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Bioelectrical Insight: Correlation Cell Count and Electrical Impedance of Whole Blood throughout Storage with an Impedance Analyzer Methode

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Abstract: Blood, a vital tissue comprising blood cells within the plasma matrix, plays a crucial role in transporting oxygen, nutrients, and functional components throughout the body. Insufficient blood levels can lead to disorders or life-threatening conditions, necessitating blood transfusions for those experiencing deficiencies. Blood banks store blood products to meet transfusion needs, emphasizing safety, donor health, patient conditions, cross-matching accuracy, and storage quality. Examining stored blood indicates an individual's physiological response to environmental changes, with quantitative and qualitative changes visible through whole blood cell count and impedance parameters. Electrical impedance spectroscopy, which measures these biological properties, shows that although blood cell count remains stable over 35 days, impedance characteristics change significantly. Analysis of the Nyquist Zriil plot reveals a consistent decrease in Zriil values, indicating reduced extracellular resistance (Res) over time. These impedance changes reflect alterations in blood morphology, providing crucial insights into the quality of stored blood. In conclusion, electrical impedance spectroscopy effectively monitors stored blood quality, detecting significant changes in extracellular resistance over extended storage periods. These findings underscore the importance of regular monitoring and proper management of stored blood to ensure its safety and effectiveness for transfusions.

Keywords: Cell count; Electrical impedance; Storage time

Introduction

Whole blood is used in medical procedures to replace lost blood volume due to injury or surgery and helps increase hemoglobin levels in patients with anemia. Blood transfusions can also be administered to patients with blood clotting disorders (Cap et al., 2018). Additionally, whole blood can be used to assess the health of patients by measuring levels of red blood cells, white blood cells, platelets, as well as glucose and protein levels in the blood (Kondoh et al., 2021). These tests can assist healthcare professionals in diagnosing a disease. Whole blood is typically stored in blood banks, found in blood transfusion centers, hospitals, or the PMI. Whole blood stored in blood banks must be consistently maintained to prevent bacterial contamination, requiring sterile packaging and the application of appropriate storage techniques (Jacobs et al., 2024; Ramirez-Arcos et al., 2023). Additionally, whole blood can be stored at a cool temperature, with the optimal range being 1-6 degrees Celsius. Blood stored at this temperature can last up to 32-35 days after the addition

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of CPDA (Citrate Phosphate Dextrose Adenin) (Gupta et al., 2019). Whole blood must be maintained in terms of temperature, pH, and calcium ion concentration, as the number of platelets continuously decreases during storage (Ichikawa et al., 2022; Mindukshev et al., 2022). Modern blood processing technologies have been developed to separate blood components to minimize rolling and blood aggregation. However, if whole blood remains one of the options for blood transfusion, efforts are needed to improve storage conditions and maintain platelet activation during longer storage periods (Widodo et al., 2024).

Blood is an electrical conductor, with its electrical conductivity depending on the concentration, type of ions, proteins, and other molecules present in the plasma and cellular components of blood. The electrical properties of blood have been studied for years, and several research studies indicate that changes in the electrical characteristics of blood can be an indication of certain diseases or conditions. For example, changes in the electrical impedance of blood have been observed in whole blood cell and packed red cell damage during storage Livshits et al. (2021), simple resistance measurements in pig blood hemolysis Walski et al. (2022), and recent research using Bioelectrical Impedance Analysis (BIA) method to monitor the quality of blood stored at 4ºC for 21 days. This research serves as a reference that bioelectric resistance values of blood can be linked to the quality of stored blood and can be used to predict platelet activation levels during storage (Branco et al., 2023).

The electrical properties of blood cells are a phenomenon that occurs due to differences in electrical potential between two parts of the blood cell. Electrical properties of blood cells can occur because of differences in ion concentrations inside and outside the blood cell (Niebur, 2010). Electrical properties of blood cells can be disrupted during the storage process, especially if blood storage is not done correctly (Welsh et al., 2024). Bioelectrical Impedance Analysis (BIA) can be a useful tool to evaluate blood quality and blood components (Quist et al., 2024). However, it is usually used in conjunction with other tests and methods to provide a more comprehensive picture of blood quality. BIA can measure various parameters that affect blood quality, such as ion concentration, protein content, and water content within cells (Tran et al., 2024; Huisjes et al., 2018). Moreover, BIA does not require a large blood sample like other tools used to measure blood quality. This makes BIA more practical and cost-effective. BIA serves as an indicator tool for living biological tissues, one of which is blood, by measuring its electrical characteristics (Pardeshi, 2023). When an electric current is passed through biological tissue, the tissue will resist or react to the current depending on its electrical properties (Long & Koyfman, 2016).

