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A Method for Determining the Dielectric Constant and the Conductivity of Membrane-Bounded Particles of Biological Relevance

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Abstract. Numerical assessment is made regarding Pauly and Schwan's theory which describes the dielectric behaviour of a suspension of "shell spheres" as a model of biological membrane-bounded particles. The results indicate that approximate expressions of the theory may give rise to serious errors when applied to particles smaller than about 1 μm in diameter. With a view to performing analysis according to a general expression of the theory, some of the characteristic responses of dielectric parameters upon changes in phase parameters are examined with particular reference to some numerical ranges of biological interest. On this basis a simplified and systematic procedure is proposed for estimating the phase parameters of particles whose shell phase can be regarded as non-conductive. As the application of the procedure proposed, a set of dielectric data of a synaptosome suspension is analyzed, so that the following three phase parameters are successfully determined: membrane capacitance (or shell phase dielectric constant), internal phase conductivity and internal phase dielectric constant. Some limitations of the procedure are discussed for the cases of conducting shells and small particles.

Key words: Dielectric Method — DK — Electrical Conductivity — Biological Suspensions — Membrane-bounded Particles.

I. Introduction

Because of technical difficulties and possible damage encountered in microelectrode impalement, the dielectric approach has frequently been employed for elucidating electrical properties of isolated cells and organelles in suspension [1, 11]. A dielectric theory, developed by Pauly and Schwan [8] for a model suspension system consisting of homogeneous shell spheres as depicted in Fig. 1, has stimulated a number of investigations along this line [5, 6, 7, 9, 13, 14, 16].

According to their theory a general expression for the complex dielectric constant ε^* of such a system with volume concentration Φ is given by

$$\frac{\varepsilon_a^* - \varepsilon^*}{2\varepsilon_a^* + \varepsilon^*} = \frac{(\varepsilon_a^* - \varepsilon_s^*)(2\varepsilon_s^* + \varepsilon_i^*) + (\varepsilon_a^* + 2\varepsilon_s^*)(\varepsilon_s^* - \varepsilon_i^*)(1 + 2d/D)^{-3}}{(2\varepsilon_a^* + \varepsilon_s^*)(2\varepsilon_s^* + \varepsilon_i^*) + 2(\varepsilon_a^* - \varepsilon_s^*)(\varepsilon_s^* - \varepsilon_i^*)(1 + 2d/D)^{-3}}\Phi . \quad (1)$$

The parameters used in this equation and in the equations presented below are defined as follows: ε is relative dielectric constant; the asterisked symbols imply complex quantities of the form, $\varepsilon^* = \varepsilon - j\varkappa/2\pi f \in_0$, where j, $\varkappa$, f and $\in_0$ are unit imaginary, conductivity, applied frequency, and dielectric constant of free space,

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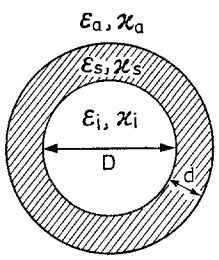

Fig. 1

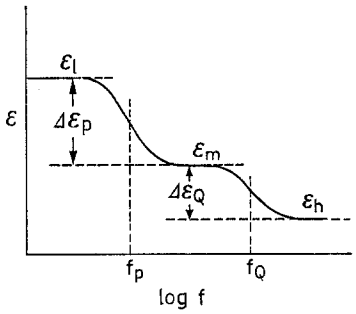

Fig. 2

Fig. 1. A dielectric model for biological particles bounded by one layer of limiting membranes

Fig. 2. Schematic diagram of frequency dependence of ε, the dielectric constant of a suspension of shell spheres, given by text Eq. (2)

respectively; and the definitions for parameters with subscripts a, s and i as well as parameters d and D are given in Fig. 1.

