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EFFECTS OF PHYSIOLOGICAL PARAMETERS ON THE ELECTRICAL PROPERTIES OF BLOOD BANK STORED ERYTHROCYTE SUSPENSIONS

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Abstract – In this study, the physiological parameters such as extracellular (SAGM + CPD + residual plasma) Na^+, K^+, Cl^-, *pH, 2,3-DPG* and *ATP* together with the Cole-Cole parameters were measured using erythrocyte suspensions from 51 male donors (31 donors form the training set and 20 donors are used for testing), on the 0^{th}, 10^{th}, 21^{st}, 35^{th} and 42^{nd} days of storage. Accordingly, electrical parameters were all correlated with Na^+, K^+, Cl^-, *pH* and *ATP*, at varying levels. By applying the multi-regression analysis, it is concluded that R_i, R_e and C_m are appropriate for modeling Na^+, K^+, Cl^-, *pH* and *ATP* during blood bank storage and predicting blood quality.

Keywords: red blood cell, blood storage lesions, bioimpedance, erythrocyte suspension.

I. INTRODUCTION

Previous studies suggest that electrical parameters can be a potential index for evaluating blood in clinical applications, especially with blood bank stored blood samples before usage [1-3]. During the "in-vitro" storage, the red blood cells undergo physiological changes that would be expected to affect the electrical impedance of blood [4-6].

The complex electrical impedance Z^* is represented by R_i and R_e, the resistances of intracellular and extracellular fluids and, the effective cell membrane capacitance C_m (Figure 1). The capacitive effects of cell membranes are lumped in a constant phase angle impedance $Z_{CPA} = K(jw)^{-\alpha}$ because of the structures of the red blood cells.

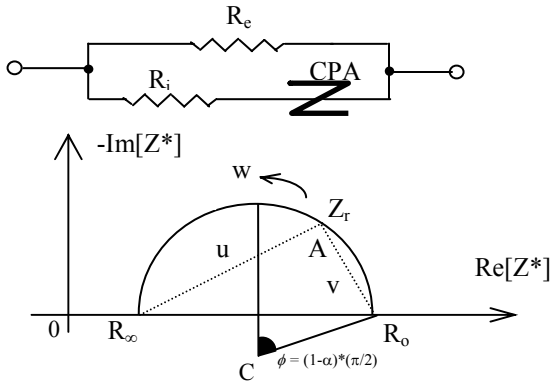


Fig. 1. Electrical equivalent circuit and the Cole-Cole Plot of tissues.

The Cole-Cole parameters, namely R_i, R_e, the characteristic frequency f_c where Im[Z*] is maximum and the phase angle α, satisfy the following equation:

$$Z^* = R_\infty + \frac{R_0 - R_\infty}{1 + \frac{R_i + R_e}{K}(j2\pi f)^\alpha} \qquad (1)$$

where $\alpha \leq 1$, K a constant, and R_0 and R_∞ the resistances at $f = 0$ and $f = \infty$ respectively: $R_0 = R_e$ and $R_\infty = \frac{R_e \cdot R_i}{R_e + R_i}$.

C_m is calculated from;

$$C_m = \frac{1}{2\pi f_c [R_i + R_e]^{1/(1-\alpha)}} \qquad (2)$$

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

II. METHODOLOGY

Materials and Method

The blood samples were collected at the Blood Bank of Marmara University Hospital from 51 healthy male donors.

Standard units (450 ± 45 mL) of blood were drawn from each donor into the main pediatric blood bag and the erythrocyte suspension samples were prepared [8]. All blood samples were stored at +4°C.

Physiological and electrical measurements of erythrocyte suspensions were performed at room temperature [9].

To evaluate changes in these parameters with storage time, Variance Analysis (ANOVA) was used. The p value of 0,001, was considered extremely significant. Means ± SDs were calculated as descriptive statistics within the data group. Physiological parameters were expressed in terms of electrical parameters by using multiple regression analysis in SPSS.

