First-principle approach to dielectric behavior of nonspherical cell suspensions

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(Received 27 October 2000; revised manuscript received 21 December 2000; published 28 June 2001)

We present a theoretical study of the dielectric behavior of cell suspensions by employing the Bergman-Milton spectral representation of the effective dielectric constant. By means of the spectral representation, we derive the dielectric dispersion spectrum in terms of the electrical and structure parameters of the cell models. Our results show that a better agreement with the experimental data can be obtained, provided that we introduce a conductivity contrast $t=\sigma_2/(\sigma_2-\sigma_1)$. We find that the conductivity of the cell cytoplasm σ_1 can be much larger than that of the suspending medium σ_2, in contrast to the previous claim that $\sigma_1 \approx \sigma_2$.

DOI: 10.1103/PhysRevE.64.012903 PACS number(s): 87.18.−h, 82.70.−y, 77.22.Gm, 77.84.Nh

I. INTRODUCTION

Recently, dielectric spectroscopy was successfully applied to real-time monitoring of cell growth in suspensions [1–3]. Real-time monitoring has advantages over conventional techniques, and would be important in investigating the dynamic behavior of cell growth. There are many factors that may influence the dielectric behavior of biological materials: structure, orientation of dipoles, surface conductances, membrane transport processes, etc. All these factors influence one another, and it is difficult to separate out the effect of a single one. However, some effects can be dominant at certain ranges of frequencies. For instance, the dielectric dispersion spectrum of living cell suspensions in radiofrequencies is mainly determined by the cell shape. The objective of this work is to develop a theory for such correlation, on which new applications in biotechnology rely.

More recently, Asami [4] reported that the dielectric dispersion spectrum of fission yeast cells in a suspension was mainly composed of two subdispersions. The experimental data revealed that the low-frequency subdispersion depended on the cell length, while the high-frequency one was independent of it. Asami adopted a shell-ellipsoid model [3], in which an ellipsoid is covered with an insulating shell as the electrical model of nonspherical biological cells. To avoid the complicated mathematics of the model, the previous theoretical analysis [3] was limited to the dilute limit in which a small concentration of cells is suspended in a medium. The comparison between model calculation [3] and experimental data [4] was far from being satisfactory.

In this work, we propose the use of a spectral representation [5] for analyzing the cell models. The spectral representation is a rigorous mathematical formalism of the effective dielectric constant of a two-phase composite material [5]. It offers the advantage of the separation of material parameters (namely, the dielectric constant and conductivity) from the cell structure information, thus simplifying the study. From the spectral representation, one can readily derive the dielectric dispersion spectrum, with the dispersion strength as well as the characteristic frequency being explicitly expressed in terms of the structure parameters and the materials parameters of the cell suspension (see Sec. II B below). Thus we can investigate their impact on the dispersion spectrum directly, without having to deal with the full dispersion spectrum.

The plan of the paper is organized as follows. In Sec. II, we will review the spectral representation theory [5], and show that the dielectric dispersion spectrum of a cell suspension can be expressed in terms of the spectral representation. In Sec. III, we will apply the spectral representation to the ellipsoidal cell model [3], and present an alternative approach. We show that a better agreement with the experimental data can be achieved. A discussion of further applications of our theory will be given.

II. FORMALISM

A. Spectral representation

We regard a cell suspension as a two-component system consisting of biological cells of a complex dielectric constant ϵ_1 dispersed in a host medium of ϵ_2. A uniform electric field $\mathbf{E}_0$ is applied. For convenience, let $\mathbf{E}_0=-\mathbf{e}_z$. We briefly review the spectral representation theory of the effective dielectric constant to establish notations.

The problem is initiated by solving the differential equation

$$\nabla\cdot\left[\left(1-\frac{1}{s}\eta(\mathbf{r})\right)\nabla\Phi(\mathbf{r})\right]=0, \tag{1}$$

where $s=\epsilon_2/(\epsilon_2-\epsilon_1)$ denotes the relevant material parameter, and $\eta(\mathbf{r})$ is the characteristic function of the cell structure. The electric potential $\Phi(\mathbf{r})$ can be solved formally [5],

$$\Phi(\mathbf{r})=z+\frac{1}{s}\int d\mathbf{r}'\,\eta(\mathbf{r}')\nabla' G_0(\mathbf{r}-\mathbf{r}')\cdot\nabla'\Phi(\mathbf{r}'), \tag{2}$$