For the practical purpose of evaluating the phase parameters of the constituent particles from dielectric measurements, a set of equations (hereafter referred to as "approximate expressions") has been derived under the conditions that $\varkappa_s/\varkappa_a \ll d/D \ll 1$ and $\varkappa_s/\varkappa_i \ll d/D \ll 1$, which appear to be a good approximation for most cases of living cells [2, 7, 8]. However, the use of the approximate expressions may lead to erroneous estimates in the case where the conditions are violated critically.

This awareness has prompted us to carry out a closer numerical examination of differences between the general expression and the approximate ones. The knowledge of limitations revealed with the latter has led to a search for a simple method to determine the parameters, ε_s, ε_i, and $\varkappa_i$, only by assuming Eq. (1). During the course of our study has appeared a similar attempt by Redwood *et al.* [10] who dealt with a case of phospholipid vesicles. Their analysis was performed by means of a totally computerized data-processing system so that there was no need for deeper comprehension of various characteristics of Eq. (1). It seems, however, not only interesting but also useful as a guide to a simpler method, to list up some characteristics shown by the general expression regarding the behaviour of the parameters included in the equation.

In this paper such a method is proposed to determine the phase parameters in Eq. (1) on the basis of the results of numerical analysis on the equation. Since a complete analysis covering all the ranges of variables is not practicable, considerations are restricted to numerical ranges of biological relevance.

II. Limitations of the Approximate Expressions

An explicit expression for ε in Eq. (1), as a function of frequency, can be written

$$\varepsilon = \frac{\Delta \varepsilon_P}{1 + (f/f_P)^2} + \frac{\Delta \varepsilon_Q}{1 + (f/f_Q)^2} + \varepsilon_h \,. \tag{2}$$

Table 1. Difference in the values of $\Delta\varepsilon_P$ and f_P between the approximate and the general expressions

Diameter D, μm	500	50	5	0.5	0.05
Ratio d/D	10^{-5}	10^{-4}	10^{-3}	10^{-2}	10^{-1}
Volume fraction Φ	Percentage error of $\Delta\varepsilon_P$[a]				
0.001	0.03	0.29	2.91	28.8	264
0.4	0.03	0.34	3.37	33.3	300
0.8	0.04	0.38	3.79	37.4	331
Φ	Percentage error of f_P[a]				
0.001	0.03	0.31	3.07	30.7	310
0.4	0.04	0.36	3.55	35.5	358
0.8	0.05	0.41	3.99	39.9	402

[a] Percentage error

$$= \frac{\text{Value by Eq. (4) (for } \Delta\varepsilon_P\text{) or Eq. (5) (for } f_P\text{)} - \text{Value by Eq. (1)}}{\text{Value by Eq. (1)}} \times 100$$

Numerical values used in the calculation are:

$$\varepsilon_a = 80, \quad \varkappa_a = 15 \text{ mS cm}^{-1},$$
$$\varepsilon_i = 60, \quad \varkappa_i = 10 \text{ mS cm}^{-1},$$
$$\varepsilon_s = 3, \quad \varkappa_s = 0 \text{ mS cm}^{-1}, \quad \text{and } d = 50 \text{ Å}.$$

where the notations used are defined as in Fig. 2, which shows schematically the frequency dependence of ε in its general pattern that can be divided into two dispersions, termed "P" and "Q", corresponding to the first and the second terms of Eq. (2). The P-dispersion which appears in the lower frequency range will be the subject of our procedure presented below.

According to the approximation made by Pauly and Schwan the following expressions are derived from Eq. (1) [2, 8]:

$$\frac{\varkappa_l}{\varkappa_a} = \frac{2(1-\Phi)}{2+\Phi}, \tag{3}$$

$$\Delta\varepsilon_P = \varepsilon_l - \varepsilon_m = \frac{9\Phi}{2(2+\Phi)^2} \cdot \frac{\varepsilon_s}{d/D}, \tag{4}$$

and

$$f_P = \frac{d/D}{\pi \varepsilon_0 \varepsilon_s} \left(\frac{1}{\varkappa_i} + \frac{1-\Phi}{2+\Phi} \cdot \frac{1}{\varkappa_a} \right)^{-1}, \tag{5}$$

where $\varkappa_l$ is the low-frequency limiting value of conductivity for a suspension.

