^+]_n$ (%) = 100 $[K^+]_{meas.}/t$ and $[Cl^-]_n$ (%) = 100 $[Cl^-]_{meas.}/t$.

After normalization, the Na^+, K^+ and Cl^- concentrations are again analysed statistically (Table 4.3). When Table 4.2 and Table 4.3 are compared, it is seen that following the normalization, the skewness of Na^+ and K^+ concentrations approximates to zero, in other words, approximates the normal distribution.

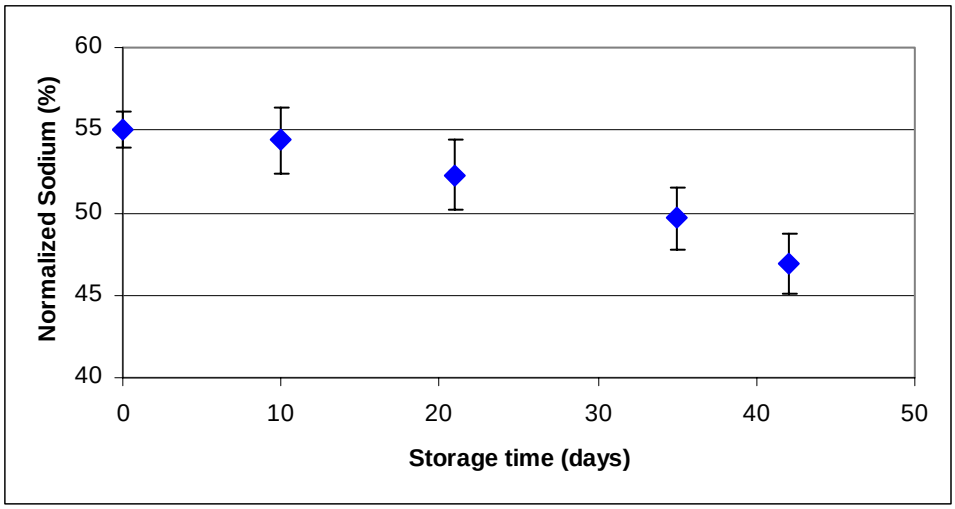
pH, 2,3-DPG and ATP data are not normalized.

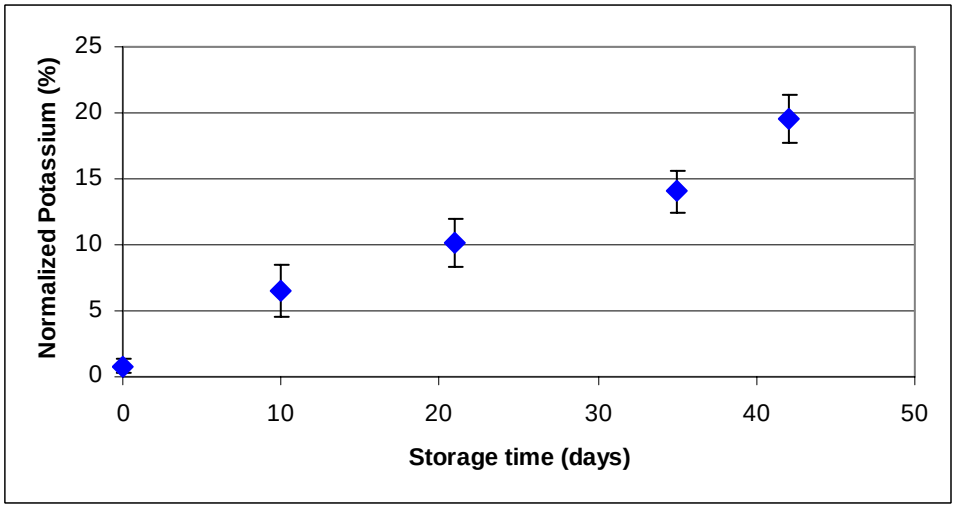
Skewness is a measure of symmetry. A distribution is symmetric when it looks the same to the left and to the right of the center point. The skewness for a normal distribution is zero, and any symmetric data should have a skewness near zero.

Table 4.3
Descriptive statistics of normalized physiological data for ES.

	Mean	*Std. deviation*	*Skewness*		*Kurtosis*	
			Statistic	*Std.Error*	*Statistic*	*Std.Error*
$[Na^+]_{norm}$ *(%)*	51,764	3,510	-,327	,156	-,719	,310
$[K^+]_{norm}$ *(%)*	10,206	6,621	,039	,153	-1,033	,304
$[Cl^-]_{norm}$ *(%)*	38,309	3,707	,341	,155	-,457	,308

By normalization, it is seen that the standard deviation of data has become smaller. The alterations of normalized physiological data (Na^+, K^+ and Cl^- concentrations) with storage time are shown in Figure 4.2.





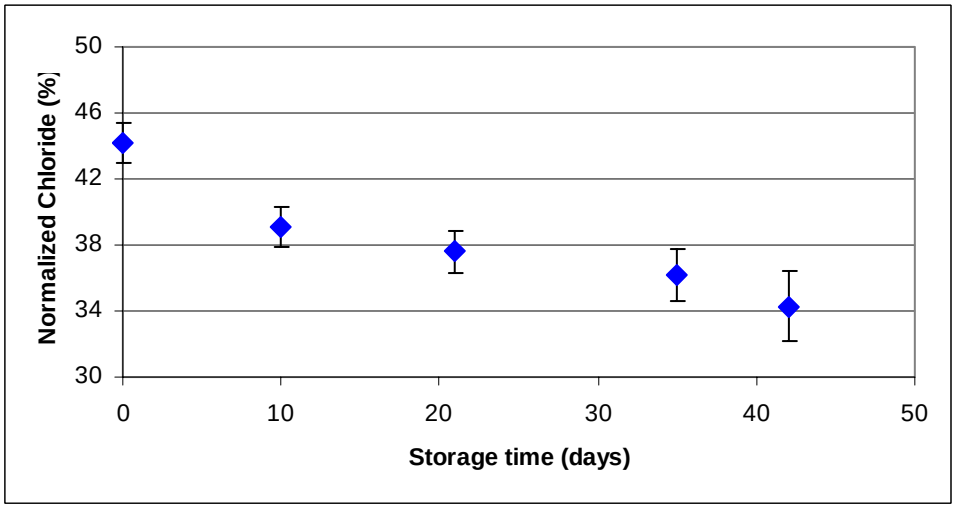


Figure 4.2 Normalized physiological data for ES under storage.

4.1.2.2 Whole Blood (WB). Like erythrocyte suspensions, physiological alterations of whole blood during storage are investigated with the raw data. Again, it is seen that the extracellular K^+ increases and the extracellular Na^+, Cl^-, pH, 2,3-DPG and ATP decreases with storage time (Figure 4.3).

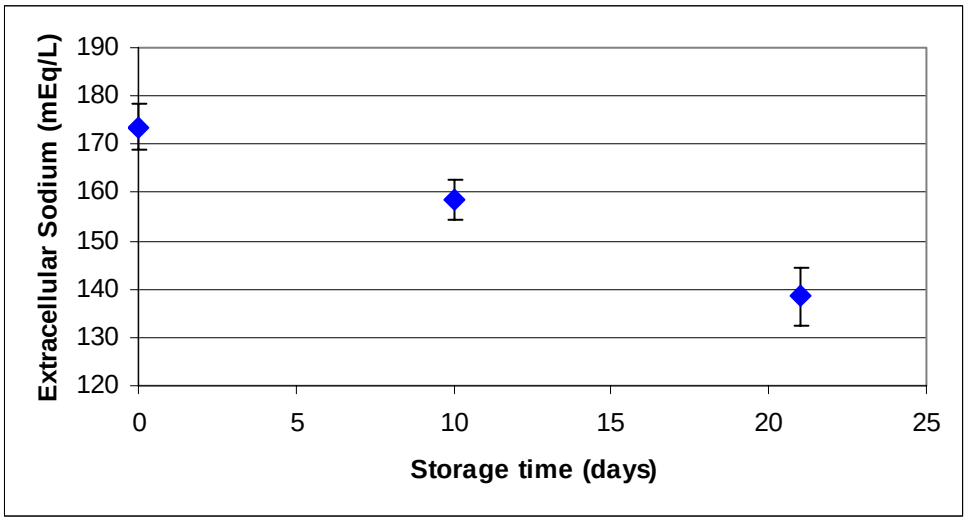
The statistics of the raw physiological data, are shown in Table 4.4.

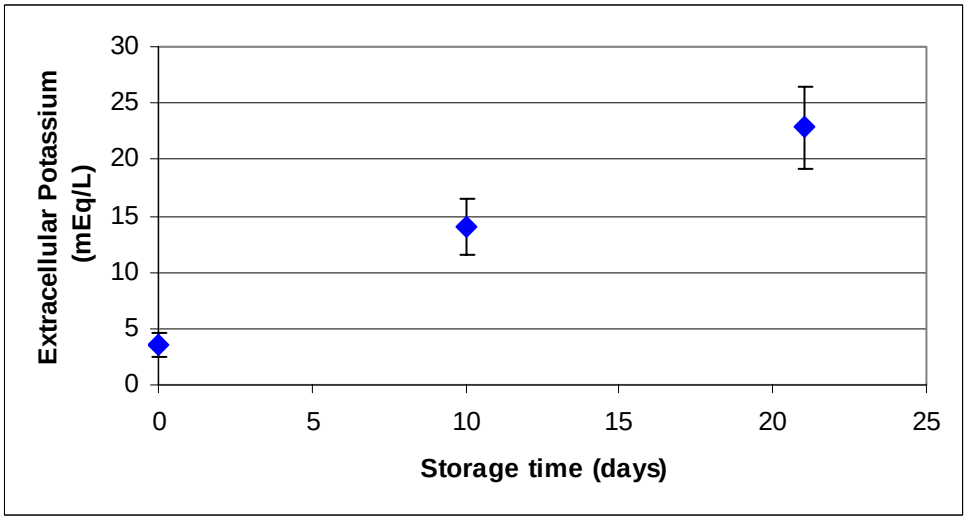
Table 4.4
Descriptive statistics of raw physiological data for WB.

	Mean	*Std. deviation*	*Skewness*		*Kurtosis*	
			Statistic	*Std.Error*	*Statistic*	*Std.Error*
Extracellular Na^+ (mEq/L)	156,900	15,245	-,229	,250	-1,025	,495
Extracellular K^+ (mEq/L)	13,449	8,360	,136	,250	-1,068	,495
Extracellular Cl^- (mEq/L)	72,671	6,343	,338	,250	-,768	,495
pH	7,193	,182	-,054	,250	-1,060	,495
2.3-DPG (mmol/L)	,990	,741	,682	,253	-1,389	,500
ATP (micromol/gHb)	13,133	3,276	-,466	,271	-,858	,535

From Table 4.4, it can be said that data distribution range is wide for Na^+, K^+ and Cl^- concentrations and their standard deviations are large.

However, raw data distribution is far from normal distribution and their skewness values show that distributions are not symmetric. The distribution of ion concentrations can be approximated to normal distribution by normalization. pH, 2,3-DPG and ATP data are not normalized.





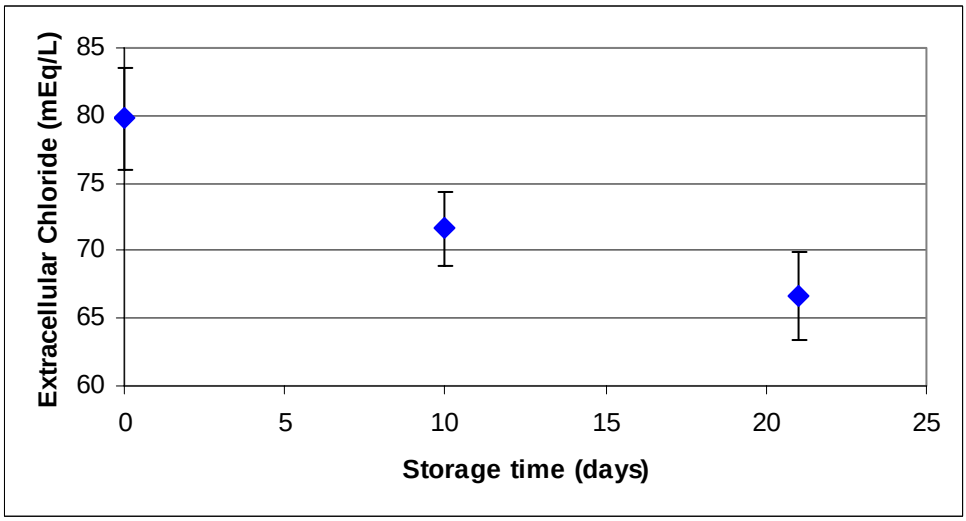
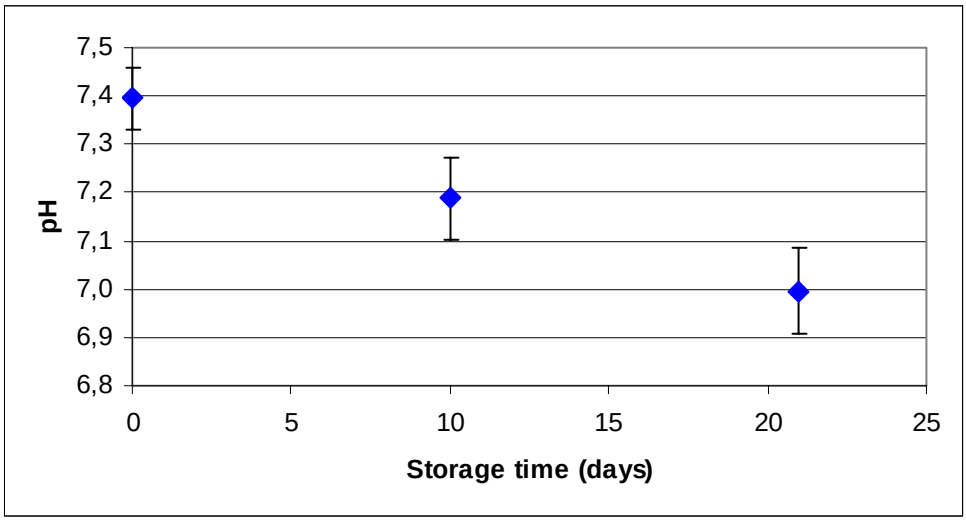
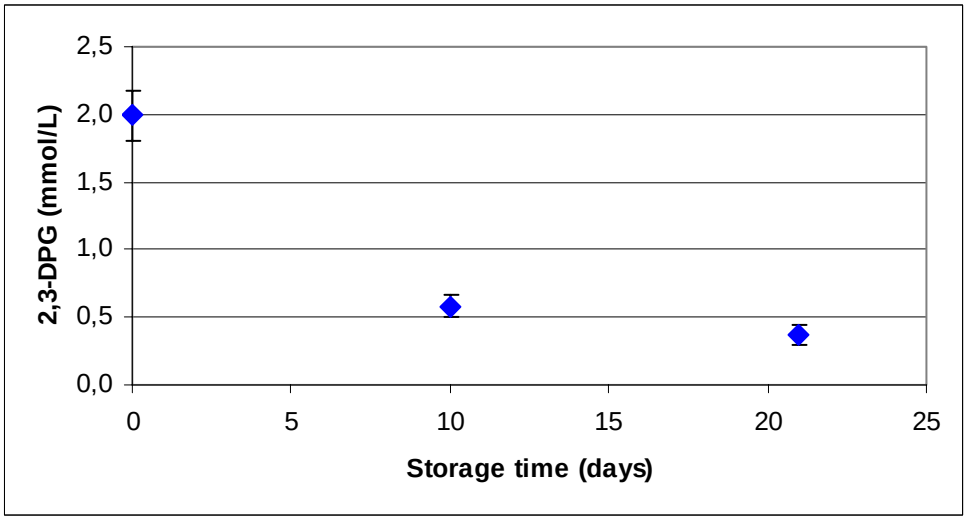


Figure 4.3 Raw physiological data for WB under storage.





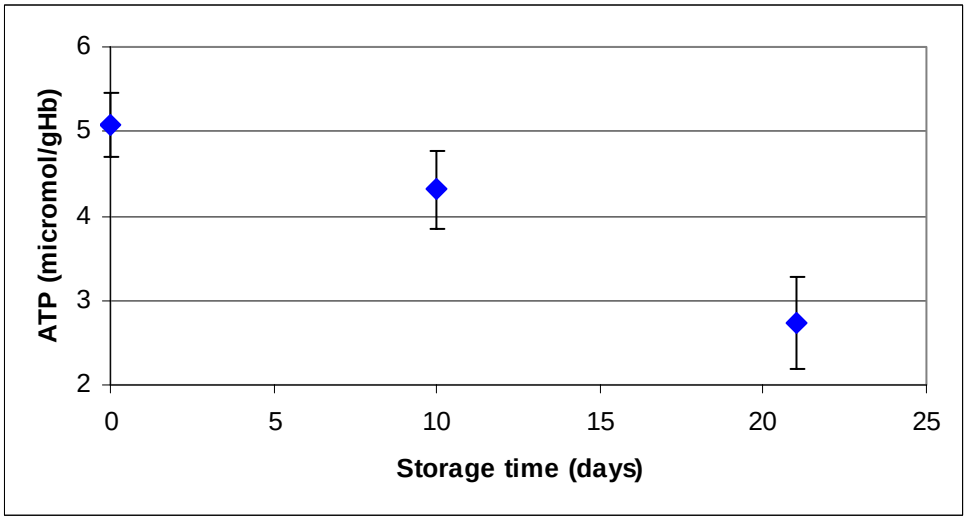


Figure 4.3 continued.

For normalization of the Na^+, K^+ and Cl^- concentrations, total ion concentration on the 0^{th} day is taken as reference: $[Na^+]_n$ (%) = 100 $[Na^+]_{meas.}$/t, $[K^+]_n$ (%) = 100 $[K^+]_{meas.}$/t and $[Cl^-]_n$ (%) = 100 $[Cl^-]_{meas.}$/t, where t = $[Na^+]+[K^+]+[Cl^-]$.

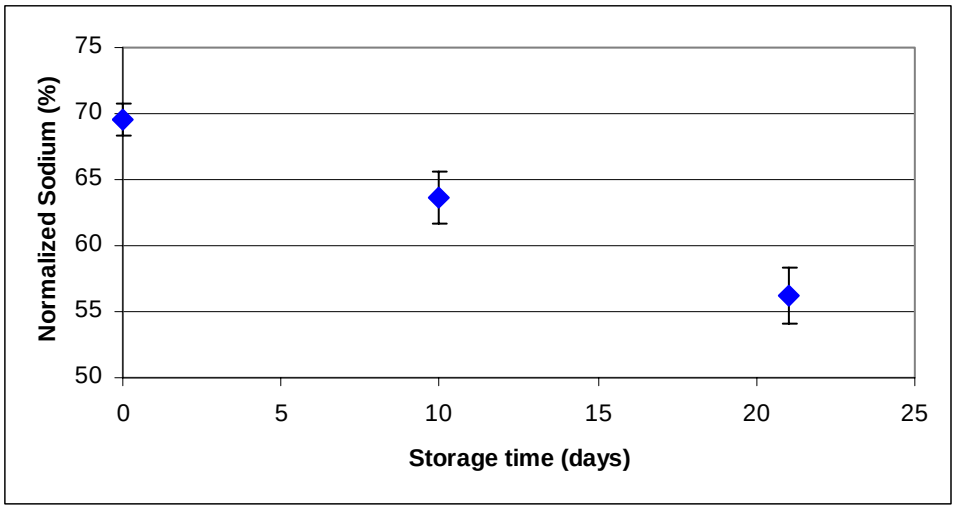
After normalization, the Na^+, K^+ and Cl^- concentrations are analysed again statistically (Table 4.5). When Table 4.4 and Table 4.5 are compared, it is seen that by normalization, only the skewness of K^+ concentration approximates to normal distribution. The Na^+ and Cl^- concentrations don't have any change.

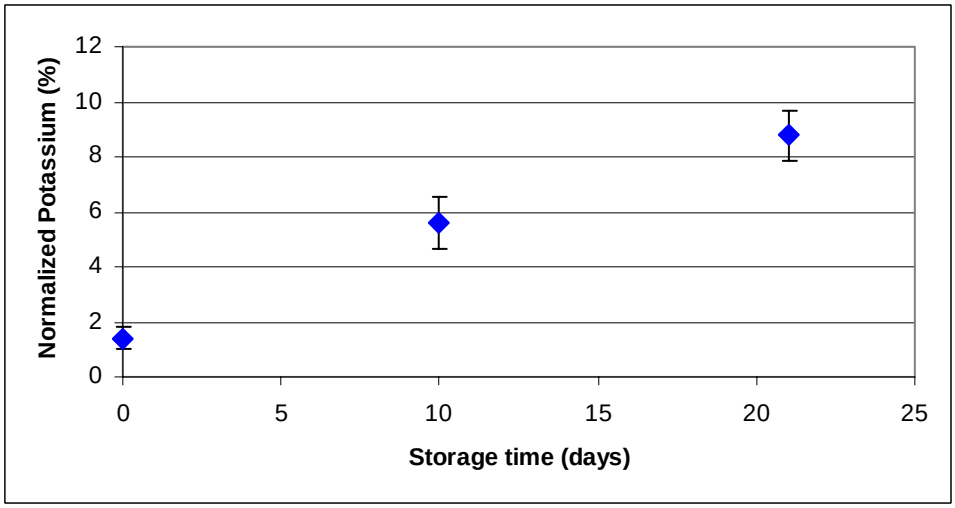
As it is expected, by normalization, data distributions approximate the normal distribution and their standard deviations become smaller.

The alterations of normalized physiological data (Na^+, K^+ and Cl^- concentrations) for whole blood during storage are shown in Figure 4.4.

Table 4.5
Descriptive statistics of normalized physiological data for WB.

	Mean	*Std. deviation*	*Skewness*		*Kurtosis*	
			Statistic	*Std.Error*	*Statistic*	*Std.Error*
$[Na^+]$norm (%)	63,36638	5,75498	-,276	,254	-1,214	,503
$[K^+]$norm (%)	5,15176	3,12819	-,009	,254	-1,381	,503
$[Cl^-]$norm (%)	29,14151	2,43155	,384	,250	-,797	,495





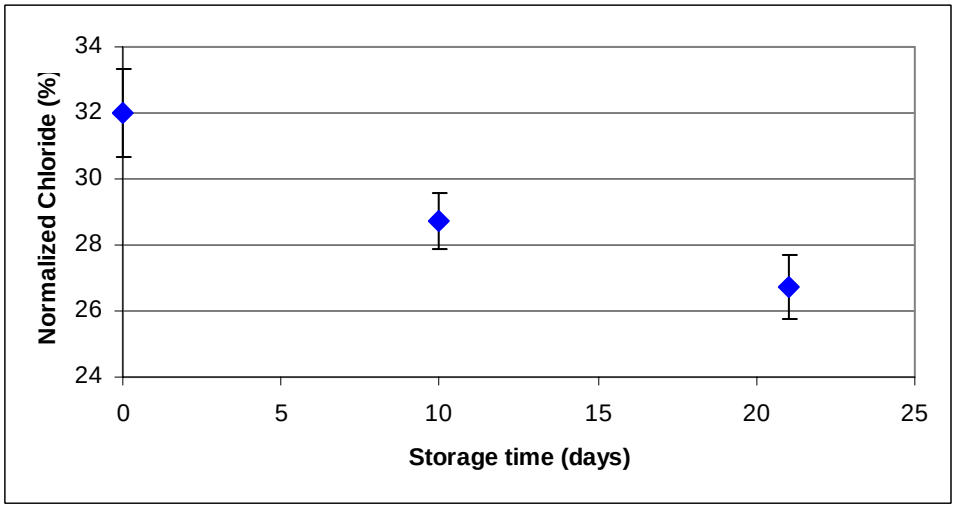


Figure 4.4 Normalized physiological data for WB under storage.

