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Electrical Impedance Study on Whole Blood Cells and Red Blood Cells during Storage in Refrigerator

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Abstract

Doctors or health laboratory workers usually carry out manual blood examinations, who cell morphology parameters by eye. Electrical impedance spectroscopy has been proposed to assist doctors in examining blood damage. The effect of storage on blood cells will be evaluated using an electrical impedance measurement system on blood cells. The results of electrical impedance measurements will be correlated with the quality and quantity of blood cells, namely the mean corpuscular hemoglobin concentration (MCHC) value. Three bags of complete blood cell packages (from different donors) and 1 bag of red blood cell packages were used as blood samples. All blood samples using citrate phosphate dextrose adenine (CPDA) anticoagulant were obtained from the Indonesian Red Cross, Malang City Branch. Electrical impedance and cell-count measurements were performed on Day 0 (before being put into the refrigerator) and on Days 2, 4, 7, 10, 17, 21 and 28 after being placed in the fridge. Electrical impedance was measured using Bio-Impedance Spectroscopic Data Acquisition (BISDAQ). As the duration of storage increases, the MCHC value tends to decrease. The total impedance tends to decrease with increasing storage duration; the total impedance on Day 28 shows the lowest value. In general, the resistance value of the solution is correlated with the MCHC value of the blood cells. The escape of some material in the cell to the solution outside the cell results in a decrease in the resistance properties of the solution, according to the study's results. The total electrical impedance decreases with increasing storage duration. Storage duration affects the quality and quantity of blood cells. The MCHC value of blood cells decreased with increasing storage duration. The value of MCHC is strongly correlated with the value of the external solution resistance of the cell.

Keywords: Electrical impedance, Blood cell storage, MCHC, Whole blood, Nyquist, EIS

Introduction

Blood is a connective tissue composed of blood cells in the plasma matrix. Blood is essential in delivering oxygen, nutrients and other functional components, such as enzymes and hormones, throughout the body. Blood cells consist of 55 % liquid components in the form of plasma and 45 % solid components in the form of blood cells. Red blood cells make up almost 98 % of blood cells, making up a substantial part of blood. New blood cells are continuously formed in the red bone marrow; white blood cells that have reached a certain age will be destroyed [1]. A blood transfusion is needed if the body lacks or requires a blood supply [2].

Technological advances in blood storage have allowed blood banks to store blood at 1 - 6 °C for approximately 28 days, depending on the preservative used. The incidence of hemolysis should be kept to less than 1 % at the end of storage. The number of cells with a 24 h posttransfusion survival rate is at least 75 %. The safety of the transfused blood product depends on the donor's health, the patient's needs and condition, the accuracy of cross-matching and the quality of storage [3].

During storage, red blood cells can undergo metabolic changes due to different situations with body conditions *in vivo*. Red blood cells experience a progressive decrease in quality manifested through biochemical and biomechanical changes that can affect their function. Laboratory tests determine the quality and quantity of red blood cells to obtain accurate information that assists doctors in making diagnoses. Currently, the quality and amount of red blood cells are determined by a hematological

examination [4]. Over time, blood or blood components stored in the refrigerator decrease in quality, one of which is hemolysis [5-8], which causes changes in MCHC values.

Some studies have shown that various conditions, such as environmental exposure or disease, can damage blood cells. In hereditary spherocytosis cases, blood cell damage occurs, where the blood cells change shape from thin layers with hollows to spherical or round. Studies have shown that this change in shape increases MCHC [9]. RBC, HCT, MCV, MCH, MCHC, RDW and platelet count are hematological markers impacted by room temperature storage. Standardized storage settings minimize pre-analytical variance and hematological parameters impacted by temperature and storage in a way that can affect clinical judgments [10]. For instance, the levels of MCHC and hemoglobin considerably increased after the air purifier intervention. Study shows imply that using an indoor air purifier could lower indoor PM levels and homemakers' risk of anemia. The effect on anemia was proven 3 months after the air purifier operation since it is a subacute effect. In conclusion, PM reduction management can enhance anemia signs [11].

