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MODELING OF DIELECTRIC PERMITTIVITY OF THE ERYTHROCYTES MEMBRANE AS A THREE-LAYER MODEL

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Abstract. The dielectric theories which used for applications of dielectric spectroscopy to investigate heterogeneous systems, such as particle suspensions, membranes, and tissue are described in this article. The dielectric constant ε', the loss ε'', and the conductivity σ of substances in a broad frequency range (1 Hz to 10^{11} Hz), with for using a combination of different method of dielectric spectroscopy applied to identical samples was described. In the present work, we analyze the dispersion regions, commonly found in biological substances and tissue, usually as termed as α, β, δ and γ-dispersions. The nature of the dispersion caused by the membrane of RBCs in the radio frequency spectrum of the dielectric properties of blood which often is ascribed to the motion of bound water molecules is not yet fully understood. Dielectric properties of RBS membrane have been studied by means of the spheroidal model, where the three shells correspond to the double lipid bilayer and the spectrin network of the inner membrane, respectively. Based on the Maxwell-Wagner model and takes into account the morphological parameters of RBS membranes of cells using appropriate approximations the some simple relations was derived. In discussing the dielectric properties of RBS membrane was considered the mathematical model which accurately fit the experimental observations. Such models have obvious applications when characterizing the data and testing it against possible physical theories.

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1. Introduction

The blood represents of heterogeneous material containing water, dissolved organic molecules, macromolecules, ions and soluble matter. The constituents of the blood are highly organized in cellular and subcellular structures forming macroscopic elements. The presence of ions in the blood plays an important role in the interaction with an electric field, providing means for ionic conduction and polarization effects. The ionic charge drift creates conduction currents and as knows as initiates polarization mechanisms through charge accumulation at structural interfaces, which occur at various organizational levels of membranes of cells of blood. Their dielectric properties will thus reflect contributions to the polarization from both structure and composition [1]. A common way to describe dielectric properties of cell of human blood or tissue between 1 Hz and 50 GHz is to fit Cole–Cole or Cole–Cole-type relaxation models to measurement data [2-5]. The measurement of dielectric properties of blood is known to be of importance for diagnosis of diseases [6-11]. Early studies of dielectric properties of the human erythrocytes are connecting with alternating current in the frequency domain were pioneered at the beginning of this century [12-16]. The frequency dependence of the dielectric properties of the RBC in suspension has been investigated by many scientists [17-28]. In the simplest model the RBC suspension is treated as an equivalent circuit for the measured capacitance and conductance [29]. The dielectric properties of RBC depend on the volume fraction (haematocrit) and the shape of cells [30, 31, 22]. Dielectric spectroscopy measurement in medical investigation depends with phenomenon known as electrode polarization [32]. In this paper we consider dielectric properties of normal blood and RBC and mechanism of relaxation processes. Based on the Maxwell-Wagner model and takes into account the morphological parameters of RBS membranes of cells using appropriate approximations the mathematical model of RBS membrane was derived.

2. Models and Data analysing

2.1. Dielectric theory: a summary

The dielectric response of a substance is the result of either dipolar or space-charge polarisation. Dipolar polarisation can be caused by the separation of a pair of opposite charges in either permanent dipoles (water) or induced dipoles in non-polar molecules. Each type of polarisable entity will exhibit its own characteristic temporal response to an imposed electric field.

This response is mathematically handled by describing the relative permittivity as a complex function of the form:

$$\varepsilon^*(\omega) = \varepsilon_\infty + (\varepsilon_S - \varepsilon_\infty) / (1 + i\omega\tau),$$

where ε_∞ is the permittivity measured, ε_S is the limiting low frequency permittivity where the polarization effect is fully realized, ω is the angular frequency of the applied electric field $\omega = 2\pi f$ (f is frequency), i is $\sqrt{-1}$, τ is the characteristic response or relaxation time.

The real and imaginary components of the complex relative permittivity can be expressed in the form

$$\varepsilon^* = \varepsilon' - i\varepsilon'' \quad (1)$$

where the real part ε' represent the dielectric constant and is given by

$$\varepsilon'(\omega) = \varepsilon_\infty + (\varepsilon_S - \varepsilon_\infty) / (1 + \omega^2\tau^2) \quad (2)$$

The imaginary component ε'', corresponding to the dissipative loss associated with the polarisable charges moving in phase with the electric field and takes the form of a loss peak, has the form

$$\varepsilon''(\omega) = (\varepsilon_S - \varepsilon_\infty)\omega\tau / (1 + \omega^2\tau^2) \quad (3)$$

