ORIGINAL ARTICLE

Physiological quality assessment of stored whole blood by means of electrical measurements

Y. Ülgen · M. Sezdi

Abstract The physiological parameters of blood such as extracellular Na^+, K^+, Cl^-, pH, 2,3-DPG and ATP and the complex electrical impedance were measured using whole blood samples from 31 male donors (21 donors form the training set and ten donors were used for testing), on the 0th, 10th and 21st day of blood bank storage. During storage, while the extracellular fluid resistance (R_e) and the intracellular fluid resistance (R_i) decreased progressively with time, the effective cell membrane capacitance (C_m) has increased. Blood bank storage resulted in a rise in K^+ and a fall in Na^+, Cl^-, pH, 2,3-DPG and ATP. Accordingly, all electrical parameters correlated with Na^+, K^+, Cl^-, pH and ATP, at varying levels. By applying the multi-regression analysis, it was demonstrated that R_i, R_e and especially C_m were appropriate for the assessment of Na^+, K^+, Cl^-, pH and ATP until the 21st day of storage.

Keywords Whole blood · Blood storage lesions · Multifrequency bioimpedance · Whole blood · Blood bank

1 Introduction

The bioimpedance measurement of the passive electrical properties of tissues is an emerging tool for the medical practice. It requires low-cost instrumentation and is safely and easily applicable in practice with online monitoring capability.

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Coulter counting, measurement of hematocrit and monitoring of cell cultures are possible. Diagnostic methods such as impedance plethysmography, impedance cardiography and impedance pneumography are based on monitoring the volume changes by electrical impedance changes of tissues [5, 29].

Multifrequency bioimpedance is used for the separation of fat and fluid compartments of the body, and also for biological tissue characterization as a potential technique for functional medical imaging [18].

It has been shown that electrical impedance measurements might be useful for monitoring red blood cell ageing and assessing the quality of stored red blood cells [44]. The quality of in vitro storage of blood focuses mainly on maintaining both viability and functional capacity as close as possible as to prior to storage conditions. Prior to release, the quality (viability) of the blood bank stored blood units could be characterized by measuring physiological parameters, especially the ATP level [44]. However, current techniques are time consuming and expensive. The studies about the electrical properties of blood show that, the electrical parameters can be a potential index for evaluating blood in clinical applications [42–46].

The electrical impedance of blood is affected by the physical and chemical changes, such as cell swelling and shape transformation, loss of surface charges and increase in ion permeability of cell membranes, during storage [6, 8, 11, 13, 15, 17, 23, 27, 37, 40, 41].

In this study, the effect of each physiological parameter on the electrical parameters was investigated and appropriate equations were obtained using multiple regression analysis. This study characterized the electrical properties of stored whole blood units as a possible means for evaluating the suitability for transfusion.

2 Materials and methods

2.1 Blood samples

The blood samples from 31 donors were collected at the Blood Bank of Marmara University Hospital in Istanbul. Only male donors were chosen to stay in a narrow range of hematocrit values. Volunteers were tested to exclude Hepatitis B, Hepatitis C, HIV and syphilis infectivities (Fig. 1).

Standard units (450 ± 45 mL) of blood were drawn from each donor into the main pediatric blood bag that contained 63 ml of citrate–phosphate–dextrose (CPD) (Fig. 2). The pediatric blood bag consists of four bags, approximately 110 ml of whole blood was transferred into the first satellite bag for the 0 day measurement.

Tenth and 21st day measurements were performed using the second and the third satellite bags respectively, with 110 ml of blood transferred into it, each time. The purpose of using pediatric bags was to have access to whole blood samples in a closed system avoiding any bacterial contamination. Blood bags were stored at + 4°C.

2.2 Method

2.2.1 Biochemical measurements

Physiological parameters (Fig. 3), the extracellular Na^+, K^+ and Cl^- concentrations, 2,3-DPG, ATP and pH were measured at room temperature as described in the earlier study [36].

