PASSIVE ELECTRICAL PROPERTIES OF MICROORGANISMS

V. Low-Frequency Dielectric Dispersion of Bacteria

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ABSTRACT Very high dielectric constants have been observed for bacteria at low frequencies. High dielectric constants such as these can be explained by a theory which has been developed for the low-frequency dielectric dispersion of porous charged particles and which has been tested successfully through measurements with ion exchange resins. The bacterial cell wall is electrically similar to an ion exchange resin. Observations show that the theory provides a quantitative explanation for the low-frequency dielectric dispersion of bacteria.

INTRODUCTION

Most of the observed dielectric properties of bacterial cells can be explained satisfactorily on the basis of a model consisting of a conducting cytoplasmic core, contained by a thin insulating membrane, which in turn is surrounded by a porous, conducting cell wall (Carstensen, 1967, 1968; Carstensen et al., 1965; Einolf and Carstensen, 1969). No quantitative explanation, however, has been given for the low-frequency dispersion which gives rise to effective dielectric constants of the order of 10^5 at frequencies below 1000 Hz (Schwan, 1957; Einolf and Carstensen, 1967, 1969). Since there is no correlation between electrophoretic mobility and magnitude of the low-frequency dielectric constant (Carstensen et al., 1965), it is apparent that the Schwarz (1962) ion cloud relaxation mechanism is not directly applicable to bacteria. In a recent study of suspensions of ion exchange resins, the Schwarz theory has been extended to include the case of porous charged particles (Einolf and Carstensen, 1971). For this case it has been demonstrated that layers of ions both inside and outside the surface of the porous charged particle are constrained to move effectively parallel to the surface. This phenomenon leads to very high dielectric constants at low frequencies. The cell wall of a bacterial cell is electrically similar to an ion exchange resin. As demonstrated here, the theory for ion exchange resins provides a satisfactory quantitative explanation for the low-frequency dielectric dispersion of bacteria.

THEORY

The distribution of mobile ions near the interface between (*a*) a solid and a liquid where the surface has a fixed charge $e_0\Sigma$ (coulombs per square meter), and (*b*) a porous region and a liquid where the porous region is characterized by a fixed charge of e_0N (coulombs per cubic meter) are illustrated in Fig. 1. The counterion densities n and coion densities p have a Maxwell-Boltzmann distribution:

$$\begin{aligned} n &= n_0 \exp\,(e_0\phi(x)/kT), \\ p &= p_0 \exp\,(-e_0\phi(x)/kT), \end{aligned} \tag{1}$$

where $n_0 = p_0$ are the ion densities in the bulk liquid, e_0 is the electronic charge, $\phi(x)$ is the electrostatic potential at a distance x from the surface resulting from the surface charge $e_0\Sigma$ or volume charge e_0N, and kT is the thermal energy. For simplicity this discussion is limited to the case of monovalent mobile ions. The potential itself falls close to zero within a distance of the order of a Debye length. Thus the influence of the surface on the distribution of ions in the liquid in most cases is limited to a distance of the order of tens of angstroms. In the first case, only ions external to the solid surface are mobile. In the second case, mobile ions on both sides of the interface are dominated in their distribution by the fixed charge N in the porous region (Mauro, 1962). The local static electric fields which maintain these ion distributions near the interfaces are very large, of the order of 10^5–10^6 V/m, for typical cell surfaces.

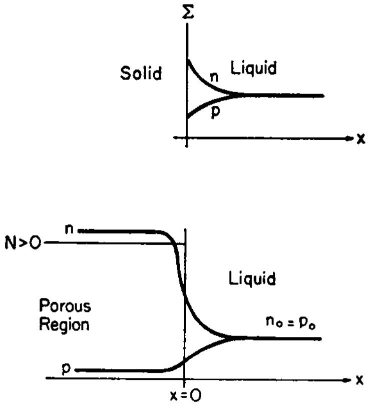

FIGURE 1 The distribution of mobile ions near the interface between a liquid and a solid with a fixed surface charge $e_0\Sigma$ (top), and a porous region with a fixed charge density e_0N (bottom).

