

# Conductivity of Normal and Pathological Human Erythrocytes (Homozygous $\beta$ -Thalassemia) at Radiowave Frequencies

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The conductivity of normal and homozygous  $\beta$ -thalassemic erythrocyte suspensions has been measured over the frequency range from 5 KHz to 100 MHz in the temperature interval from 5 to 45 °C.

The electrical parameters of the membrane, *i.e.*, the capacitance  $C_M$  and the conductance  $G_M$  per unit surface have been calculated from an expression given by Hanai for the conductivity of a suspension of ellipsoidal particles covered with a shell.

Some interesting differences between the normal and pathological state are evidentiated.

## Introduction

It is well known that biological materials [1], in particular biological cell suspensions, display dielectric and conductometric dispersions in the radio-frequency range.

These dispersions (generally termed  $\beta$ -dispersions) are explained in terms of Maxwell-Wagner mechanism [2], considering the cell as a conducting particle covered with a less conducting membrane. For values of the capacitance and conductance of the membrane of the order of  $1 \mu\text{F}/\text{cm}^2$  and  $0.1 \text{ mho}/\text{cm}^2$  respectively, this dispersion occurs at about  $10^6$ – $10^7$  Hz, depending on shape and electrical properties of the dispersed particles.

At frequencies approaching 100 MHz, the capacitance of the cell membrane has a negligible reactance and the suspension behaves, in a first approximation, as formed of inclusions dispersed in the extracellular electrolyte, displaying only the ordinary Maxwell-Wagner effect which occurs, for conductivities of the order of  $10 \text{ mmho}/\text{cm}$ , at microwave frequencies.

Among the biological cell suspensions, the red blood cells have been widely studied by a variety of experimental methods [3, 4] to make evident

possible structural alterations which might occur in pathological states.

In particular, various studies [5, 6] have provided evidence for differences in the membrane of homozygous  $\beta$ -thalassemic red blood cells either in lipid alteration or in the rigidity and in a different structuring action on the internal aqueous environment. The damaged membrane could also explain, the metabolic changes found in the cation permeability [6].

The homozygous  $\beta$ -thalassemia [7] is a genetic disorder characterized by a reduced or non-synthesis of  $\beta$ -globin chains in the human hemoglobin molecule. The excess of unstable  $\alpha$ -chains precipitates in the erythrocytes forming inclusion bodies, like the Heinz bodies, which attack the membrane thereby facilitating erythrocyte lysis.

In order to obtain further informations on the modifications occurring in the homozygous  $\beta$ -thalassemic erythrocyte membrane, conductometric measurements in the range of frequency where the  $\beta$ -dispersion occurs, may be usefully employed.

In this study, the conductometric behaviour of human erythrocyte suspensions, both in normal and pathological state (homozygous  $\beta$ -thalassemia) was observed over a frequency range from 5 KHz to 100 MHz at two different NaCl concentrations of the suspending medium, in the temperature interval

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from 5 to 45 °C. The characteristic parameters of the membrane, *i.e.*, the capacitance  $C_M$  and the conductance  $G_M$  for unit surface have been estimated both for normal and  $\beta$ -thalassemic erythrocytes and some interesting differences have been evidenced. In particular, the lower value of  $G_M$  observed in pathological membrane in comparison with that of normal one, may reflect in a reduction of the protein content and/or in a structural modification of the lipid bilayer.

## Experimental

Blood was drawn by venipuncture from normal, and homozygous  $\beta$ -thalassemic donors. Cells were washed three times in physiological saline solution (5 mM Naphosphate, pH = 7.4, 0.15 M NaCl) and then resuspended in the same solution to the desired hematocrit of 15% and 30%.

The sample with a hematocrit of 15% was suspended in a more conductive saline solution ( $\sigma = 23.5$  mmho/cm at 25 °C). Hematological characterization of the samples was performed using a Royco-Cell 920A Counter.

The conductivity measurements were carried out by means of commercially available bridges, Vector Impedance Meters HP mod. 4800A and HP mod. 4815A in the frequency range from 5 KHz to 500 KHz and 0.5 MHz to 100 MHz respectively.