Prior to extracellular (surrounding medium) Na^+, K^+ and Cl^- measurements, blood samples were pipetted into 5-ml tubes and then centrifuged for 5 min, at 2,000 g. The supernatant plasma was separated and then analyzed according to the standard methods using the 917 HITACHI chemical analyzer.

Prior to 2,3-DPG and ATP measurements, blood samples were 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°C in the freezer [12], for 3 months until the measurements were performed. 2,3-DPG analysis (UV-test at 340 nm) was
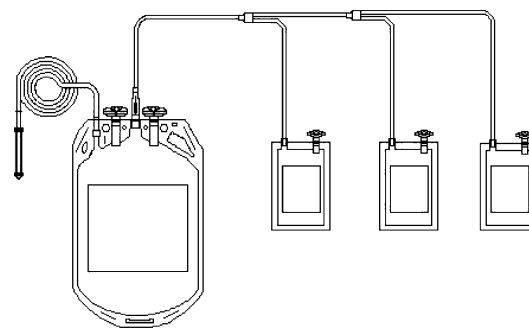

Fig. 2 Pediatric CPD blood bag system (Kansuk blood bags [22])
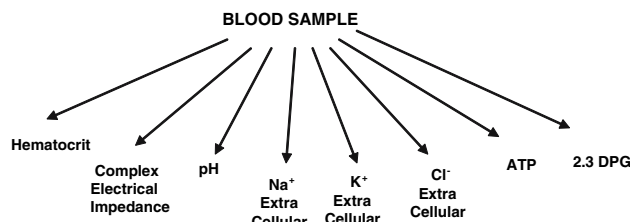

**Fig. 3** The measured parameters

performed on the PU 8620 PHILIPS spectrophotometer and the ATP analysis (bioluminescence test) was run on the Berthold luminometer by using the assay kits. Blood gas meter (Avlomni, Roche) was used for extracellular pH measurements. All measurements were performed at room temperature.

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

2.2.2 Electrical measurements

The complex electrical impedance of blood is modeled in terms of R_i and R_e, the resistances of intracellular and extracellular fluids and, the effective cell membrane capacitance C_m (Fig. 1a). Because red blood cells have non-uniform shapes, ages, structures and electrical time

Fig. 1 a Electrical equivalent circuit of biological tissues. **b** The Cole–Cole plot of the impedance between terminals A and B
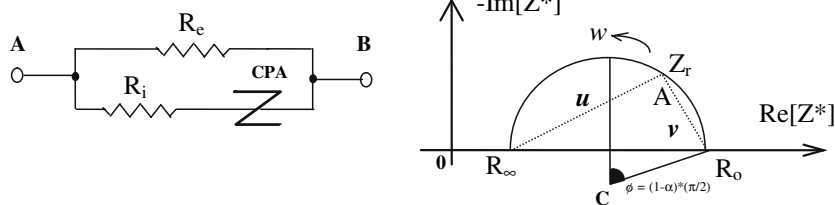
constants, the capacitive effects of cell membranes are lumped in a constant phase angle impedance $Z_{CPA} = K(j\omega)^{-\alpha}$ [19].

The imaginary component against the real part of the complex impedance in Fig. 1a, as a function of frequency fits a semicircle with the center depressed below the real axis, known as the Cole–Cole plot, for biological tissues (Fig. 1b) [30, 32, 38].

The Cole–Cole parameters, namely R_i, R_e, the characteristic frequency f_c where the imaginary part is maximum, and the phase angle α which represents the degree of homogeneity among the cells, satisfy the following equation:

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

where $\alpha \leq 1$, $R_\infty = \frac{R_e R_i}{R_e + R_i}$, $R_0 = R_e$ and K a constant.