4.2 Electrical Impedance Alterations During Storage

4.2.1 Introduction

The dielectric properties of blood were studied by early investigators and the subject still continues to be important for practical applications. Cole presented an interesting review of the early works by Hober, Fricke, and others [23]. Schanne and P. Ceretti summarized the results of the many dielectric studies performed on tissue cells [24]. Trautman and Newbower have prepared an excellent review of conductivity data from blood, including many references to earlier research works [25]. The dielectric relaxation properties of whole blood and hemoglobin have been reviewed by Schwan with emphasis on the mechanisms responsible for them [69]. Zhao has several studies about Cole-Cole parameters. He showed that Cole-Cole parameters are a potential index for evaluating blood in clinical applications [1],[11],[27],[30].

Bioelectrical impedance analysis (BIA) has many advantages over methods in that it is safe, inexpensive, portable, rapid, easy to perform and requires minimal operator training. The technique has become widely used in hospitals, health and fitness centers, and in field studies.

This technique is extended to biologic systems provided that both real and reactive impedance components are measured with sufficient accuracy over the frequency range of interest [70],[71].

4.2.2 Cole-Cole Representation of Complex Impedance

The complex series impedance $(R + jX)$ of a single cell can be represented by a plot of the imaginary component against the real part of the impedance at different frequencies to give the Cole-Cole plot [72].

The Cole-Cole plot of the impedance of a single cell is given by the following equation:

$$Z = R_{\infty} + \frac{R_0 - R_{\infty}}{1 + j\omega\tau} \tag{4.1}$$

where R_0 is the resistance at $f = 0$, R_∞ is the resistance at $f = \infty$, and τ is the time constant.

In the equivalent circuit of the single cell, the extracellular and the intracellular fluid conductivities are represented by R_{ec} and R_{ic} respectively; C_m represents the cell membrane capacitance. However, even within the same tissue, cells will have slightly different shapes and structures and will have different time constants, producing a spread of relaxation times. Cole-Cole plots of biological tissues are depressed circles with the centre below the real axis. The intersection of the Cole-Cole plot with the real axis provides R_0 and R_∞. To account for this effect, capacitive effects of cell membranes are usually lumped in a constant phase angle impedance Z_{CPA} defined by

$$Z_{CPA} = K(j\omega)^{-\alpha} \tag{4.2}$$

where K is a constant with dimensions (Ω sec $^{-\alpha}$) [72]. The equivalent circuit is shown in Figure 4.5.

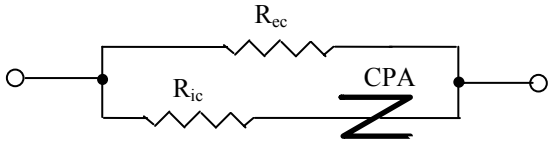


Figure 4.5 Electrical equivalent circuit of biological tissues.

The depressed semicircular locus is a representation of an impedance of the form given by Equation 4.1 where the depression angle ϕ is equal to $(\alpha.\pi/2)$ radians. The Cole-Cole equation can be written as:

$$Z^* = R_\infty + \frac{R_0 - R_\infty}{1 + \frac{R_{ic} + R_{ec}}{K}(j\omega)^\alpha} \tag{4.3}$$

In this equation above, α is an experimental parameter which has a value between 0 and 1 and shows the deviation from the pure capacitance (where α=1) [72]. The dc resistance value R_0 is equal to R_{ec} and R_∞ is given by the parallel combination of R_{ec} and

R_{ic}. The imaginary part of Z^* is maximum at the characteristic frequency ω_c and is determined by plotting $\ln\left(\frac{|v|}{|u|}\right)$ against $ln\ \omega$: $\frac{|v|}{|u|} = (\omega\tau_0)^{1-\alpha}$

The intersection of this straight line with the $ln\ \omega$ axis gives the characteristic frequency ω_c. The slope yields the quantity (1-α). The phasors u and v are defined in Fig. 4.6.

$$|u| = \sqrt{\left(\mathrm{Im}\{Z_A^*\}\right)^2 + \left(\mathrm{Re}\{Z_A^*\} - R_\infty\right)^2} \tag{4.4}$$

$$|v| = \sqrt{\left(\mathrm{Im}\{Z_A^*\}\right)^2 + \left(R_0 - \mathrm{Re}\{Z_A^*\}\right)^2} \tag{4.5}$$

Once ω_c is obtained, the effective capacitance C_{eff} is then determined from;

$$C_{eff} = \frac{1}{\omega_c \left(R_{ic} + R_{ec}\right)^{\frac{1}{1-\alpha}}} \tag{4.6}$$

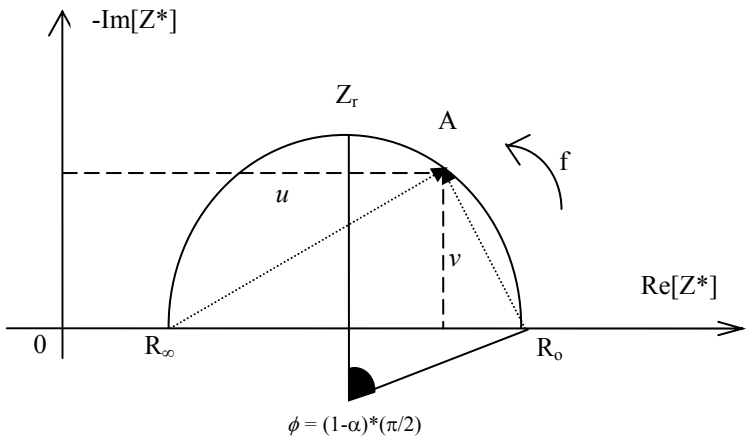


Figure 4.6 The depressed Cole-Cole plot [72].

4.2.3 Alterations During Storage

4.2.3.1 Erythrocyte Suspensions (ES). Electrical impedance alterations during storage are initially investigated with the raw and the normalized data. When using the raw data, the surrounding fluid resistance R_e and the effective cell membrane capacitance C_m increase progressively with storage time, while the intracellular resistance R_i decreases (Figure 4.7). Because each donor has a different hematocrit value and the blood electrical impedance is related to the hematocrit, the impedance data range is large, hence normalization is necessary.

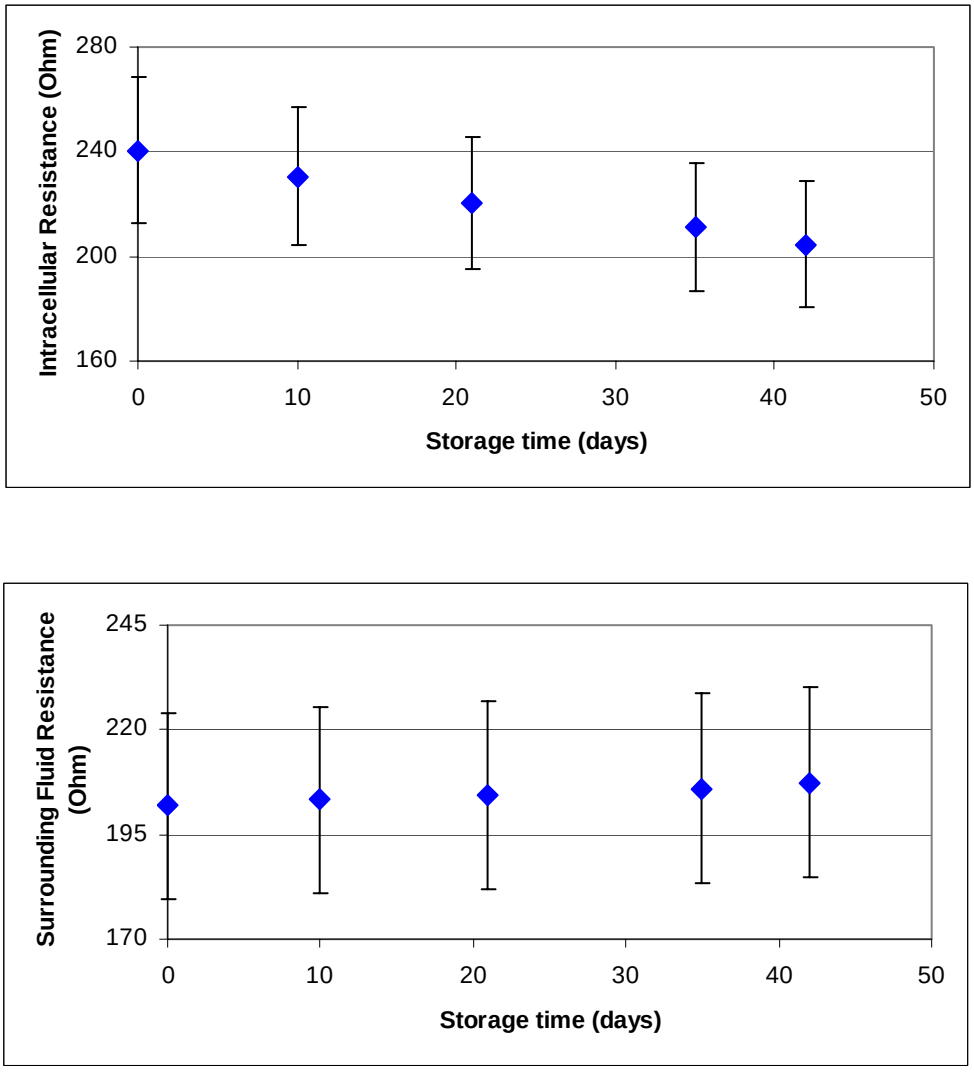
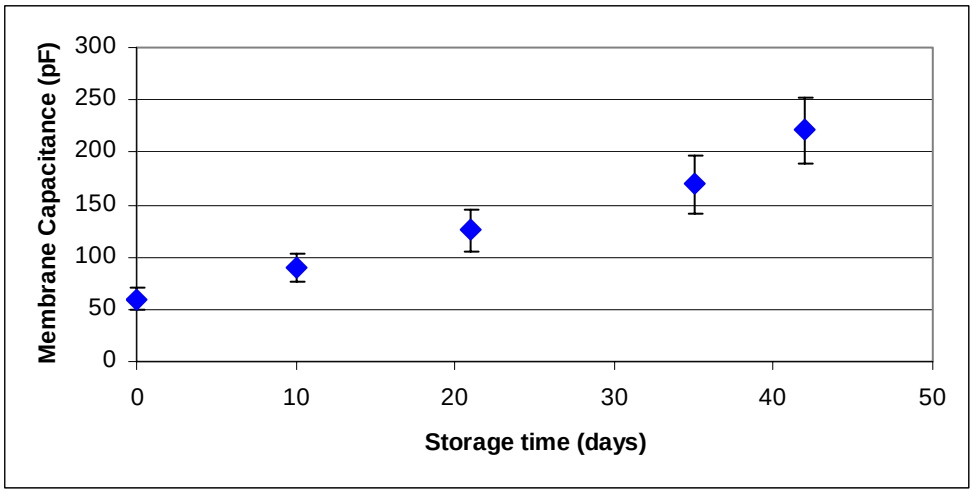
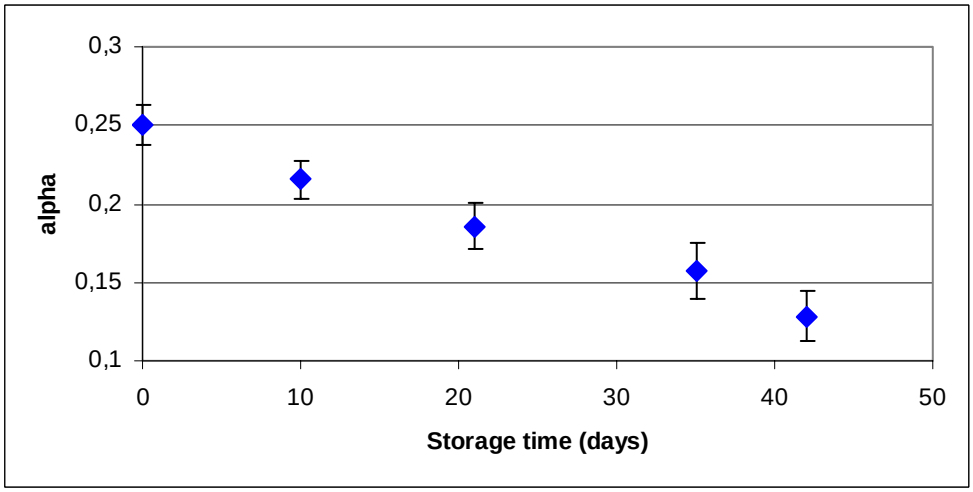


Figure 4.7 Raw electrical impedance data for ES under storage.





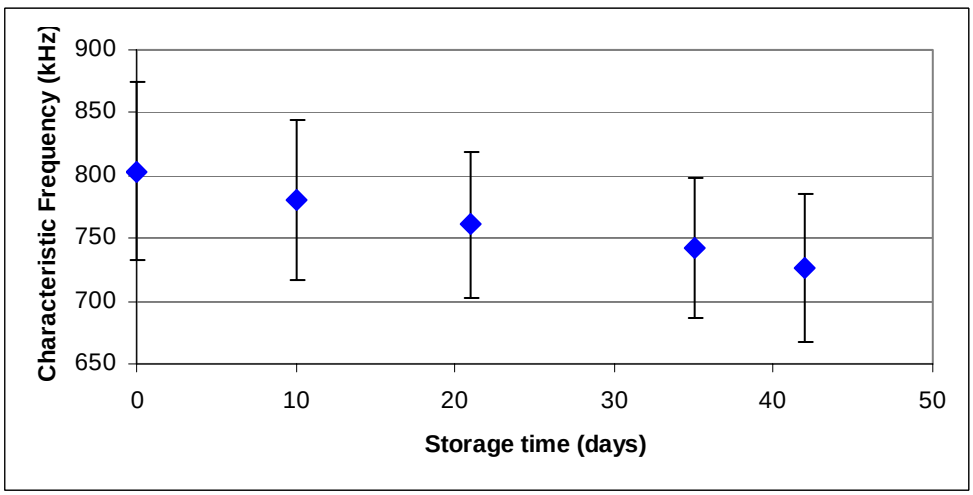


Figure 4.7 continued.

The surrounding fluid resistance (R_e) and the cell membrane capacitance (C_m) are directly; and the intracellular resistance (R_i) is inversely proportional to the hematocrit or packed cell volume (PCV) [1],[28]. Figure 4.8 shows the hematocrit dependence of R_i , R_e and C_m on the 0^{th} day.

The statistic analysis for raw electrical data (mean, minimum, maximum, standard deviation, kurtosis and skewness) with their standard errors is shown in Table 4.6.

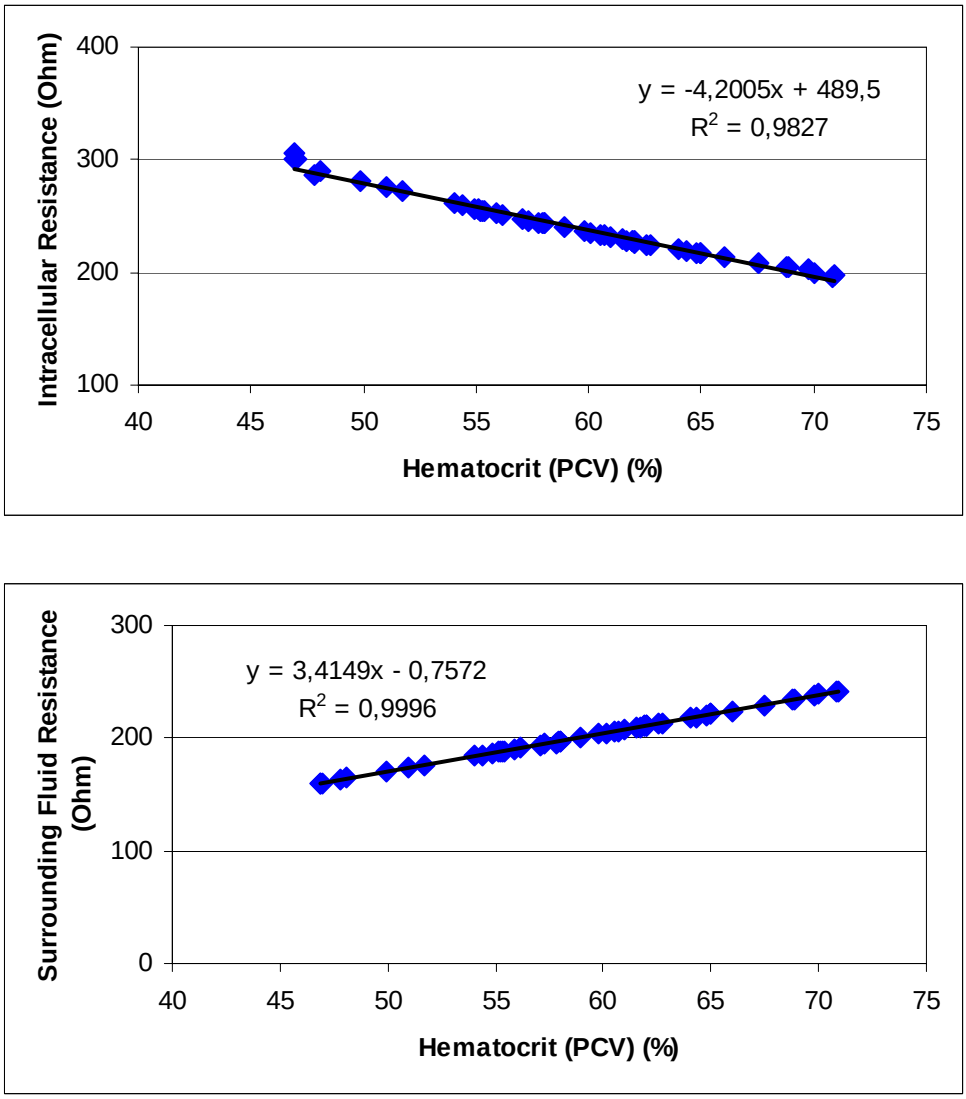


Figure 4.8 Raw electrical parameters versus the donors' hematocrit on the 0^{th} day (ES).

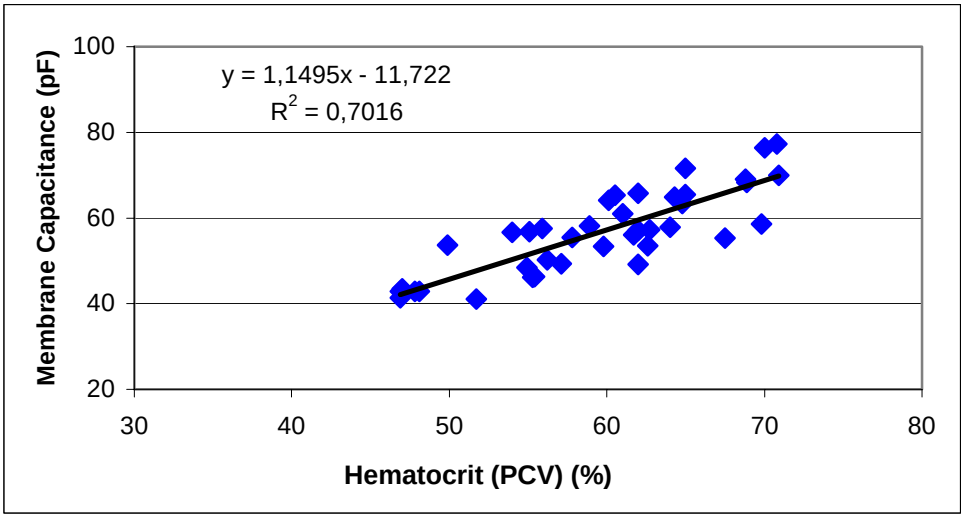


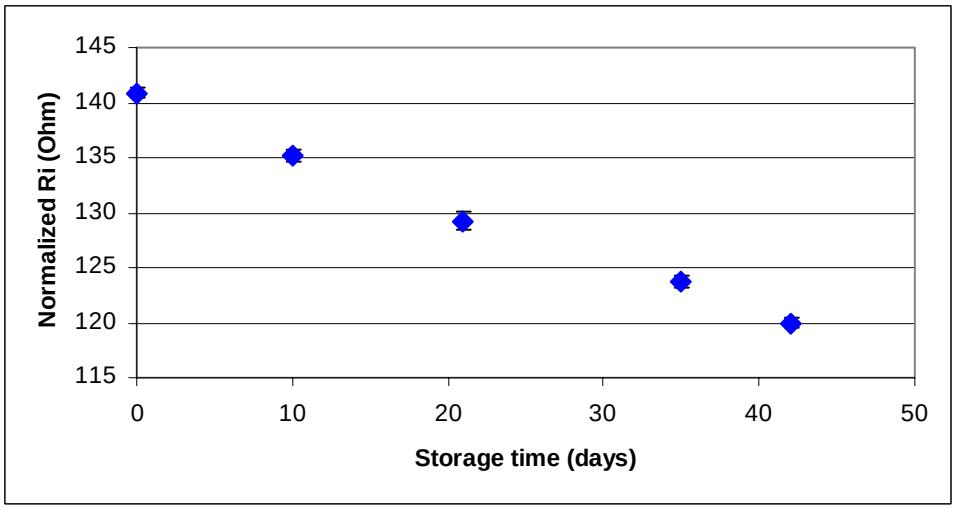
Figure 4.8 continued.

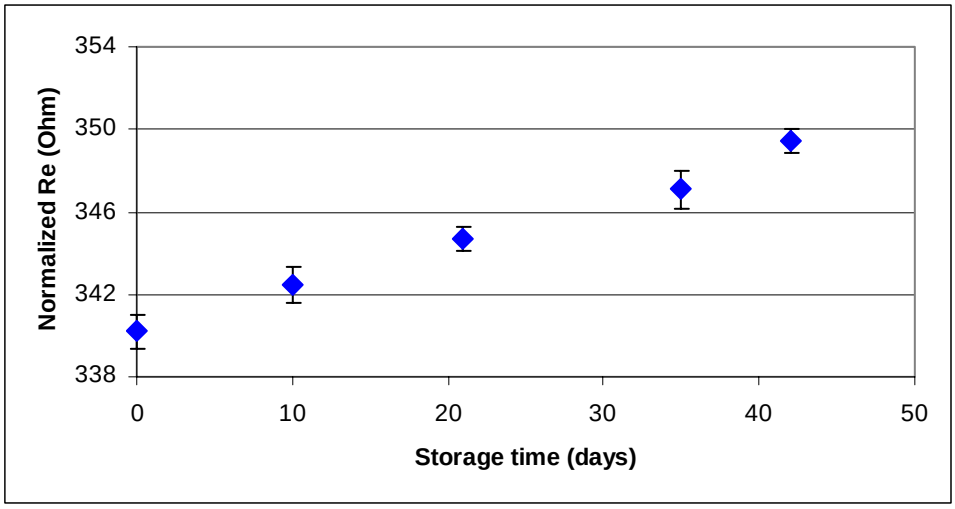
Table 4.6
Descriptive statistics of raw electrical data for ES.

	Mean	*Std. deviation*	*Skewness*		*Kurtosis*	
			Statistic	*Std. Error*	*Statistic*	*Std.Error*
R_i *(Ohm)*	221,442	28,556	,614	,153	,060	,304
R_e *(Ohm)*	204,642	22,416	-,173	,153	-,537	,304
C_m *(Ohm)*	133,160	61,510	,455	,153	-,867	,304
α	,1874	,0451	,027	,153	-1,068	,304
F_c *(kHz)*	762,947	66,982	,792	,153	-,030	,304

Normalization of electrical parameters is done with reference to their 0^{th} day hematocrit value: $(R_e)_n = (R_e)_{meas.}/h$, $(R_i)_n = (R_i)_{meas.}.h$ and $(C_m)_n = (C_m)_{meas.}/h$, with h= Hematocrit/100.

