SOME OBSERVATIONS ON THE DIELECTRIC PROPERTIES OF HEMOGLOBIN'S SUSPENDING MEDIUM INSIDE HUMAN ERYTHROCYTES

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ABSTRACT Dielectric permittivity and conductivity data for human erythrocytes exhibit two consecutive dispersions with frequency in the range from 0.1 to 250 MHz. The dispersion observed above 50 MHz yields a circle in the complex impedance plane with its center on the real axis, suggesting a Debye relaxation, for the erythrocyte interior medium, centered in the microwave frequency range as for bulk water. Moreover, the Maxwell mixture equation, indicates that this "free" water forms most of the suspending medium of hemoglobin macromolecules. These results extend to lower frequencies (earlier results obtained by Schwan) with biological tissues such as muscle, skin, or liver at microwaves frequencies.

INTRODUCTION

A general way to study electrical properties of biological material is through measurement of the frequency dependence of the permittivity and conductivity. Thus, among the published results on living tissues, Schwan and co-workers (1, 2) demonstrated that most of the tissue water has rotational mobilities similar to those in the bulk fluid. However, the electrical determination of the tissue water time constant requires difficult experiments carried out at very high frequencies (0.1–20 GHz). In this paper we propose to show that the transformation of dielectric permittivity and conductivity data into specific impedance data for a suspension of human erythrocytes does not require such high frequencies to determine essential electrical characteristics of the hemoglobin's suspending medium. The interior of erythrocytes has electrical properties in the frequency range from 10 to 200 MHz, consistent with those of normal water if the volume fraction taken by hemoglobin is appropriately considered and the rotational mobility observed in this frequency range is that of water.

METHODS

Blood was obtained by venous puncture, always from the same donor, and used as soon as possible after blood withdrawal, i.e., before 1 hr. We found several advantages in working on centrifuged samples with a high hematocrit (95% at least): First, we obtained more easily reproducible data; second, in the low frequency region the electrode polarization is lowered on account of the high impedance (3); and lastly, the small amount of trapped serum does not affect the determination of the electrical properties of the erythrocyte's interior medium by high frequency measurement.

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The sample holder has been described earlier (2); electrodes were 5 mm long and 2 mm in diameter. This sample holder allowed us to maintain a constant temperature (25 ± 0.2°C) in the bulk of the tested suspension.

Sample admittances were measured with Wheatstone-type impedance bridges. To measure a wide frequency range we had to combine two impedance bridges (Siemens Corp., Medical/Industrial Groups, Iselin, N.J., model for low frequencies <10 MHz, and Boonton "RX-meter" for higher frequencies, Boonton Electronics Corp., Parsippany, N.J.) on which we adapted the sample holder.

Experimental procedures concerning measurements, determination of the cell constants for the dielectric permittivity and conductivity, and corrections for series inductances of binding posts, are summarized by Takashima (4).

RESULTS

In Fig. 1, we present the variations of dielectric constant and conductivity data with the frequency for a sample of centrifuged human blood. Two dispersions appear on each curve: (*a*) in the lower frequency range (<20 MHz) the dispersion, termed β-dispersion by Schwan (5), is the most important and is caused by polarization of the cell membranes; (*b*) for frequencies above 50 MHz the dispersion is less pronounced. No real constant value is reached; the permittivity decreases continually whereas conductivity increases with increasing frequency. This is a strong indication of the presence of an additional relaxation process in this frequency region. This dispersion, termed γ-dispersion (5), shown in Fig. 2, is mostly the result of a relaxation process occurring inside the erythrocytes because the erythrocyte membrane appears to be practically short-circuited at frequencies above 50 MHz (2).

From this presentation of data it is difficult, if not impossible, to delimit the frequency range corresponding to each dispersion. We need to present the experimental results in a better way to draw a quantitative analysis of involved processes.

Another interesting result pertains to the dielectric constant value for frequencies above 150 MHz. The decrement vs. the water value corresponds to the amount of hemoglobin inside the erythrocytes (6).