With the aid of these relations one can evaluate the unknown phase parameters such as Φ, ε_s and $\varkappa_i$ from the data of dielectric measurements. In particular, Eq. (3) is immediately obtained by putting $\varkappa_s = 0$ in the general expression, so that this simple relation is expected to be an excellent tool for calculating the volume fraction Φ of suspensions, without any assumption on d/D, where the shell conductivity $\varkappa_s$ is negligible relative to $\varkappa_a$ or $\varkappa_i$. In this regard an experimental assessment was made by applying an "extracellular-marker" technique to suspensions of isolated mitochondria and synaptosomes, as will be reported in a separate paper. Briefly, the results from the electrical and the non-electrical

Table 2. Response of characteristic dielectric parameters to individual changes in phase parameters

Case	Phase parameter	Variation from values for the reference state[a], %				
		Dielectric parameter				
		$\varkappa_l$	ε_l	ε_h	ε_m	f_P
I	Φ [+ 10 %]	− 5.5	+ 2.2	− 1.3	− 1.3	+ 0.91
II	ε_s [− 50 %]	*0.00*	− 31	− 7.0	− 7.0	+ 55
III	ε_i [− 50 %]	*0.00*	*0.00*	− 11	− 8.3	+ 12
IV	$\varkappa_i$ [− 50 %]	*0.00*	*0.0*[b] −(0.61)	*0.00*	+ 1.8[c]	− 44

[a] The reference state is specified: $\varepsilon_a = \varepsilon_i = 80$, $\varkappa_a = \varkappa_i = 15$ mS cm^{-1}, $\varepsilon_s = 3$, $\varkappa_s \lessapprox 10^{-4}\,\varkappa_a$, $d = 50$ Å, $D = 0.5\,\mu$m, and $\Phi = 0.3$.

[b] This term increases slightly with increasing $\varkappa_s$, and shows the value given in parentheses at $\varkappa_s/\varkappa_a = 10^{-4}$. All other terms in italics remain less than 0.002 % throught the range $\varkappa_s/\varkappa_a = 0$ to 10^{-4}.

[c] This term shows small values less than 2 % over the ranges of $d/D = 10^{-4}$ to 5×10^{-1} and $\varkappa_s/\varkappa_a \lessapprox 10^{-2}$.

methods have proved to be in good agreement with each other at least for $\Phi < 0.3$. This evidence also supports the merits of Eq. (3).

In view of the conditions used to derive Eqs. (4) and (5), the extent of applicability of both equations may be a limited one depending on the values of d/D. In fact, discrepancy between the approximate expressions and the general one is evidently shown in Table 1, in which is given an example for $\Delta\varepsilon_P$ and f_P. It is seen that the differences expressed as percentage error increase in proportion to the factor d/D, and that the finite errors for a given value of d/D still remain insensitive to the full change of Φ from zero to unity.

On account of the limitations indicated above, it is strongly suggested that a quantitative analysis on biological suspensions in which the size of the constituent particles is smaller than about 1 μm should be directly based on the general equation instead of its approximated forms.

III. Proposal of a Procedure Directly Based on the General Expression

In order to establish a systematic procedure to determine the phase parameters, ε_s, ε_i, $\varkappa_i$ and Φ, it seems to be a prerequisite to get knowledge of the effects of changing one of these phase parameters on the behaviour of the characteristic dielectric parameters, ε_l, ε_m, ε_h, $\varkappa_l$ and f_P, predicted from Eq. (1). Calculation was made, on a hypothetical system having the reference-state parameters [$\varepsilon_a = \varepsilon_i = 80$, $\varkappa_a = \varkappa_i = 15$ mS cm^{-1}, $\varepsilon_s = 3$, $\varkappa_s \lessapprox 10^{-4}\,\varkappa_a$, $d = 50$ Å, $D = 0.5\,\mu$m and $\Phi = 0.3$], according to the following scheme:

Case I Increase in Φ by 10 %
Case II Decrease in ε_s by 50 %
Case III Decrease in ε_i by 50 %
Case IV Decrease in $\varkappa_i$ by 50 %.