Schwarz (1962) considered the case of a solid spherical particle with surface charge density of $e_0\Sigma$. When an external electric field is applied to a suspension of these particles, the mobile counterions are displaced by the applied field but the large, static, local field of the particle causes the counterions to move effectively along the surface of the particle. The case for a suspension of spherical porous particles with fixed volume charge e_0N is qualitatively the same. The large static electric fields near the surface have the effect of causing the counterions near the surface to move along a tangent to the surface when under the influence of an external electric field. Whereas in the first case all mobile ions are outside the solid, in the case of the porous particle there are counterions on both sides of the interface and, in fact, there is no difference electrically between a counterion just outside and one just inside of the interface.

Quantitative estimates of the number of counterions which are under the influence of the large local static field have been given previously. In the first case, the counterion concentration δ_0 per unit area is approximately equal to the surface ion density Σ (Cole, 1933). In the second case if $N \gg n_0$ (Einolf and Carstensen, 1971),

$$\delta_0 \approx \frac{1}{2e_0} (N\epsilon_i kT)^{1/2} \ln (|N| kT/\epsilon_i), \qquad (2)$$

where ϵ_i is the permittivity of the porous region. This estimate includes all mobile ions which are in the region near the interface where the static field is much greater than typical applied fields. Because the static field is such a strong function of the distance from the interface, the arbitrary choice of the limits of this region do not critically affect the value of δ_0.

After an analysis of the problem which closely parallels that of Schwarz (1962), it has been shown that the effective, homogeneous, complex conductivities σ_2^+ of the suspended particles either with fixed surface charge $e_0\Sigma$ or volume charge e_0N are (Einolf and Carstensen, 1971):

$$\sigma_2^+ = \sigma_i^+ + i\omega\epsilon_0 \frac{\Delta\kappa_2}{1 + i\omega\tau}, \qquad (3)$$

where

$$\Delta\kappa_2 = \frac{e_0^2 R\delta_0}{\epsilon_0 kT}, \qquad (4)$$

and

$$\tau = \frac{1}{2\pi f_0} = \frac{R^2}{2ukT}. \qquad (5)$$

Here σ_i^+ is the effective, homogeneous, complex conductivity of the particle neglecting surface effects, ϵ_0 is the permittivity of free space, R is the radius of the particle, and u is the mobility of the counterion.[1]

Although this theory applies to ion exchange resins and assumes a particle with uniformly distributed fixed charges, it can easily be extended to include bacteria. Carstensen (1967) has shown that the bacterial cell membrane insulates the cytoplasmic core at low frequencies. Therefore, the bacterial cell appears as an insulating core surrounded by a thick conducting layer containing fixed charges (the cell wall). If one considers the electric field equations of such a layered particle (Fricke, 1955), the field external to the particle (the field that is directly measured) is identical with that field for the ion exchange particle. Thus, the ion exchange particle is a most appropriate model for the low-frequency dielectric properties of the bacterial cell.

DISCUSSION

Data have been reported previously which can be used to provide a test of the validity of this theory in relating the low-frequency dielectric properties of bacteria to the fixed volume charge density of the bacterial cell wall (Carstensen, 1967, 1968; Einolf and Carstensen, 1967, 1969). The pertinent values are summarized in Tables I and II. Although there is at present no direct chemical method for determining the fixed volume charge density in the intact bacterial cell wall, acid-base titrations of isolated wall fragments of *Micrococcus lysodeikticus* have been performed and found to give values of N which agree within experimental error with values estimated from the effective conductivities of the walls (Carstensen, 1968). For isolated walls the values obtained from titration range from 24 to 82 meq/liter. From the conductivities of isolated walls, the estimates ranged from 70 to 95 meq/liter. In this same study it was pointed out that intact cell walls may be more highly conducting than isolated walls. On this basis we must include estimates as high as 150 meq/liter in the range of possible values for the fixed volume ion density of the intact *M. lysodeikticus* walls. The calculations in Table I use $N = 80$ meq/liter for *M. lysodeikticus*. An unnamed species of *Micrococcus* was reported which has a higher wall conductivity ($\sigma_W = 1.1$ mho/m) than *M. lysodeikticus* (Carstensen, 1967). Using this value of the conductivity and assuming the mobility of the counterions is equal to that of a sodium in free solution, one would estimate $N \approx 250$ meq/liter for the wall of *Micrococcus* species. These values for the fixed ion density are used in Eqs. 2 and 4 to predict the magnitude of the low-frequency dielectric constant for the two bacteria which have been studied. The results of the calculations are compared with observed values in Table I. Best estimates for N give reasonably good values for $\Delta\kappa_2$. The relaxation frequencies f_0 predicted by Eq. 5 are compared with the observed values in Table II. In Eq. 5 the mobility u of the sodium ion in free