The measuring cell consists of a circular cylindrical guide containing the sample which behaves as a waveguide excited far beyond its  $TM_{01}$  cut-off frequency, thus utilizing in the measurements only the stray fields of the coaxial line-waveguide transition.

The cell, similar to that employed elsewhere can be described by a capacity  $C_0$ , which acts as the dielectric probe, with a shunt capacity  $C_1$  and a series inductance  $L$ .

The input impedance is

$$Z = i \omega L + \frac{1}{i \omega (\epsilon^* C_0 + C_1)}$$

where  $\epsilon^*$  is the complex dielectric constant of the sample ( $\epsilon^* = \sigma^*/i \omega \epsilon_0$ ).

The conductivity  $\sigma$  is given by

$$\sigma = \frac{\epsilon_0 |Z| \cos \varphi}{C_0 \{ |Z|^2 - 2 \omega L |Z| \sin \varphi + \omega^2 L^2 \}}$$

where  $|Z|$  and  $\varphi$  are the measured quantities (modulus of the total impedance and phase angle),  $\epsilon_0$  is the absolute dielectric constant of vacuum,  $\omega$  the angular frequency. The parameters  $C_0$ ,  $C_1$ ,  $L$  are determined by calibration measurements with various reference liquids, following the procedure suggested by Bottomley [10].

The errors in the conductivity measurements were estimated within 2% in the whole frequency range, for conductivity of about  $10^{-3} \div 10^{-2} \Omega^{-1} \text{cm}^{-1}$ .

The temperature was varied from 5 to 45 °C within 0.1 °C. No evidence for alterations of the sample due to temperature was observed.

## Results and Discussion

Figs. 1 and 2 show the conductivity dispersions of normal and pathological erythrocyte suspensions with an hematocrit of 30 and 15% at a temperature of 25 °C.

For comparison and clarity of presentation, the measured conductivity of the physiological saline solution is also reported.

A trait-thalassemic erythrocyte suspension with an hematocrit of 15% in physiological saline solution was also examined, but no appreciable difference with the normal sample within the experimental errors, was evidenced (Fig. 2).

As can be seen, all the curves show the presence of marked electrode polarization effect in the lower frequency range (up to about 100 KHz) due to the high value of the d.c. ionic conductivity of these samples.

This phenomenon is related to the existence of an electrical double layer at the metal-solution interface that behaves electrically as a capacitance dependent on frequency of the applied field, solution conductivity, electrode surface area and perhaps other variables.

Methods for minimizing and correcting this type of polarization are not applied here, since the conductivity dispersions occur at sufficiently high frequencies, where the electrode polarization effect become negligible.

The conductivity curves were analyzed in terms of a single Debye-type dispersion

$$\sigma = \sigma_0 + \frac{(\sigma_\infty - \sigma_0) \omega^2 \tau^2}{1 + \omega^2 \tau^2}$$

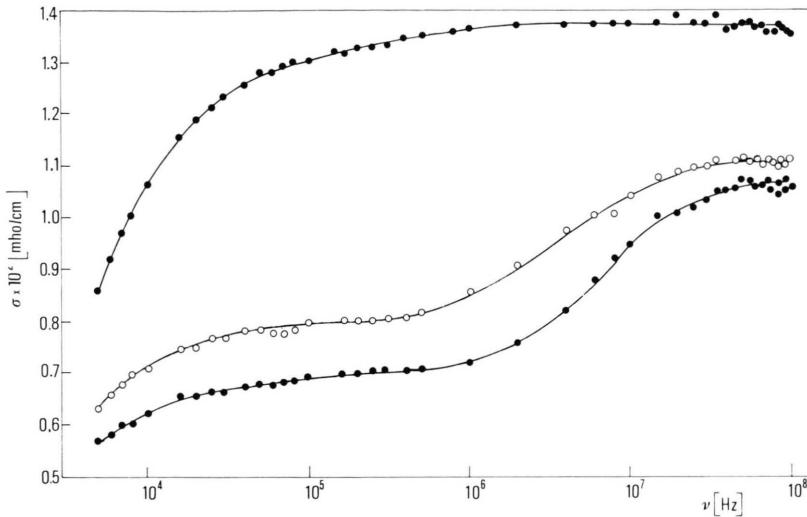


Fig. 1. Conductivity of erythrocyte cells in physiological saline solution as a function of frequency, at the temperature of 25 °C. Fractional volume of the dispersed phase  $\varphi = 0.30$ . (●) Homozygous  $\beta$ -thalassemic erythrocyte suspension; (○) normal erythrocyte suspension. The upper curve represents the conductivity of the saline solution, in the same frequency range.