A hematological examination of the blood is carried out to identify problems or abnormalities in the quantity and quality of red blood cells and thus help determine the patient's medical condition. Hematology examination uses the parameters of the number and morphology of blood cells, both normal and abnormal. The Automatic Cell Counter device has determined the number of cells, but checking blood cells' quality or morphological parameters is still done manually [12,13]. Doctors or health laboratory workers usually perform manual blood examinations and identify cell morphology parameters by eye [4].

Several factors, such as differences in physical condition, concentration and personnel knowledge, can cause differences between doctors in identifying problems or abnormalities in blood cells. This factor drives the accuracy of the morphological examination results to be low. In addition, identifying the morphology and types of blood disorders can require considerable time and many medical personnel [14]. The electrical impedance spectroscopy method is proposed to assist doctors in examining blood damage. Electrical impedance spectroscopy is a method that can be used to analyze the electrical properties of biological materials. This method applies an electric current stimulus to an object, and then the system response can be observed as a potential signal or current. Electrical impedance spectroscopy is a noninvasive method that can detect biological samples' properties when exposed to an electric field between electrodes. Samples can be characterized according to their geometric and dielectric features using the current injection method at several frequencies [15].

The electrical impedance spectroscopy method can measure cell volume at low frequencies (hundreds of kHz to 1 MHz), detect the presence of capacitive components such as cell membranes at intermediate frequencies (on the order of 1 MHz), and detect the presence of intracellular features such as cytoplasmic conductivity and permittivity at a high frequency (greater than 1 MHz) [16]. Cell morphology is an essential parameter for impedance-based single-cell analysis. The characteristic impedance is highly dependent on the geometric parameters and the dielectric properties of the cell.

Impedance is an electrical characteristic of biological materials, each with a different characteristic impedance. Impedance has been measured in single or multiple cells, suspended cells and cells attached to a substrate [17]. This technique has been applied to basic cell biology research and sorting [18-19]. Several factors, including temperature and measurement frequency and the moisture content, density, composition and material structure of the biological material, influence the characteristic impedance of natural material. In biological samples of whole blood (WB), the characteristic impedance can be affected by several factors: The number and morphology of red blood cells and blood plasma. Blood is a suspension of particles (red blood cells) with high resistivity in the conducting fluid (plasma) [20,21]. Other cells and platelets are considered to have an insignificant role in the electrical properties of blood because they are too small in size and number [22]. Therefore, it is necessary to know the effect of changes in the quantity and quality of red blood cells during storage on the characteristics of the impedance value of WB cells using the electrical impedance spectroscopy method. The storage effect of blood cells will be evaluated using an electrical impedance measurement system in blood cells.

Materials and methods

Whole-blood (WB) and packet red cell (PRC) preparation

Three bags of complete blood cell packages (from different donors) and 1 bag of red blood cell packages are used. All blood samples using CPDA anticoagulant were obtained from the Indonesian Red Cross, Malang City Branch. CPDA is an anticoagulant used to maintain erythrocyte viability, optimize pH during storage, and store blood for up to 28 days at 1 - 6 °C temperature. CPDA specimens should be mixed immediately after collection to prevent blood clots and micro clot formation. The mixing method with

inversion is 10 - 15 times. Furthermore, each blood package was placed in a 3 mL blood collection tube with 24 tubes and stored in a refrigerator at 1 - 6 °C temperature.