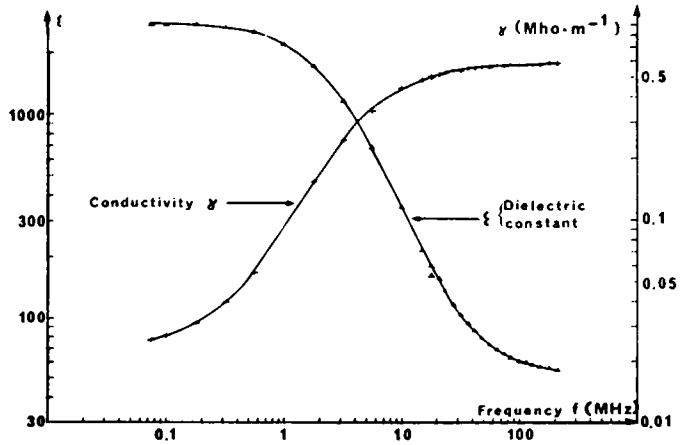

FIGURE 1 Dielectric permittivity ϵ and conductivity γ of packed human erythrocytes vs. frequency f for the whole frequency range (from 0.1 to 250 MHz).

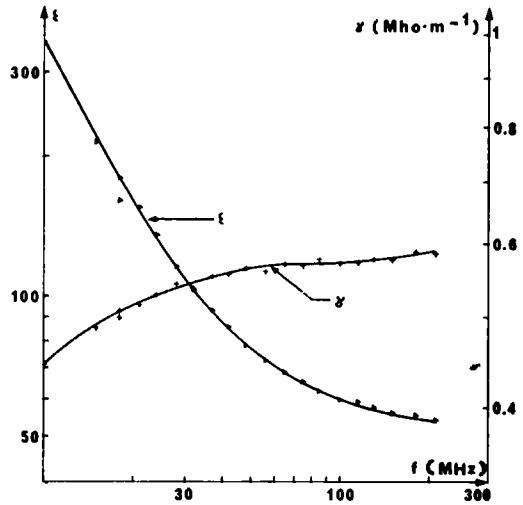

FIGURE 2 Dilelectric permittivity ϵ and conductivity γ of packed human erythrocytes vs. frequency f for the high frequency range (from 10 to 300 MHz).

ANALYSIS OF DATA

Definitions

The electrical properties of matter may be characterized in terms of an equivalent parallel combination of a conductance, G, and capacitance, ωC, the admittance of which, Y^*, is given by

$$Y^* = G + j\omega C, \tag{1}$$

where j is the imaginary operator, and $\omega = 2\pi f$ is the alternative electrical current pulsation of frequency f.

G and C are tied to conductivity, γ, and dielectric permittivity, $\epsilon\epsilon_r$, respectively by a same coefficient, k, that represents the shape and the size of the sample of matter:

$$Y^* = k(\gamma + j\omega\epsilon\epsilon_r), \tag{2}$$

with ϵ_r which is the dielectric permittivity of the free space:

$$\epsilon_r = \frac{1}{36\pi 10^9} \qquad \text{Farad} \cdot m^{-1},$$

ϵ which is the relative dielectric permittivity (or dielectric constant); it is a dimensionless term.

If one considers the admittance, Y^*, as being that of a conductor, it is therefore possible to define the complex conductivity, Γ^*

$$Y^* = k\Gamma^* \tag{3}$$

and,

$$\Gamma^* = \gamma + j\omega\epsilon\epsilon_r. \tag{4}$$

In the same way, if one considers the admittance, Y^*, as being that of a capacitor, it is possible to introduce the complex dielectric constant, ϵ^*, such that as

$$Y^* = jk\epsilon^*\epsilon_r\omega, \tag{5}$$

or

$$\gamma + j\omega\epsilon\epsilon_r = j\epsilon^*\epsilon_r\omega, \tag{6}$$

and

$$\epsilon^* = \epsilon - j\frac{\gamma}{\omega\epsilon_r}. \tag{7}$$

Moreover, the complex specific impedance, ρ^*, is defined as being the reverse of the complex conductivity

$$\rho^* = \frac{1}{\Gamma^*}, \tag{8}$$

or by separating the real part, A, and the imaginary part, B, of the complex specific impedance

$$\rho^* = A + jB, \tag{9}$$

with

$$A = \frac{\gamma}{\gamma^2 + (\omega\epsilon\epsilon_r)^2}, \tag{10A}$$

$$B = \frac{-\omega\epsilon\epsilon_r}{\gamma^2 + (\omega\epsilon\epsilon_r)^2}, \tag{10B}$$