The equations (2) and (3) are commonly known as Debye Dispersion formulae [33]. The equations (2) and (3) are referred to the situation where equilibrium is attained exponentially with time when a constant external electric field is imposed on a dielectric. The loss factor ε'' can be defined in terms of a frequency-dependent conductivity as

$$\varepsilon''(\omega) = \sigma(\omega) / \omega\varepsilon_0 = (\sigma_0 + \sigma_d(\omega)) / \omega\varepsilon_0 \quad (4)$$

where σ_0 is the steady-state conductivity arising mainly from mobile ions, $\sigma_d(\omega)$ is the frequency-dependent conductivity arising from dielectric polarization losses. Because the energy in the electric field is either stored or lost, conductivity and permittivity are related. The conductivity gives a measure of its ability to conduct, i.e. let charge pass through it, whereas the permittivity gives a measure of the polarizability of the material, i.e. to store charge.

Often the form of the above equations can be derived from defining the

$$\Delta\varepsilon = \varepsilon_S - \varepsilon_\infty .$$

From the real (2) and imaginary (3) components of dielectric permittivity we then obtain the relationships

$$\varepsilon'(\omega) = \varepsilon_\infty + \Delta\varepsilon / (1 + (f / f_r)^2 \quad (5)$$

and

$$\sigma(\omega) = \sigma_S + 2\pi\varepsilon_0 f^2 \Delta\varepsilon \,/\, f_r(1 + (f \,/\, f_r)^2) \tag{6}$$

where the relaxation frequency is $f_r = 1/2\pi\tau$, σ_S is the low-frequency limit of the conductivity. The factor σ_S is the parameter which includes the steady-state conductivity and dielectric losses connected with any polarization processes having relaxation frequencies well below that defined by relaxation frequency. For frequencies very much greater than f_r

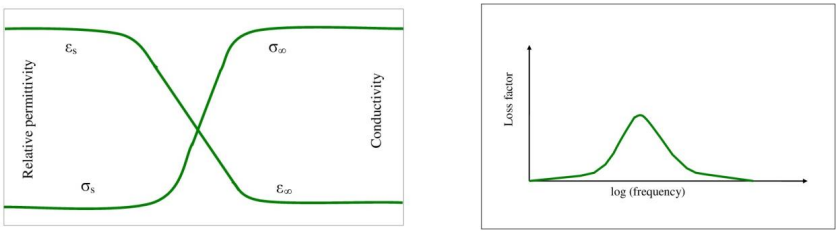
$$\Delta\sigma = \sigma_\infty - \sigma_S = 2\pi\varepsilon_0 f_r \Delta\varepsilon \tag{7}$$

The increment in conductivity is directly proportional to the permittivity change and can be used as a check on the validity of experimental data. The relaxation times can be rewritten as $\tau = \varepsilon_0 \Delta\varepsilon \,/\, \Delta\sigma$.

A simple model, which can be used to describe dielectric relaxation processes that involve dipolar molecules, is one that considers the dipoles to be spheres whose rotation is opposed by the viscosity of the surrounding medium. The relaxation time for such a sphere is $\tau = 8\pi\eta R^3 \,/\, 2kT$, where k is Boltzmann's constant, T is the absolute temperature, R rigid sphere of radius, η is macroscopic viscosity. The classical model of dielectric relaxation of the Debye type cannot adequately describe the relaxation phenomena and kinetics. Fig. 1 shows the variation in the permittivity, loss factor, and conductivity with frequency for a single time constant relaxation for an idealized monomolecular polar substance with no residual frequency-independent conductivity. The best example of such material is pure water. At the relaxation frequency, the permittivity is halfway between its limiting values and the loss factor at its highest. In the case of a single time constant as described in Fig. 1 the conductivity is halfway between its limiting values at the relaxation frequency.

The Debye expression does not include the effect of conduction currents as would arise from the drift of free ions in static fields. Numerous empirical distribution functions and models have been proposed to model the experimental data without elaboration of the underlying mechanisms. Simple exponential relaxation laws describes in such complex systems as Cole-Cole (CC) behaviour (it is one of the most commonly used models, a modified version of the Debye expression) [34]:

$$\varepsilon^*(\omega) = \varepsilon_\infty + \frac{(\varepsilon_S - \varepsilon_\infty)}{1 - (j\omega\tau)^{1-\alpha}} = \varepsilon' - j\varepsilon'', \; 0 \le \alpha \le 1 \tag{8}$$



(a) (b)

Fig.1. Ideal dielectric relaxation of the Debye type. (a) Frequency dependence of relative permittivity ($(\varepsilon' - \varepsilon_\infty) / (\varepsilon_S - \varepsilon_\infty)$) and conductivity ($\omega\varepsilon_0\varepsilon'' / (\varepsilon_S - \varepsilon_\infty)$); (b) Frequency dependence of loss factor ($\varepsilon'' / (\varepsilon_S - \varepsilon_\infty)$), for a single time constant relaxation plotted against f / f_r.