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[1] A frequency-independent contribution of surface conductance has been omitted in Eq. 3 (Einolf and Carstensen, 1971). For bacteria this term is small by comparison with the cell wall conductivity which is contained in $\overset{+}{\sigma_i}$.

TABLE I

COMPARISON OF THE MEASURED LOW-FREQUENCY DIELECTRIC CONSTANT FOR TWO SPECIES OF *MICROCOCCUS* WITH VALUES PREDICTED FROM ESTIMATES OF FIXED VOLUME CHARGE DENSITY IN THE CELL WALL USING EQS. 2 AND 4*

Organism	N	δ_0	R	$\Delta\kappa_2$	
				Predicted	Measured
	meq/liter	m^{-2}	μm		
M. lysodeikticus	80	$1.3 \cdot 10^{18}$	0.4	$3.2 \cdot 10^{6}$	$3.2 \pm 0.2 \cdot 10^{6}$
Micrococcus species	250	$2.1 \cdot 10^{18}$	0.4	$5.3 \cdot 10^{6}$	$6.0 \pm 0.4 \cdot 10^{6}$

* See text for discussion of the choice of values of the fixed ion density N used in the calculations. Data are obtained from Carstensen, 1967 and 1968, and Einolf and Carstensen, 1967 and 1969.

TABLE II

RELAXATION FREQUENCIES IN THE LOW-FREQUENCY DIELECTRIC DISPERSION FOR TWO SPECIES OF *MICROCOCCUS**

Organism	Predicted f_0	Observed	
		Dielectric constant f_κ	Conductivity f_σ
	kHz	*kHz*	*kHz*
M. lysodeikticus	3	0.8	5
Micrococcus species	3	2	10

* The mobility of the counterion is assumed to be that of a sodium ion in free solution ($3.4 \cdot 10^{11}$ $m^2/C\text{-}V\text{-}s$).

solution is used (3.4×10^{11} $m^2/C\text{-}V\text{-}s$). As indicated by Schwarz (1962), it is probable that there is actually a distribution in the mobilities for the counterions. Therefore, the dielectric constant data tend to emphasize the low relaxation frequencies. The conductivities tend to be dominated by the high relaxation frequencies. Table II illustrates this effect. The relaxation frequency for the dielectric constant f_κ is lower than f_0 whereas the relaxation frequency for the conductivity f_σ is greater than f_0.

In this development it has been assumed that the cell wall is a homogeneous, porous layer surrounding the cytoplasmic membrane. No attempt has been made to consider complexities which might arise if the wall itself were an inhomogeneous structure. Evidence from electron microscopy indicates that *Micrococcus* walls are comparatively simple. For these organisms the data which are available are in reasonably good agreement with predictions. The basic phenomenon, which depends upon counterion movement along interfaces which separate regions with large static potential differences, probably is involved in the low-frequency dispersion of all microorganisms.

SUMMARY

Low-frequency dielectric measurements give two independent indications of the volume ion density N in the cell walls of bacteria. To a first approximation the low-frequency limit of the effective, homogeneous dielectric constant should be proportional to the square root of N and inversely proportional to the radius of the cell; it appears to be independent of the counterion mobility, environmental ion concentration, and the amount of space which the wall occupies. On the other hand, the effective homogeneous conductivity σ_2 of the cell at low frequencies is a function of wall conductivity σ_W and the volume fraction $(1 - p)$ of the cell which the wall occupies, specifically,

$$\sigma_2 = \sigma_W \frac{1 - p}{1 + p/2}. \tag{6}$$

The wall conductivity, in turn, depends upon the fixed charge concentration N, the counterion mobility, and also the environmental ion concentration.

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