III. RESULTS AND DISCUSSION

R_e and C_m are directly, and R_i is inversely proportional to the donors hematocrit *Ht* or packed cell volume *PCV* [1]. The normalization of the electrical parameters was done with reference to their 0^{th} day hematocrit value, i.e. volume fraction of red blood cells in the surrounding medium

(SAGM + CPD + residual plasma): $(R_e)_n = (R_e)_{meas}/h$, $(R_i)_n = (R_i)_{meas}.h$ and $(C_m)_n = (C_m)_{meas}/h$, *with* $h = Ht/100$.

As seen from Figure 2, the Cole-Cole plot of erythrocyte suspensions has shifted upwards with decreasing □. Very little change is seen in the characteristic frequency f_c as the storage period increased.

It has been observed that, measured parameters such as *2,3-DPG, ATP, pH*, extracellular [Na^+], [K^+] and [Cl^-] were within the range of published data (Fig. 3) [10-11].

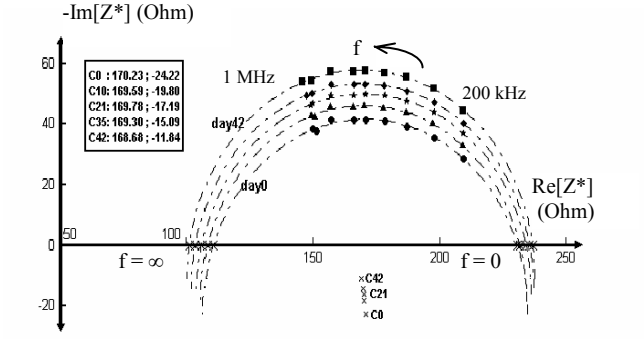


Fig. 2. Cole-Cole plot of erythrocyte suspensions under storage.

Stored red blood cells (RBC) were depleted of *2,3-DPG,* following the 1st or 2nd week of storage and they have a left-shifted oxygen dissociation curve, greater oxygen affinity, and supply less oxygen to tissues [12]. The mean value of *2,3-DPG* decreased to 16% of its initial value on the 21st day and then slowly decayed until the 42nd day. When the cell ionic structure is considered, as expected, the surrounding medium (extracellular) Na^+ and Cl^- have decreased and K^+ increased. Decreasing *ATP* during storage affected the Na^+- K^+ pump [5]. On day 0, the mean extracellular K^+ was 2,1 ± 1,0 mEq/L and this has increased to 45,3 ± 3,7 mEq/L on the 42nd day (Figure 3(a)).

The surrounding fluid *pH* has decreased as it can be seen from Figure 3(b) because of glycolysis with lactic acid formation [13]. The mean extracellular *pH* of 7,5 ± 0,2 (day 0) diminished slightly to 6,5 ± 0,2 by day 42. As shown in Figure 3(g), in storage conditions, *ATP* fell down remarkably as the red blood cells have no mitochondria and they can not regenerate adenosine triphosphate [11]. The mean *ATP* value on day 0 was 5,4 ± 0,5 μmol/gHb and a gradual decrease was observed with 1,6 ± 0,5 μmol/gHb (30%) on day 35. By the 42nd day, only 10% of the mean initial value was measured.

During storage, the extracellular resistance and the cell membrane capacitance increased progressively while the intracellular resistance decreased as seen from Figure 4(a), 3(c) and 3(e).

Results of the regression analysis applied to electrical and physiological parameters of erythrocyte suspensions are given in Figure 3(a)-3(i).

Figure 3(a) and Figure 3(c) illustrate the correlations of R_i and R_e with the surrounding K^+ ion concentrations; R_i and R_e were highly correlated with K^+.

All electrical parameters were significantly correlated with *pH* and *ATP*. Since the *pH* level controls the movement of cell ions, electrical parameters were affected by the *pH* shifts. Fig. 3(b), 3(d) and 3(f) illustrate the strong *pH* dependence of R_i, R_e and C_m . The effects of *ATP* on the Na^+-K^+ pump are given in Fig. 3(g), 3(h) and 3(i).