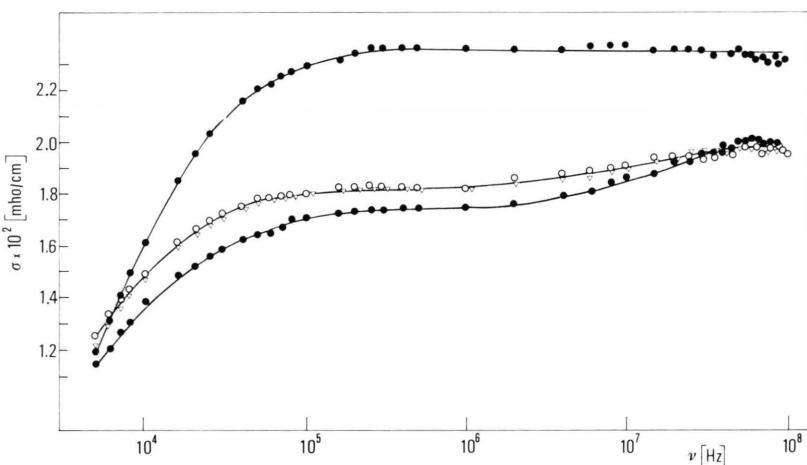


Fig. 2. Conductivity of erythrocyte cells in saline solution as a function of frequency, at the temperature of 25 °C. Fractional volume of the dispersed phase  $\varphi = 0.15$ . (●) Homozygous  $\beta$ -thalassemic erythrocyte suspension; (▽) trait-thalassemic erythrocyte suspension; (○) normal erythrocyte suspension. The upper curve represents the conductivity of the saline solution in the same frequency range.

where  $\sigma_0$  and  $\sigma_\infty$  are the limiting values at low and high frequency respectively, and  $\tau$  is the relaxation time characteristic of the dispersion.

In Figs. 3–5 these three parameters are shown as a function of temperature for normal and pathological suspensions of the two different hematocrit values.

The conductivity of a suspension of ellipsoidal particles covered with a shell is given by [11]

$$\frac{\sigma^* - \sigma_m^*}{\sigma^* + 2\sigma_m^*} = \frac{\Phi}{9} \sum_{i=1}^3 \frac{\bar{\sigma}_i^* - \sigma_m^*}{\sigma_m^* + (\bar{\sigma}_i^* - \sigma_m^*) A_i} \quad (1)$$

where

$$\bar{\sigma}_i^* = \sigma_s^* \left\{ 1 + \frac{n(\sigma_p^* - \sigma_s^*)}{\sigma_s^* + (\sigma_p^* - \sigma_s^*)(1-n)A_i} \right\} \quad (2)$$

$$(i = 1, 2, 3)$$

is the equivalent complex conductivity of the particle with the shell;  $\sigma^*$  and  $\sigma_m^*$  are the complex conductivities of the suspension and the suspending medium respectively,  $\sigma_s^*$  is the complex conductivity of the shell,  $\Phi$  is the fractional volume of the dispersed particle;  $A_i$  are the polarization factors