MCV characterizes the estimate of the ruddy blood cells and is communicated as femtoliters. The normal values for MCV are 87 ± 7 fl. MCH evaluates the sum of hemoglobin per red blood cell. The ordinary values for MCH are 29 ± 2 picograms (pg) per cell. MCH measures the sum of hemoglobin per red blood cell. The typical values for MCH are 29 ± 2 pg per cell. And MCHC shows the sum of hemoglobin per unit volume. In differentiation to MCH, MCHC relates the hemoglobin substance with the volume of the cell. It is communicated as g/dL of red blood cells or as rate esteem. The typical values for MCHC are 34 ± 2 g/dL. Calculation of MCV, MCH and MCHC by [23]:

$$\text{MCH} = \frac{\frac{\text{Hemoglobin in } gram}{\text{1,000 mL of blood}}}{\text{Red Blood Cell count in millons/mL}} \text{pg/cell}$$

$$\text{MCV} = \frac{\frac{\text{Volume of packed cells}}{\text{1,000 mL of blood}}}{\text{Red blood cell count in millons/mL}} \text{fl}$$

$$\text{MCHC} = \frac{\frac{\text{Hemoglobin in } gram}{\text{100 mL of blood} \times 100}}{\text{Volume of packed cells/100 mL of bld}} \text{g/dL}$$

Electrical impedance spectroscopy measurements

Electrical impedance and cell count measurements started on Day 0 (before being put into the refrigerator) and continued on Days 2, 4, 7, 10, 17, 21 and 28 after being placed in the fridge. Complete blood cell counts were performed using an ABX Micros 60 Hematology Analyzer. Electrical impedance measurement was performed using BISDAQ [24]. The electrical impedance measurement is performed by injecting a current of 10 µA into a blood sample in the frequency range of 100 Hz - 100 kHz. The frequency response test was carried out in the system calibrated to the AC source. Interdigitated electrodes were used, and the measurement mechanism was presented in previous studies [20,21]. The EIS Spectrum Analyzer application analyzes measurement results using an analogy model of an electrical circuit, as shown in **Figure 1**.

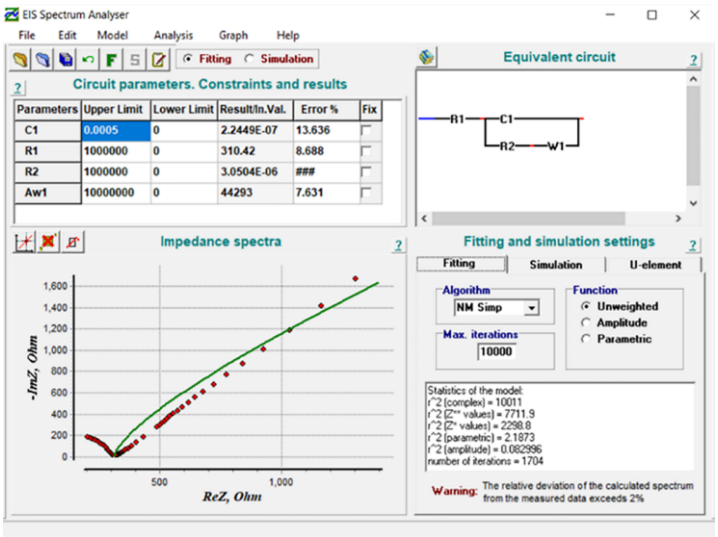

Figure 1 EIS spectrum analyzer (analysis and simulation of impedance spectra) [25]. The electrical circuit analogy model for a blood sample (where R1 is the solution resistance of the cell, and W1 is the Warburg impedance (infinite diffusion layer thickness).

Results and discussion

The quality and quantity of blood cells can be determined based on the following criteria: Average red blood cell size (MCV), hemoglobin amount per red blood cell (MCH) and the amount of hemoglobin relative to the size of the cell (hemoglobin concentration) per red blood cell name as MCHC. Based on the

blood cell count results, the MCHC value was obtained, as shown in **Table 1**. As the duration of storage increases, the MCHC value tends to decrease.

Table 1 MCHC value of the blood sample. (MCH: Mean corpuscular hemoglobin and MCV: Mean corpuscular volume.