where $\varepsilon^*(\omega)$ is the complex dielectric permittivity spectrum with low frequency ($\varepsilon_S = \varepsilon^*(\omega \to 0)$), and high frequency ($\varepsilon_\infty = \varepsilon^*(\omega \to 0)$) limits, $\Delta\varepsilon = \varepsilon_S - \varepsilon_\infty$ is dielectric strength and α is an empirical exponent, referred to as a measure of the peak broadening, for $\alpha = 0$ the model reverts to Debye equation, j is ($-1^{1/2}$). Usually the relaxation time τ, the exponent α, the dielectric strength $\Delta\varepsilon$ are strictly dependent on the structure, the temperature, the pressure and other controlled physical parameters [35]. The real and imaginary parts can be rewritten as

$$\varepsilon' = \varepsilon_\infty + \frac{(\varepsilon_S - \varepsilon_\infty)\left[1 - (\omega\tau)^{1-\alpha}\sin(\alpha\pi / 2)\right]}{1 + (\omega\tau)^{2(1-\alpha)} + 2(\omega\tau)^{1-\alpha}\sin(\alpha\pi / 2)}$$

$$\varepsilon'' = \frac{(\varepsilon_S - \varepsilon_\infty)(\omega\tau)^{1-\alpha}\cos(\alpha\pi / 2)}{1 + (\omega\tau)^{2(1-\alpha)} + 2(\omega\tau)^{1-\alpha}\sin(\alpha\pi / 2)}$$

Indicating that a plot of ε' against ε'' is a semicircle with its center below the real axis. The deviation is formulated by various empirical equations proposed by Cole and Cole [34], von Schweidler [37], Fuoss and Kirkwood [38], Davidson and Cole [39], Havriliak and Negami [39], Williams and Watts [40], Jonscher [42] and so forth. These equations are used not only for classifying dielectric relaxation of various materials and substance but also for extracting the relaxation parameters from dielectric relaxation data. They do serve a useful purpose in enabling the parameterization of the experimental data, albeit with very limited clarification of the underlying mechanisms. One of the challenges in dielectric spectroscopy today is to

uncover a physical mechanism underlying the CC behaviour in complex systems [36]. For analysis of dielectric relaxation can be used the complex plane plot (or the Cole–Cole plot) usually. In the complex plane plot the loss factor is plotted against the relative permittivity ε', tracing a semicircle if the dielectric relaxation has a single relaxation time, namely, the Debye type relaxation. The complex plane plots, however, often deviate from a semicircle, which indicates a distribution of relaxation times (Fig. 2).

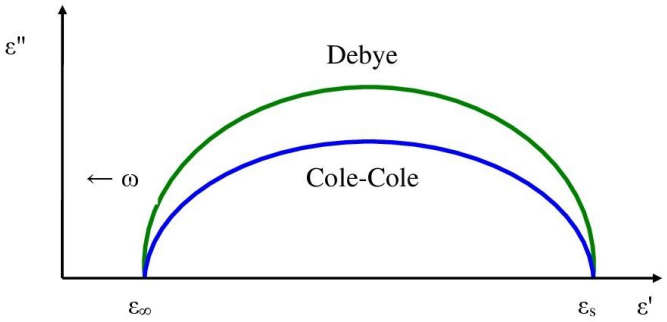


Fig. 2. The complex plane plot. (a) Cole-Cole plots of the Debye and Cole-Cole equations. Plot of normalized permittivity against loss factor showing a semicircle with its center on the real axis in the case of the Debye and an arc of a semicircle with its center below the real axis in the case of the Cole–Cole; the apex of the arc corresponds to the mean relaxation frequency

The Cole–Cole plot of the Cole–Davidson model is an asymmetric curve intercepting the real axis at different angles at high and low-frequencies. The distribution of relaxation times is also asymmetric (Fig. 3).

Theories proposed by researchers on the nature of polarization processes in the living structures are reduced to next basic physical mechanisms:

1. Dipolar orientation of the molecules
2. Macrostructural polarization the living structures
3. Ordering of water structure
4. Counter ion polarization
5. Interfacial polarization
6. Delocalisation of electrons.