where $G_0(\mathbf{r}-\mathbf{r}')=1/4\pi|\mathbf{r}-\mathbf{r}'|$ is the free space Green's function. By denoting an operator

$$\Gamma = \int d\mathbf{r}'\, \eta(\mathbf{r}')\nabla' G_0(\mathbf{r}-\mathbf{r}')\cdot\nabla', \tag{3}$$

and the corresponding inner product

$$\langle\Phi|\Psi\rangle = \int d\mathbf{r}\, \eta(\mathbf{r})\nabla\Phi^*\cdot\nabla\Psi, \tag{4}$$

it is easy to show that Γ is a Hermitean operator. Let s_n and $\Psi_n(\mathbf{r})$ be the nth eigenvalue and the eigenfunction of the Γ operator, respectively; then we write the effective dielectric constant $\bar{\epsilon}$ in the Bergman-Milton representation [5]:

$$\begin{aligned}\bar{\epsilon} &= -\frac{1}{V}\int dV\, \epsilon(\mathbf{r})E_z\\ &= \frac{1}{V}\int dV\, \epsilon_2\left[1-\frac{1}{s}\eta(\mathbf{r})\right]\frac{\partial\Phi}{\partial z}\\ &= \epsilon_2\left(1-\frac{1}{V}\sum_n \frac{|\langle\Psi_n|z\rangle|^2}{s-s_n}\right)\\ &= \epsilon_2\left(1-\sum_n \frac{f_n}{s-s_n}\right).\end{aligned} \tag{5}$$

The spectral representation offers a separation of the material parameter s from the structure parameters s_n and f_n. The structure parameters s_n and f_n satisfy simple properties that $0\leqslant s_n\leqslant 1$ and $\Sigma f_n = p$, where p is the volume fraction of the suspending cells [5].

B. Dielectric dispersion spectrum

For cells of arbitrary shape, the eigenvalue problem of the Γ operator can only be solved numerically. However, analytical solutions can be obtained for isolated spherical and ellipsoidal cells. For dilute suspensions of prolate spheroidal cells under a weak applied field, the cells can be regarded as noninteracting and randomly oriented. The problem is simplified to a calculation of s_n and Ψ_n with a single cell, which can be solved exactly. Only two of the f_n's are nonzero, due to the orthogonality of the Ψ_n with z. The corresponding eigenfunctions are $\Psi_1 = \mathbf{r}\cdot\mathbf{e}_z/v$ and $\Psi_2 = \mathbf{r}\cdot\mathbf{e}_{x(or\ y)}/v$, where $\mathbf{e}_z$ and $\mathbf{e}_{x(or\ y)}$ are unit vectors along the major and minor axes of the prolate spheroid, respectively, and v is the volume of the cell. By averaging over all possible orientations of the cells, we obtain the effective complex dielectric constant $\bar{\epsilon}$ of the suspensions,

$$\bar{\epsilon} = \epsilon_2\left(1-\frac{1}{3}\frac{p}{s-s_1}-\frac{2}{3}\frac{p}{s-s_2}\right), \tag{6}$$

where s_1 and s_2 are the depolarization factors along the z axis and the x axis (or y axis) of the prolate spheroid. In what follows, we will show that from the spectral representation, one can readily derive the dielectric dispersion spectrum.

Substituting $\epsilon_1 = \varepsilon_1 + \sigma_1/j2\pi f$ and $\epsilon_2 = \varepsilon_2 + \sigma_2/j2\pi f$ (ε and σ being the real and imaginary parts of the complex dielectric constant) into Eq. (6), defining a new parameter $t = \sigma_2/(\sigma_2-\sigma_1)$, and redefining $s = \varepsilon_2/(\varepsilon_2-\varepsilon_1)$, we rewrite the effective dielectric constant $\bar{\epsilon}$ after simple manipulations,

$$\bar{\epsilon} = \epsilon_H + \frac{\Delta\epsilon_1}{1+jf/f_1^c} + \frac{\Delta\epsilon_2}{1+jf/f_2^c} + \frac{\sigma_L}{j2\pi f}, \tag{7}$$

where ϵ_H and σ_L are the high-frequency dielectric constant and the low-frequency conductivity, respectively; $\Delta\epsilon_1$ and $\Delta\epsilon_2$ are the dispersion magnitudes and f_1^c and f_2^c are the characteristic frequencies. They are given by

$$\Delta\epsilon_1 = \frac{1}{3}p\varepsilon_2\frac{s_1(s-t)^2}{s(s-s_1)(t-s_1)^2}, \tag{8}$$