R_0 and R_∞ are the resistances at $f = 0$ and $f = \infty$, respectively. C_m is obtained from;

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

Four probe complex electrical impedance measurements were done in a conductivity cell of volume 3.0 cm³ [20, 25, 31, 39], consisting of a cylindrical plastic tube, with four stainless steel needle electrodes placed by piercing the plastic tube at equal interelectrode distances (Fig. 4) [34]. The impedance of blood between lines +V and –V in the conductivity cell is represented by the electrical circuit in Fig. 1a. The conductivity cell was calibrated using salines of varying concentrations.
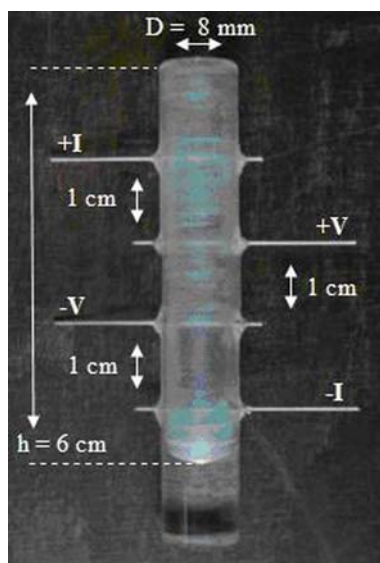
Measurements were performed at room temperature in the frequency range from 100 kHz to 1 MHz using the 4284A HP LCR Meter [1, 2, 7, 44].

The measurements were all corrected for gain-phase characteristics of the preamplifier and fitted into the Cole–Cole model [33].

Electrical properties of blood change with the orientation and deformation of red blood cells [10, 28]. The blood samples used in this study are isotropic as they are stationary with the red blood cells randomly aligned in the conductivity cell.

To evaluate changes in blood parameters with storage time, variance analysis (ANOVA) was used. Data significance was determined by the Student–Newman–Keuls Multiple Comparisons Test, using the SPSS (version 11) statistical package. A P value of 0.001 is considered extremely significant. Mean ± SD was calculated as descriptive statistics within the data group.

3 Results

R_e and C_m are directly, and R_i is inversely proportional to the donors' hematocrit (Ht) of blood samples [44]. The influence of the hematocrit is eliminated by normalization of electrical parameters with reference to their 0th 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.

As seen from Fig. 5, Cole–Cole plots of whole blood shift upwards decreasing α and suggesting a change in f_c, as the storage period increased.

The characteristic frequency of stored erythrocytes which corresponds to the largest imaginary part on the Cole–Cole plot is 600–800 kHz, within the measurement frequency range. In earlier studies [3, 4, 24, 43], the characteristic frequency always stayed below 1 MHz.
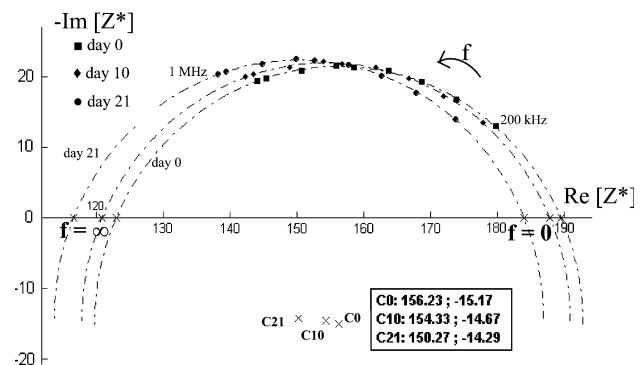

Fig. 4 The conductivity cell

Fig. 5 Cole–Cole plot of whole blood under storage (*C0*, *C10* and *C21* refer to the centers of the circles on storage days 0, 10 and 21, respectively)

Stored RBCs were depleted of 2,3-DPG (Table 1) and they have greater oxygen affinity, and supply less oxygen to tissues [14]. 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 [14]. Table 1 shows that 2,3-DPG mean value decreased to 19% of its initial value on the 21st day.

As shown in Table 1, in storage conditions, ATP fell down remarkably as the red blood cells have no mitochondria and they could not regenerate adenosine triphosphate [16]. The mean ATP value on day 0 was 5.1 ± 0.4 μmol/gHb and on the 21st day, 54% of the mean initial value was presented. The extracellular pH has decreased as it can be seen from Table 1 because of glycolysis with lactic acid formation [27]. The mean extracellular pH of 7.4 ± 0.1 (day 0) decreased to 7.0 ± 0.1 by day 21.