The relative variation in the dielectric parameters from those for the reference state is shown in Table 2. The most remarkable point is that all six terms in the lower-left part of the Table are zero.

After further calculation, such a tendency was ascertained to be the case for wider ranges of the phase parameters specifying the reference states [$\varepsilon_a = \varepsilon_i = 3$ to 100, $\varkappa_a = \varkappa_i = 0.1$ to 100 mS cm^{-1}, $\varepsilon_s = 3$ to 50, $\varkappa_s = (0$ to $10^{-4}) \times \varkappa_a$, $d = 50$ to 100 Å, and $D = 0.01$ to 10 μm], if we allow the zeros in the italic terms in Table 2 to be replaced by a figure of 0.2%. This remark is the basis of our method presented below. The numerical ranges given above for the phase parameters appear to cover most of the numerical values of biological interest.

Inspection of Table 2 leads us rather straightforwardly to a statement that the unknown phase parameters can be uniquely determined when the determination proceeds along a diagonal line in Table 2, *i.e.*, from the upper left to the lower right. To proceed in this direction, one may determine each parameter stepwise without affecting the results of the preceding steps. Thus the procedure to be proposed is summarized in the five steps:

Step 1 To put temporarily $\varepsilon_i = \varepsilon_a$ and $\varkappa_i = \varkappa_a$ together with $\varepsilon_s = 3$ and $\varkappa_s = 0$.

Step 2 To find a proper value of Φ so that the calculated value of $\varkappa_l$ may fit in with the observed value of $\varkappa_l$.

Step 3 To find ε_s so that the calculated ε_l may fit in with the observed ε_l.

Step 4 To find ε_i so that the calculated ε_m may fit in with the observed ε_m.

Step 5 To find $\varkappa_i$ so that the calculated f_P may fit in with the observed f_P.

Some remarks must be made on the practice of the procedure. First, if *Step 5* eventually affects the result of *Step 4* because of the appearance of a non-zero term with superscript c in Table 2, these two steps should be repeated until the best compromise is attained in between. Second, whenever a finite value of $\varkappa_s$ is available from any independent measurements, *Step 1* is to start with the finite value instead of $\varkappa_s = 0$, and furthermore, *Steps 3* to *5* should be repeated for a better fit with ε_l, since the relative variation of ε_l (the term with superscript b in *Case IV*, Table 2) grows up to -0.5 to -0.9% and to -4 to -8%, respectively, when $(\varkappa_s/\varkappa_a)/(d/D) \approx 10^{-2}$ and $\approx 10^{-1}$.

IV. Example of Application

Finally, an example is shown in Fig. 3 to visualize each *Step* of the procedure as applied to a realistic analysis on a biological specimen. The specimen used was a suspension of pinched-off nerve ending particles or the so-called "synaptosomes". The details of the experiments are described in the following paper [3]. The morphological parameters employed were: $D = 0.678$ μm and $d = 50$ Å, the former being a mean value estimated by electronmicroscopy while the latter a mere assumption for the effective membrane thickness. ε_l, ε_m and f_P were all estimated reasonably from the complex plane plots of loss factor against dielectric constant observed.

By using the values determined experimentally for $\varkappa_l$, $\varkappa_a$ and ε_a, *Steps 1* to *3* were performed to give $\Phi = 0.383$ and $\varepsilon_s = 3.64$. At this stage the overall profile of ε, as a function of frequency, calculated from Eq. (1), is depicted by Curve A in Fig. 3. Fitting to the experimental points (open circles) was yet to a highly limited extent, that is, only to a point for ε_l. Then *Step 4* gave rise to Curve B and $\varepsilon_i = 35.7$. After the last *Step* was conducted yielding $\varkappa_i = 3.96$ mS cm^{-1}, satisfactory fitting was obtained at three points, ε_l, f_P and ε_m, the result being indicated by Curve C.