BIA has been widely used in clinical research to evaluate body composition, nutritional status, and health conditions related to body water and fluid. Additionally, BIA can also be used to monitor blood quality during storage. The characteristic impedance values of whole blood during storage can provide crucial information about changes in blood quality during storage (De Beukelaar & Mantini, 2023). However, there is still limited research investigating the impact of storage time on the impedance characteristics of whole blood using the BIA method. Therefore, this study will focus on analyzing the impedance characteristics of whole blood during storage (0, 2, 7, 14, 21, 28, 35) days using the BIA method. In this research, it is expected to identify impedance characteristics of whole blood during storage that can provide a deeper understanding of blood quality conditions and improve storage conditions to meet required safety and quality standards.

The results of this study can also contribute significantly to the development of new technologies for monitoring blood quality during storage and enhancing the effectiveness and safety of blood transfusions. This research aims to evaluate changes in blood characteristics during storage and determine whether blood impedance values can be used as an indicator of blood quality during storage.

Method

The tools used in this research include a thermometer, humidity meter, syringe, and vacuum tubes. For microscopic examination of blood cells, an optical microscope, spreader, and glass slides are used. A blood cell counter is employed for cell count analysis. Viscosity testing utilizes a rotary viscometer, and for Bioelectrical Impedance Analysis (BIA) measurements, a BISDAQ testing kit, computer, and a set of IDT electrodes are used. The material used in this study is whole blood (Antonacci et al., 2024; Constable et al., 2024). The research will be conducted using a BISDAQ testing kit as the main tool. This study involves injecting a current of 10 μA into the sample to generate voltage values dependent on frequency.

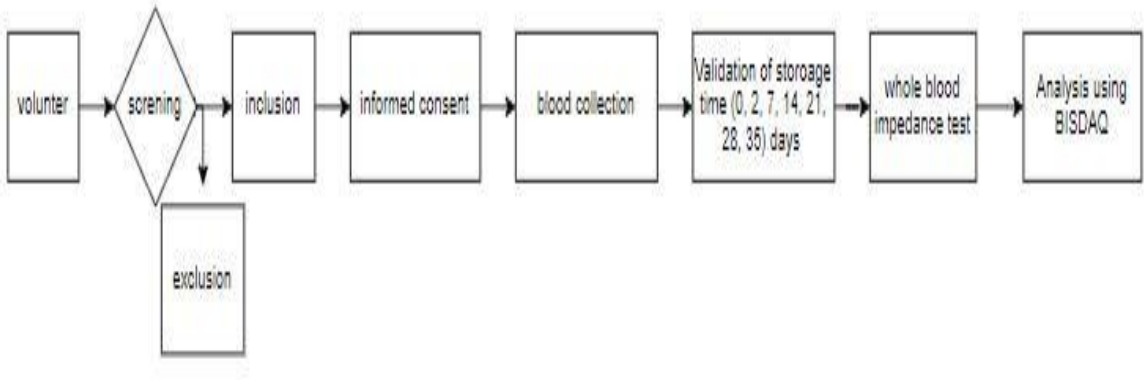

Figure 1. Diagram methodology of the research

The sample preparation stage consists of preparing the sample for microscopic examination, blood cell count, and measuring blood cell impedance. Blood samples are collected by medical personnel at the PMI branch in the city of Malang. The collected blood samples are processed according to the standard operating procedures (SOP) established by the Malang City PMI using the CPDA anticoagulant. CPDA is an anticoagulant used to maintain the viability of whole blood, optimize pH during storage, and preserve blood for up to 32 days at a temperature of 1-6 °C. The citrate compound in the blood bag prevents coagulation by binding calcium in the donor's plasma. Phosphate acts as a buffer to maintain blood pH and prevent a decrease in 2,3-diphosphoglycerate (DPG). Dextrose is added to the blood bag to meet the energy needs of cells, serving as a substrate to produce ATP. Adenine functions to regenerate adenosine triphosphate (ATP). CPDA specimens must be mixed immediately after blood collection to prevent blood clotting and the formation of microclots, specifically by inverting the mixture 10-15 times (Gupta et al., 2019).