2,3-DPG was not correlated at all with any of the electrical parameters, *2,3-DPG* does not have any measurable effect on electrical parameters.

Changes in ion concentrations, *pH* and *ATP* affected the electrical properties of blood directly.

During storage, increased permeability of the membrane resulted 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 [14].

Lower *ATP* results in expulsion of lactate to the surrounding medium, decreasing the *pH*. The movement of band 3-1 spectrin inside the phospholipids of the cell membrane which is due to reduced *pH*, generates ionic currents, namely Cl^- ions. While Cl^- ions enter the cell, bicarbonate ions leave the cell [4],[6]. The cell swells with Cl^- and a shape change occurs from discocyte to spheroechinocyte shape, resulting in an increased form factor [9]. The extracellular resistance is inversely proportional to this form factor. The observed increase in R_e of erythrocyte suspensions indicates that ion transportation is dominant during storage. 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 have influenced C_m negatively by being adsorbed to the surface of the membrane [3].

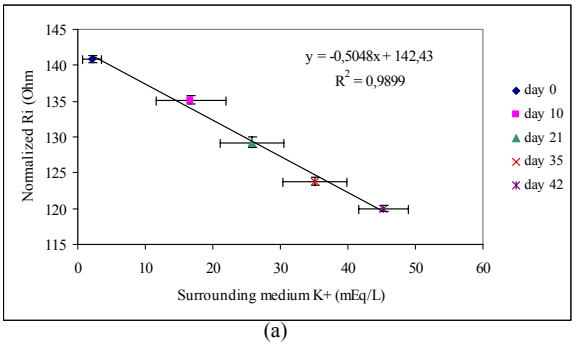
The Multiregression Analysis

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 physiological parameters of blood bank stored blood. Before modeling, multicollinearity was investigated between the independent variables R_i, R_e, C_m and α. Multicollinearity is the undesirable situation where the correlations among the independent variables are strong.

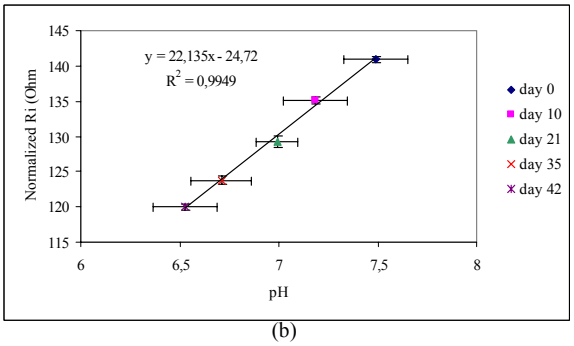
The indicators of multicollinearity (tolerance, variance inflation factor (VIF), the eigenvalues and variance decomposition proportions) were statistically examined and it is seen that only C_m and α are correlated.

Hence, the independent parameters R_i, R_e, C_m and their higher order products were considered in the multiple regression analysis with the SPSS.

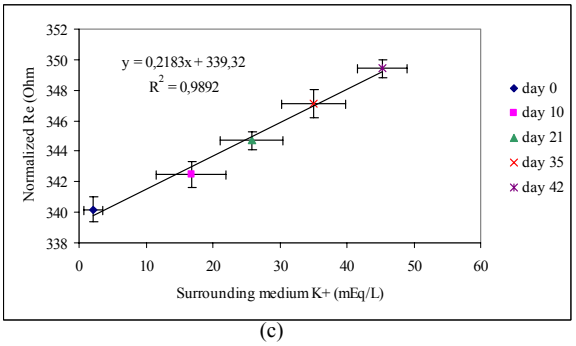
The following general equation was derived by applying the standard 'backward elimination' variable selection procedure to the data from the training set. Y(R_i, R_e, C_m) represents here the physiological variable of interest:



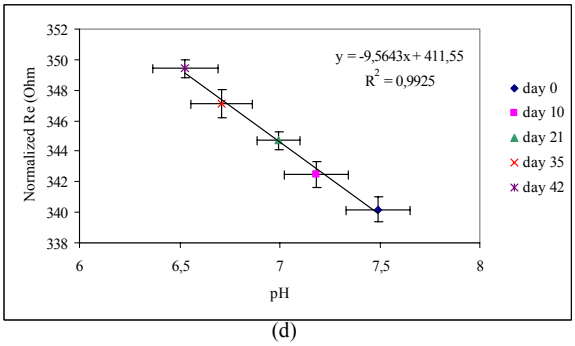
(a)



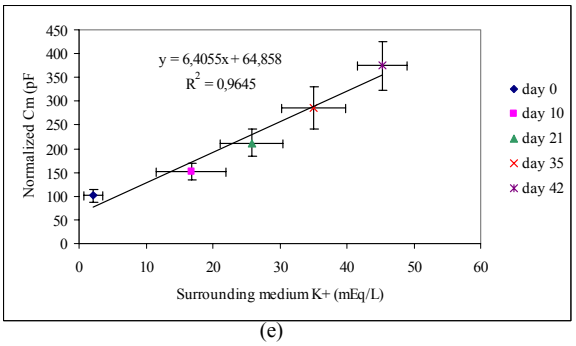
(b)



(c)



(d)



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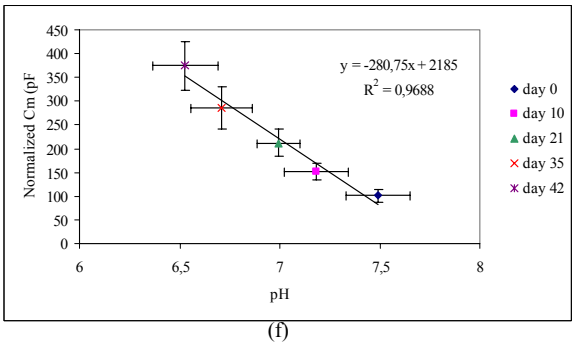

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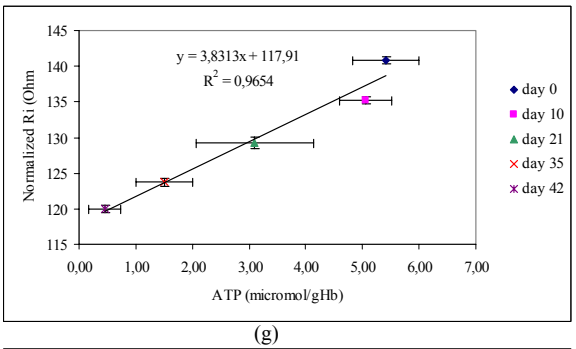
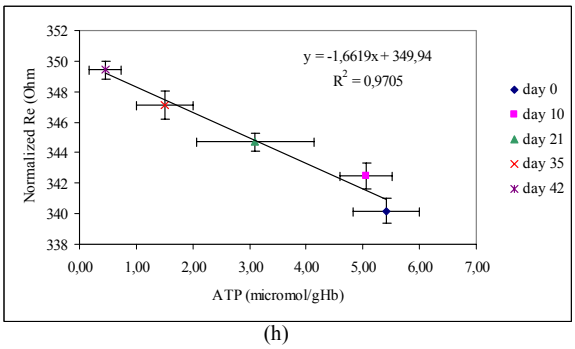


(g)



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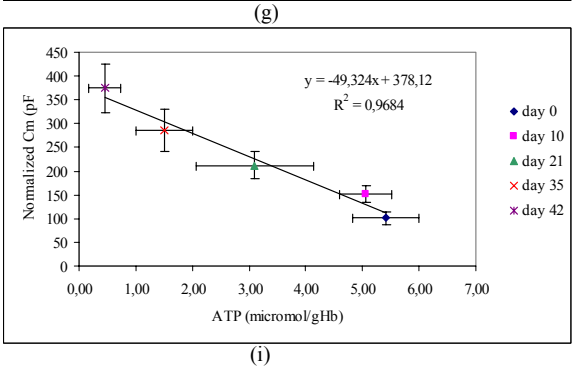

(i)

Fig.3. Electrical v.s. physiological parameters of erythrocyte suspensions. Each data point is mean ± SD of 51 donors.

$$Y(R_i,R_e,C_m) = a_0 + a_1R_i^3 + a_2R_e^3 + a_3C_m^3 + a_4R_iR_e^2 + a_5R_i^2C_m + a_6R_eC_m^2 \quad (3)$$

The units of the electrical parameters are (Ohm) for R_i and R_e, (pF) for C_m. The constants of Equation 3 are given in Table 1.