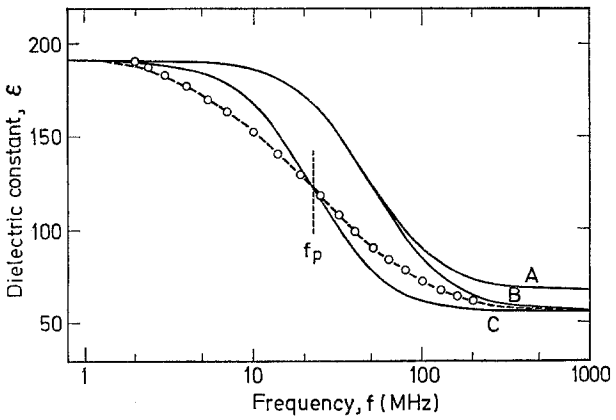

Fig. 3. Illustration of the procedure to evaluate the phase parameters, ε_s, ε_i and $\varkappa_i$, by means of a stepwise curve-fitting method based on the Pauly-Schwan general expression. Experimental points (○) refer to measurements on a synaptosomal suspension. The measured points below 2 MHz are omitted because the dielectric constant showed rapid increase owing to electrode polarization

For this system the Q-dispersion at higher side of frequency is assessed to be vanishingly small in the light of the consideration given in the later section. The discrepancy remaining between the experimental points and Curve C is attributable not to the overlapping of the P- and the Q-dispersion but to the distribution of relaxation times of the measured specimen. The membrane capacitance C_M was estimated to be 0.646 μF cm^{-2} by using the relation

$$C_M = \frac{\epsilon_0\, \varepsilon_s}{d} \cdot \frac{1 + 2\, d/D}{(1 + d/D)^2} \,. \tag{6}$$

Numerical calculation was made with a programmable calculator, Yokogawa-Hewlett-Packard Model 10.

V. Discussion

The major objective of the present work was to develop a simple and convenient procedure which enables us to apply the dielectric theory of Pauly and Schwan [8] to practical analysis for biological systems. This has been accomplished to some extent in that the determination of phase parameters except $\varkappa_s$, the shell conductivity, could be made on the basis of the general equation as far as only the P-dispersion was concerned. Some immediate questions arise, however, as to the applicability of the procedure proposed in the preceding section.

Assumption for $\varkappa_s$

We have so far made the assumption that the conductivity of shell phase is vanishingly small relative to those of the aqueous phases separated by the shell, so that we chose the condition $\varkappa_s = 0$ throughout the analysis presented above. This is apparently an oversimplification for a variety of existing biological membranes. To prove the validity of the procedure proposed, it is necessary to examine the

Table 3. Errors in the phase parameters estimated by assuming null conductivity for shell phase

$\varkappa_s/\varkappa_a$	Error in %[a]			
	Φ	ε_s	ε_i	$\varkappa_i$
10^{-5}	0.08	0.15	0.00	0.02
10^{-4}	0.73	1.5	0.15	0.28
10^{-3}	6.8	14	1.6	2.6
3×10^{-3}	17	34	3.9	6.9
10^{-2}	37	70	2.9	11

[a] $\dfrac{\text{(Values estimated with } \varkappa_s = 0) - \text{(Values compatible with given } \varkappa_s)}{\text{(Values compatible with given } \varkappa_s)} \times 100$

possible errors resulting from the use of such a simplifying condition. These errors were estimated in the following way.