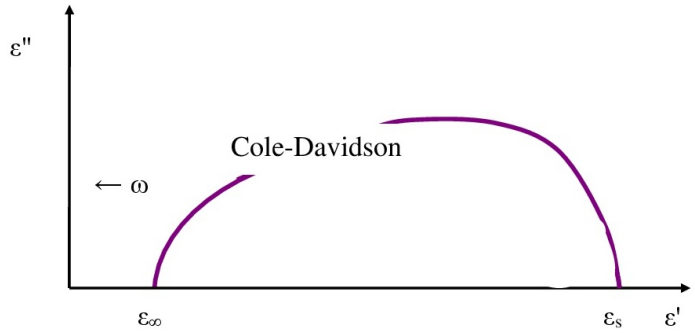


Fig. 3. Plot of normalized loss factor against permittivity showing the characteristic Cole–Davidson skewed arc where the maximum in ε'' does not correspond with $\omega\tau = 1$; this point is found at the interception of the bisector of the high-frequency limiting angle with the data plot

Three relaxation processes that are responsible for the dielectric properties in living structure are known as a) interfacial polarization; b) dipolar orientation and c) counter ion polarization.

2.2. Dielectric Properties of Biological Materials

2.2.1. Dielectric Dispersions: theory

The dielectric properties of a biological tissue result from the interaction of electromagnetic radiation with its constituents at the cellular and molecular level. By their very nature biological materials are not homogenous. A simple example of a heterogeneous biological system is that of blood corpuscles. The relative permittivity of a tissue may reach values of up to 10^6 or 10^7 at frequencies below 100 Hz. It decreases at high frequencies in three main steps known as the α, β, δ and γ -dispersions [17]. Other dispersions may also be present. The dispersions are rather broad, indicating the possible overlap of discrete relaxations arising from the polarization mechanisms encountered in the complex biological environment (Fig. 4). Permittivity is often expressed as the relative permittivity ε_r (or dielectric constant, dimensionless), which is defined as the permittivity relative to that of vacuum ($\varepsilon_0 = 8.854 \cdot 10^{-12}$ F/m), can be find as $\varepsilon_r = \frac{\varepsilon}{\varepsilon_0}$. The step changes in ε_r are called dispersions and are due to the loss of particular

polarization processes as frequency increases. The α-dispersion is due to the tangential flow of ions across cell surfaces, the β-dispersion results from the build-up of charge at cell membranes due to the Maxwell–Wagner effect, the δ-dispersion is produced by the rotation of macromolecular side-chains and 'bound' water, and the γ-dispersion is due to the dipolar rotation of small molecules particularly water [43]. The α, β, δ and γ dispersions may therefore be described by total dielectric decrements $\Delta\varepsilon_\alpha$, $\Delta\varepsilon_\beta$, $\Delta\varepsilon_\delta$ and $\Delta\varepsilon_\gamma$ respectively. Each dispersion region may be described by the relaxation, the spread of relaxation times being determined by the different physical processes involved.

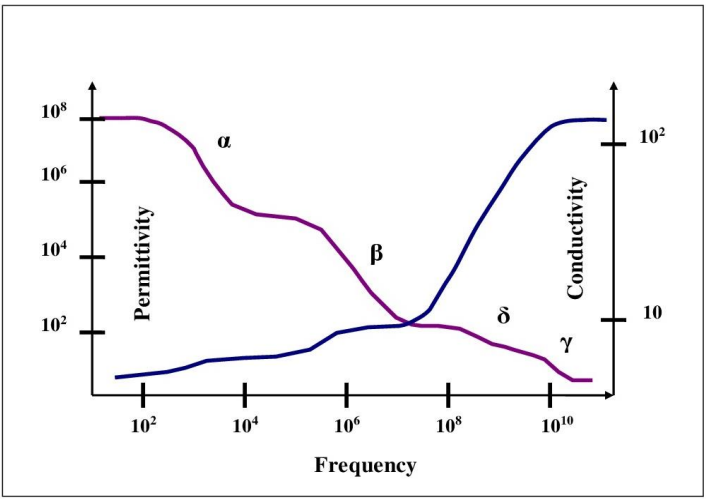


Fig. 4. Complex permittivity spectrum of biological cells. α-dispersions are to the tangential flow of ions across cell surfaces. β-dispersions are results from the build-up of charge at cell membranes due to the Maxwell–Wagner effect. δ-dispersions are produced by the rotation of macromolecular side-chains and "bound" water. γ-dispersions are to the dipolar rotation of small molecules.

2.2.2. α-dispersion

The α or low-frequency (10 Hz–10 kHz, $\tau = 1.6$ ms) dispersion is characterized by very high permittivity values and a large dielectric decrement, both of the order of 10^6. The α-dispersion can be ascribed, at least par-

tially, to counterion diffusion effects. Tissues have finite ionic conductivities commensurate with the nature and extent of their ionic content and ionic mobility. Such large values of dispersions are predicted by theories of ionic diffusion in heterogenous media.