The alterations in R_i, R_e and C_m for erythrocyte suspensions during storage are shown in Figure 4.9.





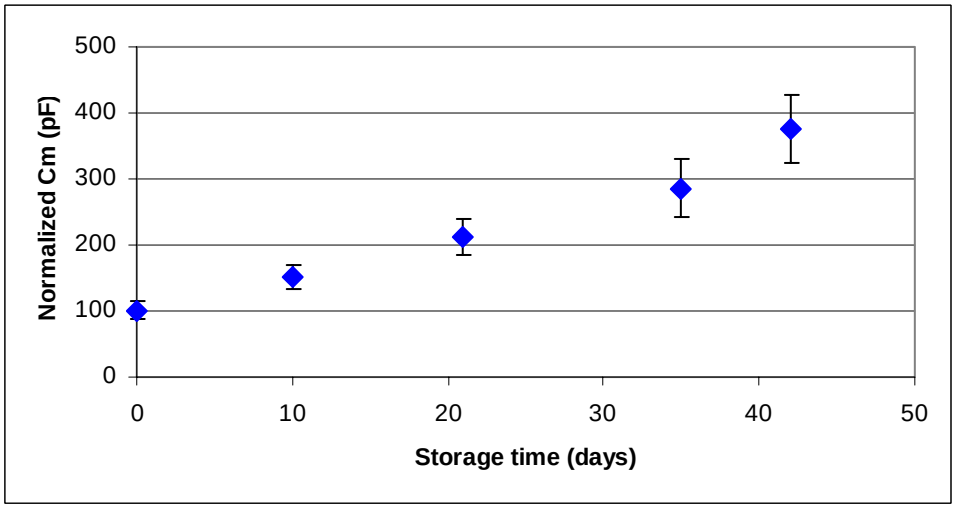


Figure 4.9 Normalized electrical data for ES under storage.

After normalization, R_i, R_e and C_m are again analyzed statistically (Table 4.7). When comparing Table 4.6 with Table 4.7, it is seen that after normalizations, the standard deviations in R_i and in R_e are decreased.

Table 4.7
Descriptive statistics of normalized electrical data for ES.

	Mean	*Std. deviation*	*Skewness*		*Kurtosis*	
			Statistic	*Std.Error*	*Statistic*	*Std.Error*
Rinorm (Ohm)	129,812	7,589	,158	,153	-1,362	,304
Renorm (Ohm)	344,779	3,360	,008	,153	-1,206	,304
Cmnorm (Ohm)	224,963	103,031	,486	,153	-,790	,304

The red blood cells with non-uniform shapes and structures loose this assortment with time and, by swelling completely, finally become spheres. This is also reflected in the Cole-Cole diagrams of blood: the circles gradually shifted upwards decreasing α and suggesting very little change in F_c, as the storage period has increased (Figure 4.10). The characteristic frequency of stored erythrocytes which represents the largest imaginary part on the Cole-Cole plot is from 600 kHz to 700 kHz, within the measurement frequency range.

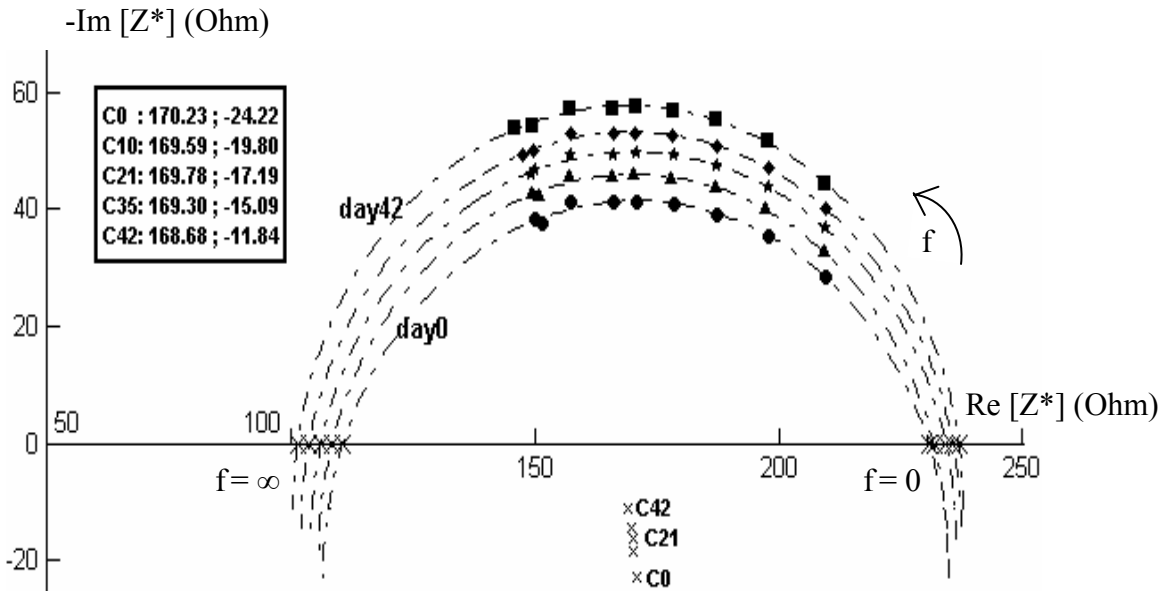
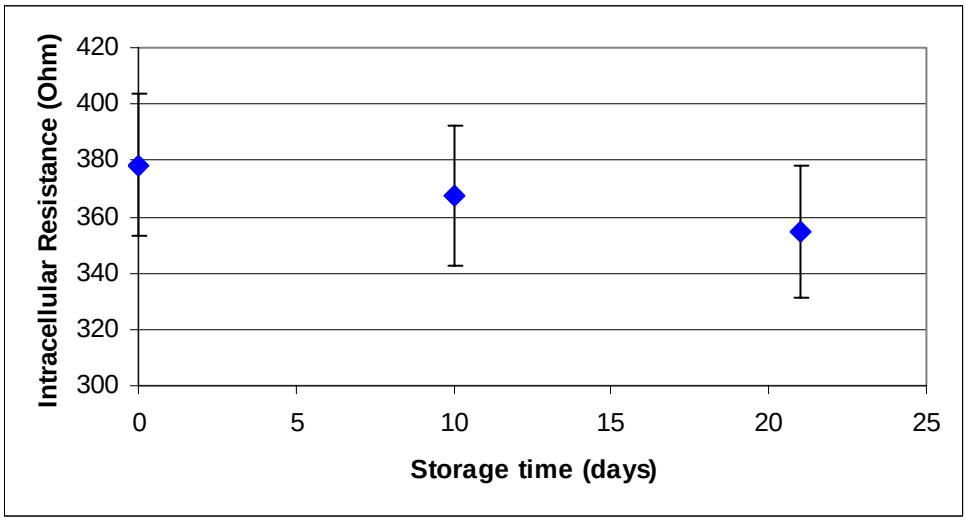


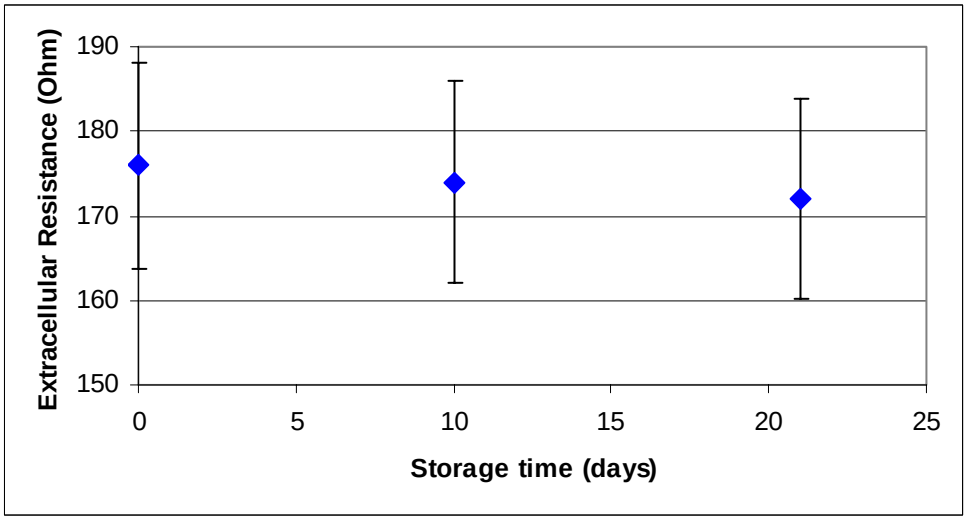
Figure 4.10 Cole-Cole curves shift upwards with storage time for erythrocyte suspensions.

4.2.3.2 Whole Blood (WB). When studying the raw data, the effective cell membrane capacitance C_m of whole blood samples increased progressively with storage time while the intracellular resistance R_i has decreased. Unlike erythrocyte suspensions, the extracellular resistance R_e has decreased (Figure 4.11). Descriptive statistics of raw electrical data are shown in Table 4.8.

Table 4.8
Descriptive statistics of raw electrical data for WB.

	Mean	*Std. deviation*	*Skewness*		*Kurtosis*	
			Statistic	*Std.Error*	*Statistic*	*Std.Error*
R_i *(Ohm)*	366,750	26,127	-,110	,250	-,714	,495
R_e *(Ohm)*	173,940	11,964	,409	,250	-,753	,495
C_m *(pF)*	120,652	32,494	,589	,250	-,358	,495
α	,171	,0232	-,068	,250	-,395	,495
F_c *(kHz)*	689,520	56,268	-,136	,250	-,817	,495





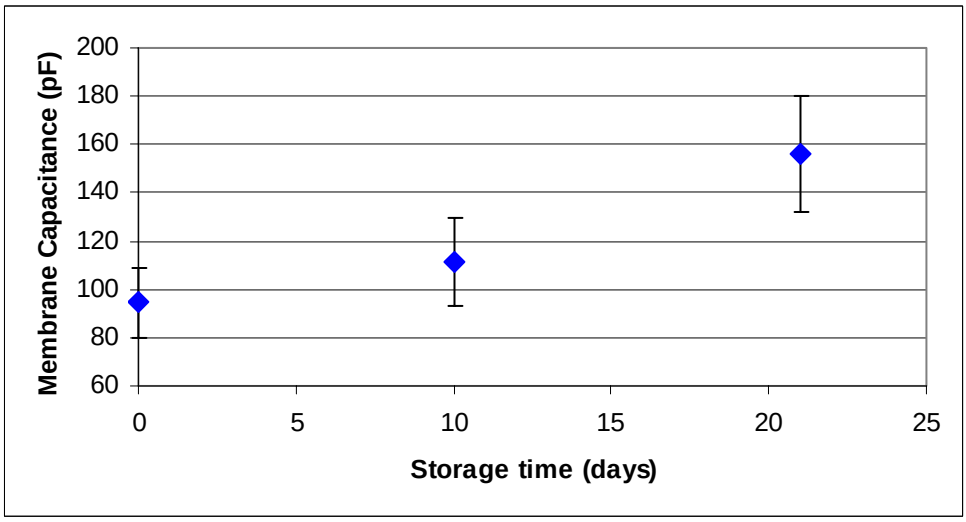
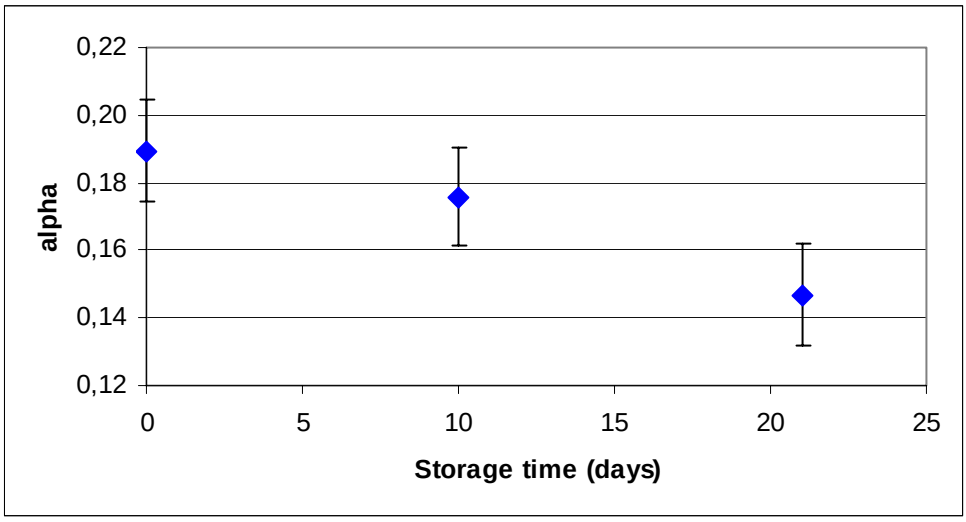


Figure 4.11 Raw electrical impedance data for WB under storage.



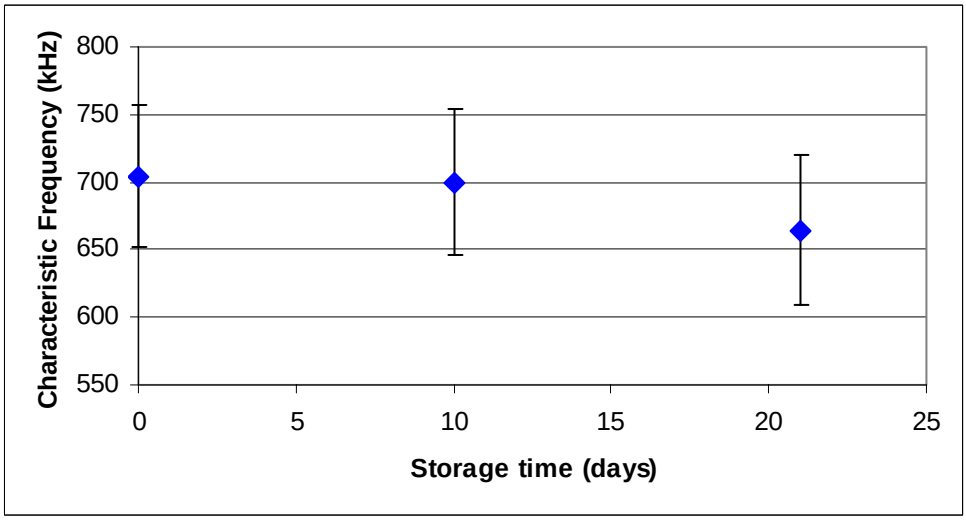
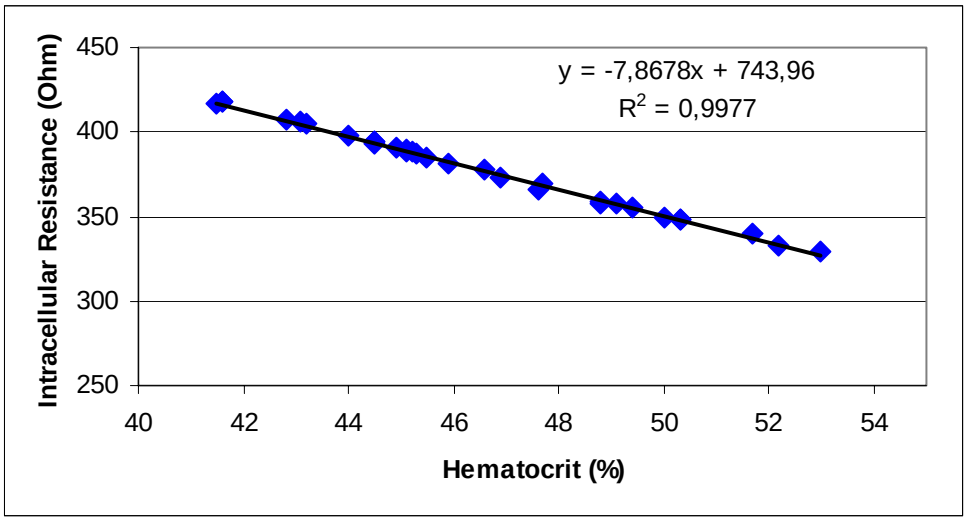


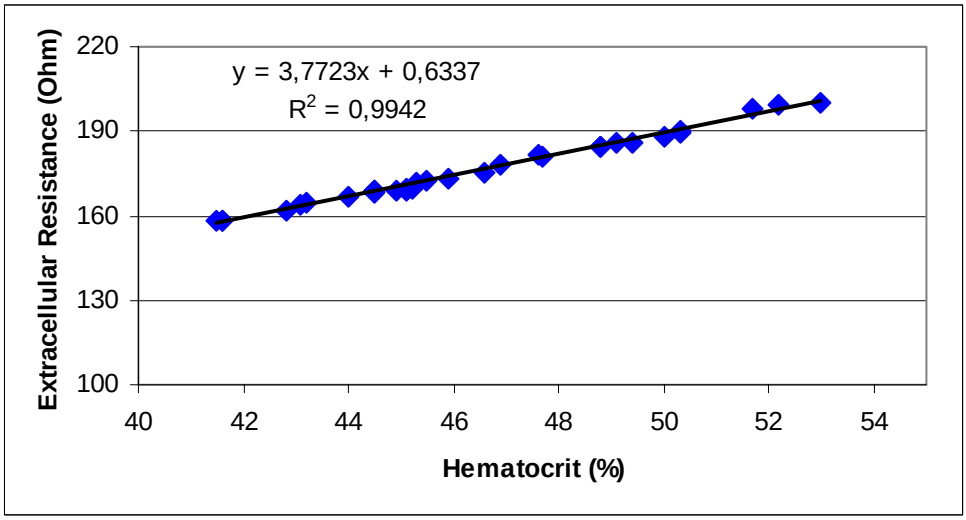
Figure 4.11 continued.

The relationships between raw electrical parameters for whole blood and hematocrit on 0^{th} day measurement are shown in Figure 4.12. From these graphs, it is clear that a normalization against the hematocrit is required.

The normalization factor: h= Hematocrit/100 and the normalized values; $(R_e)_n = (R_e)_{meas.}/h$, $(R_i)_n = (R_i)_{meas.}.h$ and $(C_m)_n = (C_m)_{meas.}/h$.

The normalized electrical data (R_i, R_e and C_m) for whole blood during storage are shown in Figure 4.13.





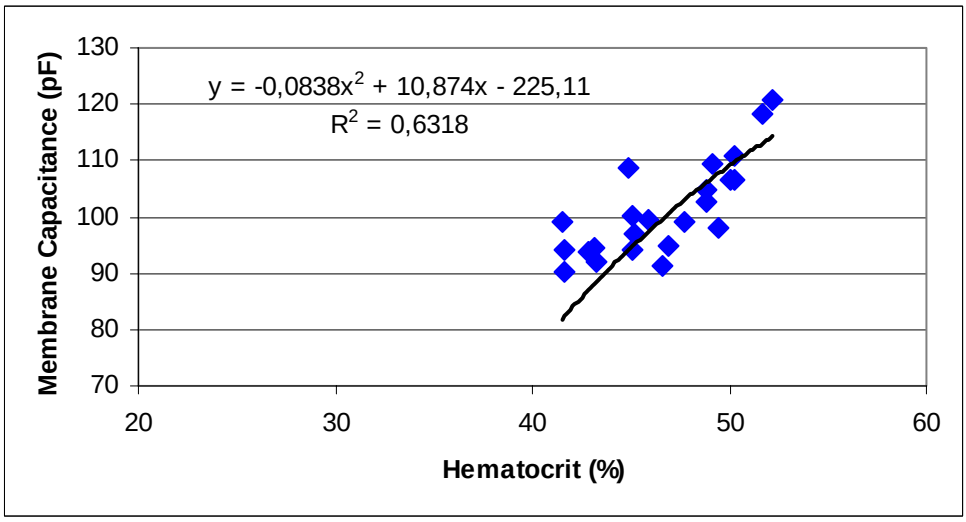
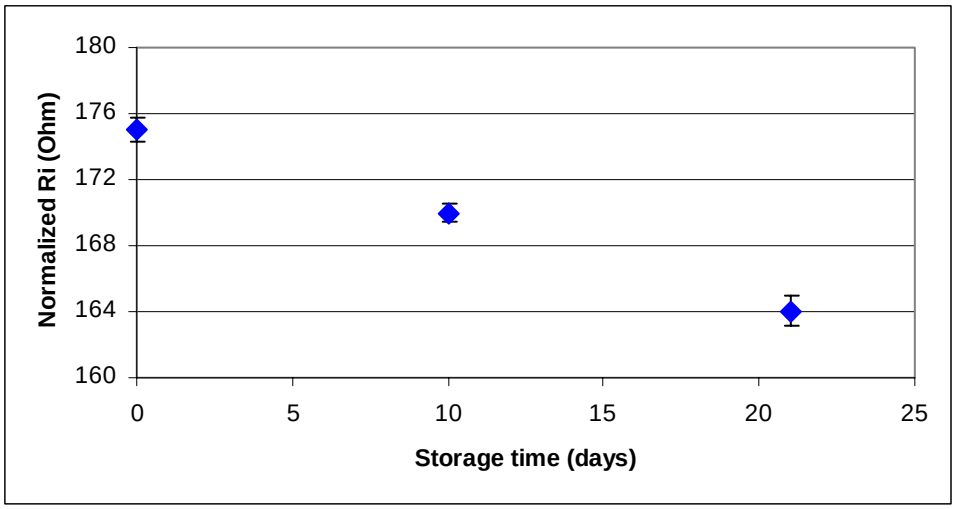
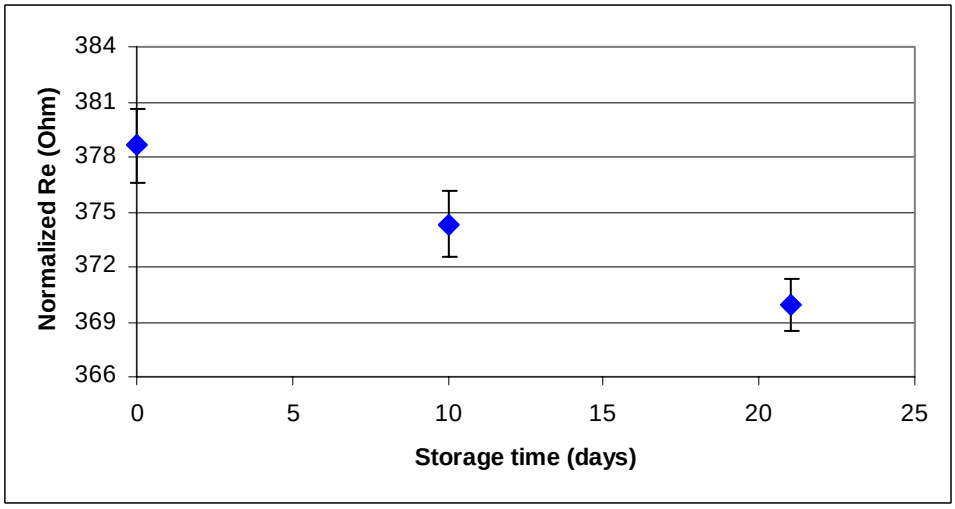


Figure 4.12 Raw electrical parameters versus the donors' hematocrit on the 0th day (WB).