Suppose a system specified by: $\varepsilon_a = 80$, $\varepsilon_i = 60$, $\varepsilon_s = 3$, $\varkappa_a = 15$ mS cm^{-1}, $\varkappa_i = 10$ mS cm^{-1}, $\varkappa_s =$ (finite value) $\times \varkappa_a$, $d = 50$ Å, $D = 0.5\,\mu$m, and $\Phi = 0.3$. Application of these numerical values to Eq. (1) yields a set of characteristic dielectric parameters, with which we may commence search for the most plausible set of phase parameters by applying *Steps 1* to *5*. The values of parameters thus obtained can be compared with the original ones. An example for errors due to gradually augmented $\varkappa_s$ in the estimates is tabulated in Table 3. The errors were found to be an increasing function of $\varkappa_s/\varkappa_a$. The most sensitive to the misuse of $\varkappa_s$ was the estimate for ε_s; yet the errors as a whole were less than 0.2% where a condition $\varkappa_s/\varkappa_a \lesssim 10^{-5}$ was fulfilled.

On the other hand, some typical values for the highest conductance reported on living membranes are 10^2 mS cm^{-2} in order of magnitude [1, 4], this value yielding the ratio $\varkappa_s/\varkappa_a \simeq 10^{-5}$ when $\varkappa_a = 10$ mS cm^{-1} and $d = 100$ Å. These values for $\varkappa_a$ and d were chosen in view of the conductivity of conventional physiological solutions and the current estimates of membrane thickness, respectively. Furthermore, calculations so far made have shown no appreciable differences in the results of the determination procedure between the choice of $\varkappa_s = 0$ and of $\varkappa_s/\varkappa_a = 10^{-5}$. Thus the possibility that the simplest choice of $\varkappa_s = 0$ invalidates totally the procedure proposed can be excluded. In addition, such a simplification is practically allowable even for $\varkappa_s/\varkappa_a \simeq 10^{-4}$ if small errors of 2% in the results are of no consequence.

Errors Due to Uncertainty in the Estimate of d

The second source of errors to be considered is uncertainty in estimate for d, the dielectrically effective shell thickness. Thickness determination in general is much more difficult and unreliable as compared with the measurement of D. In fact, morphological evidence currently available on the biological membrane thickness only scatters over a range 50 to 110 Å [15, 17]. Possible errors due to such unreliable estimates for d were calculated, on three systems having different values of D, in a manner similar to the foregoing case of $\varkappa_s$. The results are shown

Table 4. Errors due to the use of a twice as large value of shell thickness

D, μm	d/D	Error in %[a]			
		ε_s	ε_i	$\varkappa_i$	C_M
5	10^{-3}	100	0.14	0.20	— 0.0015
0.5	10^{-2}	100	1.1	1.7	— 0.11
0.05	10^{-1}	88	— 6.9	— 1.9	— 7.7

[a] $\dfrac{\text{(Values estimated with } d = 100 \text{ Å)} - \text{(Values corresponding to } d = 50 \text{ Å)}}{\text{(Values corresponding to } d = 50 \text{ Å)}} \times 100$

in Table 4 as percentage errors that were caused by overestimating the d value twice as thick as the true one, *i.e.*, 100 Å instead of 50 Å.

It was found that the relative inaccuracy in estimating the thickness d did not affect seriously the determination of ε_i, $\varkappa_i$ and C_M as far as d was less than about 1 % of D, while an error in d was directly reflected on the ε_s values estimated. This finding suggests that the value of ε_s should not be referred to if it were not for any reliable value of d available, but that the membrane capacitance C_M, instead of ε_s, is still obtainable with excellent accuracy.

Relationship between the P- and the Q-Dispersion

The procedure proposed above is impracticable when the experimental assessment of ε_m and f_P is difficult because of overlapping between the P- and the Q-dispersion. It therefore becomes important, in practivce of the procedure, to take note of the following *Relations:*

Relation A: $f_P \ll f_Q$ and *Relation B:* $\Delta\varepsilon_P \gg \Delta\varepsilon_Q$.

Relation A implies very little overlapping between the P- and the Q-dispersion. *Relation B* indicates that the dielectric dispersion observed may be assigned, regardless of *Relation A*, entirely to the P-dispersion in view of very little Q-dispersion. It follows thus that the procedure is applicable to all the cases except for simultaneous violation of the two *Relations*. In other words, a criterion of the applicability is: Do the dielectric data satisfy either of *Relations A* and *B*? In order to prevent any misleading application of the procedure, one should examine whether the data in question conform to the criterion or not by substituting the results of analysis into the general equation.