Analytical Expressions of the Observed High Frequency Dispersion

The variations with the frequency of the specific impedance modulus, $|\rho^*|$, defined as such as

$$|\rho^*| = \sqrt{A^2 + B^2}, \tag{11}$$

are presented in Fig. 3.

The two previously cited dispersions appear to be shifted toward a lower frequency range making their distinction easier. However, the accurate determination of the "limit" values for each dispersion necessitates the representation of the reactance, $(-B)$, vs. A in the complex impedance plane; this yields a diagram comprising two consecutive circle arcs corresponding to the two relaxation processes (Fig. 4).

An enlargment of the lower left area on the Fig. 4 is presented in Fig. 5. The circle arc,

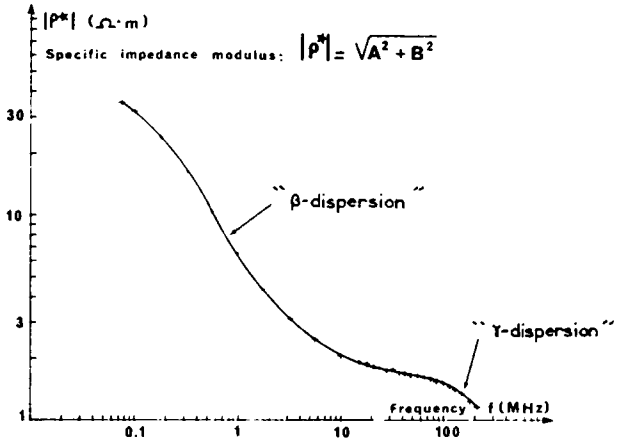

FIGURE 3 Specific impedance modulus $|\rho^*|$ of packed human erythrocytes vs. frequency f.

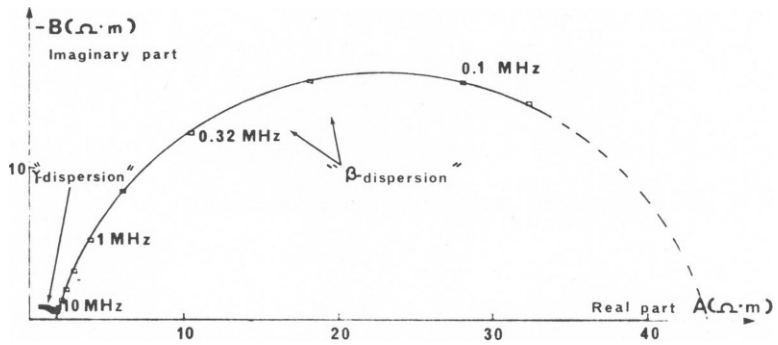

FIGURE 4 Specific impedance diagram of packed human erythrocytes.

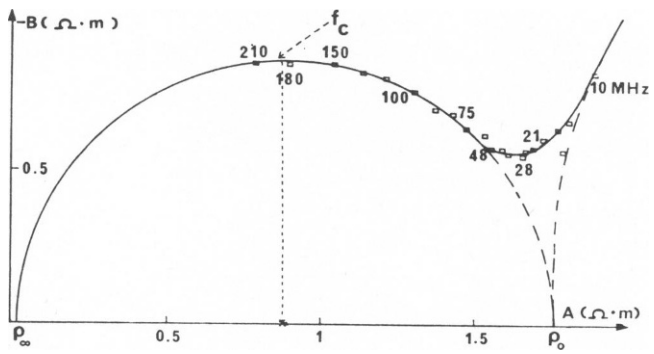

FIGURE 5 Specific impedance diagram of packed human erythrocytes drawn for high frequencies (In fact it is an enlargement of the lower left area on the Fig. 4).