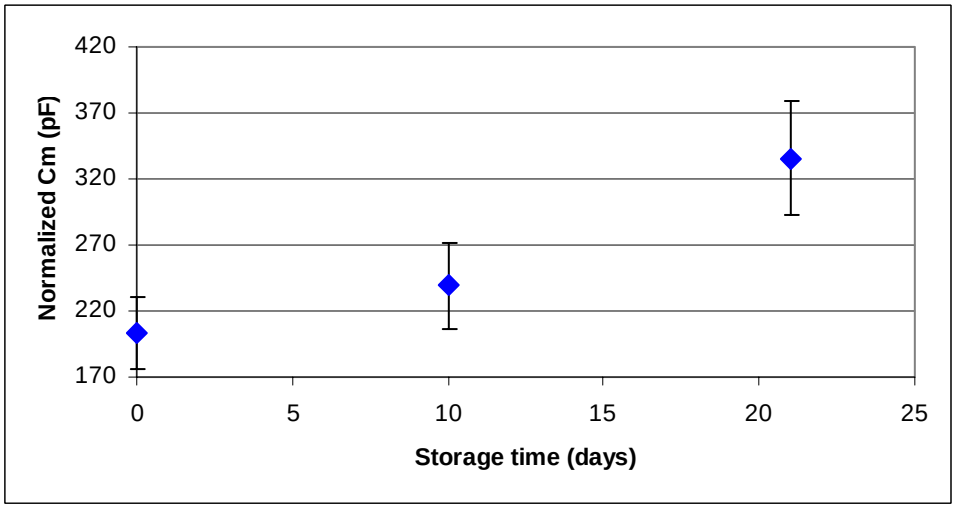


Figure 4.13 Normalized electrical impedance data for WB under storage.

Table 4.9 displays the statistics of electrical data for whole blood after normalization.

Table 4.9
Descriptive statistics of normalized electrical data for WB.

	Mean	*Std. deviation*	*Skewness*		*Kurtosis*	
			Statistic	*Std.Error*	*Statistic*	*Std.Error*
Rinorm (Ohm)	169,684	4,580	-,122	,250	-1,394	,495
Renorm (Ohm)	374,286	3,969	,115	,250	-1,050	,495
Cmnorm (pF)	259,262	65,920	,510	,250	-,632	,495

With storage time, the Cole-Cole curves for whole blood shifted upwards with F_c remaining almost constant and the phase angle α being reduced (Figure 4.14)

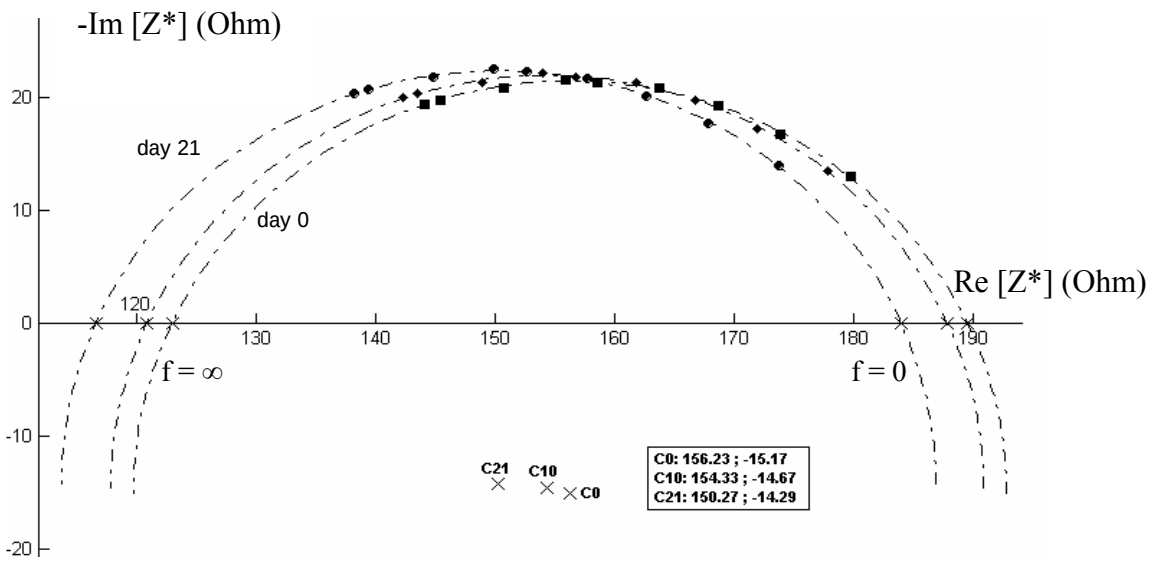


Figure 4.14 Cole-Cole curves shift upwards with storage time for whole blood.

5. BIOPHYSICAL INTERPRETATION OF ELECTRICAL IMPEDANCE CHANGES

5.1 Introduction

When analyzing the data for the health sciences disciplines, it is often desirable to determine the relationship between the variables by regression. In regression analysis, the relationship of one variable with another is estimated by expressing it as a linear or a more complex function of the other. It is also used to predict some variables in terms of the others [73].

In this study, the electrical parameters (R_i, R_e, C_m, alpha and F_c) and the physiological parameters (Na^+, K^+, Cl^- concentrations, pH, 2,3-DPG and ATP), are measured and it is desired to investigate the interdependence between these variables.

By applying the regression model in SPSS, it is seen that the intracellular resistance (R_i) is the most influenced electrical parameter with both erythrocyte suspensions (ES) and whole blood (WB) samples. Although R_i, R_e, C_m and α are correlated with Na^+, K^+, Cl^-, pH and, more significantly with ATP, F_c measurements do not show any significant correlation with the physiological parameters since it did not significantly changed with storage time.

The R_i-K^+ dependence shows the strongest correlation between them. It is also observed that K^+, among other ionic currents (Na^+ and Cl^-) and ATP, the energy source of cells, has the greatest effect on electrical parameters. Since the pH level controls the movement of cell ions, Cole-Cole parameters are also affected by the pH shifts. But, 2,3-DPG has no effect on electrical parameters.

Experiments suggest that, the Cole-Cole parameters may serve as a useful indicator in the quality prediction of stored blood samples.

5.2 Effects of Physiological Changes on Cole-Cole Parameters

5.2.1 Erythrocyte Suspensions Under Storage

As the first step, to investigate the relationship between the physiological parameters and the electrical parameters of erythrocyte suspensions, initially the correlation between the parameters is examined. Correlation analysis is concerned with measuring the strength of the relationship between variables. The results are given in Table 5.1.

Table 5.1
The correlation between blood parameters for ES.

	R_i	R_e	C_m	F_c	α
$[Na^+]$	1	1	1	0	1
$[K^+]$	2	2	2	0	2
$[Cl^-]$	2	2	1	0	1
pH	2	2	2	0	2
2,3-DPG	1	1	0	0	1
ATP	2	2	2	0	2

Correlation is significant at the 0,01 level [61].
0: No significant effect (Pearson correlation coefficient: 0.0 – 0.69)
1: Weak correlation (Pearson correlation coefficient: 0.70 - 0.84)
2: Strong correlation (Pearson correlation coefficient: 0.85 – 1.0)

As seen from Table 5.1, for erythrocyte suspensions R_i, R_e, C_m and α are correlated with $[K^+]$, pH and ATP.

Graphical representation of the relationships between the Cole-Cole parameters and the physiological parameters $[Na^+]$, $[K^+]$, $[Cl^-]$, pH, 2.3-DPG and ATP are given in Figures 5.1, 5.2, 5.3,....., 5.10.

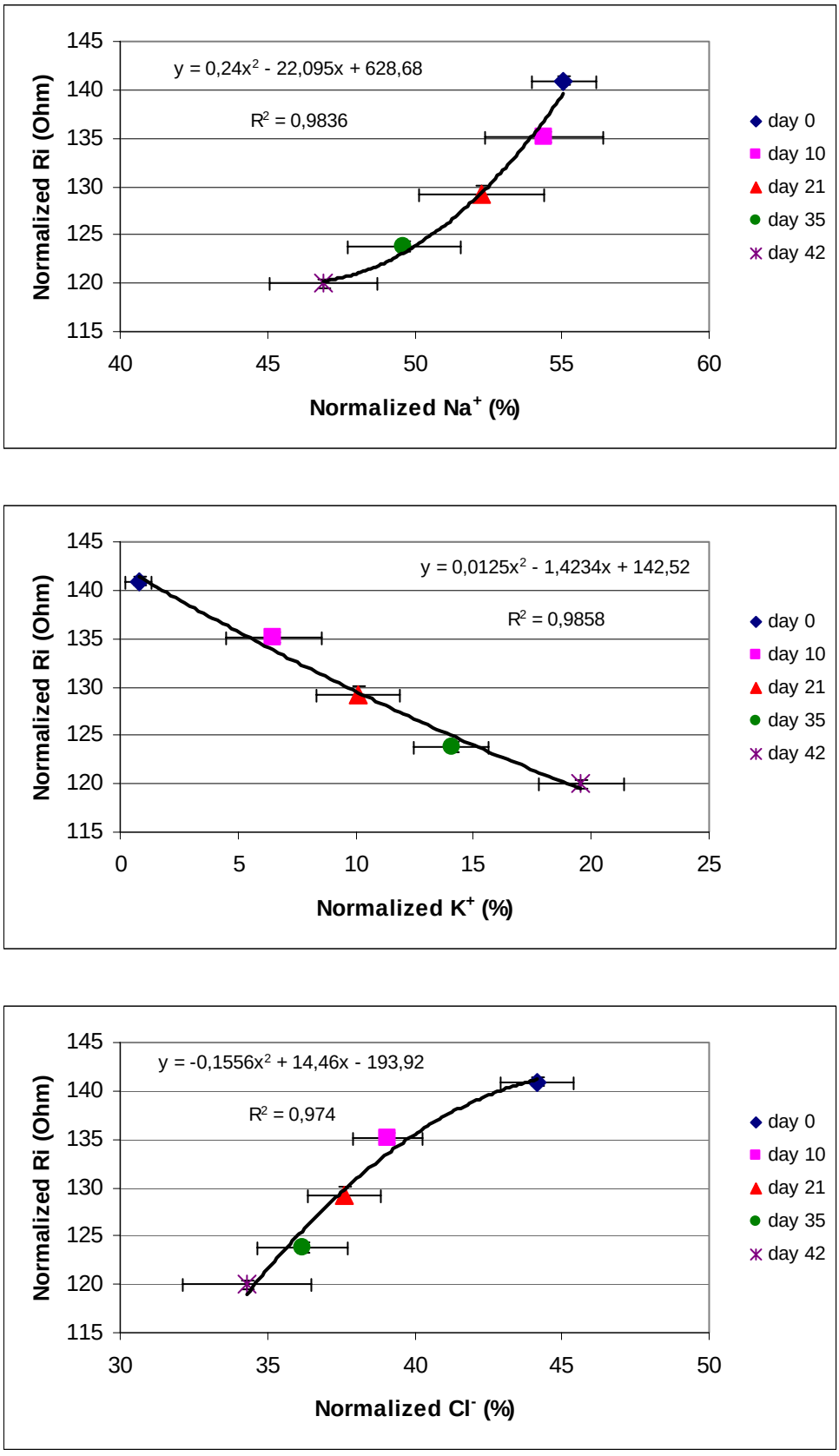


Figure 5.1 The relationship between R_i and Na^+, K^+ and Cl^-, for ES under storage.

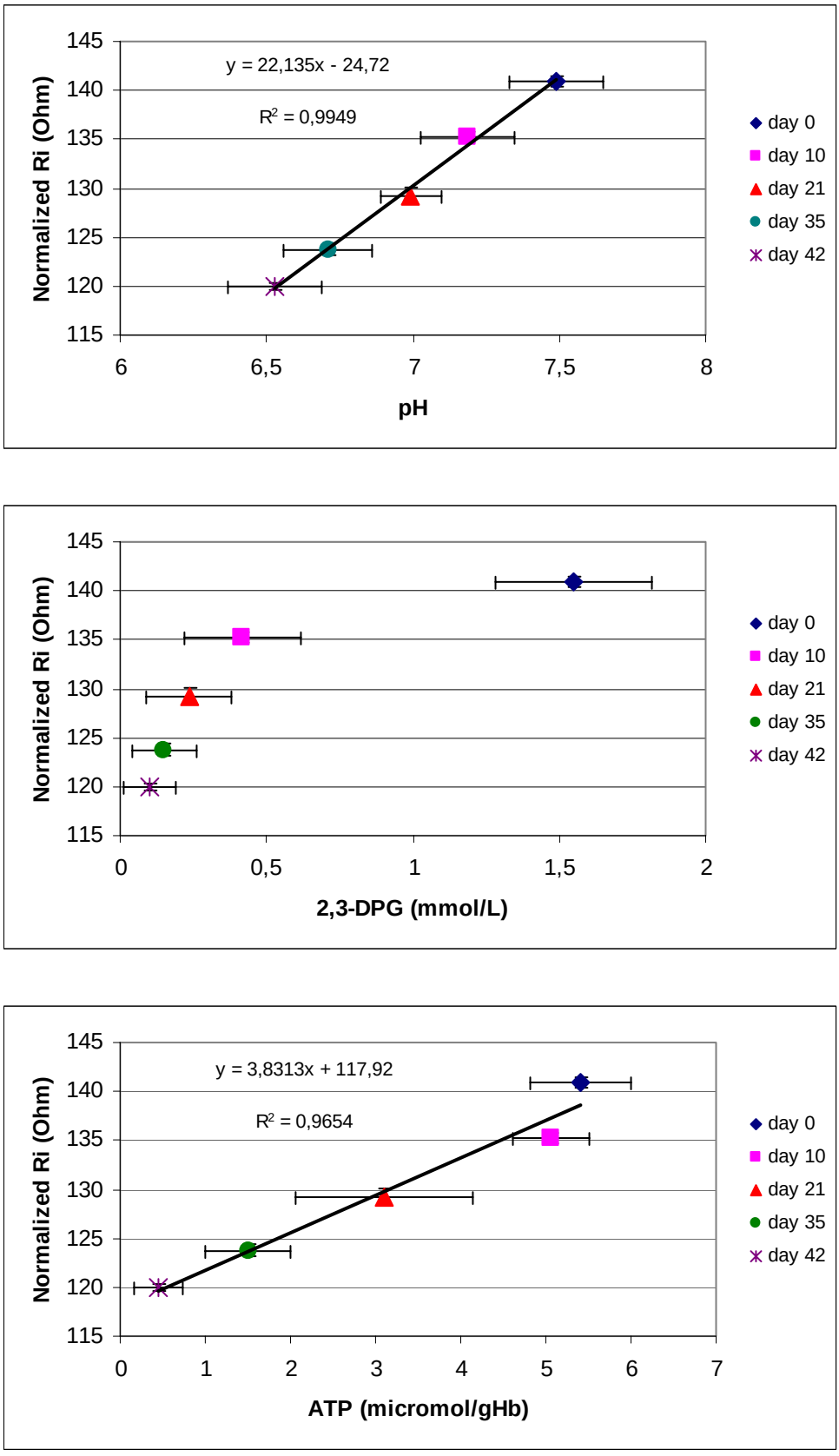


Figure 5.2 The relationship between R_i and pH, DPG and ATP, for ES under storage.

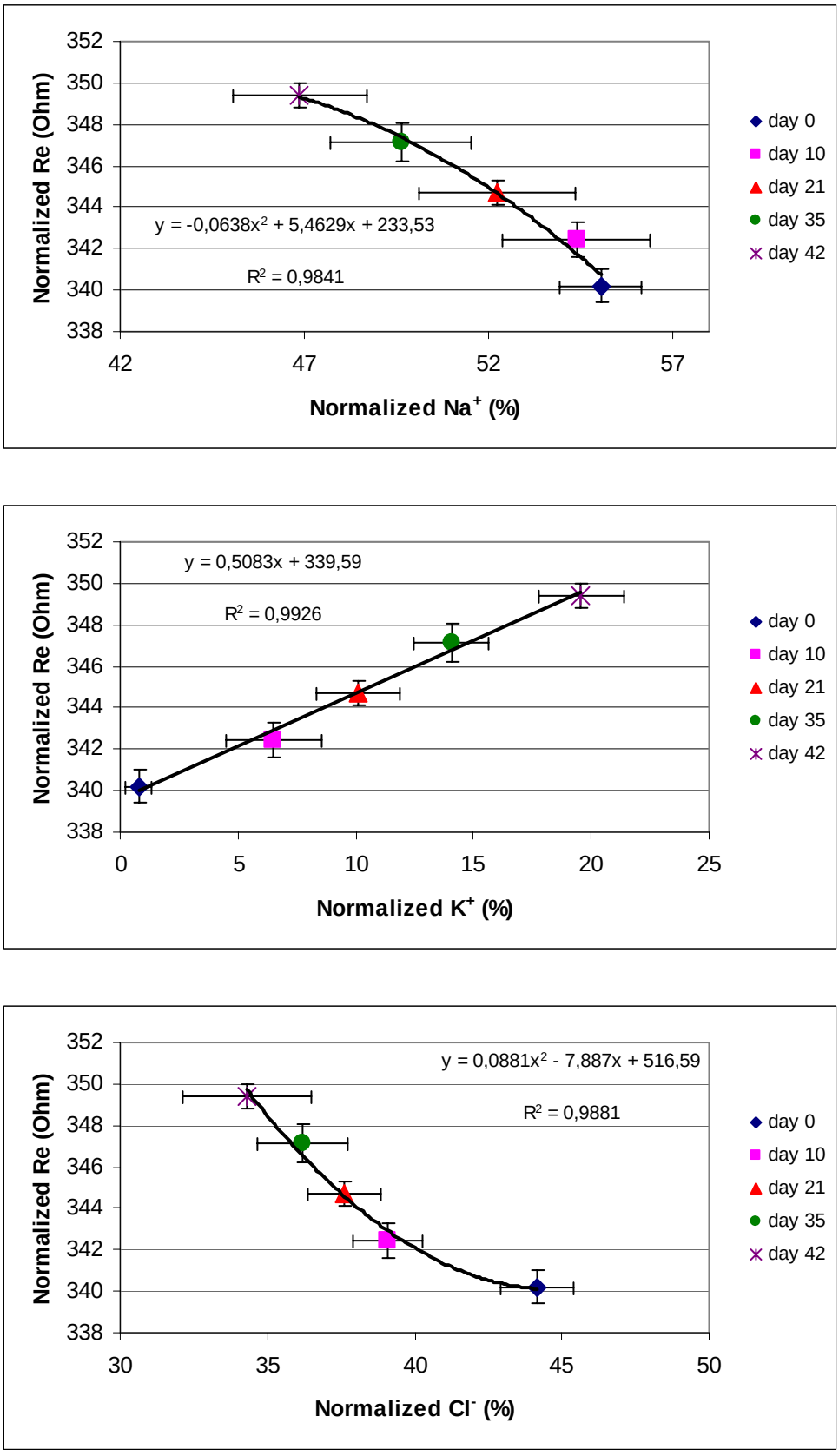
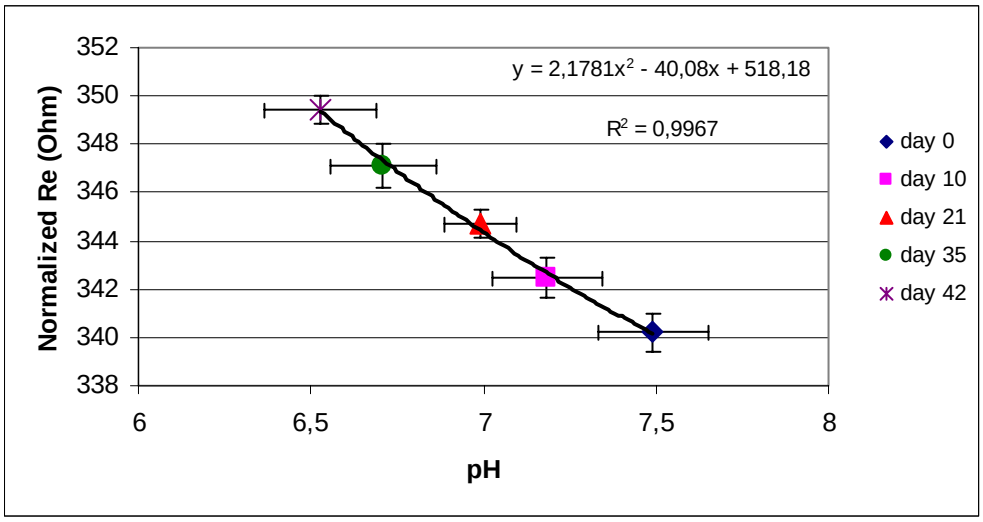
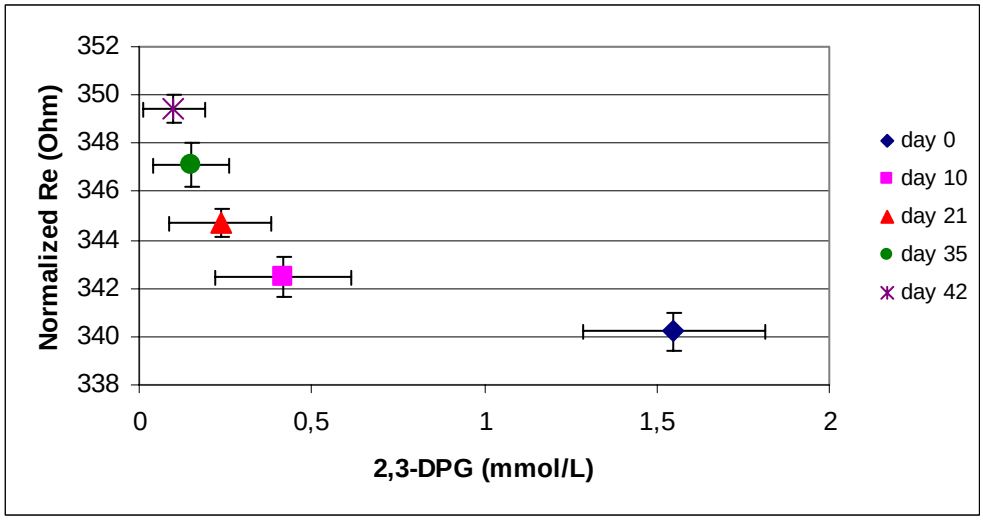


Figure 5.3 The relationship between R_e and Na^+, K^+ and Cl^-, for ES under storage.





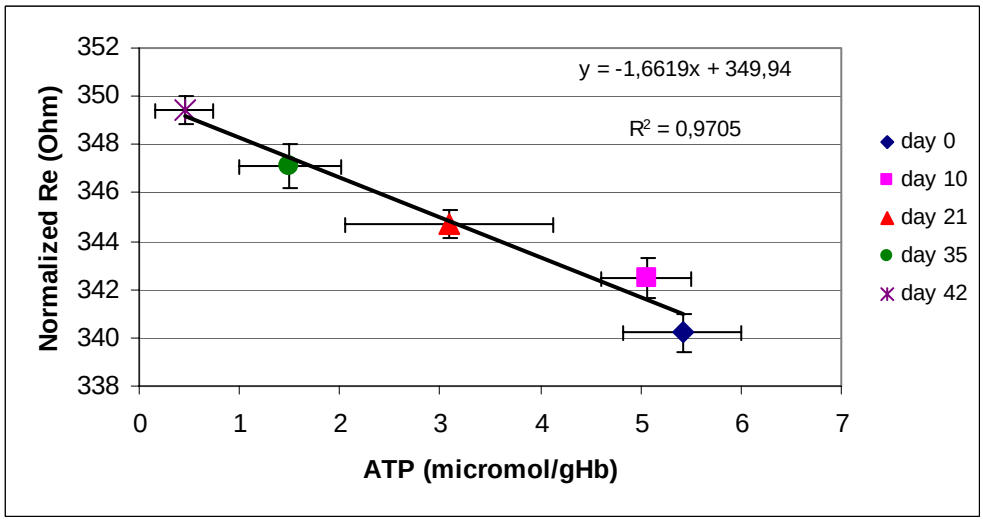


Figure 5.4 The relationship between R_e and pH, DPG and ATP, for ES under storage.