Other theories to describe to the α-dispersion as the interactions in the vicinity of the cell membrane. Membrane-related mechanisms that are thought to contribute to the α-dispersion include the charging of intracellular membrane-bound organelles and frequency dependence in the impedance of the cell membrane itself. As known the cell membrane is a complex, dynamic structure comprising a phospholipid bilayer. The lipid, hydrophobic ends of the phospholipids form a middle layer; the hydrophilic groups cover the inner and outer surfaces. The lipid bilayers form the basis of the membranes and cholesterol could regulate the fluidity of the membrane. Various proteins play a significant role in the functions of cell membrane, such as ion transport and signal transduction. The movements of charged ions and electrons across the membrane are restricted. They can go through the membrane from the specialized ion channels and membrane spanning protein semiconductors respectively. Cell membranes contain ionizable acidic and basic groups, although for most cells so far studied the acidic groups are dominant and the membranes carry a net negative charge. Cell membrane is the most significant portion of the cell as it separates each cell from the surrounding world. It is about 4-10 nm in thickness. The ionic balance between the intra- and extracellular media maintains a 60-to 70-mV potential difference between them of about 10 kV/mm across the membrane. An important reason for the uncertainty in the understanding of this dispersion is the paucity of error-free dielectric data in its frequency range [44]. The α-dispersion has a very large permittivity increment. The corresponding decrement in conductivity is small. ; this, however, does not contravene the principle of causality and the Kramers–Kronig relations [45], which predict a change in conductivity of about 0.005 S/m for a 106 increment in permittivity and relaxation frequency of 100Hz.

2.2.3. β-dispersion

The β-dispersion occurs at intermediate frequencies (10 kHz–10 MHz, $\tau = 300$ ns) and originates mostly from the capacitive charging of the cellular membranes and those of membrane-bound intracellular bodies. The current can pass through the cytoplasm while membranes

are electrically shorted. The impedance decreases due to the accessibility of the cytoplasm as a current path, which forms the β-dispersion. This phenomenon, also known as interfacial polarization, associated with the heterogeneous structure inherent in membrane-electrolyte structures, has been studied theoretically and experimentally. At the interface between two dissimilar dielectrics there is a build-up of charge and this gives rise to interfacial, or Maxwell-Wagner, polarizations. The magnitudes of these polarizations are dependent on the conductivity, permittivity and geometry of the separate components of the heterogeneous structure. With increasing frequency, the more resistive components are neutralized by their associated parallel capacitances. The structure therefore becomes progressively more (electrically) homogeneous. It was established experimentally that damage to the cell membrane changes the features of the β-dispersion. The dependence of the β-dispersion upon the integrity of cell membranes was clearly shown by Pauly and Schwan [46], who measured the effect of digitonin in lysing the fiber membranes of bovine eye lens. Biomedical engineering are based on the variation of the parameters of the β-dispersion with pathological conditions involving changes in cell physiology and morphology. In the frequency range of the β-dispersion the tissue with directed, anisotropic cellular structure would exhibit an anisotropic dielectric response. The electrodynamics modeling the of a simplified tissue-like system, for example, suspensions of spherical inclusions in conductive media, has established theoretical grounds for the presence of the β-dispersions. This calculations to enables the computation of an effective permittivity of similar order of magnitude to the β-dispersion. These interfacial polarizations are boundary effects that occur in addition to other polarizations that may occur in the components of the system. β-dispersion is characterised by dielectric decrement of $\Delta\varepsilon_\beta \approx 104$. Blood displays this dispersion at higher frequencies $f = 3\text{MHz}$, $\tau = 50$ ns with a dielectric decrement of $\Delta\varepsilon_\beta \approx 2000$. This effect is thought to be caused by the charging of cell membranes with smaller contributions arising from the dipolar relaxation of proteins in tissue. This latter effect is sometimes analysed as a separate dispersion, called the $\beta 1$-dispersion [47]. The larger permittivity values observed in tissues compared to blood are due to larger cell sizes. Observation of the $\beta 1$-dispersion can give valuable information on the coupling of externally imposed fields and field strengths in tissue [48].

2.2.4. δ -dispersions

Tissues and other biological materials may exhibit dispersions other than the three main ones. The δ -dispersion, identified in some protein solutions between the β and γ. δ -dispersions occurs in the frequency range 0.1 to 5 GHz; when present, its magnitude is small compared to the adjacent ones. Its dielectric decrement is typically $\Delta\varepsilon_\delta \approx 15$.