drawn for high frequencies (>50 MHz), is the real image of a relaxation process located in the erythrocyte interior medium (2). It cuts diametrically across the real axis of the complex impedance plane in two singular points corresponding respectively to the low (ρ_0) and high (ρ_∞) frequency limit of the dispersion under consideration. Such a circular diagram can be verified to represent a dispersion having a single time constant (7), i.e. a Debye-type dispersion, (8), obeying the relation:

$$\rho^* = \rho_\infty + \frac{\rho_0 - \rho_\infty}{1 + j\omega T}, \tag{12}$$

where T is the time constant, linked to the characteristic frequency, f_c, by the relationship:

$$2\pi f_c T = 1. \tag{13}$$

where f_c is the frequency for which the reactance, $(-B)$, is maximum.

This circular presentation in the complex impedance plane, of course, demands a circular presentation in the complex admittance plane.

From the Eq. 8 and 12 we are able to write the complex conductivity

$$\Gamma^* = \frac{1}{\rho_\infty} + \frac{\frac{1}{\rho_0} - \frac{1}{\rho_\infty}}{1 + j\omega T\left(\frac{\rho_\infty}{\rho_0}\right)}. \tag{14}$$

It is the equation of a dispersion having a single time constant (8), T_1:

$$T_1 = T\left(\frac{\rho_\infty}{\rho_0}\right). \tag{15}$$

The real part, γ, of the complex conductivity is also frequency dependent:

$$\gamma = \frac{1}{\rho_0} + \left(\frac{1}{\rho_0} - \frac{1}{\rho_\infty}\right)\frac{(\omega T)^2\left(\frac{\rho_\infty}{\rho_0}\right)^2}{1 + (\omega T)^2\left(\frac{\rho_\infty}{\rho_0}\right)^2}, \tag{16}$$

or

$$\gamma = \gamma_0 + (\gamma_\infty - \gamma_0)\frac{(\omega T_1)^2}{1 + (\omega T_1)^2}, \tag{17}$$

after we have checked that

$$\gamma_0 = \frac{1}{\rho_0}, \tag{18A}$$

and

$$\gamma_\infty = \frac{1}{\rho_\infty}, \tag{18B}$$

for the frequency limit values of the dispersion under consideration.

Moreover, it is possible to get from the imaginary part, $j\omega\epsilon\epsilon_r$, of the complex conductivity Γ^*, an expression representing the frequency dependence of the dielectric constant, ϵ, with the same time constant, T_1.

This analysis leads to the important result that a proportionality factor, (ρ_∞/ρ_0), links the relaxation time constant, T_1, to the time constant of the impedance dispersion, T. So, we can write that the critical relaxation frequency, f_{c_1}, corresponding to a maximum of absorbed energy in the sample of matter (9), is related by the ratio (ρ_0/ρ_∞) to the characteristic frequency, f_c, defined only from the maximum value of the reactance $(-B)$:

$$f_{c_1} = f_c\left(\frac{\rho_0}{\rho_\infty}\right). \tag{19}$$

This general result can be used with data obtained from measurements carried out on solutions or suspensions insofar as the "limit" values can be extrapolated from their representation.

Evaluation of the Critical Relaxation Frequency, f_{c_1}

We have applied this analysis to our problem of impedance measurements on a sample of packed human erythrocytes. From Fig. 5, we have been able to extrapolate the values of f_c, ρ_0, and ρ_∞, this latter being not very accurate: f_c = 190 MHz, ρ_0 = 1.75 ± 0.01 Ωgm, π_∞ = 0.02 ± 0.01 Ω·m, and finally we find a critical value for the conductivity dispersion in the microwave frequency range, f_{c_1} = 16.5 GHz, a value roughly identical to that of pure water at 25°C (10–12).

The identically computed values from measurements carried out on several different aqueous solutions with different ionic strengths (KCl, 0.1 M; NO_2Na, 0.1 M; KOH at pH 8 . . .), agree with the values for bulk water.[1]

Thus, Fig. 1 and 2, on which are plotted only the dielectric permittivity and conductivity variations with the frequency, simply indicate an additional relaxation process over the β-dispersion for both the conductivity and the dielectric permittivity of tightly packed human erythrocytes. On the other hand, the specific impedance diagram in the complex plane reflects with more significance the two dispersions and permits calculation of the time constant of the γ-dispersion. The value obtained in this way for an erythrocyte suspension is comparable to that found by Schwan and Foster (1) after consideration of data obtained by measurements at very high frequencies (0.1–10 or more GHz) on such heterogeneous tissues as muscle, skin, liver, etc. . . .