Day	WB			PRC		
	MCV (μm^3)	MCH (pg/cell)	MCHC (g/dL)	MCV (μm^3)	MCH (pg/cell)	MCHC (g/dL)
0	89.80	28.98	32.27	95.14	28.78	30.25
2	89.69	28.88	32.20	95.05	28.61	30.10
4	90.11	28.69	31.84	95.70	28.46	29.73
7	89.34	28.99	32.45	94.57	29.01	30.67
10	91.20	28.79	31.56	96.80	29.16	30.12
17	89.68	27.61	30.79	94.33	27.54	29.19
21	90.18	27.88	30.90	94.44	27.25	28.85
28	89.14	26.55	29.79	94.40	26.69	28.28

The impedance measurement results for WB and PRC are shown in **Figure 2**. In general, the total impedance decreases with increasing frequency. This indicates that the resistive nature of the sample is dominant at low frequencies, and at higher frequencies, it is dominated by capacitive properties. The total impedance tends to decrease with increasing storage duration; it can be seen on day 28 that the total impedance shows the lowest value. Measurements in the frequency range of 100 - 10 kHz showed a significant difference in the total electrical impedance of the samples in different storage durations. Meanwhile, at frequencies above 10 kHz, the system could not distinguish them.

Based on the measured total impedance shown in **Figure 2**, it can be abstracted in terms of the real impedance axis and the imaginary axis, commonly referred to as the Nyquist plot. The Nyquist plot method describes the system's frequency response and stability. In **Figure 2**, the electrolyte concentration significantly affects the slope, according to the intermediate EIS frequency observation results. In other words, the slope of the nonvertical line can indicate whether the conduction process is controlled by the arrangement (large slope) or limited by the diffusion of ions in the electrolyte (slight incline).

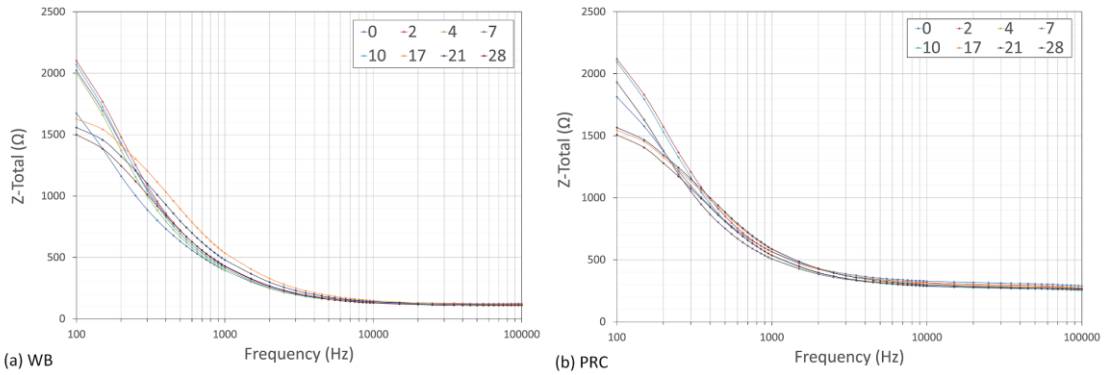

Figure 2 Total electrical impedance (Z-total) of (a) WB and (b) PRC at various storage times. (Storage time at 0 - 28 days).

In **Figure 3**, the slope generally increases with increasing storage duration. The real value of electrical impedance in the PRC sample has a higher value (**Figure 3**(**b**)) than the real value of the electrical impedance in the WB sample. The slope of the chart on the 17th, 21st and 28th days, generally, has a very significant difference compared to the previous few days.