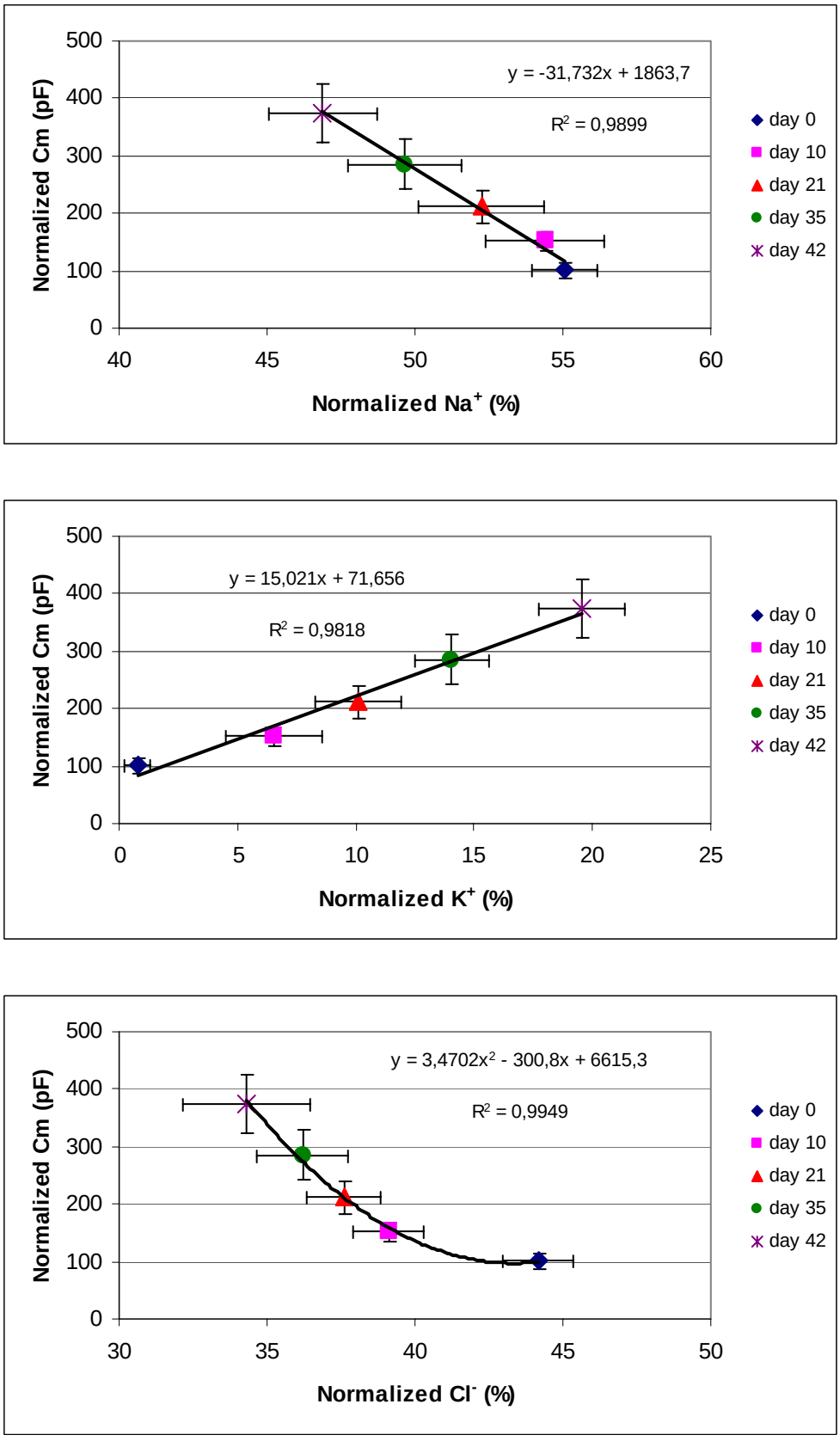
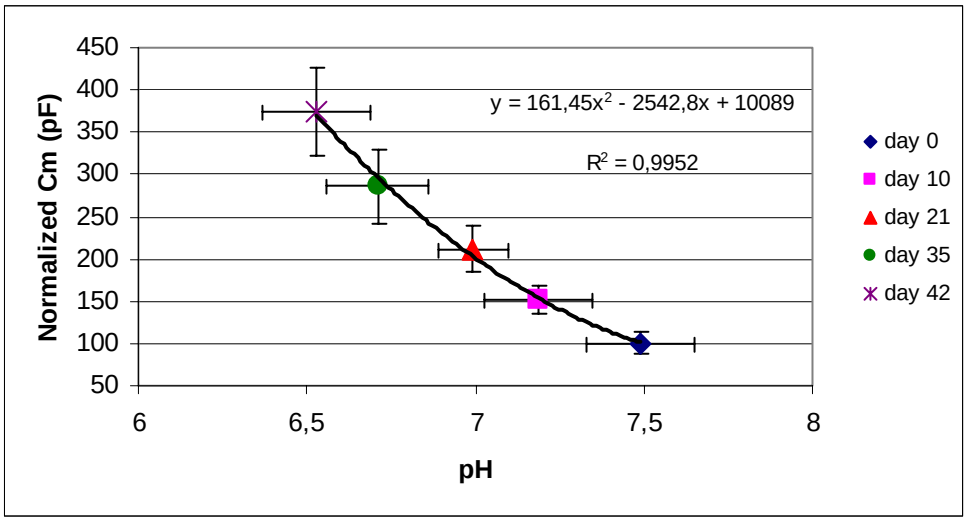
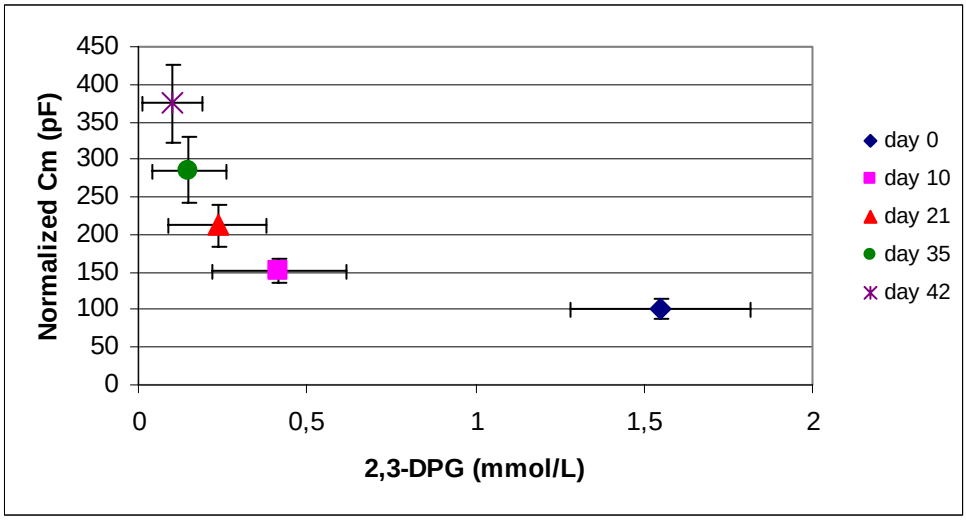


Figure 5.5 The relationship between C_m and Na^+, K^+ and Cl^-, for ES under storage.





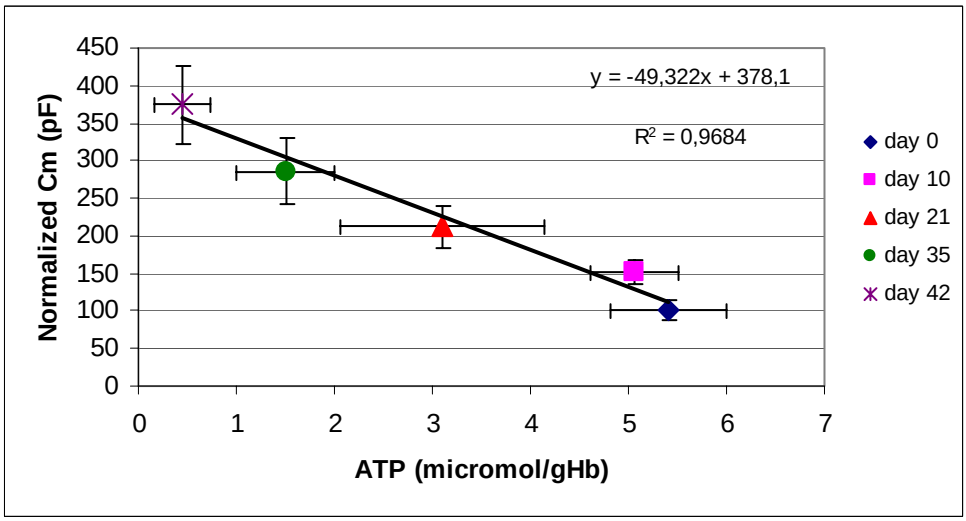


Figure 5.6 The relationship between C_m and pH, DPG and ATP, for ES under storage.

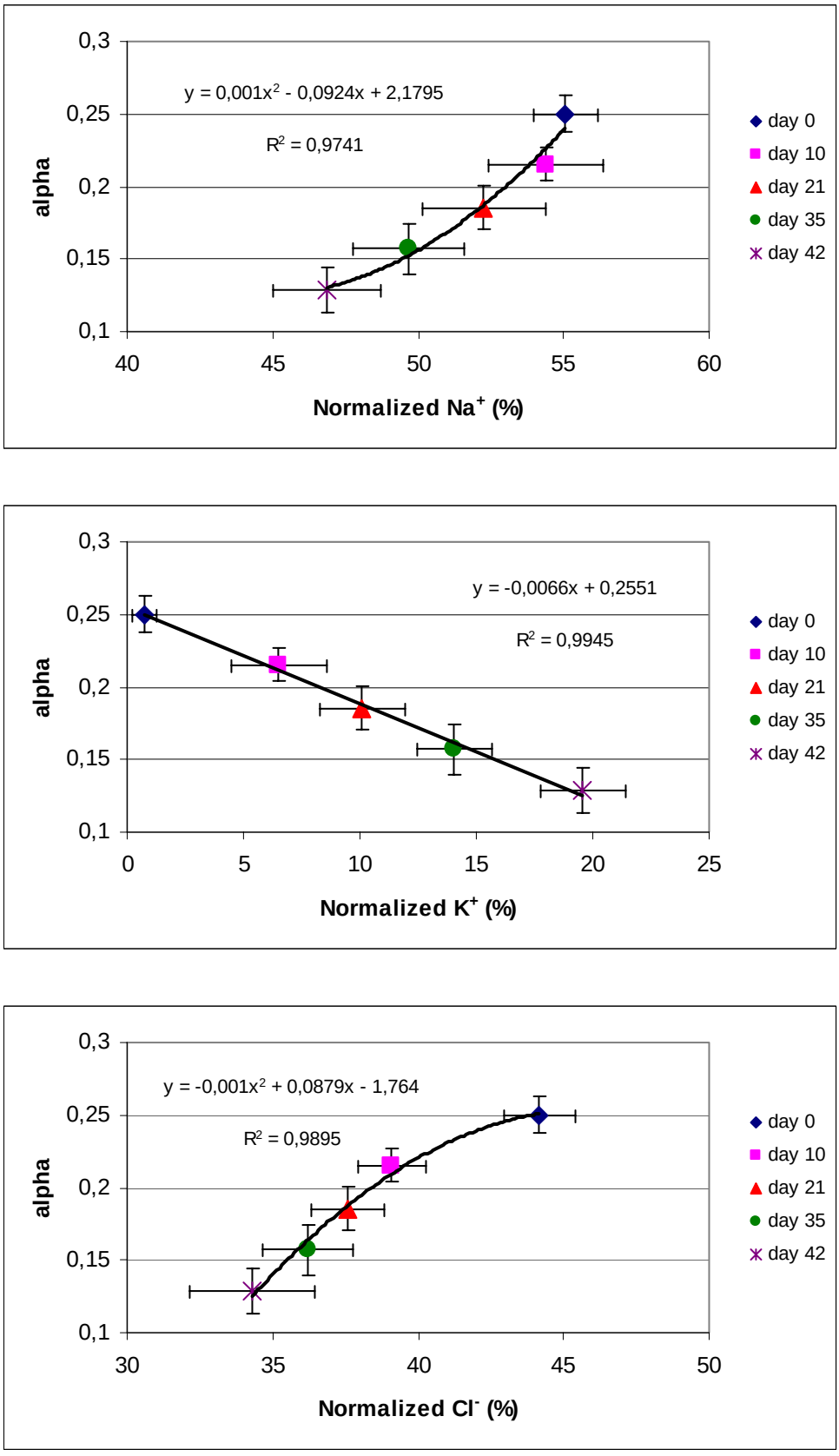


Figure 5.7 The relationship between alpha and Na^+, K^+ and Cl^-, for ES under storage.

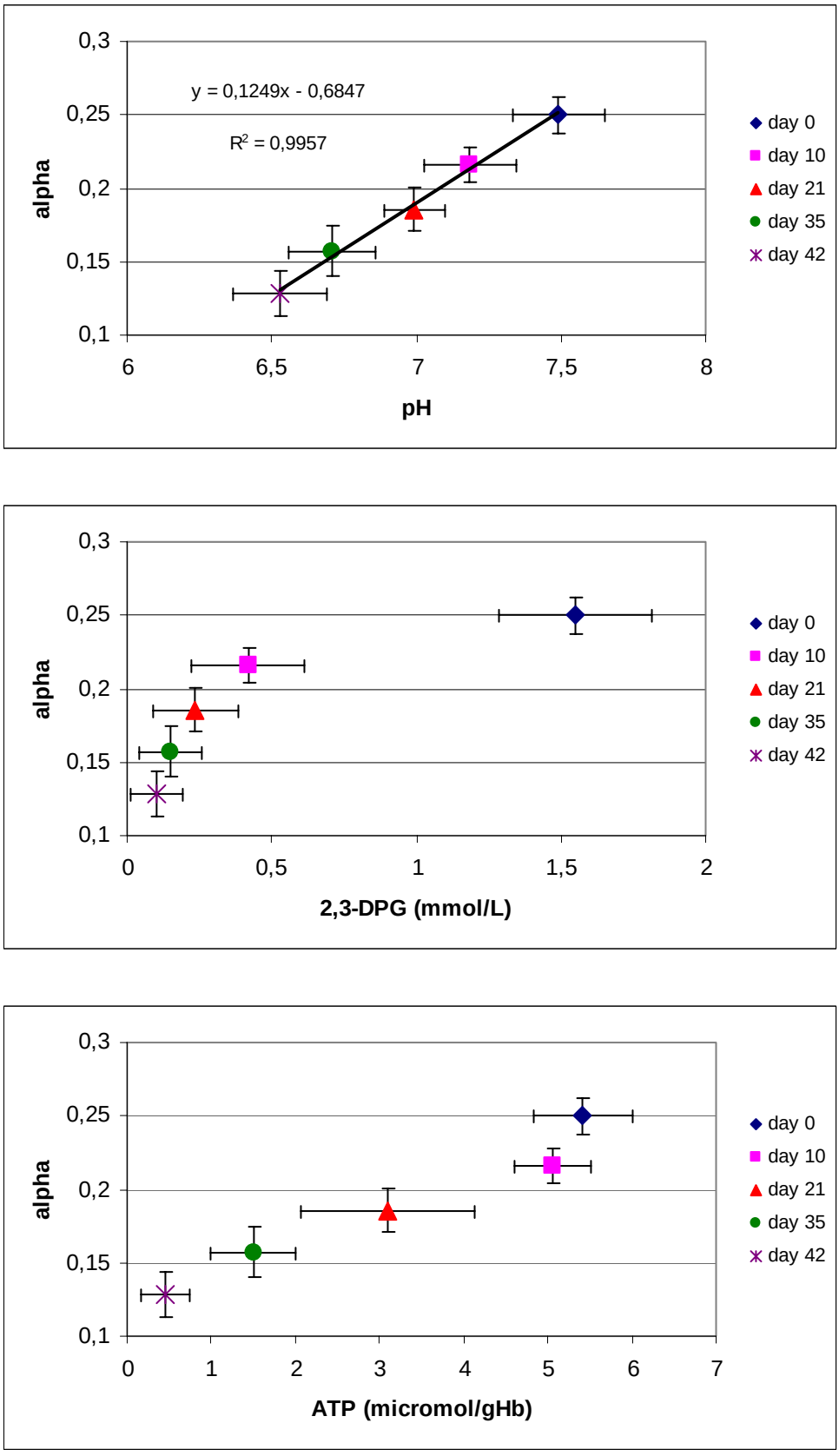
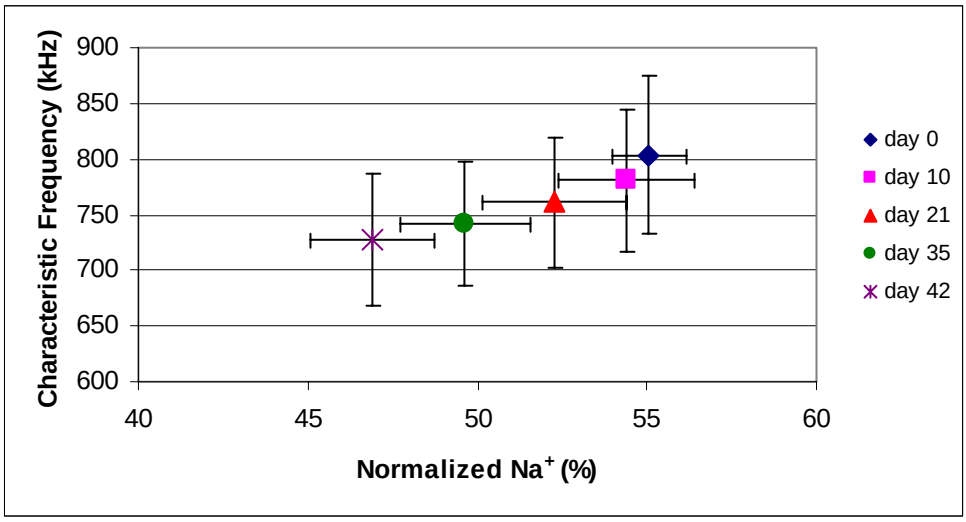
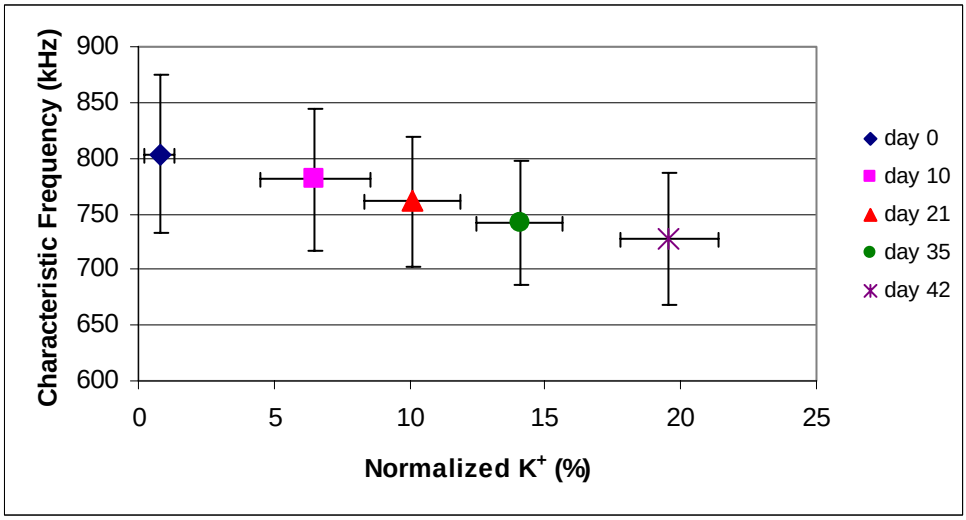


Figure 5.8 The relationship between alpha and pH, DPG and ATP, for ES under storage.





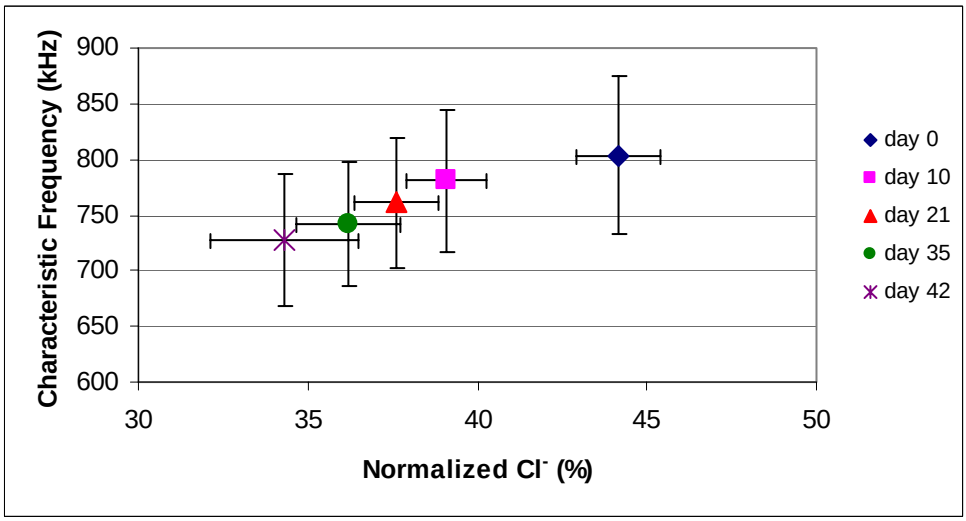
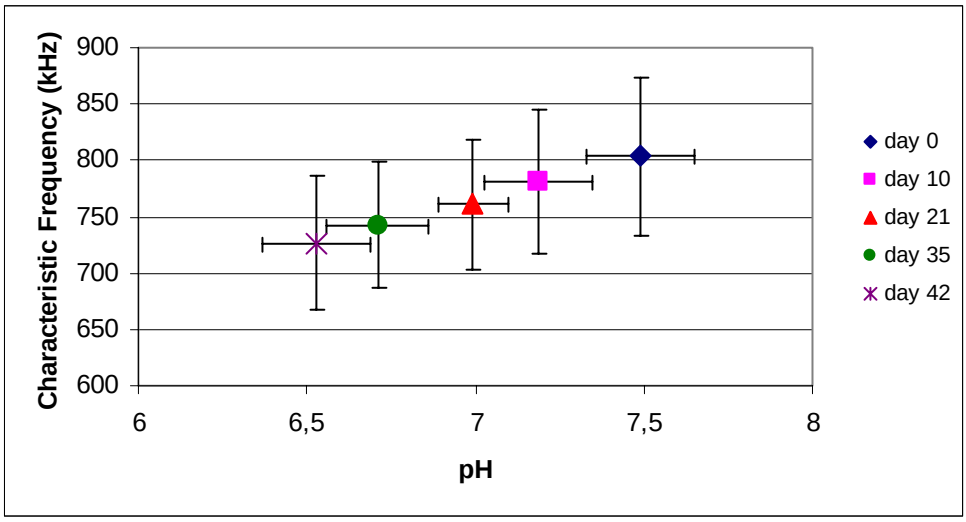
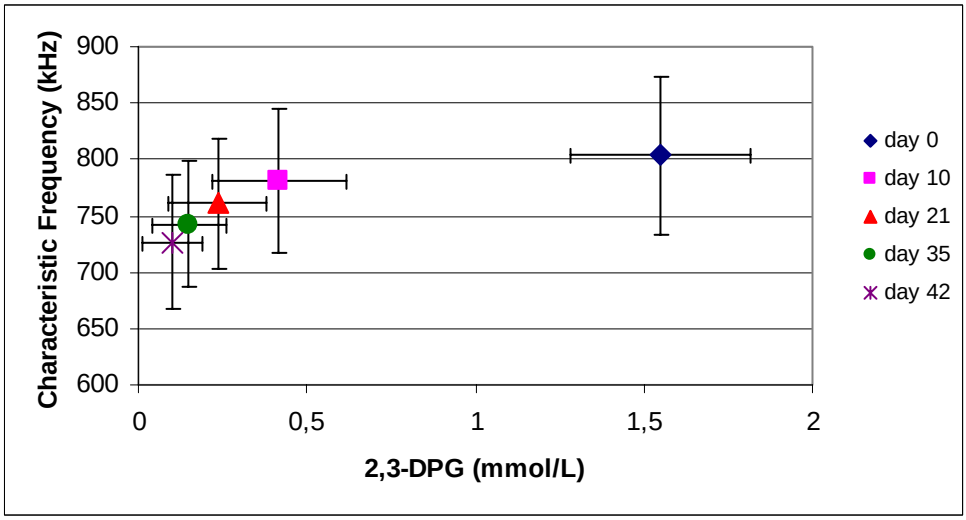


Figure 5.9 The relationship between F_c and Na^+, K^+ and Cl^-, for ES under storage.