A total of 25 samples were collected from normal whole blood donors. After blood collection, the samples underwent immediate microscopic examination, blood cell counting, and blood cell impedance measurement on the day of sample collection or storage day 0 as control data. Subsequently, the blood samples were stored for 35 days at a controlled temperature of 1-6º C, and all tests were conducted on specific days. Donor selection at the PMI Blood Transfusion Unit (UTD) in Malang. Donors should be aged between 17 and 60 years old with a minimum weight of 45 kg. Their body temperature should range from 36.6°C to 37.5°C. Good blood pressure is required, including a systolic pressure of 110-150 mmHg and a diastolic pressure of 70-90 mmHg. Additionally, their pulse rate should be approximately 50-100 beats per minute.

Venous blood with CPDA anticoagulant is used because the mixture of blood and anticoagulant is essential in creating blood smears. The stored blood samples are placed in a blood storage cabinet at a temperature of approximately 1-6ºC. Microscopic examination control data is collected on day 0 of storage or on the day of blood collection. Subsequently, blood samples that have been collected are prepared into blood smears on predetermined days, namely 0, 2, 7, 14, 21, 28, 32, and 35 days.

The data analysis to be conducted will involve creating graphs illustrating the relationship between impedance values and frequency for each blood sample. Impedance values are obtained from measuring sinusoidal voltage, which is then converted using physics equations. The impedance values from blood samples will be measured by converting the sinusoidal waveform voltage values into impedance values using physics equations (Huynh et al., 2018; Ain et al., 2018). The voltage values used are the peak-to-peak voltage of the waveform. To analyze the data, graphs will be created to show the relationship between impedance values and frequency for each blood sample. Additionally, graphs will be generated to depict the relationship between the duration of storage and impedance values for each sample. These graphs will be created by extracting impedance values at specific frequencies. Through the analyzed graphs, it is expected to differentiate the characteristics of blood impedance values in each blood type.

Result and Discussion

Impedance measurements are conducted in the frequency range of 100 Hz to 100 kHz with a current injection of 10 µA. The Bode plot graph is presented in Figure 5.1. This graph illustrates the measured impedance spectra based on the frequency function. The impedance spectra are obtained from 25 whole blood

samples. The measured impedance values for the 25 samples fall within the range of 1000 Ω to 4000 Ω. Based on Figure 5.1, it can be observed that the measured impedance values from the 25 whole blood samples tend to remain relatively stable when the provided frequency is above 5 kHz. The phase shift values for the four samples can be seen in the following figure:

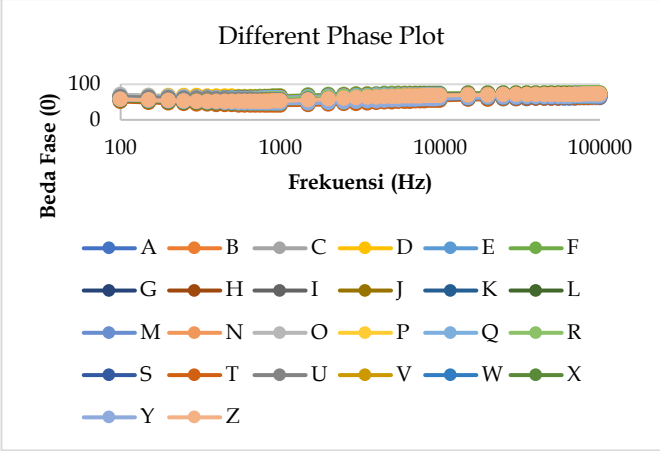

Figure 2. Different Phase Plot

The differences in impedance values for the 25 samples will be more detailed when the impedance values for each sample are plotted at specific frequencies. Figure 2 shows several impedance plots at frequencies of 1 kHz, 5 kHz, 10 kHz, and 50 kHz for the 25 whole blood samples.

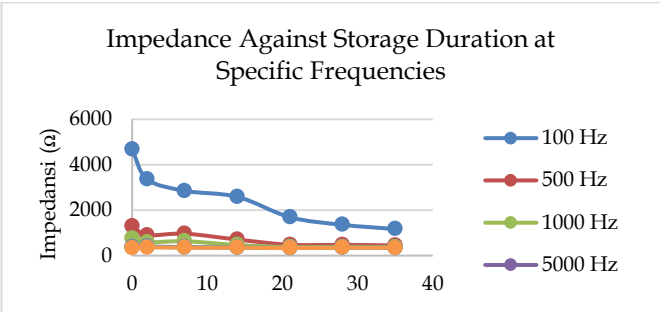

Figure 3. Impedance Against Storage Duration at Specific Frequencies

The Nyquist plot is a graphical representation of the relationship between the imaginary part Zim as a function of the real part Zre of the complex impedance. The first point on the x-axis of the Nyquist plot Zriil at a frequency of 30 kHz indicates the presence of a series of medium resistances. These resistances usually originate from solution resistance in electrolyte resistance, separators, and external circuits [15]; (Chen et al., 2021). Twenty-five samples were tested and exhibited similar components. Therefore, this medium resistance can be associated with the resistance of extracellular fluid or plasma. Plasma has lower resistivity compared to red blood cells or erythrocytes. The interface capacitance of the electrode appears at low frequencies as indicated on the Nyquist plot by a straight line with a certain slope. This arises as a result of ion diffusion occurring at the electrode interface layer (Cappabianca et al., 2023; Hess et al., 2021).