$$\Delta\epsilon_2 = \frac{2}{3}p\varepsilon_2\frac{s_2(t-s)^2}{s(s-s_2)(t-s_2)^2}, \tag{9}$$

$$f_1^c = \frac{\sigma_2 s(t-s_1)}{2\pi\varepsilon_2 t(s-s_1)}, \tag{10}$$

$$f_2^c = \frac{\sigma_2 s(t-s_2)}{2\pi\varepsilon_2 t(s-s_2)}. \tag{11}$$

Thus we are able to obtain the dispersion strengths as well as the characteristic frequencies explicitly in terms of the structure parameters and material parameters of the cell suspension. To compare with experiment [4], it is instructive to compute the ratios $\Delta\epsilon_1/\Delta\epsilon_2$ and f_2^c/f_1^c:

$$\frac{\Delta\epsilon_1}{\Delta\epsilon_2} = \frac{s_1(s-s_2)(t-s_2)^2}{2s_2(s-s_1)(t-s_1)^2}, \tag{12}$$

$$\frac{f_2^c}{f_1^c} = \frac{(t-s_2)(s-s_1)}{(t-s_1)(s-s_2)}. \tag{13}$$

Thus, by using the spectral representation, we obtain very simple expressions for comparison with the experimental data.

TABLE I. The dispersion strengths and characteristic frequencies calculated from the experimental data of Ref. [4].

$q(l/d)$	$\Delta\epsilon_1$	f_1^c (kHz)	$\Delta\epsilon_2$	f_2^c (MHz)	$\Delta\epsilon_1/\Delta\epsilon_2$	f_2^c/f_1^c
3.46	1750	190	790	1.7	2.21	8.9
7.17	6920	73	800	2.0	8.65	27.3
10.24	13100	38	800	2.0	16.4	52.6

III. COMPARISON WITH EXPERIMENTS

Table I lists the parameters calculated from the experiment [4], which was done by using a temperature sensitive cell division cycle mutant of fission yeast, cdc25-22, whose cell length increases in proportion to the cultivation time at the restrictive temperature, while keeping the cell diameter

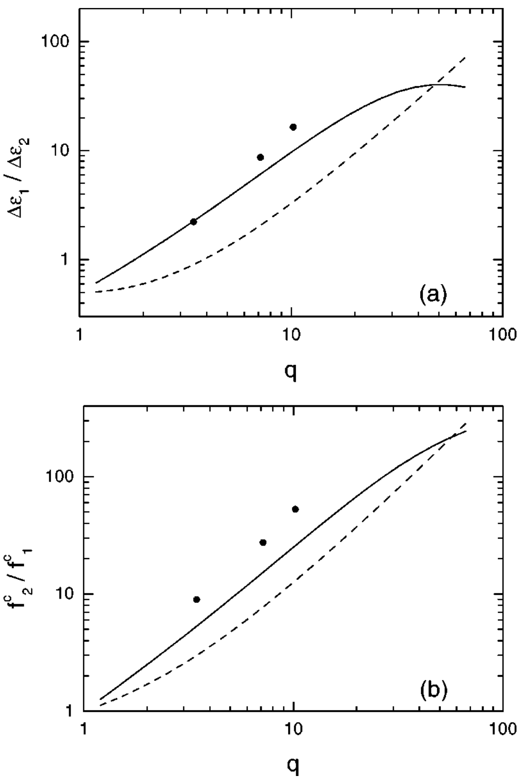

FIG. 1. (a) Ratio of the dispersion magnitudes plotted against the axial ratio (cell length vs diameter): solid line (present theory), dashed line (Asami's theory), and filled circles (experimental data). (b) Ratio of the dispersion frequencies plotted against the axial ratio: solid line (present theory), dashed line (Asami's theory), and filled circles (experimental data).