Table 1. Coefficients of Equation 3. (R^2 is the regression coefficient.)

	Na^+ mEq/L	K^+ mEq/L	Cl^- mEq/L	pH	DPG mmol/L	ATP µmol/gHb
a_0	*-184,9*	*56,81*	*109,4*	*3,02*	*-4,87*	*-3,74*
a_1	*-3,26 10^{-4}*	*3,25 10^{-5}*	*-4,29 10^{-5}*	*7,82 10^{-7}*	*9,26 10^{-6}*	*-9,27 10^{-7}*
a_2	*-3,92 10^{-5}*	*7,81 10^{-6}*	*-1,12 10^{-5}*	*2,06 10^{-9}*	*8,81 10^{-7}*	*-4,46 10^{-7}*
a_3	*-2,55 10^{-6}*	*2,65 10^{-7}*	*-1,37 10^{-6}*	*3,85 10^{-9}*	*-3,38 10^{-8}*	*1,37 10^{-7}*
a_4	*1,73 10^{-4}*	*-2,82 10^{-5}*	*3,60 10^{-5}*	*1,19 10^{-7}*	*-3,33 10^{-6}*	*1,73 10^{-6}*
a_5	*-2,64 10^{-5}*	*5,29 10^{-6}*	*-1,31 10^{-5}*	*1,41 10^{-7}*	*-1,63 10^{-7}*	*8,66 10^{-7}*
a_6	*5,57 10^{-6}*	*- 6,37 10^{-7}*	*2,88 10^{-6}*	*-1,2 10^{-8}*	*5,83 10^{-8}*	*-1,24 10^{-8}*
R^2	0,88	0,80	0,83	0,92	0,76	0,85

To test the validity of these equations, an external blood sample set from 20 donors were considered. The resistance of the extracellular and intracellular fluids and, the cell membrane capacitance were all normalized with respect to the 0^{th} day hematocrit value of the test sample, calculated from the equation:

$$Ht\ (\%) = -4{,}2\ R_i\ (Ohm) + 489{,}5 \quad (R^2 = 0{,}98) \quad (4)$$

Normalized electrical parameters were then plugged into the model equations (Table 1) to predict the physiological parameters on the day of withdraw and during 10th, 21st, 35th and 42nd days of storage. When compared with the already measured physiological data, *Na^+, K^+, Cl^-, pH* and *ATP* values were in close agreement with a rms error of less than 6,1% (Table 2). However, *2,3-DPG* can not be estimated at all, at any time.

Clearly, there exists dependence between the electrical parameters and physiological behaviour of RBC's and it has been shown that, it is possible to calculate the physiological parameters such as *Na^+, K^+, Cl^-, pH* and *ATP* with the Cole-Cole parameters obtained from multifrequency complex electrical impedance measurements of erythrocyte suspensions.

Hence, the impedance measurement technique may become a very useful technique in the quality prediction of the blood bank stored erythrocyte suspensions prior to their usage.

Table 2. The (%) rms errors.

Days	Na^+	K^+	Cl^-	pH	2,3-DPG	ATP
0	3,5%	5,2%	4,3%	1,8%	31,7%	5,1%
10	3,0%	5,1%	6,5%	2,5%	31,1%	4,6%
21	3,0%	6,1%	3,6%	1,7%	62,2%	4,9%
35	5,6%	4,5%	5,1%	2,6%	60,4%	5,0%
42	5,9%	4,7%	5,8%	3,5%	47,9%	5,2%

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