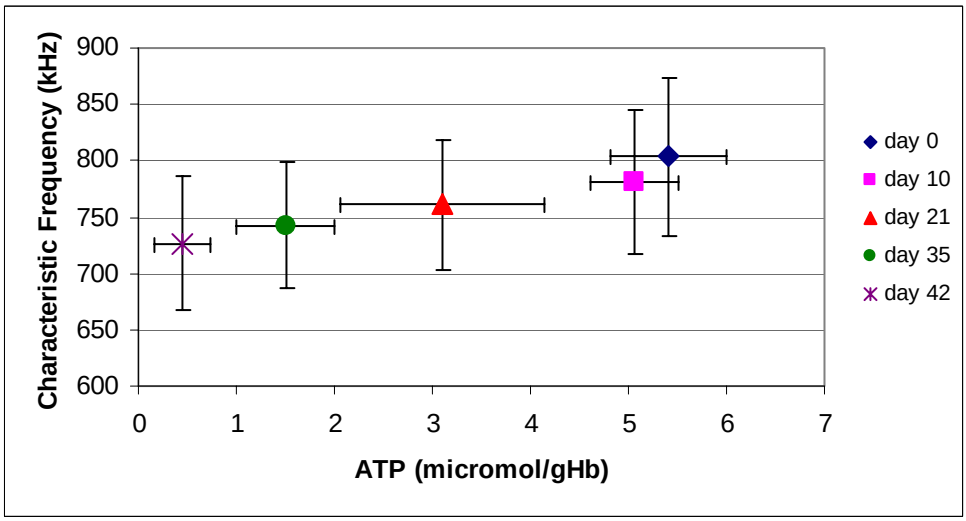


Figure 5.10 The relationship between F_c and pH, DPG and ATP, for ES under storage.

5.2.2 Whole Blood Under Storage

The results of correlation analysis with the whole blood samples are given in Table 5.2.

Table 5.2
The correlation between blood parameters for WB.

	R_i	R_e	C_m	F_c	α
$[Na^+]$	2	1	1	0	1
$[K^+]$	2	2	1	0	1
$[Cl^-]$	2	1	1	0	0
pH	2	2	1	0	0
2,3-DPG	1	1	0	0	0
ATP	2	1	1	0	1

Correlation is significant at the 0,01 level [61].
0: No significant effect (Pearson correlation coefficient: 0.0 – 0.69)
1: Weak correlation (Pearson correlation coefficient: 0.70 - 0.84)
2: Strong correlation (Pearson correlation coefficient: 0.85 – 1.0)

It is seen from Table 5.2 that for whole blood, R_i, R_e, C_m and α are correlated with $[K^+]$, pH and ATP.

The relationship between the physiological parameters and electrical parameters of whole blood is given in Figures 5.11, 5.12, 5.13,....., 5.20.

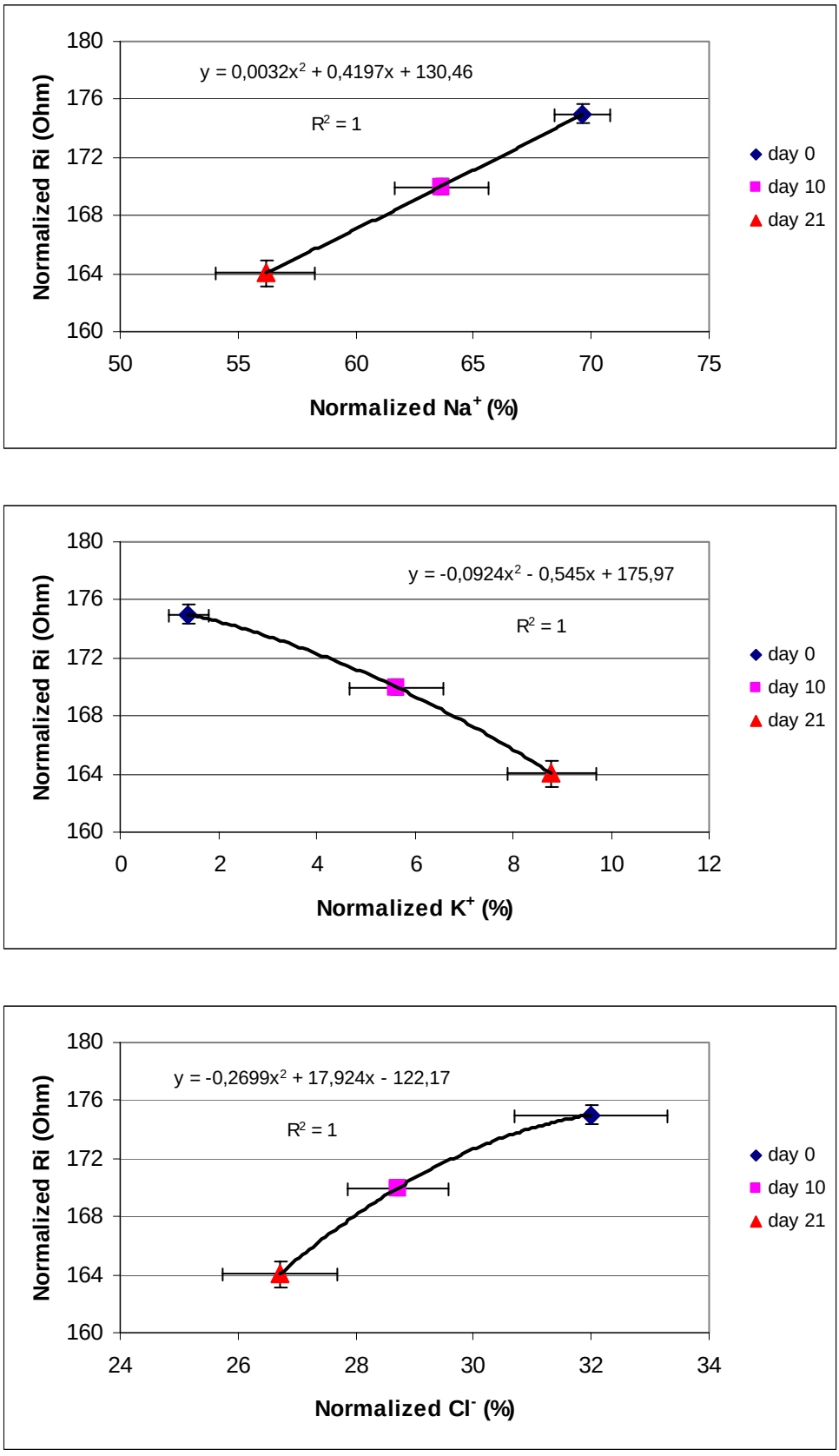


Figure 5.11 The relationship between R_i and Na^+, K^+ and Cl^-, for WB under storage.

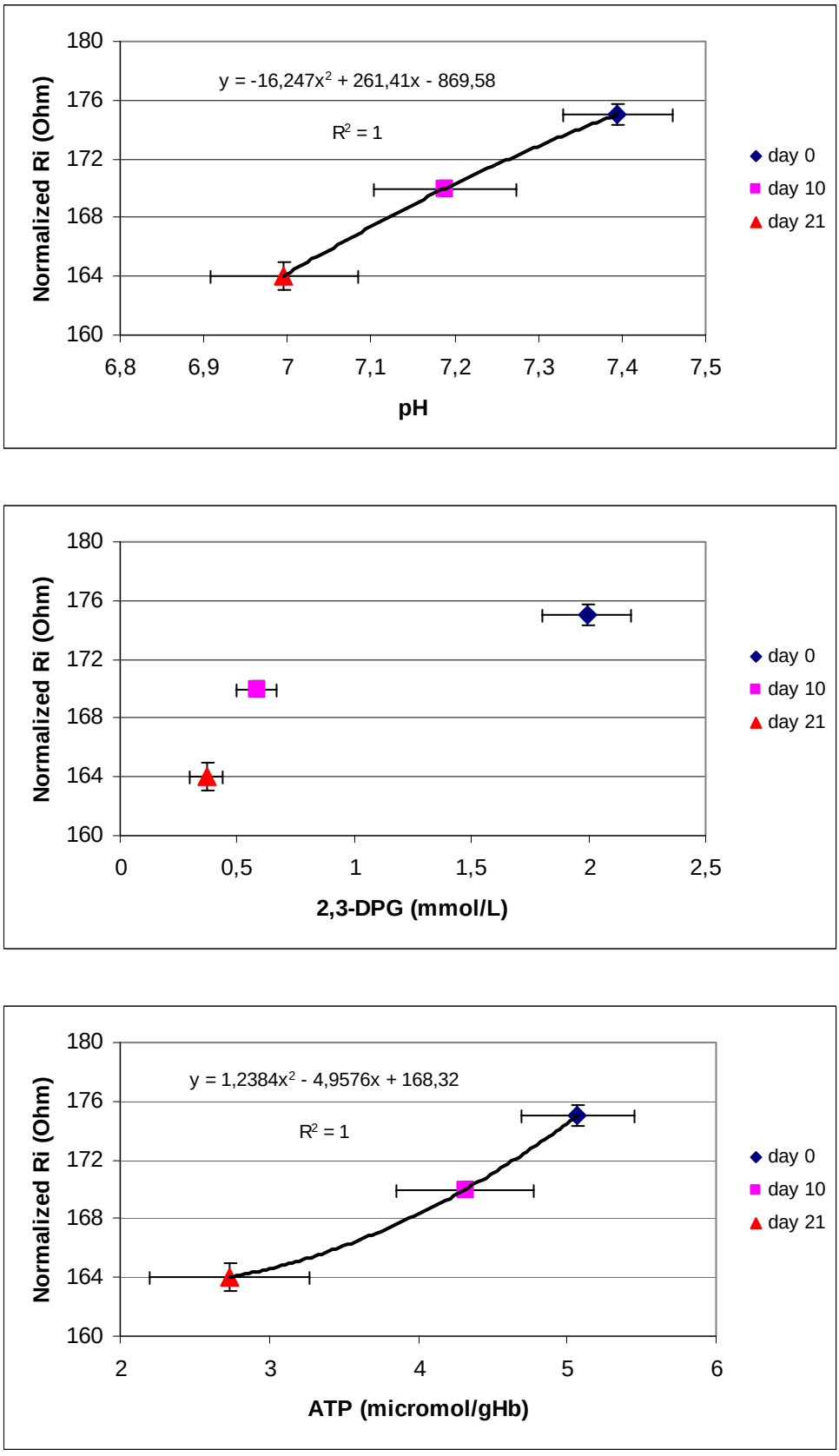


Figure 5.12 The relationship between R_i and pH, DPG and ATP, for WB under storage.

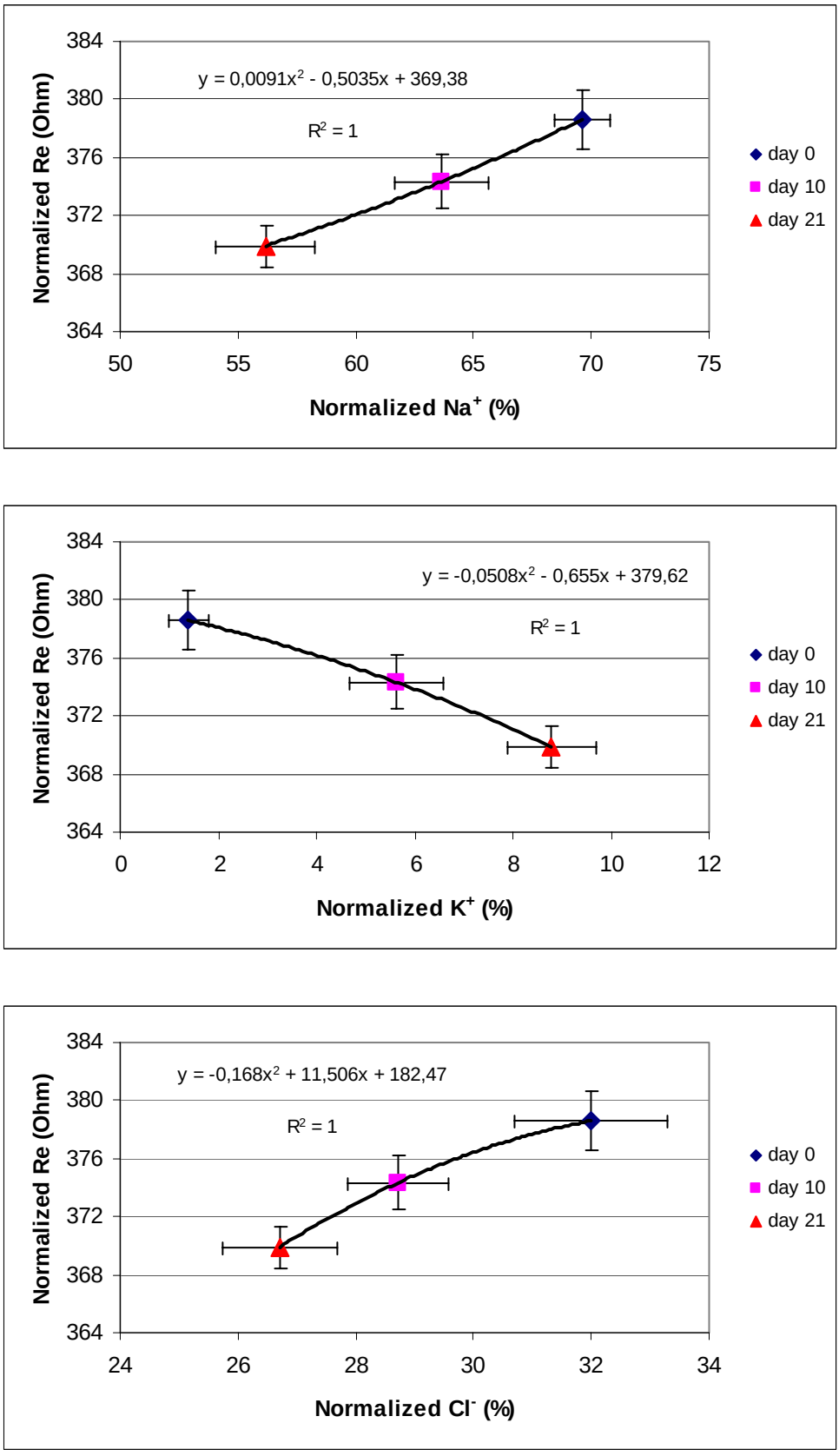


Figure 5.13 The relationship between R_e and Na^+, K^+ and Cl^-, for WB under storage.

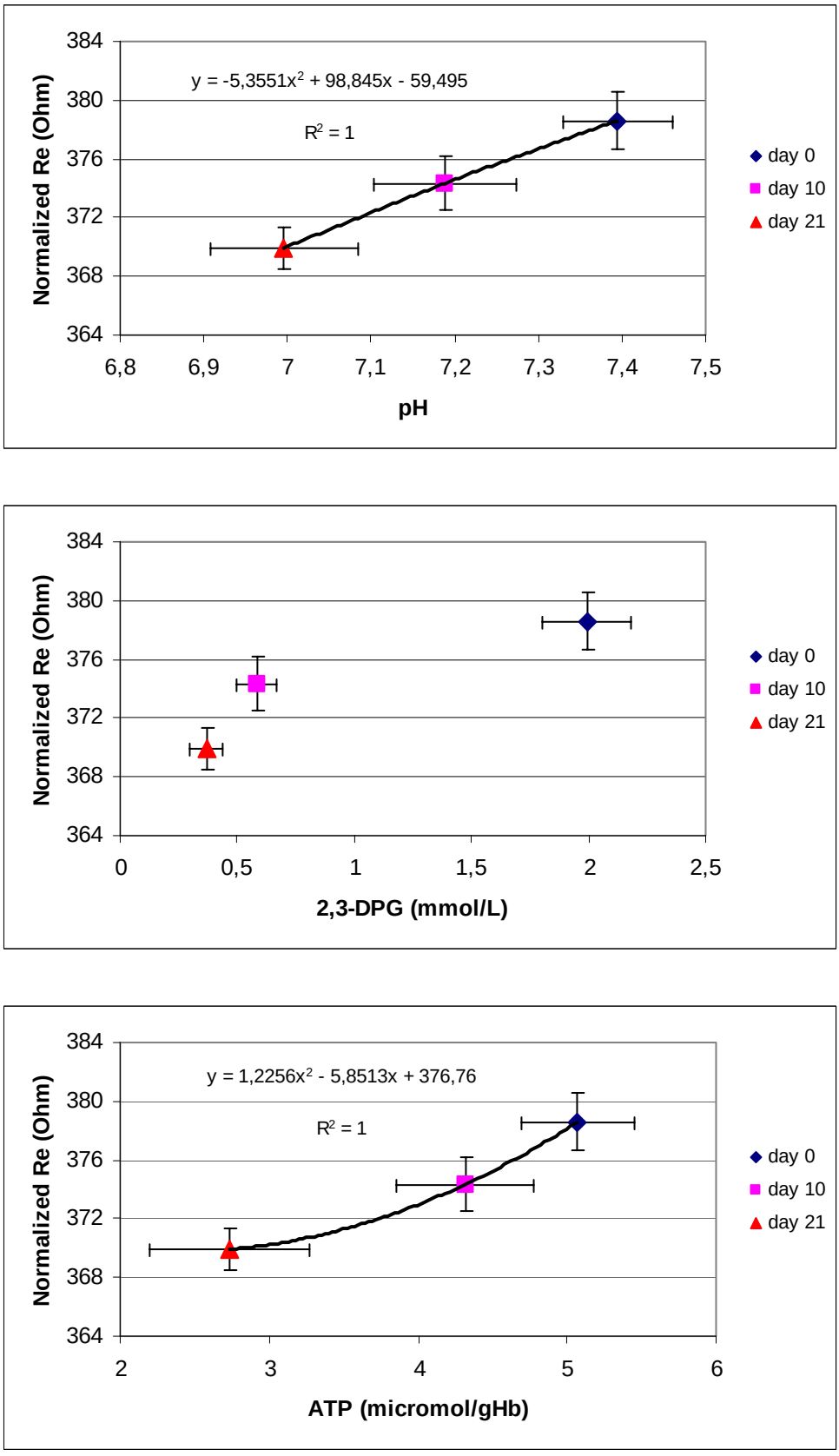


Figure 5.14 The relationship between R_e and pH, DPG and ATP, for WB under storage.

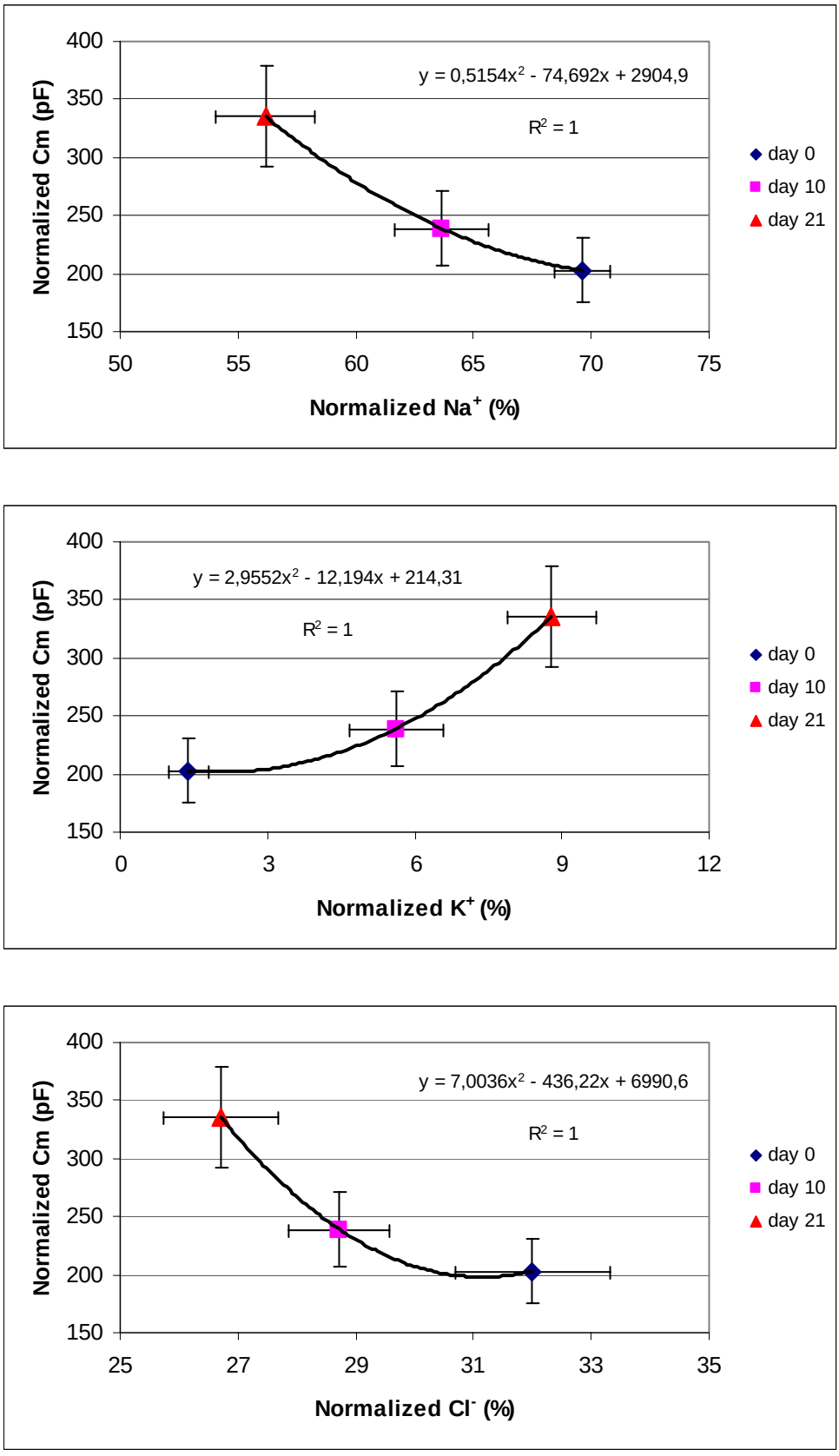


Figure 5.15 The relationship between C_m and Na^+, K^+ and Cl^-, for WB under storage.