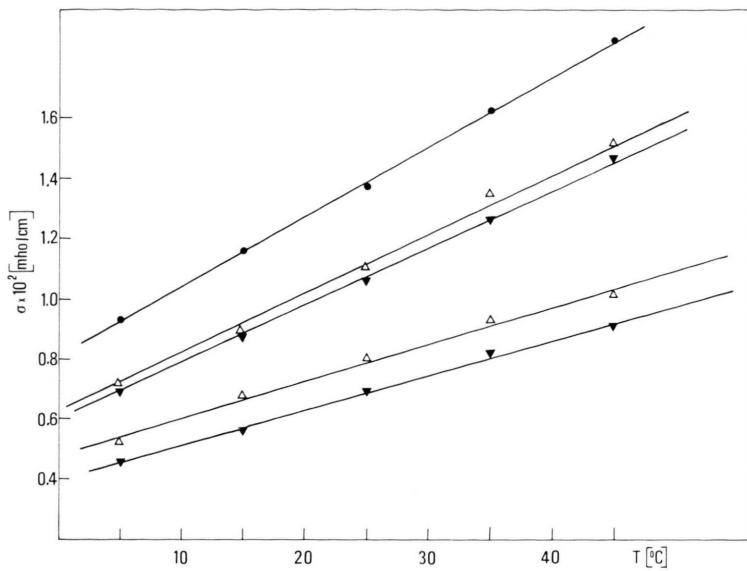


Fig. 3. Limiting conductivity parameters  $\sigma_0$  and  $\sigma_\infty$  as a function of temperature for suspensions with hematocrit of 30%. (▼) Homozygous  $\beta$ -thalassemic erythrocytes; ( $\Delta$ ) normal erythrocytes; ( $\bullet$ ) physiological saline solution.

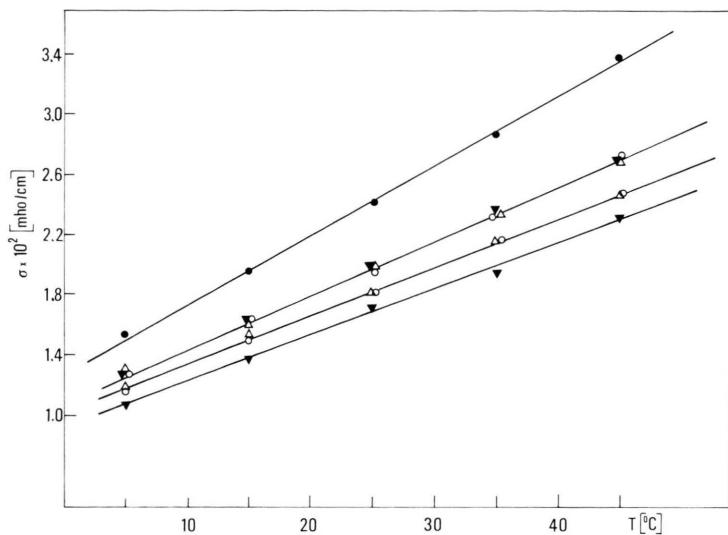


Fig. 4. Limiting conductivity parameters  $\sigma_0$  and  $\sigma_\infty$  as a function of temperature for suspensions with hematocrit of 15%. (▼) Homozygous  $\beta$ -thalassemic erythrocytes; ( $\circ$ ) trait-thalassemic erythrocytes; ( $\Delta$ ) normal erythrocytes; ( $\bullet$ ) saline solution.

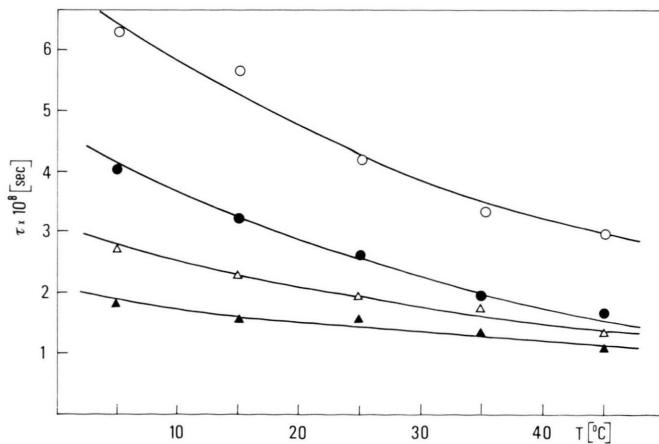


Fig. 5. Relaxation time  $\tau$  of erythrocyte suspensions as a function of temperature. Homozygous  $\beta$ -thalassemic erythrocytes: ( $\bullet$ )  $\varphi = 30\%$ ; ( $\blacktriangle$ )  $\varphi = 15\%$ ; normal erythrocytes: ( $\circ$ )  $\varphi = 30\%$ ; ( $\Delta$ )  $\varphi = 15\%$ .