During storage, as expected, the extracellular Na^+ and Cl^- decreased and K^+ increased, respectively, as shown in Table 1. Sodium levels fell while potassium levels increased [37]. On day 0, the mean extracellular K^+ was 3.5 ± 1.0 mEq/L and this has increased to 22.8 ± 3.6 mEq/L by day 21. On the 21st day of storage, the initial mean values of 173.6 ± 4.7 mEq/L of Na^+ and 79.8 ± 3.8 mEq/L of Cl^- have decreased to 138.5 ± 5.9 mEq/L and 66.6 ± 3.3 mEq/L, respectively.

Relative absolute changes in electrical and biochemical parameters during storage are displayed in Table 2. Besides K^+, it is seen that C_m and 2,3-DPG are the most outstanding parameters showing the largest variations during the 21 day period. C_m parameter seemed to be the one best suited for electronically evaluating blood samples.

Results of the regression analysis applied to electrical and physiological parameters of whole blood are given in Figs. 6, 7 and 8. Figures 6d–f and 7d–f illustrate the correlations of R_i and R_e with the surrounding ion concentrations (Na^+, K^+ and Cl^-); R_i and R_e were highly correlated with Na^+, K^+ and Cl^-.

Among the ions, only Na^+ had a second degree quadratic regression with R_i and R_e (Figs. 6d, 7d). Figure 8d, f shows that, C_m had a second degree quadratic regression with Na^+ and Cl^-. C_m was highly correlated with Cl^- ($R^2 = 1.0$) compared to other electrical parameters ($R^2 = 0.97$).

All electrical parameters were also significantly correlated with pH and ATP. Since the pH level controls the movement of cell ions, electrical parameters were affected by the pH shifts. Figures 6c, 7c and 8c 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 Figs. 6b, 7b and 8b.

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

4 Discussion

The physiological changes affect the electrical properties of blood directly. The measured impedance is mainly affected by the resistivity and volume of each fluid and by

Table 2 Relative changes in electrical and biochemical parameters

Parameter	Storage time (%)	
	10 days	21 days
R_i (Ω)	–2.9	–6.3
R_e (Ω)	–1.1	–2.3
C_m (pF)	17.9	65.4
Na^+ (mEq/L)	–8.6	–20.2
K^+ (mEq/L)	301.7	554.4
Cl^- (mEq/L)	–10.2	–16.5
pH	–2.7	–5.3
2,3-DPG (mmol/L)	–70.9	–81.4
ATP (μmol/gHb)	–14.8	–46.0

Table 1 The actual data of measured parameters (biochemical parameters were measured in plasma and electrical measurements performed on whole blood)

Parameter	Day 0		Day 10		Day 21	
	Mean	σ	Mean	σ	Mean	σ
R_i (Ω)	378.31	25.31	367.43	24.66	354.51	23.47
R_e (Ω)	175.95	12.16	173.96	11.95	171.91	11.83
C_m (pF)	94.43	14.59	111.30	18.10	156.23	24.25
Na^+ (mEq/L)	173.56	4.66	158.60	4.22	138.54	5.91
K^+ (mEq/L)	3.49	1.07	14.02	2.52	22.84	3.59
Cl^- (mEq/L)	79.78	3.76	71.62	2.70	66.61	3.26
pH	7.39	0.07	7.19	0.09	7.00	0.09
2,3-DPG (mmol/L)	1.99	0.19	0.58	0.09	0.37	0.07
ATP (μmol/gHb)	5.07	0.38	4.32	0.46	2.74	0.54