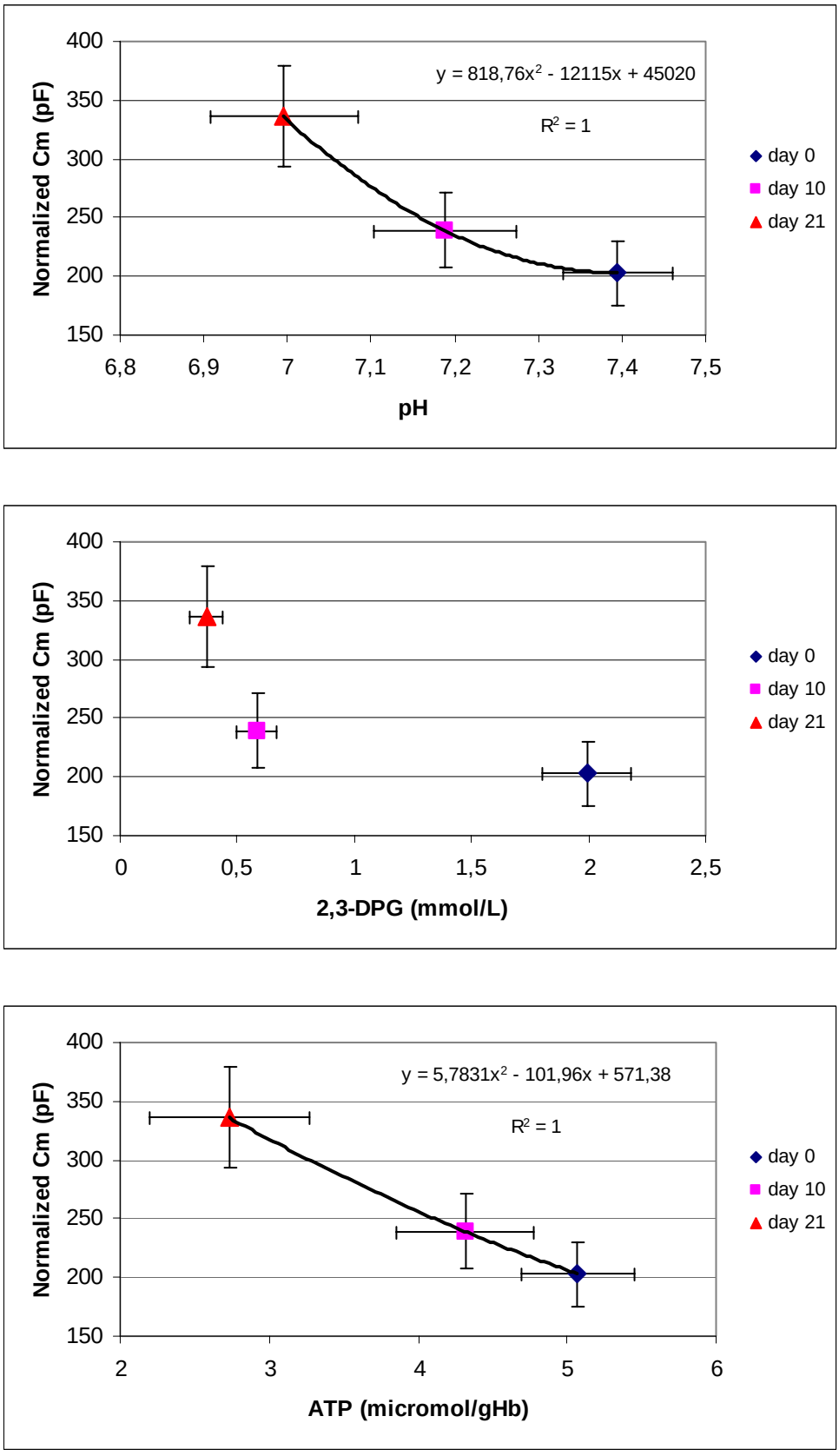


Figure 5.16 The relationship between C_m and pH, DPG and ATP, for WB under storage.

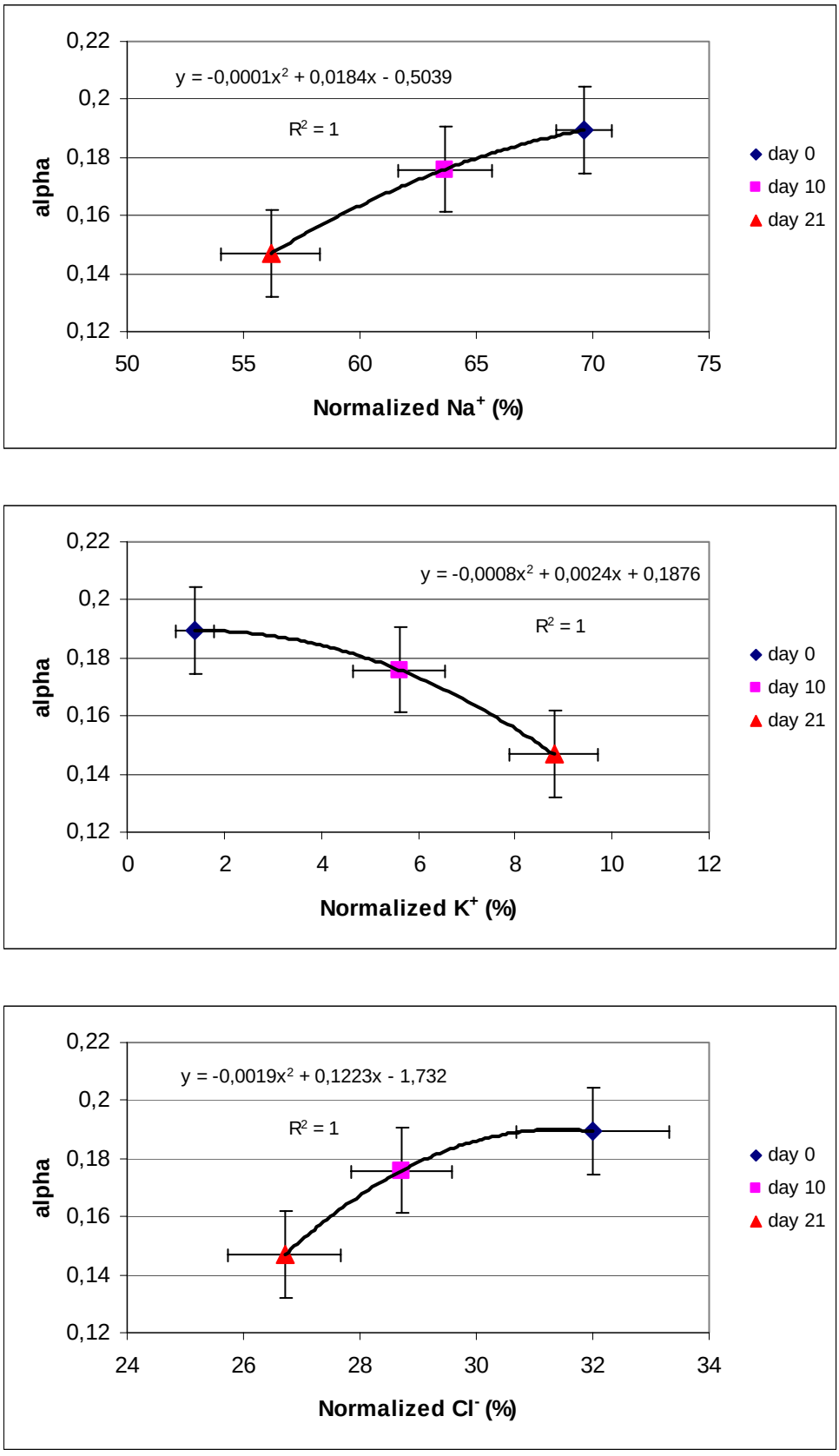


Figure 5.17 The relationship between alpha and Na^+, K^+ and Cl^-, for WB under storage.

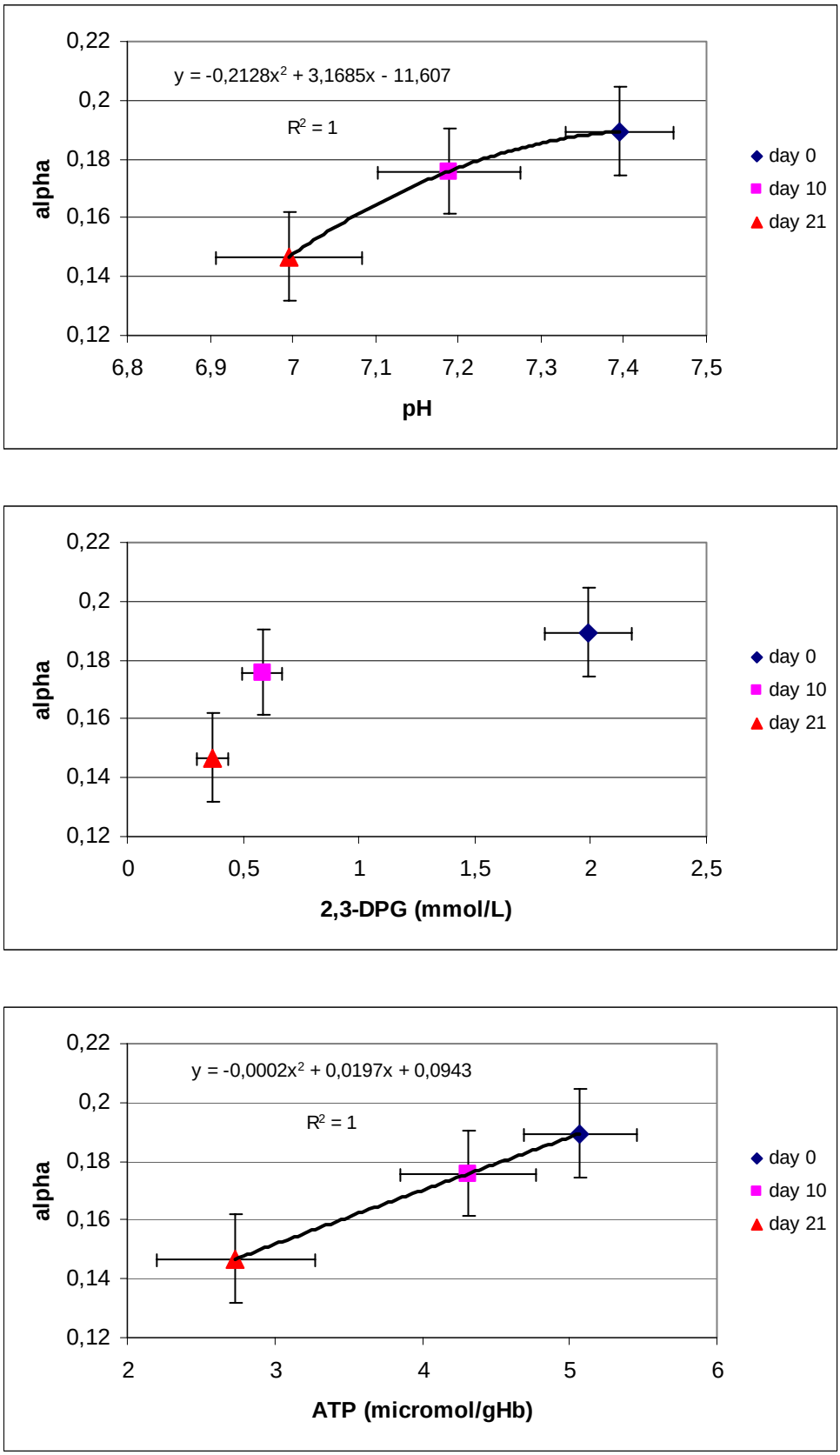


Figure 5.18 The relationship between alpha and pH, DPG and ATP, for WB under storage.

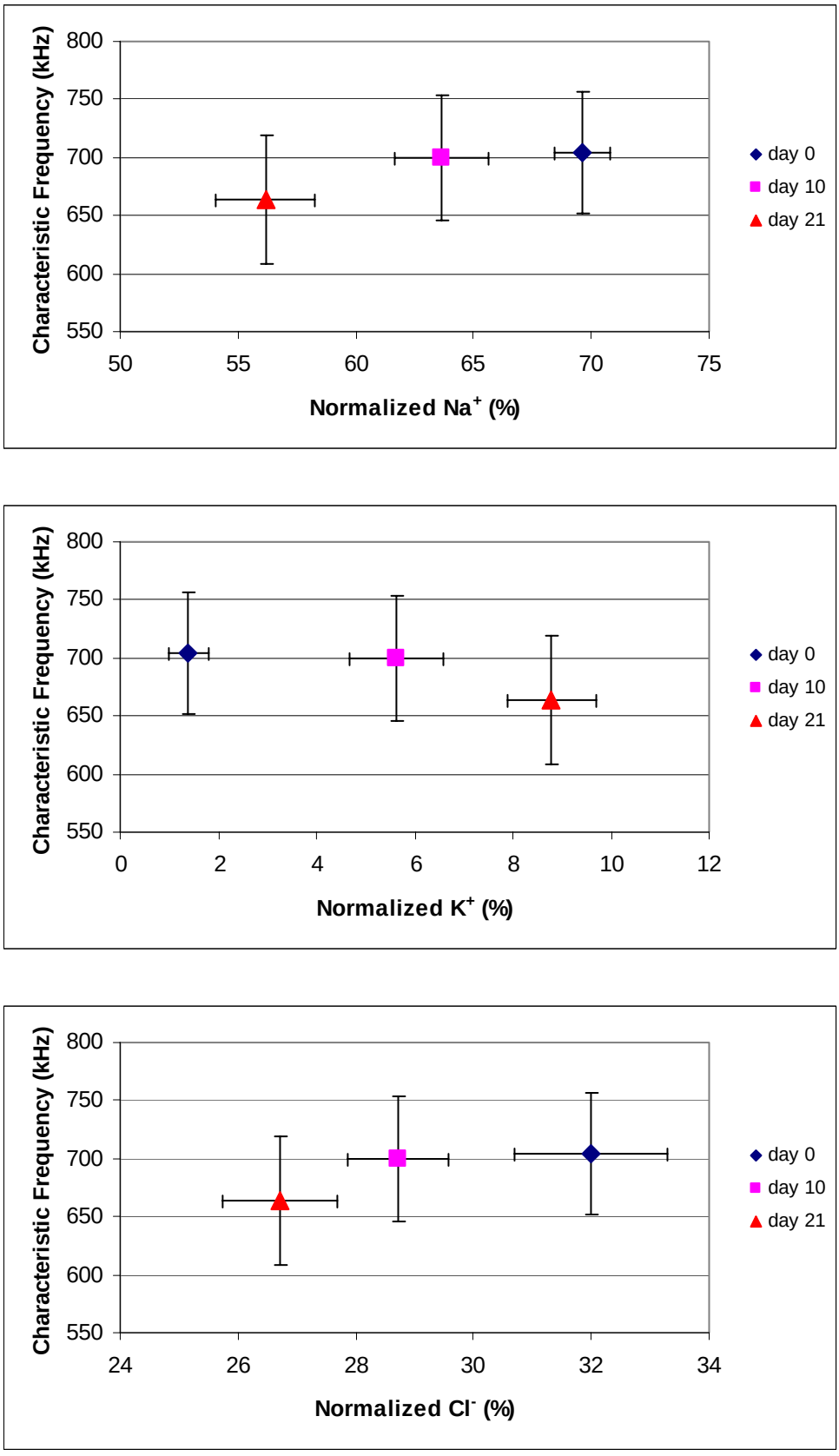
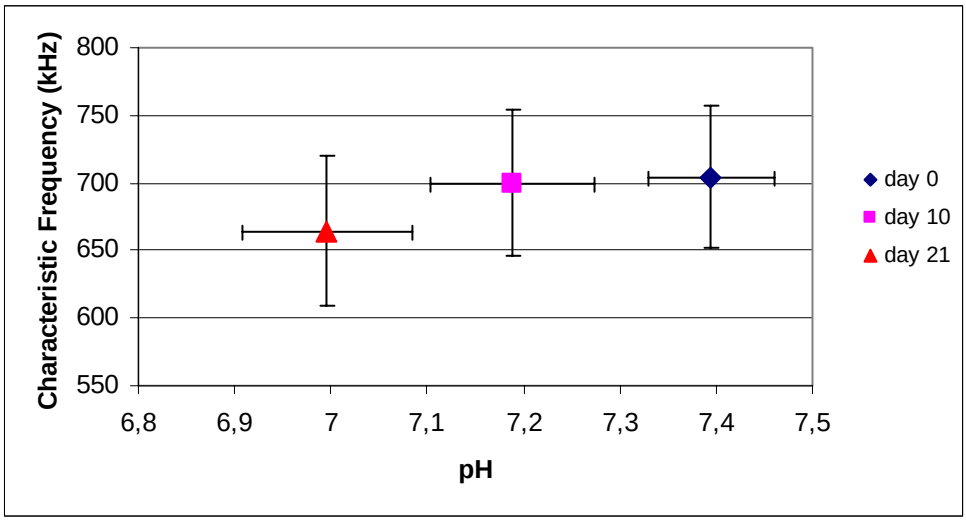
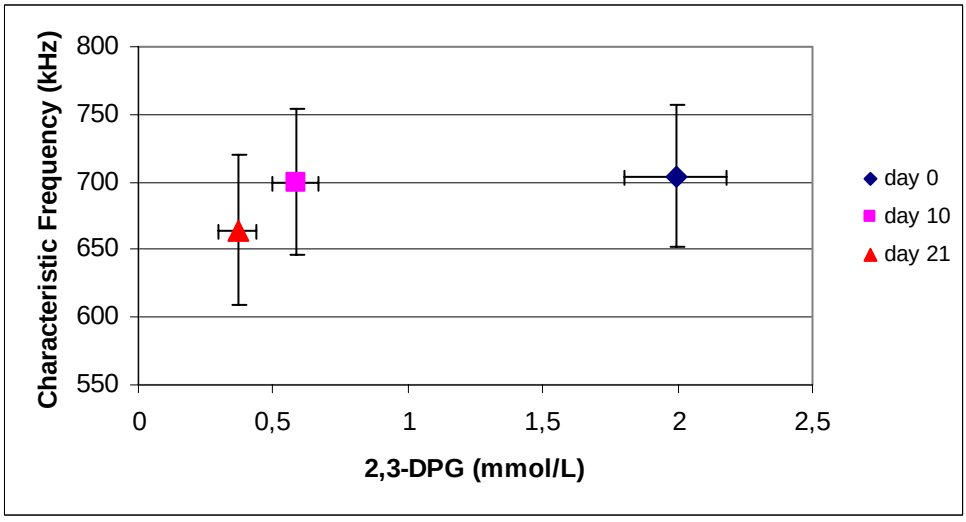


Figure 5.19 The relationship between F_c and Na^+, K^+ and Cl^-, for WB under storage.





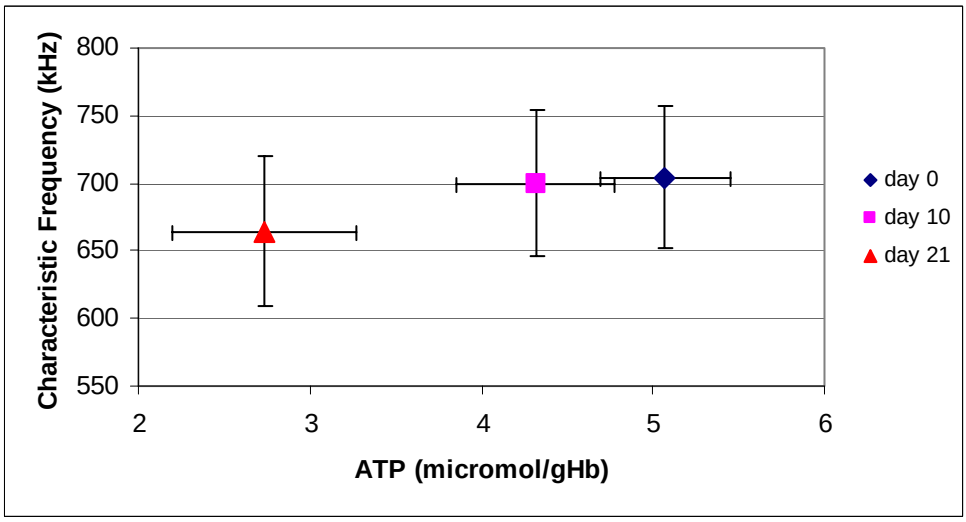


Figure 5.20 The relationship between F_c and pH, DPG and ATP, for WB under storage.

6. COLE-COLE PARAMETERS AS PREDICTORS OF BLOOD QUALITY

6.1 Importance of ATP and pH in Assessing the Blood Quality

All blood and blood components (red cell concentrate, red cell suspension, leucocyte-depleted red cells) should comply with the production standards and are subject to appropriate quality assurance checks. Both storage and transport conditions must comply with the recommmendations of the Blood Transfusion service. It is also worth noting that blood, like any other biological variables, displays a range in storage-dependent physico-chemical changes which the Blood Transfusion Service has taken into account when setting criteria of acceptability for any of the blood products issued.

The quality of the red blood cells that are transfused would be observed both in their function and ability to survive in the microcirculation.

Inevitably, some loss of quality occurs during storage. The physiological parameters may be assessed as a measure of storage quality, but as none of these, except possibly the cellular ATP content and the pH, correlates well with the red cell survival after transfusion.

Red blood cell viability is very much affected by the ATP concentration. ATP is the chief source of energy for red blood cells and ensures normal functioning of the sodium pump, thereby preventing erythrocyte swelling. ATP may also ensure normal spectrin function essential to membrane plasticity, and is a major factor in the preservation of adequate RBC flow in the microcirculation, where capillary diameter is sometimes half that of the erythrocyte [74].

A significant correlation between ATP and RBC viability was indicated in early studies by Rapoport and later shown by others [75].

It has been observed that, a 24 hour survival of 75% of red blood cells is possible with an ATP concentration of 50% [58]. However, ATP levels as low as 20% are also acceptable.

In 1994, Mazor et al. showed that the RBC ATP concentration was most directly affected by the pH and the adenine and phosphate content [76]. Higher pH and adenine and phosphate concentrations initially drive RBC ATP synthesis. By decreasing the pH level of blood during storage, glycolysis that is closely related to the red cells' acid-soluble phosphorus compounds, can not proceed. Hence, ATP formation can not occur. Because ATP is an important co-enzyme of glycolysis, RBCs with very low ATP levels cannot phosphorylate glucose in energy circle and hence are fated to die.

In our study, all physiological parameters showed high correlation with R_i, R_e, C_m and α. If these parameters, especially ATP and pH that determine the life criteria of stored blood, can be expressed in terms of the Cole-Cole parameters, it could be possible to obtain a quick assessment of the quality of blood bank stored blood samples; the electrical parameters could be used as predictor of viability.

For this approach, it is required to obtain a multiple regression model between the physiological parameters and the electrical parameters (R_i, R_e, C_m and α).

6.2 Modeling of Physiological Parameters using Multiple Regression

Na^+, K^+ and Cl^- concentrations, pH and ATP all showed strong dependence on R_i, R_e, C_m and α. By expressing physiological parameters in terms of these electrical parameters, it could be possible to obtain models for predicting the physiological parameters of stored blood.

6.2.1 Selection of Independent Variables in the Multiple Regression Model

The most important point during multiple regression is that, there must not be any relationship between the independent variables, that are R_i, R_e, C_m and α.

Before modelling, multicollinearity is investigated between independent variables. Multicollinearity is the undesirable situation where the correlations among the independent variables are strong [77], [78], [79].

Statistically, the indicators of multicollinearity are tolerance and variance inflation factor (VIF). Tolerance is a statistical parameter used to determine how much the independent variables are linearly related to one another (multicollinear). The proportion of a variable's variance not accounted for by other independent variables in the equation. A variable with very low tolerance contributes little information to a model and can cause computational problems [78].

The variance inflation factor (VIF) is the reciprocal of the tolerance. As the variance inflation factor increases, so does the variance of the regression coefficient, making it an unstable estimate. Large VIF values are an indicator of multicollinearity.

For the selection of independent variables of our multiregression model, to examine multicollinearity, tolerance and VIF are studied and it is seen that there is a relationship among independent variables (R_i, R_e, C_m and α) with small tolerance and large VIF values.

However, multicollinearity is investigated by studying the eigenvalues and variance-decomposition proportions. On the same eigenvalue, the variables with high variance proportion has a relationship between them.

In our modelling, it is seen that C_m and α are correlated together for both ES and WB. To obtain this result, multicollinearity is examined.

For example, let's assume pH is dependent and R_i, R_e, C_m and α are independent variables for erythrocyte suspension. Descriptive statistics and correlations of variables are given in Table A.1 and Table A.2 (see Appendix). For the pH (R_i,R_e,C_m,α) model, the regression coefficient is $R^2 = 0{,}824$ (Table A.3).