δ -dispersions were first characterized by Pethig [49]. δ-dispersions are faint sub-dispersions occurring between the β and γ -dispersions and are attributed to the dipolar moments of large molecules, such as proteins. They are therefore related to biopolymers and cellular organelles [44], proteins and protein-bound water [50]. Possible mechanisms include the dipolar relaxation of "bound" water (water of hydration), relaxation of small dipolar segments or side chains of biological molecules, and counterion diffusion along small regions of the charged surface. Under these conditions it is difficult to isolate and, in view of the multiplicity of possible mechanisms, difficult to interpret. It is often treated as the tail end of the β -dispersion or a broadening of the γ -dispersion.

The large proportion of the water in tissues has rotational properties similar to those of normal bulk water. Approximately 10% of water in tissues, dependent on organ/tissue type, is termed "bound" water [51] i.e. it is rotationally hindered. The "bound" water has a relaxation frequency some 50 to 150 times lower than that of free water. The relaxation of bound water leads to the δ -dispersion, which is very much weaker than the γ -dispersion, and is centered at 100MHz.

This effect (the effect of "bound" water) results from the fact that, within suspensions, the electrical properties of water in the immediate vicinity of particles is perturbed by its proximity to the particles, and so differs slightly from those of free water. The differences in properties are dependent on the molecule to which the water is bound, its viscosity and temperature. There appears to be a transition at around 100MHz, above which the capacitive impedance shorts out and the resistivity is approximately that of 0.9% saline solution, which is the ionic strength of cellular fluid. Above 100MHz the dielectric properties of tissues are consistent with those expected for a suspension of low conductivity particles (cells) in an aqueous electrolyte and the distinction between tissue types becomes lost [52]. A less pronounced lowering of the water relaxation frequency was observed for ocular tissues [53]. The relaxation frequency of normal bulk water at 37°C is 25 GHz,

whereas retina (89% water content) exhibited a relaxation frequency of around 2 GHz, and for the lens nucleus (65% water) this was reduced to around 9GHz. Observed the lowering of the relaxation frequency depends to decrease water content was an interesting feature of these studies. In vivo measurements of the dielectric properties of skeletal muscle, brain cortex, spleen and liver of live cats and rats (for 0.1–10 GHz) obtained that the dielectric properties correlated well with the various tissue water contents that above 1 GHz, and that was deduced that practically all the water in skeletal muscle was in the form of bulk water [54].

For the other tissues, both free and bound water were deduced to be present, with spleen having a total tissue water content (volume basis) composed of 69% free water and 9% bound water. For liver, the total water content (80%) was found to consist of 62% free water and 18% bound water. For the other tissues, both free and bound water were deduced to be present, with spleen having a total tissue water content (volume basis) composed of 69% free water and 9% bound water. For liver, the total water content (80%) was found to consist of 62% free water and 18% bound water. Relaxations of such bound water, as mentioned above, produce a relatively weak dielectric dispersion, called the δ-dispersion, centered around 100 MHz and hence is located roughly midway between the β and γ-dispersions.

Most of the strongly bound water in tissues will be incorporated directly into the overall structure of the globular and membrane-associated proteins. It could be that perturbing the strongly bound water molecules induces changes in those physiological processes that depend on the functional behavior of the substrate proteins.

2.2.5. γ-dispersions

γ-dispersions, first characterized by Foster and Schwan, 1989 [48] are caused by the aqueous content of the biological species and the presence of small molecules. The dielectric properties of tissues above 100MHz (the dispersion, in the gigahertz region) are determined by the intra-cellular electrolytes, principally, water (0.9% saline solution) (is due to the dipole polarization of water molecules). At frequencies in excess of a few hundred megahertz, where the response of tissue water is the dominant mechanism, the complex permittivity may be expressed as Cole–Cole plus a conductivity term to simulate the dipolar dispersion of water and the contribution of the electrolytes, can be written as

$$\varepsilon^*(\omega) = \varepsilon_\infty + \frac{(\varepsilon_S - \varepsilon_\infty)}{1 + (j\omega\tau)^{(1-\alpha)}} + \frac{\sigma}{j\omega\varepsilon_0}$$

where σ is the conductivity due to ionic currents and to the lower-frequency polarization mechanisms. These properties are consistent with those from suspensions of low conductivity, low permittivity particles (i.e. cells) in an aqueous electrolyte. In the microwave band, tissue properties can be attributed to their "free" water content and the dispersion for normal bulk water. The γ-dispersion occurs at microwave frequencies (f = 25GHz, $\tau = 6$ ps) $\Delta\varepsilon_\gamma \approx 50$. The value of the distribution parameter α is significant for most tissues and negligible for body fluids. The mean relaxation time τ is generally longer than the value for water, indicating a restriction in the rotational ability of at least some of the tissue water molecules. The lengthening of the relaxation time of water in biological material is a well studied hypothesis; the effect is common to most organic solutes Various early measurements of the electrical properties of blood contributed significantly to unravel the constitution of red blood cells (RBC) [55, 56]. The results by Höber [16] provided the first indications of a dispersion (i.e. frequency dependence), caused by the membrane of RBCs, in the radio frequency (RF) spectrum of the dielectric properties of blood [57]. This relaxation process is indentified as being of Maxwell-Wagner type [58, 59] and termed, is known to increase with solute concentration [60], and has previously been observed in tissues [61].