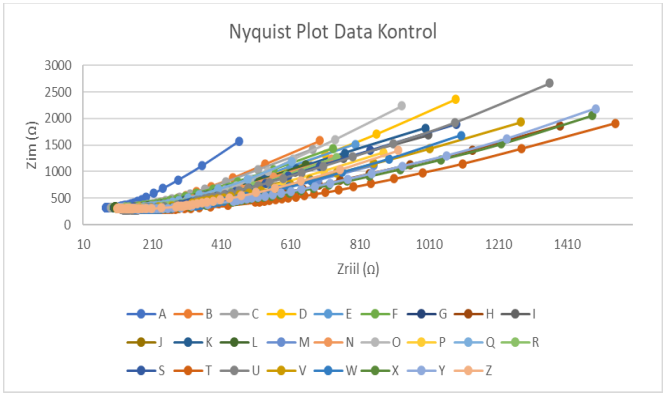

Figure 4. Nyquist Plot

The impedance measurements plotted as a function of frequency and varying storage time are shown in figure 4. Across different storage durations, it can be observed that the measured impedance spectra do not undergo significant changes or can be considered relatively stable. The same relatively stable impedance spectra measurements were obtained across 25 samples.

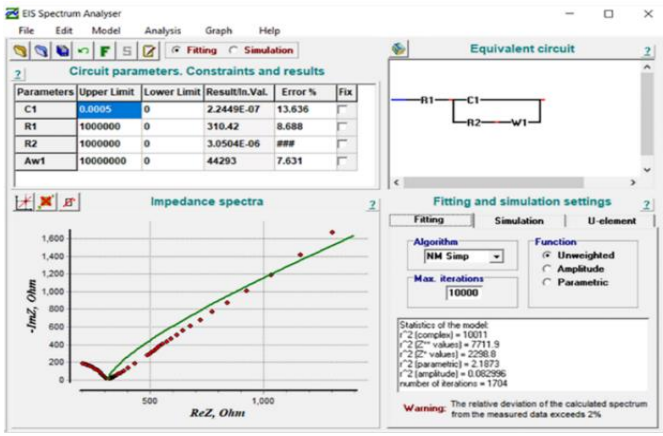

Figure 5. EIS spectrum analyzer (analysis and simulation of impedance spectra)

The Nyquist plot results obtained in this study represent something different from the Bode plot results. Figure 6 presents the average Nyquist plot of 25 samples against storage time (0, 2, 7, 14, 21, 28, and 35 days). At medium frequencies, a straight line with a certain slope appears; this impedance reflects ion diffusion phenomena within the electrode surface structure. At low frequencies, the measured sample impedance reflects behavior like a capacitor in a pure electrical double layer, characterized by a vertical line

(Rahmawati et al., 2022; Asaad and Maghdid, 2022). The first intersection point on the real axis at a frequency of 30 kHz indicates a series of medium resistances. The measured medium resistance based on the first intersection point on the real axis at a frequency of 30 kHz decreases as the sample is stored longer [19]; (Günter et al., 2018; Arts & Van Den Broek, 2022).

During the storage process, changes in red blood cell morphology occur, causing most of the intracellular fluid to move out into the plasma (Geekiyanage et al., 2020). This leads to changes in the composition of ions and molecules in the plasma during storage. The decreasing value of Rs with longer storage indicates that the longer the storage, the lower the extracellular resistance values due to increased plasma conductivity (Van Buren et al., 2020). The internal conductivity of red blood cells is mostly caused by its main ion content consisting of K+, Na+, Mg2+, Cl, and bear ions, namely, the dominant dissociated hemoglobin molecules. Hemoglobin is a protein molecule containing iron (Fe) and is found in the intracellular fluid of erythrocytes (Vogt et al., 2021).