To give an example here again, such an examination was carried out for the synaptosomal specimen with emphasis on the possible errors caused by uncertainty in estimates for d and $\varkappa_s$. The results are shown in Fig. 4. It is apparent from the figure that *Relation A* does not necessarily hold for $d/D \approx 10^{-2}$ (corresponding to $d \approx 68$ Å) or for $\varkappa_s/\varkappa_a \approx 10^{-4}$ (corresponding to $\varkappa_s \approx 10^{-6}$ S cm^{-1}), whereas *Relation B* holds for the same abscissa values. In the light of the criterion mentioned above the present data on synaptosomes (Fig. 3) are assigned exclusively to the P-dispersion, the application of the procedure thus being justified.

At the first glance it appears that the broadening of the observed P-dispersion shown in Fig. 3 might have been a resultant relaxation of the P- and the Q-dispersion. From the consideration given above, however, with respect to Fig. 4,

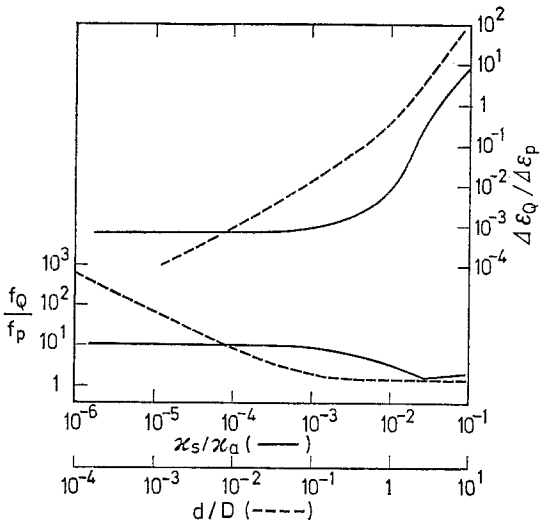

Fig. 4. Dependence of the ratios f_Q/f_P and $\Delta\varepsilon_Q/\Delta\varepsilon_P$, both predicted from text Eq. (1), on the size factor d/D (broken line) and on the conductivity ratio $\varkappa_s/\varkappa_a$ (solid line). Parameters used: $\varepsilon_a = 74$, $\varepsilon_s = 3.64$, $\varepsilon_i = 35.7$, $\varkappa_a = 10.27$ mS cm^{-1}, $\varkappa_i = 3.96$ mS cm^{-1}, $D = 0.678\,\mu$m and $\Phi = 0.383$; $\varkappa_s = 10^{-5}\,\varkappa_a$ for calculation with varying d/D; $d = 50$ Å for calculation with varying $\varkappa_s/\varkappa_a$

the Q-dispersion is estimated to be vanishingly small in comparison with the P-dispersion. Hence the broadening as seen in Fig. 3 may be attributed to distribution of globule size and internal conductivity or to the presence of intracellular organelles. Further interpretation on the mechanism of the broadening will be given in our subsequent paper on synaptosomes [3].

Concluding Remarks

The procedure proposed is applicable to suspensions, of which the size and the phase parameters fall within the following ranges:

$d = 50$ to 100 Å, $d/D \lesssim 10^{-2}$, $\varepsilon_a = 3$ to 100, $\varepsilon_s = 3$ to 50, $\varepsilon_i = 3$ to 100, $\varkappa_a = 10^{-1}$ to 10^{-2} mS cm^{-1}, $\varkappa_i = (10^{-2}$ to $10^{1}) \times \varkappa_a$, $\varkappa_s \lesssim 10^{-4} \times (\varkappa_a$ and $\varkappa_i)$.

Numerical calculation for the procedure can be made by use of a relatively simple calculator.

Both *Relations A* and *B* gradually break down for a set of phase parameters outside the ranges shown above. In such cases one should have recourse to a more extensively generalized procedure such as reported by Redwood *et al.* [10].

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