3. Methods

Mathematical modelling of RBC cells according to the Maxwell–Wagner interfacial polarization theory.

4. Results and Discussion

4. 1. Dielectric response and erythrocyte morphology

Various early measurements of the electrical properties of blood contributed significantly to unravel the constitution of red blood cells (RBC). The first indications of a dispersion (i.e. frequency dependence), caused by the membrane of RBC, was provided in the radio frequency (RF) spectrum of the dielectric properties of blood [12]. This relaxation process is indentified as being of Maxwell-Wagner type [59, 60] and termed β-relaxation [60, 61]. Most the theoretical models which describe the cell suspen-

sions focus on β and γ -relaxations [17, 14, 61, 62], including the often employed Pauly-Schwan model [63], them account for the non-spherical shape of cells. The diluted solutions and whole blood with a hematocrit value of 86% have to be treated differently. The model must account the membrane and inner medium of RBC. It should be noted that RBC's are far from being of spherical shape and in principle for shelled ellipsoidal particles up to six relaxations can be expected [64]. Furthermore, the hemoglobinmolecules within the RBC's should show all the typical complex dynamics as found in other proteins.

RBCs are disc-shaped when not subjected to external stress. The biconcave discocyte RBC has a flexible membrane with a high surface to volume ratio. That form cells facilitates large reversible elastic deformation of the RBC as it repeatedly passes through small capillaries (blood vessels as small as 2–3 μm in diameter) during microcirculation. RBC deformability is basic for circulation, which is necessary to transport oxygen and carbon dioxide. Pathological conditions affecting RBCs can lead to significant alterations to the discocyte shape. Changes to the RBC surface area or membrane can in some instances obstruct circulation of the cells in blood. The consequences of altered circulation are observed as clinical symptoms that range from benign to lethal (from obstruction of capillaries and restriction of blood flow to tissues to necrosis and organ damage). The discocyte shape of human RBCs is approximately 7.5 to 8.7 μm in diameter and 1.7 to 2.2 μm in thickness. Hemoglobin molecules, essential for gas transport within the circulation, are contained in the RBC cytosol. The membrane of the RBC comprises a phospholipid bilayer and an underlying two-dimensional network of spectrin molecules. The phospholipid bilayer has little shear resistance but contributes to bending resistance and helps to maintain cell surface area. Integral and peripheral proteins connect the bilayer and spectrin network. The spectrin network or cytoskeleton is largely responsible for the shear elastic properties of the RBC [24, 56]. Since the membrane of RBC has the three layers have different electric properties, a spheroidal model with three shells may be suited for RBC cells.

4.2. Dielectric model of RBC membrane

Based on the Maxwell-Wagner model [60, 61], the Pauly and Schwan takes into account the membranes of cells [67, 68] using appropriate approximations some simple relations are derived:

$$\varepsilon*(\omega)=\varepsilon_{out}\frac{\frac{2}{3}\sum_{k=x,y,z}\frac{\varepsilon^*_{pk}-\varepsilon^*_a}{\alpha_k\varepsilon^*_{ph2k}+(1-\alpha_k)\varepsilon^*_{out}}+1}{1-\frac{1}{3}\sum_{k=x,y,z}\frac{\varepsilon^*_{pk}-\varepsilon^*_a}{\alpha_k\varepsilon^*_{ph2k}+(1-\alpha_k)\varepsilon^*_{out}}} \quad (1)$$

The equivalent complex relative permittivity for the disc-shaped form RBCs along axis x, y, z is represented as

$$\varepsilon^*_{ph2k}=\varepsilon^*_{ph1}\frac{\beta(1-\nu_1)\varepsilon^*_{ph1}+(1+\beta_k\nu_1)\varepsilon^*_{ph1k}}{(\beta_k+\nu_1)\varepsilon^*_{ph1}+(1-\nu_1)\varepsilon^*_{ph1k}} \quad (2)$$

with

$$\varepsilon^*_{ph1k}=\varepsilon^*_{ph2}\frac{\beta_k(1-\nu_2)\varepsilon^*_{ph2}+(1+\beta_k\nu_2)\varepsilon^*_{ph2k}}{(\beta_k+\nu_2)\varepsilon^*_{ph2}+(1-\nu_2)\varepsilon^*_{ph2k}} \quad (3)$$