Fig. 6 Intracellular resistance versus **a** 2,3-diphosphoglycerate concentrations, **b** ATP concentrations, **c** pH, **d** extracellular Na$^+$ concentrations, **e** extracellular K$^+$ concentrations, **f** extracellular Cl$^-$ concentrations. Each data point is mean ± SD of 31 donors
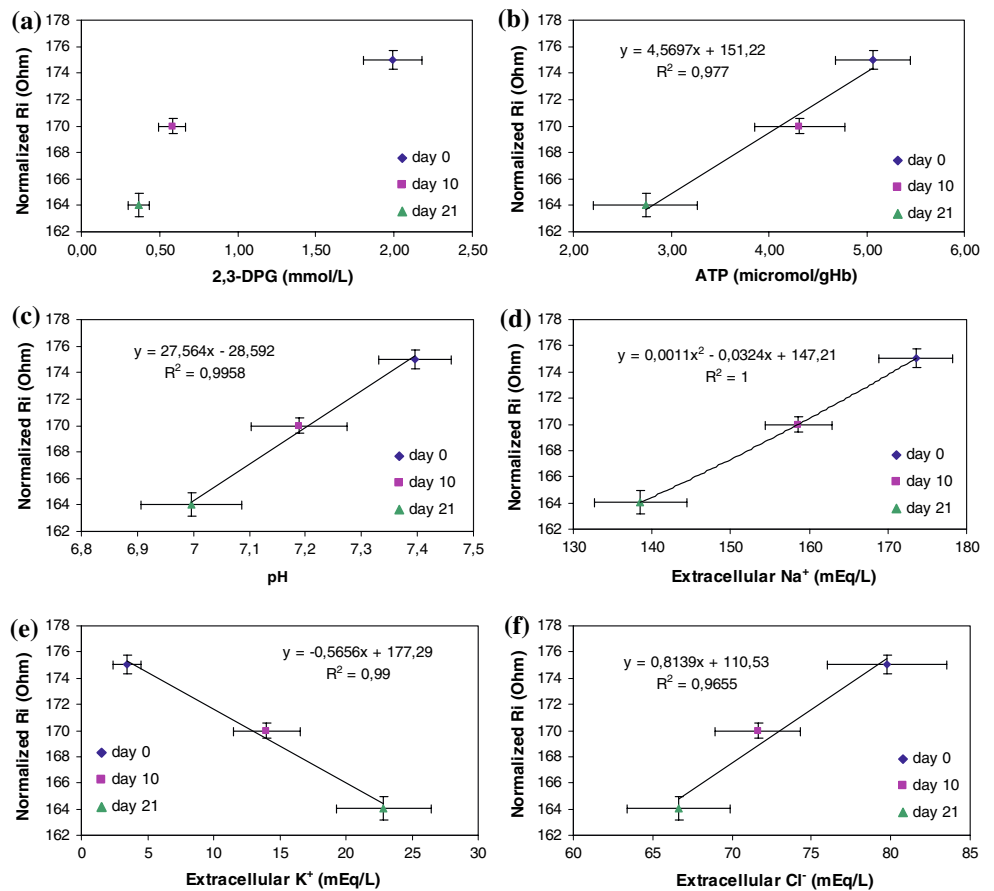
the geometrical shape of the cells [10, 21]. The decrease in the resistance of the extracellular fluid can also be explained by the cell shape changes.

During storage, Cl$^-$ ions enter the cell, the cell swells and its shape changes from discocyte to spheroechinocyte, resulting in an increased form factor in the Maxwell–Fricke equation [9, 13]. 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 the dominant factor during storage. The effective membrane capacitance C_m augmented with storage time progressively as a 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 [44].

4.1 The multiregression analysis

Na$^+$, K$^+$ and Cl$^-$ concentrations, pH and ATP all showed strong dependence on R_i, R_e, C_m and α. The physiological parameters are expressed in terms of electrical parameters. 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 eigen values 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 are considered in the multiple regression analysis using the SPSS. The following general equation is derived by applying the standard "backward elimination" variable selection procedure to the data from the training set (21 donors). Y (R_i, R_e, C_m) represents the physiological variable of interest:

$$Y(R_i, R_e, C_m) = a_0 + a_1 R_i^3 + a_2 R_e^3 + a_3 C_m^3 + a_4 R_i^2 C_m + a_5 R_e C_m^2 + a_6 R_i$$

$$(3)$$

The units of the normalized electrical parameters are (Ω) for R_i and R_e, pF for C_m.