Finally, we can conclude that the free water mainly influences the frequency dependence of the conductivity in the high frequency region of the spectrum brought into play for the erythrocyte suspension measurements.

SOME COMMENTS ON THE AMOUNT OF FREE WATER INSIDE THE ERYTHROCYTES

When the frequency increases over 200 MHz, we can see in Fig. 2 that the dielectric permittivity reaches a value, close to 50 U, which is not due to the specific structure of the

[1]Jenin, P. On the transformation of admittance data into impedance. Personal communication.

erythrocyte suspension (2). The decrement of 27 U compared with water can be explained by the amount of hemoglobin inside the erythrocytes. Indeed, with the hypothesis that hemoglobin's suspending medium has the same dielectric properties as free water, we are able to estimate a theoretical value for the dielectric constant of the erythrocyte interior. For the high frequency limit of the dispersion under consideration and for spherical suspended particles, the Maxwell mixture equation (13) is valid in the form:

$$\frac{\epsilon - \epsilon_w}{\epsilon + 2\epsilon_w} = p\frac{\epsilon_p - \epsilon_w}{\epsilon_p + 2\epsilon} \tag{20}$$

where ϵ represents the dielectric permittivity of the erythrocyte interior; ϵ_w represents the dielectric permittivity of the continuous hemoglobin's suspending medium; we take $\epsilon_w = 78.3$, a recent value proposed for water at 25°C (12); ϵ_p is the dielectric permittivity of the core of suspended particles, we choose $\epsilon_p = 2.5$, generally cited value for dry hemoglobin (14); p is the volume occupied by hemoglobin in the erythrocyte interior medium, always, the hemoglobin concentration is of ~35–37 g for 100 cm^3 of erythrocytes; considering an hematocrit of 95% at least, if we assume a specific volume of 0.75 for hemoglobin (15), the volume fraction of dry hemoglobin in the sample is ~0.25–0.265.

Finally, the calculation gives value for ϵ between 52.1 and 55.4, slightly above the experimental limit value (cf., Fig. 2). These values correspond to dielectric decrements that roughly agree with the results of Schwan and Li (6) at microwaves frequency concerning the specific decrement of solutions of hemoglobin in water. The small discrepancy between theoretical values and the experimental limit value arises because we did not take into account the water bound to hemoglobin. Indeed, because the Maxwell mixture equation implies that the suspended particles do not influence the dielectric properties of the suspending medium, the calculation is concerned only with erythrocyte free water content. Therefore, the agreement between theoretical values and experimental values means the bound water has only a small effect on the dielectric permittivity of the erythrocyte interior. Thus, the dielectric measurements, carried out on concentrated suspensions of erythrocytes, show that most of the erythrocyte water content exhibits rotational mobilities similar to those in the pure liquid.

CONCLUSIONS

Dielectric permittivity and conductivity data which pertain to a sample of concentrated erythrocytes are presented for the frequency range from 0.1 to 250 MHz. The originality of this work consists in showing that the transformation of data into specific impedance allows a determination of the time constant of relaxation processes occurring at very high frequencies (20 GHz for example). The following conclusions can be drawn: (*a*) In the complex impedance plane the arc circle with its center on the real axis corresponds to a conductivity dispersion located in the microwave frequency range as for bulk water. (*b*) Assuming the hemoglobin's suspending medium has the same dielectric properties as free water and using the generally cited values for the hemoglobin concentration inside erythrocytes, we calculate a theoretical value of the dielectric permittivity that roughly agrees with the experimental high frequency limit value of the dielectric permittivity. The small discrepancy is due to the fact

that we did not take into account the water bound to hemoglobin. (*c*) Not only at microwave frequencies but also at lower frequencies cell water behaves as free water.

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