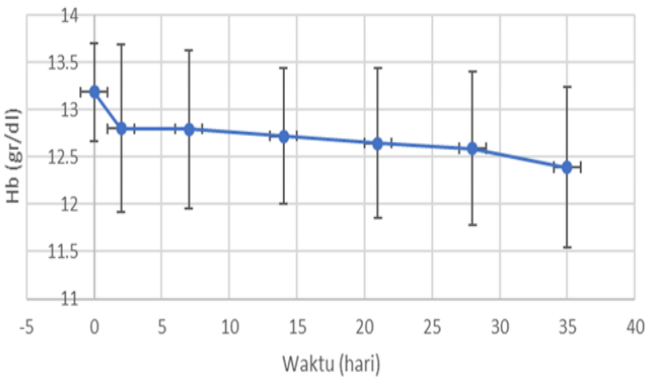

Figure 6. Average amount of hemoglobin at various storage times

Decreasing hemoglobin levels indicate oxidation, leading to structural changes in hemoglobin molecules. Electrical changes in the storage environment can affect the ions present in the blood sample (Pawlik-Sobecka et al., 2020). The presence of certain ions can accelerate the oxidation process of hemoglobin. The oxidative properties of these ions can damage the structure of hemoglobin and cause a decrease in hemoglobin levels. Unstable electrical conditions or electrochemical reactions occurring during storage can impact hemoglobin molecules. Under certain conditions, electrochemical reactions can stimulate changes in the heme and globin groups of hemoglobin. Additionally, electrolysis produces by-products that can interact with hemoglobin and cause a decrease in hemoglobin levels (Carrola et al., 2023).

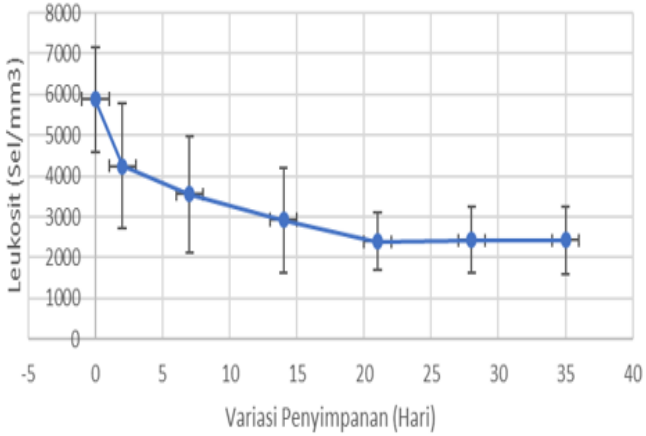

Figure 7. The average number of leukocytes at various storage times

Leukocytes can undergo apoptosis or cell death during storage. This process can be influenced by prolonged storage time. Decreasing leukocyte counts mean there are fewer white blood cells contributing to the overall blood composition. The reduced cell concentration can affect the electrical conductivity in the blood, thus altering its impedance (Zhbanov & Yang, 2015; Jafarinia et al., 2024).

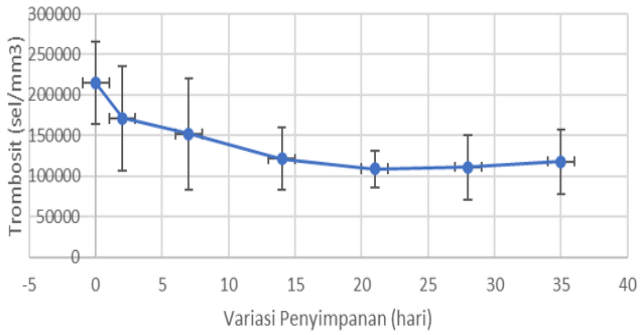

Figure 8. Platelet count at varying storage times

Thrombocytes, or platelets, undergo changes during storage in whole blood. Prolonged storage can lead to platelet activation and aggregation, affecting their functional properties. This alteration may impact clotting ability and overall blood quality over time (Weisel & Litvinov, 2019; Litvinov et al., 2021; Packebush et al., 2023).

Conclusion

A skeletal system learning application design with skull bone objects has been developed using the Rapid Application Development (RAD) software development method. User model control in the form of user interaction provided such as rotate, zoom, scaling and

grabbing objects. A 3D-VR application development environment experiment has been conducted and the results of the case study object development trial in the unity work environment.

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Author Contributions
Conceptualization, V. Q. A.; methodology, C. S. W.; validation, E. R.; formal analysis, V. Q. A; investigation, C. S. W.; resources, E. R.; data curation, V. Q. A.: writing—original draft preparation, C. S. W; writing—review and editing, M.: visualization, E. R. All authors have read and agreed to the published version of the manuscript.

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Conflicts of Interest
The authors declare no conflict of interest.

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