To test the validity of these equations, an external set of ten donors were considered. The resistance of the extracellular and intracellular fluids and, the cell membrane

Fig. 7 Extracellular resistance versus **a** 2,3-diphosphoglycerate concentrations, **b** ATP concentrations, **c** pH, **d** extracellular Na$^+$ concentrations, **e** extracellular K$^+$ concentrations, **f** extracellular Cl$^-$ concentrations. Each data point is mean ± SD of 31 donors
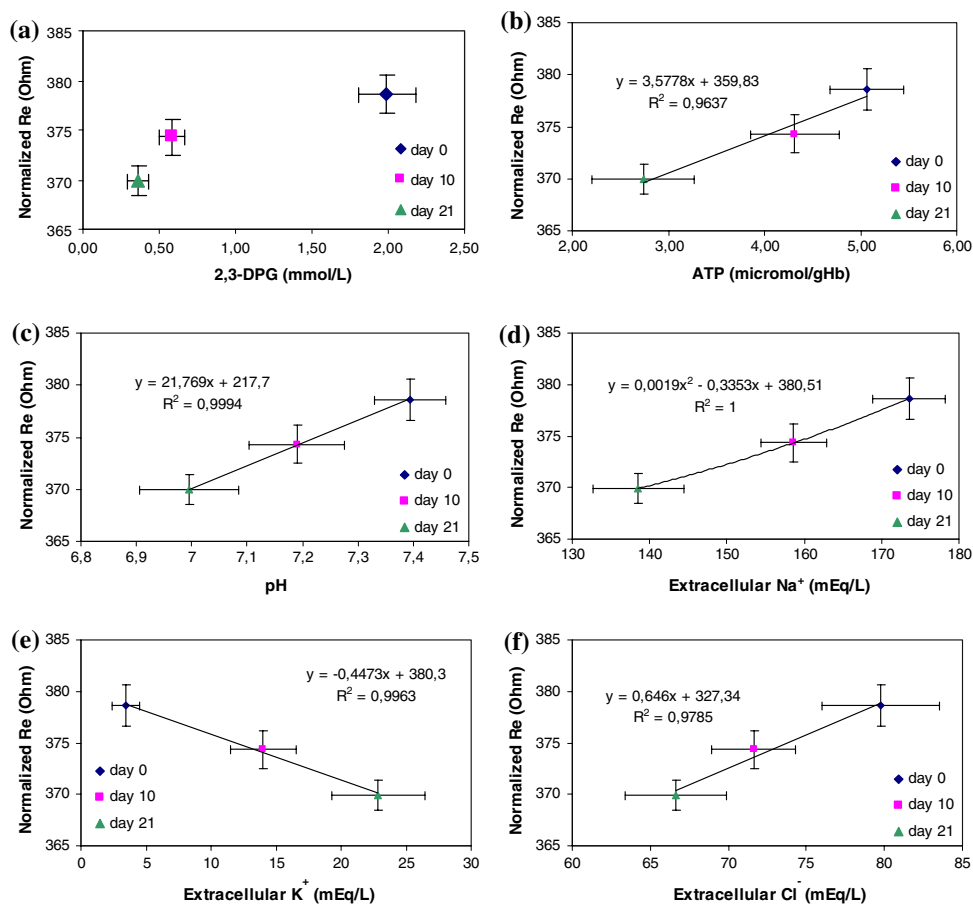
capacitance were all normalized with respect to the 0th day hematocrit value of the test samples [35]:

$$Ht(\%) = -7.9 R_i(\Omega) + 743.96 \ (R^2 = 0.99) \qquad (4)$$

Normalized electrical parameters were then plugged into Eq. (3) for the assessment of the physiological parameters, on the day of withdrawl and on the 10th and the 21st days of storage.

When compared with the measured physiological data, calculated Na$^+$, K$^+$, Cl, pH and ATP values were in close agreement, with a rms error of less than 7.0%. 2,3-DPG, however, cannot be predicted at all, at any time.