and  $n$  takes into account for the volume of the shell;  $n = (a_0 - d)(b_0 - d)^2/a_0 b_0^2$  where  $d$  is the membrane thickness. For an oblate spheroid with semiaxes  $a_0 < b_0 = c_0$ ,  $A_i$  reduce to

$$A_1 = \frac{a_0 b_0^2}{3} \int_0^\infty \frac{d\xi}{(a_0^2 + \xi)^{3/2} (b_0^2 + \xi)};$$

$$A_2 = A_3 = \frac{1}{2} (1 - A_1).$$

In the derivation of the above relations, the outer and inner surface of the shell are considered two confocal ellipsoids, and consequently, the shell thickness is assumed not uniform. Moreover, it was assumed that the axial ratio for the outer surface is nearly equal to that of the inner surface. These requirements are fulfilled in biological cells, since the membrane thickness, is generally negligible in comparison with the cell diameters.

In the most general case, the medium inside the cell, the external medium and the cell membrane show complex conductivities including both dielectric constant  $\epsilon$  and d.c. conductivities  $\sigma$ , *i.e.*

$$\begin{aligned} \sigma_m^* &= \sigma_m + i\omega\epsilon_0\epsilon_m; \\ \sigma_s^* &= \sigma_s + i\omega\epsilon_0\epsilon_s; \\ \sigma_p^* &= \sigma_p + i\omega\epsilon_0\epsilon_p. \end{aligned} \quad (3)$$

Here, the dielectric constants are assumed as real quantities, since this analysis is carried out at radiowave frequencies and the dipolar relaxation of the aqueous phase occurs at microwave frequencies.

Substituting of Eq. (3) into Eqs. (1) and (2) yields the conductivity properties of the suspensions completely determined. In particular the dependence on  $\omega$  in Eq. (1) (second order polynomial in  $\omega$ ) suggests two separate frequency dependences of the relaxation process, corresponding to distinct Maxwell-Wagner polarization effects at the three phase boundaries.

In the case under test however, the dispersion due to the two bulk media, owing to their conductivity values (of the order of  $10^{-2} \div 10^{-3} \Omega^{-1} \text{cm}^{-1}$ ), occurs at very high frequencies (at about  $10^3 \text{ MHz}$ ), well beyond the frequency range experimentally investigated in this work.

Thus, the observed dispersion at lower frequency must be recognized as the  $\beta$ -dispersion.

The analysis of the data, according to Eq. (1), was carried out as follows.

The erythrocyte is simulated [11] with an oblate spheroid of  $8 \mu\text{m}$  in major diameter and  $2.4 \mu\text{m}$  in minor diameter, and the thickness of the membrane is assumed to be  $50 \text{ \AA}$ .

The phase parameters  $\epsilon_s$ ,  $\sigma_s$ ,  $\sigma_p$  have been determined by fitting the theoretical curve to the observed dispersion data with respect to the three parameters  $\sigma_0$ ,  $\sigma_\infty$ ,  $\tau$  which are characteristic of the dispersion curve.

The conductivity  $\sigma_m$  of the physiological saline suspensions was measured and  $\epsilon_m$  was assumed equal to that of pure water.

The value of  $\epsilon_p$  was derived from the analysis of dielectric data on erythrocyte suspensions carried out by Asami *et al.* [11] and a value of 66 at  $25^\circ\text{C}$  was assumed. This value, derived from dielectric data on a wide low-frequency range seems to be more appropriate than that calculated by Cook [12] applying the Fricke relation to whole blood at microwave frequencies.

The dielectric constant of the inner phase is lower than the suspending medium owing to membranous organelles, lipid granules and proteins inside the cell.

It must be noted however, that  $\epsilon_p$  and  $\epsilon_m$  have little influence on all the other conductivity phase parameters, in particular on  $\sigma_s$  and, even if in a different way, on  $\epsilon_s$ .