$$\varepsilon^*_{spk}=\varepsilon^*_{sp}\frac{\beta_k(1-\nu_3)\varepsilon^*_{sp}+(1+\beta_k\nu_3)\varepsilon^*_{in}}{(\beta_k+\nu_3)\varepsilon^*_{sp}+(1-\nu_3)\varepsilon^*_{in}} \quad (4)$$

$$\beta_k=(1-\alpha_k)/\alpha_k$$

The depolarization factors αx, αy, αz along the x-, y- and z axes for prolate spheroids ($R_z \succ R_x = R_y$) are given by

$$\alpha_z=\frac{1}{q^2-1}+\frac{q}{(q^2-1)^{3/2}}\ln\left\{q+(q^2-1)^{1/2}\right\},$$

$$\alpha_x=\alpha_y=\frac{1}{2}(1-\alpha_z),$$

where q is the axial ratio defined as $q = R_z / R_x$. The volume ratios Q_{ph1} , Q_{ph2} and Q_{sp} were approximately written as:

$$Q_{ph1}=\frac{(R_Z-d_{ph1})(R_x-d_{ph1})^2}{R_zR_x^2}, \quad (5)$$

$$Q_{ph2}=\frac{(R_Z-d_{ph1}-d_{ph2})(R_x-d_{ph1}-d_{ph2})^2}{(R_z-d_{ph1})(R_x-d_{ph2})^2}, \quad (6)$$

$$Q_{sp}=\frac{(R_Z-d_{ph1}-d_{ph2}-d_{sp})(R_x-d_{ph1}-d_{ph2}-d_{sp})^2}{(R_z-d_{ph1}-d_{ph2})(R_x-d_{ph1}-d_{ph2})^2} \quad (7)$$

In the three-shell model the thickness of each shell is non-uniform, but we assume uniform shell thicknesses d_{ph1} , d_{ph2} and d_{sp} because d_{ph1} , d_{ph2} and $d_{sp} \ll R_x$, R_y . In the three-shell spheroidal model, there are many variables. Some of the parameters were found to be of less influence on

the dielectric parameters and therefore were appropriately fixed to reduce the number of variables. Were R_x, R_y, R_z – the semi-axes of the outermost ellipsoid along x-, y-, z-axes, $R_z \succ R_x = R_y$; d_{ph1} – the thickness of the part phospholipids (1) of the membrane RBC; d_{ph2} – the thickness of the part phospholipids (2) of the membrane RBC; d_{sp} - the thickness of the two-dimensional network of spectrin molecules of the membrane RBC; ε^* – complex relative permittivity defined as $\varepsilon^* = \varepsilon' - jk / \omega\varepsilon_0$; ε - relative permittivity; k - conductivity; ω – angular frequency; ε_0 – the permittivity of vacuum; ε^*_{ph1} – the complex relative permittivity of the part phospholipids (1) of the membrane RBC ε^*_{ph2} – the complex relative permittivity of the part phospholipids (2) of the membrane RBC; ε^*_{sp} – the complex relative permittivity of the two-dimensional network of spectrin molecules of the membrane RBC; ε^*_{in} – the complex relative permittivity of the inner medium of the cell; ε^*_{out} – the complex relative permittivity of the external medium of the cell.

5. Conclusions

The use of dielectric spectroscopy allows researching the dielectric parameters' of blood cells on different frequency, carry out real time observations and to extract a wealth of information about their physiological properties, reveal a rich variety of dynamic processes. Understanding of these processes is the interest for relevance of biomedical research. This review covers the different methods of interpreting dielectric investigation and progress made in applications of spectroscopy for human cells observations by covering a frequency range from 1 Hz to 10^9 GHz.

The ultra-broadband data of the complex effective permittivity show that the spectrum can be fitted with a one Debye and some Cole-Cole models. The high frequency response above 1 GHz can be directly related to the orientational relaxation of "bound" water and thus to the volume fraction of free pore water. The Maxwell-Wagner effect is almost negligible in the low frequency range but dominates the dispersion in the high frequency range above 1 MHz.

From the biological point of view, the erythrocyte can be considered the as cell with no organelles, but its physical behavior in blood is complex and can strongly influence the results from dielectric spectroscopy. Many factors might contribute to the high capacitance values observed for the erythrocyte in normal blood. The values calculated should be considered

as an apparent one, reflecting the dielectric response of red blood cells. The model study showed that the three-shell spheroidal model of the RBCs membrane can be described as the inner part of the membrane which consist of spectrin molecules and an outer part consisting of the phospholipids bilayer. In addition, the phospholipids bilayer is much more permeable to sugar and ions than the spectrin molecules, and is not regarded as a barrier for ions but as a filter to exclude large molecules.

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