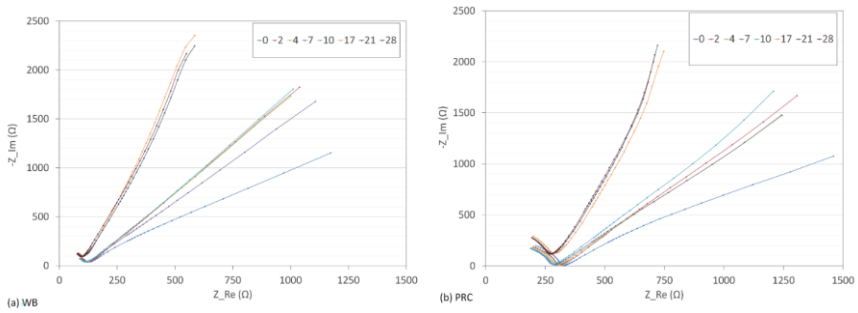

Figure 3 Nyquist plot of the electrical impedance of (a) WB and (b) PRC at various storage times. (Storage time at 0 - 28 days; Z-Re: Real Value of electrical impedance; Z-Im: Imaginary Value of Electrical Impedance).

As shown in **Figure 1**, on the EIS Spectrum Analyzer system, the implementation of the circuit model obtained values for R1, R2, C1 and W1. In this study, the importance of R2, C1 and W1 was relatively the same. At the same time, the value of R1, which describes the value of the solution resistance in the cell, changed considerably for sample storage with different durations, as shown in **Table 2** for the WB sample and **Table 3** for the PRC.

Table 2 The value of each component of the fitting results using the EIS Spectrum analyzer for WB samples.

Component	Days							
	0	**2**	**4**	**7**	**10**	**17**	**21**	**28**
C1 (F)	1.48E-07	2.78E-07	2.99E-07	2.36E-07	2.87E-07	2.25E-07	2.48E-07	2.80E-07
R_Sol (Ω)	122.3	140.5	133.12	129.92	132.14	75.758	83.638	82.57
R2 (Ω)	2.94E-06	1.24E-05	1.10E-05	1.04E-05	2.58E-05	6.46E-05	1.48E-04	2.03E-03
Aw1	28591	52930	49463	44888	51891	99849	93527	92458

Table 3 The value of each component of the fitting results using the EIS Spectrum analyzer for a PRC samples.

Component	Days							
	0	**2**	**4**	**7**	**10**	**17**	**21**	**28**
C1 (F)	2.73E-07	2.24E-07	2.49E-07	2.41E-07	2.87E-07	2.47E-07	2.45E-07	2.74E-07
R_Sol (Ω)	327.06	310.42	298.4	288.25	132.14	257.74	246.65	248.3
R2 (Ω)	3.99E+02	2.40E-06	7.04E-06	1.62E-06	2.79E-06	9.21E-05	7.04E-05	1.91E-05
Aw1	24438	44293	37410	37213	51891	81833	85390	82863

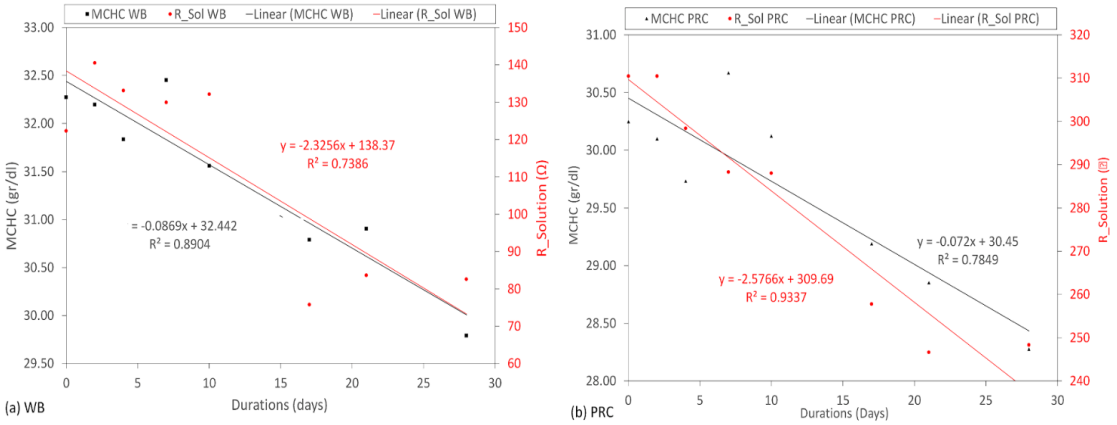

Figure 4 Fitting plot of MCHC and R_Solution from the EIS analyzer of (a) WB and (b) PRC (R_Solution: Resistance value of sample solution).