Following the procedure suggested by Hanai *et al.* [13] we have examined the influence on the limiting value of the conductivity and the relaxation time caused by changes in  $\epsilon_p$  and  $\epsilon_m$ . The results are that 30–40% variation on  $\epsilon_p$  and  $\epsilon_m$  reflects in a change of the dispersion parameters within 5%.

The membrane capacitance  $C_M$  and the membrane conductance  $G_M$  were calculated from the dielectric constant  $\epsilon_s$  and conductivity  $\sigma_s$  of the membrane by means of the equations  $C_M = \epsilon_0 \epsilon_s / d$  and  $G_M = \sigma_s / d$  when is assumed  $d \ll a_0, b_0$ .

The phase parameters obtained with the above procedure are listed in Table I for the normal and  $\beta$ -thalassemic erythrocyte suspensions examined.

As can be seen, the estimated values of  $C_M$  and  $G_M$  are independent, within the limit of the fitting procedure accuracy, of the volume fraction of the suspension and of the ionic conductivity of the outer medium.

These results observed both for normal and  $\beta$ -thalassemic erythrocyte suspensions agree with the circumstance that these parameters refer to the

Table I. Phase parameters  $C_M$ ,  $G_M$ ,  $\sigma_p$  obtained from Eq. (1) for normal and  $\beta$ -thalassemic erythrocyte suspensions.

$\varphi = 15\%$	$T [^\circ\text{C}]$	$\sigma_m [\text{m}\mu/\text{cm}]$	Normal erythrocyte suspension			Homozygous $\beta$ -thalassemic erythrocyte suspension		
			$C_M [\mu\text{F}/\text{cm}^2]^a$	$G_M [\text{v}/\text{cm}^2]^b$	$\sigma_p [\text{m}\mu/\text{cm}]$	$C_M [\mu\text{F}/\text{cm}^2]^a$	$G_M [\text{v}/\text{cm}^2]^b$	$\sigma_p [\text{m}\mu/\text{cm}]$
$\varphi = 15\%$	5	15.2	1.12	0.5	3.5	0.53	0.10	3.0
	15	19.0	1.10	1.7	5.2	0.53	0.11	5.0
	25	23.5	1.10	2.0	5.0	0.53	0.16	5.8
	35	28.7	1.08	2.8	5.0	0.56	0.20	6.0
	45	33.6	1.03	3.0	4.6	0.44	0.26	4.2
	$\varphi = 30\%$	5	9.4	1.12	3.0	0.53	0.10	2.8
	15	11.6	1.10	1.4	4.7	0.62	0.10	4.0
	25	13.8	1.10	2.2	6.4	0.53	0.16	5.3
	35	16.5	1.08	2.6	8.2	0.35	0.20	6.4
	45	18.6	1.03	3.0	9.6	0.44	0.26	8.1

<sup>a</sup> The membrane capacitance is calculated from  $C_M = \epsilon_0 \epsilon_{s/d}$ .

<sup>b</sup> The membrane conductance is calculated from  $G_M = \sigma_{s/d}$ .

cell membrane and consequently they must remain unchanged regardless of the bulk parameters of the system.

It should be noted, moreover, that the conductivity  $\sigma_p$  of the aqueous phase inside the cells are lower than those of the external medium at the corresponding temperature.

Since the erythrocyte cells include intracellular organelles, and a different ion content, particularly dissociated hemoglobin molecules at high concentration, in comparison with the saline solution, a direct comparison of  $\sigma_p$  with  $\sigma_m$  can not be discussed directly because of the obviously great structural complexity of this cell. On the other hand, Pauly and Schwan [14] have measured at 25 °C a value of 5.18 mmho/cm for the internal conductivity of erythrocyte cell, in good agreement with the value estimated from our measurements. The same results were also obtained by Hanai *et al.* from dielectric measurements on yeast cells [15], on erythrocytes [11] and on *Escherichia coli* suspensions [13] and by Schwan and Morowitz on pleuropneumonia-like organism [16]. As pointed out by Pauly and Schwan [14], the "ideal" internal conductivity of human erythrocytes, valued from the concentration and the limiting ionic conductance of the ionic species, should be at 25 °C about 14.5 mmho/cm.