almost unchanged [4]. The experimental dielectric dispersion curves show two main steps for all three cell populations with a different axial ratio q (length l/diameter d), which is in fairly good agreement with the form of Eq. (7). The changes of the dispersion magnitude $\Delta\epsilon_i$ and the characteristic frequency f_i^c are attributed to the change of the structure parameters s_n. For the prolate-spheriodal cell, s_n is given by

$$s_1 = -\frac{1}{q^2-1} + \frac{q\ln[q+(q^2-1)^{1/2}]}{(q^2-1)^{3/2}}, \tag{14}$$

$$s_2 = (1-s_1)/2. \tag{15}$$

Note that s_1 tends to zero as q goes to infinity. Both $\Delta\epsilon_1/\Delta\epsilon_2$ and f_2^c/f_1^c increase rapidly as q increases. Equations (12) and (13) imply that t should be smaller than or at least of the same magnitude as s_1, which is approximately $[\ln(2q)-1]/q^2$ for $q^2 \gg 1$. Physically, t should also be negative. We estimate t and s by fitting Eqs. (12) and (13) to the experimental ratio of $\Delta\epsilon_1/\Delta\epsilon_2$ and f_2^c/f_1^c, and we obtain $t = -0.0014$ and $s = 5.0$. This means that $\sigma_1 \approx 700\sigma_2$ and $\varepsilon_1 \approx 0.80\varepsilon_2$. The enhanced conductivity of cell cytoplasm is attributed to the membrane potential. The result is in contrast to the previous (unjustified) claim that $\sigma_1 \approx \sigma_2$.

Figure 1 shows the experimental [4] and theoretical [Eqs. (12) and (13)] ratios $\Delta\epsilon_1/\Delta\epsilon_2$ and f_2^c/f_1^c versus the axial ratio q. The theoretical calculations of Ref. [4] are also plotted for comparison. From the figure, it is evident that our theory gives a better comparison with experiment. The reason for the better fit lies in our ability to introduce the conductivity contrast t naturally via the spectral representation. Because of the small t value obtained, a large value of σ_1 should be used.

Next we consider the effect of the cell membrane. For shelled spherical and spheroidal cells, one can adopt treatments similar to these in Refs. [6,7]. For a thin membrane of a few nanometers, the dispersion magnitudes remain essentially unchanged.

IV. DISCUSSION AND CONCLUSION

In this work, we have applied a spectral representation to the dielectric dispersion of suspensions of fission yeast cells. The large cytoplasmic conductivity is a key result of our investigation. We believe that the large cytoplasmic conductivity is reasonable because the cells have to maintain a higher ion concentration in their cytoplasm to avoid the shrinkage of cells due to a loss of water across the cell membrane. However, to our knowledge, there exists no direct experimental mesurement of the cytoplasmic conductivity. In our work, we propose a convenient and practical means of determining the cytoplasmic conductivity from the dielectric spectroscopy data. We should remark that while the membrane transport is worth studying, it is a complex living process and we do not have a detailed physical model at present.

Although a better agreement with experiment has been obtained, there are some discrepancies. As mentioned by Asami [4], the discrepancies could be attributed to the rodlike cell shape. For cells of nonconventional shape, however, there exists no available cell model in the literature, and we must develop the spectral representation from first principles. The present approach should be applicable to cells of nonconventional shape.

More precisely, we will develop a Green's function formalism [8,9] for calculating the spectral representation of rods of finite length. A similar formalism was adopted for biological cells near their subdivision point [10–12]. We propose modeling the rodlike cells as spherocylinders, i.e., circular cylinders with two hemispherical caps at both ends. We will solve the spectral representation of the effective dielectric constant from first principles. We propose to investigate if the spherocylinder model would yield a better description of the experimental data.

Moreover, in the presence of a higher applied electric field (either dc or ac), the dipole moments of the rodlike cells can be reoriented in favor of the applied field. In this case, the effective dielectric constant can be anisotropic. In a previous work [13], we showed that the anisotropic microstructure can have a significant impact on the spectral function. We naturally propose to examine how the anisotropy would change the dielectric dispersion spectrum accordingly.

In conclusion, we have presented a first-principles study of the dielectric behavior of cell suspensions by employing a spectral representation of the effective dielectric constant. By means of this spectral representation, we have derived the dielectric dispersion spectrum in terms of the electrical and structure parameters of the cell models. Our results have shown a better agreement with the experimental data.

Note added in proof: After the acceptance of this paper, we received a letter from Dr. Koji Asami of the Institute for Chemical Research at Kyoto University, who informed us that the approximation in his previous calculation was not appropriate and that the calculation without that approximation provides a good simulation for his experimental results with fission yeast [14]. Asami and his co-workers also calculated the circular cylinder model by the boundary-element method and found that the result is almost the same as that of the spheroidal model. Similar conclusions were obtained by us [15].

ACKNOWLEDGMENTS

This work was supported in part by a Direct Grant for Research of the Research Committee and in part by the Research Grants Council of the Hong Kong SAR Government under Grant No. CUHK4284/00P. K.W.Y. acknowledges useful conversation with Professor G. Q. Gu.

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