As it is seen from Table A.4 (see Appendix), tolerance values are very small and VIF values are very high. Therefore, it can be said that independent variables (R_i,R_e,C_m, and α) have a relationship together. But, to identify parameters that are related, collinearity is studied. In Table A.5 (see Appendix), C_m has a variance proportion of 0,96 and alpha has 0,83 for the 3rd eigenvalue. It means that 96% of C_m is effected from a little variance, while 83% of alpha is affected from it. It means that C_m and alpha are correlated. Multicollinearity are also examined for whole blood and again, the same result is obtained. Shortly, C_m and alpha are related together.

In this situation, since it is required to include only one of related variables in the model, (R_i, R_e, α) variables are appropriate to the model.

In this model, by supposing not only the independent variables are effective on the dependent variables but also their higher order products, variable selection for multiple regression is used in SPSS.

Method selection allows us to specify how independent variables are entered into the analysis. Using different methods (enter, forward selection, backward elimination and stepwise) we can construct a variety of regression models from the same set of variables.

Forward variable selection enters the variables in the block, one at a time, based on entry criteria. Backward variable elimination enters all of the variables in the block in a single step, and then removes them one at a time based on removal criteria. Stepwise variable entry and removal examines the variables in the block at each step for entry or removal [79].

All independent variables selected are added to a single regression model.

For the model with the independent parameters (R_i, R_e, α), by adding the independent variables' squares, cubes and products of them into the model, variable selection for multiple regression is used in SPSS. Forward selection, backward elimination, stepwise and enter methods are applied to the model (R_i, R_e, α, R_i^2, R_i^3, R_e^2, R_e^3, α^2, α^3, $R_i^2R_e$, $R_iR_e^2$, $R_i^2\alpha$, $R_i\alpha^2$, $R_e^2\alpha$, $R_e\alpha^2$, R_iR_e, $R_i\alpha$, $R_e\alpha$, $R_iR_e\alpha$) and the results are compared with each other. The best appropriate models are accepted as multiple regression model between the physiological parameters and the electrical parameters (R_i, R_e and α).

As a result of comparison of these four methods, it is seen that the standard errors of the models are approximately identical. Because the backward elimination and enter method give the same model equations, backward elimination is used for modeling of the physiological parameters.

6.2.2 Model Equations for Erythrocyte Suspensions

By applying the standard 'backward elimination' variable selection procedure, the following equations are derived by applying the data from 31 donors (training set):

$$Ht\ (\%) = 0{,}2927\ R_e + 0{,}24 \qquad (R^2 = 0{,}99) \tag{6.3}$$

$$Na^+\ (\%) = -28{,}390 - 5{,}86x10^{-5}\ R_i^{\,3} - 5{,}93x10^{-6}\ R_e^{\,3} + 2{,}983x10^{-5}\ R_i R_e^{\,2} - 7{,}58x10^{-4}\ R_e^{\,2}\alpha + 0{,}758\ R_e \alpha^2 \qquad (R^2 = 0{,}90) \tag{6.4}$$

$$K^+\ (\%) = 47{,}440 + 3{,}397x10^{-5}\ R_i^{\,3} + 4{,}930x10^{-6}\ R_e^{\,3} - 2{,}06x10^{-5}\ R_i R_e^{\,2} + 5{,}383x10^{-4}\ R_e^{\,2}\alpha - 0{,}672\ R_e \alpha^2 \qquad (R^2 = 0{,}85) \tag{6.5}$$

$$Cl^-\ (\%) = 80{,}950 + 2{,}460x10^{-5}\ R_i^{\,3} + 9{,}983x10^{-7}\ R_e^{\,3} - 9{,}19x10^{-6}\ R_i R_e^{\,2} + 2{,}192x10^{-4}\ R_e^{\,2}\alpha - 8{,}56x10^{-2}\ R_e \alpha^2 \qquad (R^2 = 0{,}87) \tag{6.6}$$

$$pH = 4{,}050 + 4{,}590x10^{-7}\ R_i^{\,3} - 6{,}21x10^{-8}\ R_e^{\,3} + 3{,}074x10^{-7}\ R_i R_e^{\,2} - 1{,}71x10^{-6}\ R_e^{\,2}\alpha - 1{,}75x10^{-2}\ R_e \alpha^2 \qquad (R^2 = 0{,}92) \tag{6.7}$$

$$2,3\text{-}DPG\ (mmol/L) = -4{,}777 + 9{,}555x10^{-6}\ R_i^{\,3} + 9{,}616x10^{-7}\ R_e^{\,3} - 3{,}59x10^{-6}\ R_i R_e^{\,2} - 3{,}62x10^{-6}\ R_e^{\,2}\alpha + 4{,}985x10^{-3}\ R_e \alpha^2 \qquad (R^2 = 0{,}70) \tag{6.8}$$

$$ATP\ (\mu mol/g\ of\ Hb) = -2{,}992 - 5{,}71x10^{-6}\ R_i^{\,3} - 1{,}17x10^{-6}\ R_e^{\,3} + 4{,}338x10^{-6}\ R_i R_e^{\,2} - 1{,}90x10^{-5}\ R_e^{\,2}\alpha + 7{,}955x10^{-3}\ R_e \alpha^2 \qquad (R^2 = 0{,}83) \tag{6.9}$$

In these equations, the units of R_i and R_e are Ohm, and α has no unit.

To test the validity of these equations, an external set of 20 donors is selected. The resistance of the extracellular and intracellular fluids and, the cell membrane capacitance are all normalized with respect to the 0^{th} day hematocrit value of the test samples, calculated from Equation 6.3. Normalized electrical parameters are then plugged into the model equations (Equations 6.3-6.9) to predict the physiological parameters on the day of withdraw and on the 10th, 21st, 35th and 42^{nd} days of storage. The results are then compared with the measured physiological data, as shown in Table 6.1.

It is seen from Table 6.1 that, Na^+, K^+, Cl^- and pH values are in close agreement with a rms error of less than 7%. ATP estimation is limited to the 0^{th}, 10^{th}, 21^{st} and 35^{th} days of storage, since the error exceeds 10% on the 42^{nd} day. However, the Equation 6.8 can not estimate 2,3-DPG at all, at any time.

Table 6.1
The rms estimation errors (%) for erythrocyte suspensions.

Days	Na^+	K^+	Cl^-	pH	2,3-DPG	ATP
0	2,6	6,2	3,1	1,8	27,4	6,1
10	3,0	5,6	4,8	2,6	25,7	6,4
21	3,8	5,7	3,2	1,7	26,1	6,8
35	4,8	5,7	5,2	2,6	30,5	5,1
42	5,0	6,3	6,7	3,5	26,8	25,0

6.2.3 Model Equations for Whole Blood

By applying the standard 'backward elimination' variable selection procedure, the following equations are derived by applying the data from 11 donors (training set):

$$Ht\ (\%) = 0{,}2635\ R_e + 0{,}104 \qquad (R^2 = 0{,}99) \tag{6.10}$$

$$Na^+\ (\%) = -309{,}699 + 2{,}304\ R_i + 171{,}854\ \alpha - 1{,}55\times10^{-5}\ R_i^3 + 8{,}303\times10^{-7}\ R_e^3 - 1{,}312\ R_e\ \alpha^2 \qquad (R^2 = 0{,}89) \tag{6.11}$$

$$K^+\ (\%) = -94{,}416 + 1{,}458\ R_i - 11{,}872\ \alpha - 2{,}27\times10^{-5}\ R_i^3 - 6{,}65\times10^{-7}\ R_e^3 + 3{,}708\times10^{-2}\ R_e\ \alpha^2 \qquad (R^2 = 0{,}84) \tag{6.12}$$

$$Cl^-\ (\%) = 65{,}817 - 0{,}463\ R_i - 249{,}646\ \alpha + 9{,}767\times10^{-6}\ R_i^3 + 2{,}987\times10^{-7}\ R_e^3 + 1{,}864\ R_e\ \alpha^2 \qquad (R^2 = 0{,}85) \tag{6.13}$$

$$pH = 27{,}113 - 0{,}207\ R_i - 0{,}110\ \alpha + 2{,}773\times10^{-6}\ R_i^3 + 3{,}130\times10^{-8}\ R_e^3 - 3{,}59\times10^{-3}\ R_e\ \alpha^2 \qquad (R^2 = 0{,}92) \tag{6.14}$$

$$2,3\text{-DPG (mmol/L)} = 263,382 - 2,440\, R_i + 8,966\, \alpha + 2,963x10^{-5}\, R_i^3 + 1,137x10^{-7}\, R_e^3 - 8,77x10^{-2}\, R_e \alpha^2 \quad (R^2 = 0,75) \tag{6.15}$$

$$ATP\ (\mu mol/g\ of\ Hb) = -246,559 + 2,008\, R_i + 47,362\, \alpha - 2,19x10^{-5}\, R_i^3 + 2,297x10^{-7}\, R_e^3 - 0,291\, R_e \alpha^2 \quad (R^2 = 0,82) \tag{6.16}$$

In these equations, the units of R_i and R_e are Ohm, and α has no unit.

This model for whole blood is tested in a similar fashion; with the measurements on the last 20 donors. The 0^{th} day hematocrit value of the test sample that is used to normalize electrical parameters, is calculated from Equation 6.10. Test results are given in Table 6.2. All physiological parameters, except for 2,3-DPG, are derived from electrical measurements with less standard error (s.d. < 7%).

Table 6.2
The rms estimation errors (%) for whole blood.

Days	Na^+	K^+	Cl^-	pH	2,3-DPG	ATP
0	2,5	5,3	5,6	0,7	11,4	6,3
10	3,3	6,9	3,7	1,6	10,9	4,7
21	4,9	7,0	5,9	1,7	17,1	5,5

By using these formulae, it seems possible to calculate the physiological parameters and their level changes for the assessment of the viability of stored blood. Electrical measurements of blood on the day of withdraw are taken as reference. The normalized electrical parameters are used to estimate the blood physiological values. To evaluate the quality of stored blood, prior to transfusion, electrical measurements are repeated and the physiological values derived from the model equations, are compared with the reference values.

If the changes are within the acceptable range, it can be said that this blood sample fulfills the life criteria of stored blood.

7. CONCLUSION AND DISCUSSION

Current quality standards for in vitro storage of blood focus mainly on maintaining both viability and functional capacity as close as to the "prior to storage" conditions. Survival of blood cells, usually 24 hours after transfusion, should be at least 75% of the transfused cells.

All physiological parameters that become abnormal during storage such as Na^+, K^+ and Cl^- concentrations, pH, 2,3-DPG and ATP, are counter indicative of red cell quality; especially ATP and pH are used to judge on the red blood cell quality.

In the first part of this study, physiological and electrical parameter changes with storage time are investigated on 82 male donors (51 donors for erythrocyte suspension and 31 donors for whole blood).

From measurements, it is observed that, parameters such as 2,3-DPG, ATP, pH, extracellular Na^+, K^+ and Cl^- concentration are measured within the range of published data (Figure 4.1 and Figure 4.3) [43, 60, 80, 81, 82, 83].

Stored RBCs are depleted of 2.3-DPG, following the 1^{st} or 2^{nd} week of storage (Figure 4.1 and Figure 4.3) and they have a left-shifted oxygen dissociation curve, greater oxygen affinity, and they supply less oxygen to tissues [84]. The concentration of 2,3-DPG in the cell is regulated by an enzyme at high pH levels and because of the decreasing pH level during storage, 2,3-DPG could not be regulated [84]. Figure 4.1 shows that the 2,3-DPG mean value of erythrocyte suspensions decreased to 16% of its initial value on the 21^{st} day and then slowly decayed until the 42^{nd} day for the erythrocyte suspensions. For whole blood, by the 21^{st} storage day the 2,3-DPG mean value diminished to 19% of its initial value.

In storage conditions, ATP fell down remarkably in erythrocyte suspensions and whole blood, because the red blood cells have no mitochondria and they can not regenerate adenosine triphosphate [43]. As shown in Figure 4.1, for the erythrocyte suspensions, the mean ATP value on day 0 is 5,4 ± 0,5 μmol/gHb and a gradual decrease is observed with 1,6 ± 0,5 μmol/gHb (30%) on day 35. By the 42^{nd} day, only 10% of the mean initial value

is measured. Figure 4.3 shows that the mean ATP value of whole blood on the 0^{th} day is 5,1 ± 0,4 μmol/gHb and on the 21^{st} day, 54% of the mean initial value is presented.

The extracellular pH has decreased as it can be seen from Figure 4.1 and Figure 4.3 because of glycolysis with lactic acid formation [75]. The mean extracellular pH of 7,5 ± 0,2 (day 0) diminished slightly to 6,5 ± 0,2 by day 42 for the erythrocyte suspension. During 21 storage day for whole blood, the mean extracellular pH of 7,4 ± 0,1 (day 0) decreased to 7,0 ± 0,1.

When the cell ionic structure is considered, as expected, the extracellular Na^+ and Cl^- decreased and K^+ increased as shown in Figure 4.1 and Figure 4.3, respectively. Decreasing ATP during storage affected the Na^+- K^+ pump. Na^+ and K^+ that leak during storage have not been actively pumped back across the red cell membrane and sodium level fell while potassium level rised [66]. On day 0, for the erythrocyte suspension, the mean extracellular K^+ is 2,1 ± 1,0 mEq/L and increased to 45,3 ± 3,7 mEq/L by day 42. On the 42^{th} day of storage, the initial mean values of 147,7 ± 4,2 mEq/L of extracellular Na^+ and 118,5 ± 6,2 mEq/L of extracellular Cl^- decreased to 107,2 ± 9,9 mEq/L and 79,6 ± 6,0 mEq/L, respectively. All these proportional changes are also observed with ion concentrations of whole blood.

During storage, the extracellular resistance and the cell membrane capacitance of the erythrocyte suspension increased progressively while the intracellular resistance decreased as seen in Figure 4.9. For whole blood, the extracellular resistance R_e did not show any increase (Figure 4.12). This result can be explained by other storage lesions.

Changes in ionic concentrations, pH and ATP affect the electrical properties of blood directly. During storage, increased permeability of the membrane results in an enhanced interchange of intra-and extracellular fluids. The measured impedance is mainly affected by the resistivity and volume of each fluid and by the geometrical shape of the cells [85],[86]. Since the intracellular resistivity is two to three times that of the plasma [87], the progressive solute transportation across the cell membranes led to an increased extracellular resistance and a decreased intracellular resistance. The increase in the resistance of the extracellular fluid can also be explained by the decrease in the extracellular fluid volume between the swelled red blood cells [85].

Besides ionic movements, electrical properties are also affected by shape changes in cells. Lower ATP results in expulsion of lactate to the extracellular medium, decreasing the extracellular pH. The movement of band 3-1 spectrin inside the phospholipids of the cell membrane, caused by reduced pH, generates ionic currents, namely Cl^- ions. While Cl^- ions enter the cell, bicarbonate ions leave the cell [58]. The cell swells with Cl^- and a shape change occurs from discocyte to spheroechinocyte, resulting in an increased form factor in the Maxwell-Fricke equation [88]. The extracellular resistance is inversely proportional to this form factor. The observed decrease in R_e on whole blood indicates that the shape change might be dominant factor during storage in contrast to increase of R_e on erythrocyte suspension resulted from ion transportation.

The effective membrane capacitance C_m augmented with storage time progressively as the result of radius increase and shape transformation of blood cells. Substances in the plasma might also influence C_m negatively by being adsorbed to the surface of the membrane [1].

During storage, the phase angle, α is decreased with storage time in both erythrocyte suspensions and whole blood. But, F_c did not significantly change with storage.

The red blood cells with non-uniform shapes and structures loose this assortment with time and, by swelling completely, they finally become spheres. This is also reflected in the Cole-Cole diagrams of blood: the circles gradually shifted upwards with F_c remaining almost constant with the phase angle α being reduced.

In the second part of this study, effects of physiological parameters on the electrical parameters are thoroughly investigated. Clearly, there existed dependence between the electrical parameters and physiological behaviour of RBC's.

Results of the regression analysis applied to electrical and physiological parameters of erythrocyte suspensions and whole blood are given in Figures 5.1, 5.2, 5.3,...., 5.10 and Figures 5.11, 5.12, 5.13,...., 5.20, respectively.

It is clear from all these figures, that R_i is the most influenced electrical parameter in both erythrocyte suspensions and whole blood. Figure 5.1 and Figure 5.3 illustrate the

correlations of R_i and R_e with the extracellular ion concentrations (Na^+, K^+ and Cl^-) for erythrocyte suspensions and, Figure 5.11 and Figure 5.13 show it for whole blood. R_i and R_e are highly correlated with Na^+, K^+ and Cl^-. As it can be seen from Figure 5.1 and Figure 5.11, the R_i - K^+ dependence showed the strongest correlation.

C_m is highly correlated with K^+ (R^2 = 0,83) compared to other ion parameters (R^2 = 0,64) on erythrocyte suspensions (Figure 5.5). For whole blood, while C_m was correlated with Na^+ and K^+ with the same regression coefficient (R^2 = 0,63), C_m-Cl^- regression has a regression coefficient of 0,54 (Figure 5.15).

As it is seen from Figure 5.7 and Figure 5.8, the physiological parameters of erythrocyte suspensions have a dependence on the phase angle α, like C_m. C_m and α showed similar regregression with the physiological parameters.

All electrical parameters are significantly correlated with pH and ATP. Since the pH level controls the movement of cell ions, electrical parameters are affected by the pH shifts. As it can be seen from Figure 5.2 and Figure 5.4, R_i and R_e of the erythrocyte suspension is correlated to pH with the regression coefficients, R^2 = 0,82 and R^2 = 0,78, respectively. Figure 5.12 and Figure 5.14 compare R_i and pH, and, R_e and pH for whole blood, respectively. These relationships had the same correlation coefficient of 0,76. But, Figure 5.6 shows a relationship between C_m and pH with a correlation coefficient of 0,72 for erythrocyte suspension.

The correlations between R_i and ATP, and, R_e and ATP of erythrocyte suspension had a coefficient of 0,85 as it can be seen from Figure 5.2, and Figure 5.4, showing the effect of ATP on the Na^+-K^+ pump. ATP dependence on the electrical parameters of whole blood is lower than erythrocyte suspension's ATP dependence (Figures 5.12, 5.14 and 5.16).

2,3-DPG is not correlated at all with electrical parameters because it is not possible to fit any regression model. Hence, it is seen that 2,3-DPG did not have any measurable effect on electrical parameters.

None of the physiological parameters are correlated with the characteristic frequency (F_c), because F_c did not significantly change with storage time (Figures 5.9, 5.10, 5.19 and 5.20).

The correlation between parameters on erythrocyte suspension is generally better than those on whole blood, because whole blood consists of several different particles (leucocytes, platelets, plasma proteins) that may affect our measurements.

Finally, in this study, model equations are derived by using multiple regression model to predict the physiological behaviour of erythrocyte suspensions and whole blood from their electrical measurements.

It has been shown that, it is possible to estimate physiological parameters (Na^+, K^+, Cl^- concenrations, pH and ATP) from the electrical parameters of whole blood at all storage time. But, for erythrocyte suspensions, it is limited to the 35^{th} day of storage. Stored blood samples are normally consumed within the first two weeks of storage. Hence, by performing the complex impedance measurements, stored blood can be controlled and an assesment of viability can be made.

For the application of the physiological parameters' model, firstly, the electrical measurements on the day of withdraw are accepted as reference. The hematocrit values on the 0^{th} day of the blood that will be stored, are derived from the hematocrit equation in the model and they are used to normalize electrical measurements. By using the normalized electrical parameters, the reference blood physiological values on the day of withdraw are estimated from the model equations. To evaluate the quality of stored blood, prior to transfusion, after the electrical measurements are repeated and normalized, the physiological values derived from the model equations by using the new normalized electrical data , are compared with the reference values.

If the changes of the physiological parameters' values are within the acceptable range, it can be said that this blood sample fulfills the life criteria of stored blood.

There are no available data reporting reference ATP and pH values of stored blood that indicate blood quality. It has been observed that, a 24 hour survival of 75% of red blood cells is possible with an ATP concentration of 50% [58]. However, ATP levels as

low as 20% are also acceptable. It has been generally believed that a maximum alteration of 20% in the pH level is usually acceptable during storage.

Consequently, the results indicate that the complex electrical impedance measurement might provides a useful method for investigating the storage lesions, and for monitoring the quality of red cells during storage.

Physiological measurements of blood, especially ATP measurements, are time consuming and too expensive for routine studies. On the contrary, electrical measurements can be realized within few seconds. By providing a practical measurement tool, impedance measurement technique may become an inevitable procedure for the quality of blood during storage and may serve future implications. The most attractive advantage for this method is that the impedance could be measured on an intact bag used for storage by implanting electrodes somewhere inside the bag during manufacturing.

APPENDIX

Table A.1
Descriptive statistics for pH (R_i,R_e,C_m,α) model of ES.

	Mean	Std. Deviation	N
pH (erythrocyte sus.)	6,981407	,3718168	255
Rinorm (Ohm)	129,8116	7,58882	255
Renorm (Ohm)	344,7794	3,36040	255
Cmnorm (pF)	224,9626	103,03141	255
Alpha	,187374	,0450977	255

Table A.2
Correlations of variables for pH (R_i,R_e,C_m,α) model of ES.

		pH (erythrocyte sus.)	Rinorm	Renorm	Cmnorm	Alpha
Pearson Correlation	pH (erythrocyte sus.)	1,000	,907	-,882	-,840	,848
	Rinorm	,907	1,000	-,974	-,928	,945
	Renorm	-,882	-,974	1,000	,918	-,931
	Cmnorm	-,840	-,928	,918	1,000	-,979
	Alpha	,848	,945	-,931	-,979	1,000
Sig. (1-tailed)	pH (erythrocyte sus.)	,	,000	,000	,000	,000
	Rinorm	,000	,	,000	,000	,000
	Renorm	,000	,000	,	,000	,000
	Cmnorm	,000	,000	,000	,	,000
	Alpha	,000	,000	,000	,000	,
N	pH (erythrocyte sus.)	255	255	255	255	255
	Rinorm	255	255	255	255	255
	Renorm	255	255	255	255	255
	Cmnorm	255	255	255	255	255
	Alpha	255	255	255	255	255

Table A.3
Regression coefficient and significance for pH (R_i,R_e,C_m,α) model of ES.

					Change Statistics					
Model	R	R Square	Adjusted R Square	Std. Error of the Estimate	R Square Change	F Change	df1	df2	Sig. F Change	Durbin-Watson
1	,908[a]	,824	,821	,1572555	,824	292,493	4	250	,000	1,575

a. Predictors: (Constant), Alpha, Renorm, Cmnorm, Rinorm

Table A.4
Coefficients for pH (R_i,R_e,C_m,α) model of ES.

Model		Unstandardized Coefficients		Standardized Coefficients	t	Sig.	95% Confidence Interval for B		Collinearity Statistics	
		B	Std. Error	Beta			Lower Bound	Upper Bound	Tolerance	VIF
1	(Constant)	-,146	5,243		-,028	,978	-10,472	10,181		
	Rinorm	4,934E-02	,007	1,007	7,554	,000	,036	,062	,040	25,238
	Renorm	3,571E-03	,013	,032	,270	,787	-,022	,030	,049	20,276
	Cmnorm	-6,14E-04	,000	-,170	-1,305	,193	-,002	,000	,041	24,135
	Alpha	-1,979	1,221	-,240	-1,621	,106	-4,383	,426	,032	31,140

Table A.5
Collinearity diagnostics for pH (R_i,R_e,C_m,α) model of ES.

Model	Dimension	Eigenvalue	Condition Index	Variance Proportions				
				(Constant)	Rinorm	Renorm	Cmnorm	Alpha
1	1	4,773	1,000	,00	,00	,00	,00	,00
	2	,226	4,594	,00	,00	,00	,02	,00
	3	1,031E-03	68,039	,00	,01	,00	,96	,83
	4	3,014E-04	125,837	,00	,32	,00	,02	,16
	5	1,993E-06	1547,282	1,00	,68	1,00	,00	,00

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