The discrepancy with the measured value of about 5 mmho/cm is pronounced, thus suggesting that the ionic mobility in the aqueous phase inside the cells is lower than that of the outer medium or, perhaps, the erythrocyte cell membrane exerts

electrostatic effects which reduce the ionic conduction.

In the samples under investigation this parameter assumes approximately the same value both for normal and  $\beta$ -thalassemic erythrocyte cells.

A value of about 1.1  $\mu\text{F}/\text{cm}^2$  independent of temperature is obtained for the membrane capacitance of the normal cells. This value is in agreement with the mean value reported in literature for most biological cells ranging somewhere between 0.8  $\div$  1.3  $\mu\text{F}/\text{cm}^2$ .

On the other hand, a value of about 0.5  $\mu\text{F}/\text{cm}^2$  is estimated for the membrane of  $\beta$ -thalassemic erythrocyte cells. It is noteworthy that this value approaches that of a bilayer lipid membrane.

As pointed out by Fettiplace *et al.* [17], the difference between the membrane capacitance value ( $\sim 1 \mu\text{F}/\text{cm}^2$ ) of biological cells and lipid bilayer ( $0.4 \div 0.6 \mu\text{F}/\text{cm}^2$ ) may be due to the presence of proteins immersed in the hydrocarbon region of membranes. Thus, the lower value of  $C_M$  observed in pathological membrane suggests a decrease in the protein content of the cell membrane or conversely in a different protein interaction with the membrane lipids by means of hydrophobic associations of the non-polar residues with the lipid bilayer.

Typical membrane conductance values  $G_M$  range from 0.1 to 1 mho/ $\text{cm}^2$ .

For normal erythrocyte cells, we have estimated a somewhat greater value, approximately from 0.5 to 3.0 mho/ $\text{cm}^2$  as the temperature is increased from 5 to 45 °C.

It must be noted, however, that it may be difficult to extract this parameter with accuracy from the bulk data of the suspensions, since Eq. (1) yields an approximately constant conductivity increment for value of  $\sigma_s$  lower than  $5 \times 10^{-7} \Omega^{-1} \text{cm}^{-1}$ , when internal and external conductivities are of the order of  $5 \div 10 \text{ mmho/cm}$ . Consequently, changes in the  $\sigma_s$  values towards of  $10^{-7} \div 10^{-8} \Omega^{-1} \text{cm}^{-1}$  reflect into very low increase of the conductivity increment, which can not be experimentally observed.

Nevertheless, for the pathological erythrocytes, a value of  $G_M$  lower than about one order of magnitude, must be taken into account in comparison with those of normal cells. Moreover, its dependence on temperature is less pronounced.

This characteristic may be connected with defects or alterations, at a membrane level, present in the pathological state.

In a previous study [18], on the erythrocyte membrane in homozygous  $\beta$ -thalassemia carried out by means of electron spin resonance spectroscopy, it has been observed that the membrane outer surface is less fluid than in normal cell.

Since the lipid composition and concentration in pathological cell is the same as that in normal one, it is reasonable to suppose that the different fluidity and the lower membrane conductance must be due either to a different protein composition or perhaps to a different lipid distribution.

Since the transport properties of red cell membrane are partially governed by the membrane

proteins, which span the hydrophobic core of the lipid bilayer, a reduction in the membrane conductance may reflect a decreasing in the anion transport which is influenced by the transmembraneous proteins. On the contrary, the low value for the electrical conductance would not affect the transport of electroneutral complexes. Moreover, it must be noted that the inclusion bodies which attached themselves to the membrane, may contribute in the lowering the membrane conductance occluding the microchannels in the lipid bilayer and thus affecting the ionic transport equilibrium between cell and medium.

In conclusion, in this work we have shown how conductometric measurements in the frequency range where the  $\beta$ -dispersion occurs may offer suggestions on the structural state of the cell membrane in the  $\beta$ -thalassemia.

Further studies are in progress on the specific interaction of the cell membrane with cations different from those of the physiological solution, to investigate if the affected values of  $C_M$  and  $G_M$  influence the transport processes across the membrane.

#### Acknowledgement

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