In general, the resistance value of the solution is relevant to the MCHC value of the blood cells, as shown in **Figure 4**. The decrease in MCHC values in the PRC group was faster than in the WB group. The reduction in the resistance of the solution describes the increase in the conductance properties of the solution. This is likely because of the additional material that comes from the cell.

During storage, the RBC membrane experiences substantial modifications. RMP production, osmotic fragility, hemolysis and changes in deformability will all be affected by the duration of storage. These modifications to RBC quality *in vitro* could be a factor in transfusion responses and unfavorable posttransfusion outcomes. Red blood cells are broken down or destroyed during hemolysis, which releases the oxygen-carrying pigment hemoglobin into the surrounding tissue.

Hemoglobin and other intracellular erythrocyte components are typically released into the extracellular area of blood during hemolysis. Both *in vivo* and *in vitro* hemolysis are possible. *In vitro*, hemolysis is brought on by incorrect or careless handling of specimens during specimen collection, while *in vivo* hemolysis results from various conditions and disorders (inherited or acquired hemolytic anemias). *In vitro*, testing is unreliable because it can affect the results' accuracy and laboratory testing's reliability. Hemolysis is the most common preanalytical artifact encountered in laboratory specimens.

Hemolysis is common in clinical laboratories, ranging from 3.3 to 40 %. This means that hemolysis makes up to 70 % of all samples unsuitable. Taking blood samples increases the chance of clotting by nearly 5 times. This could be due to inadequate collection procedures, insufficient volume, or incorrect samples. *In vitro* hemolysis is the most common cause of specimen rejection in outpatient and inpatient samples and urgent and routine specimens [26].

The study on Hemolysis During the Storage of Blood findings concluded that hemolysis is crucial in determining how well-preserved red blood cells are. However, the hemolysis amount did not exceed the 0.8 % maximum allowed for up to 42 days of storage. Using the additive solution SAGM and DEHP as a plasticizer in blood bags for storage may be responsible for the decreased hemolysis in blood units at this facility. Although a visual examination of the blood unit is a simple and quick way to find hemolysis, it also tends to overestimate hemolysis. Therefore, routine quantitative analysis for hemolysis in a blood sample must be performed using the TMB method to prevent the unintentional loss of valuable RBC units. With storage, the potassium and plasma LDH levels gradually increase, which can be utilized as a monitoring tool and as a sign of developing hemolysis [6].

The hemoglobin concentration in red blood cells decreased in female donors when samples were stored *in vitro*. Female donors had lower MCH, MCHC and plasma antioxidant capacity than male donors throughout storage. However, baseline levels were similar between the sexes [7].

The effect of storage on MCHC values was confirmed by measuring the electrical impedance of blood cells. The escape of some cell materials into the solution outside the cell results in a decrease in the resistance properties of the solution, according to the study's results, as shown in **Figure 4**. At frequencies up to 10 kHz, the system can distinguish the occurrence of hemolysis, which is indicated by the decrease in the value of MCHC.

Conclusions

The total electrical impedance decreases with increasing storage duration. Storage duration affects the quality and quantity of blood cells. The MCHC value of blood cells decreased with increasing storage duration. The value of MCHC is strongly correlated with the value of the external solution resistance of the cell. The decrease in the MCHC value results in a reduction in the value of the external solution resistance of the cell. The difference between our results using electrical impedance analysis and other studies is that changes in the external solution components of cells due to hemolysis can be detected by the electrical impedance-based measurement system used.

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