For the determination of blood bank stored blood quality, in general, the ion concentrations are detected by hemolysis. Hemolysis, however, can lead to deterioration of its quality due to hemoglobin and potassium leakage from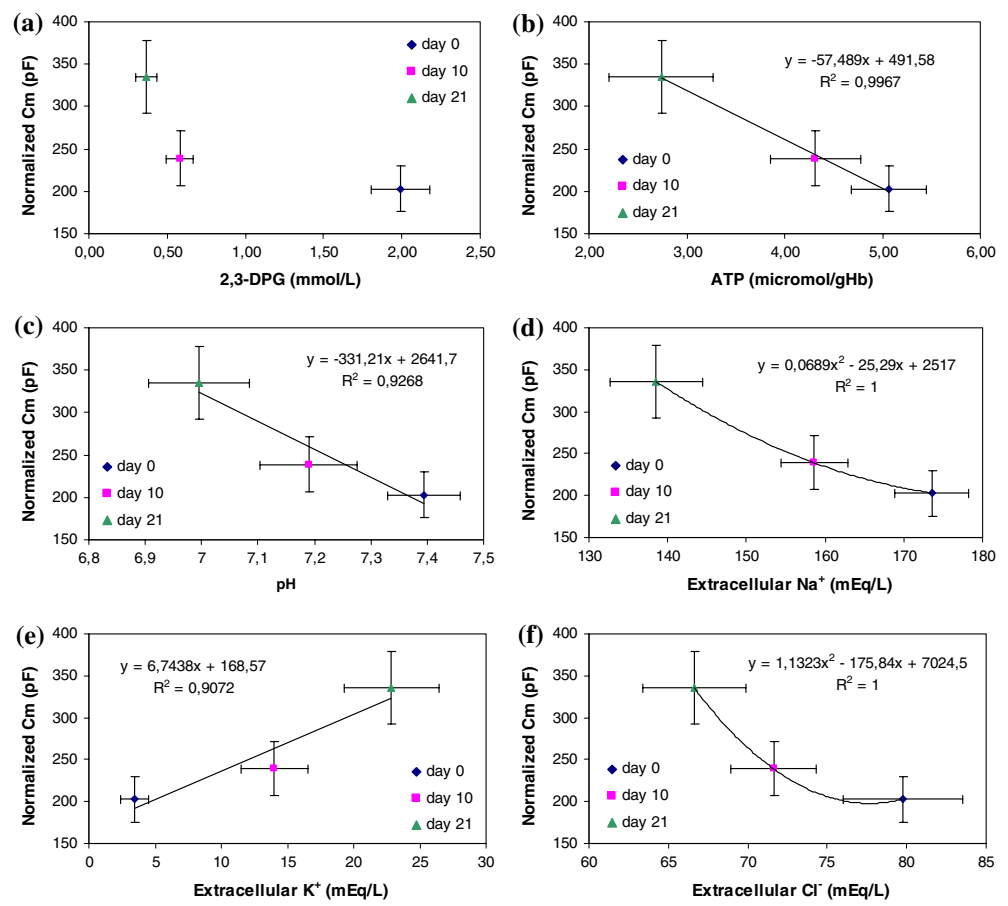 erythrocytes [26]. By establishing relationship between the physiological and the electrical parameters of blood, bioimpedance technique can be suggested as an alternative method.

Bioimpedance of stored blood should also be taken into account when impedance cardiography is post-operatively used to determine cardiac output in patients with blood transfusion, since the ion balance will be disturbed [5].

5 Conclusion

Since the electrical impedance measurement method has many advantages over the physiological measurement techniques, because of its low-cost, rapid and easy performance, impedance measurement technique may become a very useful technique for predicting the quality of blood bank storage whole blood samples.

Fig. 8 Membrane capacitance versus **a** 2,3-diphosphoglycerate concentrations, **b** ATP concentrations, **c** pH, **d** extracellular Na$^+$ concentrations, **e** extracellular K$^+$ concentrations, **f** extracellular Cl$^-$ concentrations. Each data point is mean ± SD of 31 donors

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