Determination of electric parameters of cell membranes by a dielectrophoresis method

P. Marszalek,*‡ J. J. Zielinsky,‡ M. Fikus,§ and Tian Y. Tsong*

*Department of Biochemistry, University of Minnesota, St. Paul, Minnesota, 55108, USA; ‡Department of Fundamental Research in Electrotechnics, Electrotechnical Research Institute, Pozaryskiego 28, 04-703 Warsaw, Poland; and §Institute of Biochemistry and Biophysics, Polish Academy of Sciences, Rakowiecka 36, 02-532 Warsaw, Poland

ABSTRACT Marszalek, P., J. J. Zielinsky, and M. Fikus (1989. *Bioelectrochem. Bioenerg.* 22:289–298) have described a novel design for measuring the complete dielectrophoretic spectrum of a single cell. From the analysis of the dielectrophoretic spectrum, the membrane conductivity, σ_{membr}, and the membrane dielectric permittivity, ϵ_{membr}, of the cell may be determined according to the theory of dielectrophoresis described by Sauer, F. A. (1985. Interactions between Electromagnetic Field and Cells. A. Chiabrera, C. Nicolini, and H. P. Schwan, editors. Plenum Publishing Corp., New York. 181–202). At F_0, the net force experienced by a single shell sphere in a nonuniform periodic field is zero, and the sphere ceases to move in the field. In other words, at F_0, the effective polarizability, χ_{eff}, of the sphere (the polarizability of sphere minus the polarizability of the medium) is equal to zero. For biological cells in a high conductivity medium, e.g., the isotonic saline, σ_{membr} falls below 2×10^{-6} S m^{-1}, where F_0 becomes insensitive to σ_{membr}, and the method becomes impractical. In a low conductivity medium, 0.3 M sucrose, σ_{membr} of cells is generally higher and the method may be applied. Assuming a membrane thickness of 9 nm, ϵ_{membr} of *Neurospora crassa* slime cells was determined to be in the range of 8.3–9.4 ϵ_0, and of myeloma Tib9 to be 9.4 ϵ_0, ϵ_0 being the dielectric permittivity of the free space. The values for the slime cells were compared with values obtained by the dielectric spectroscopy method which measures average values for cells in suspension.

INTRODUCTION

Dielectrophoresis denotes the motion of polarizable particles under the influence of a nonuniform electric field and the phenomenon has been known for some time (1). Dielectrophoresis of cells has gained recent popularity among cell biologists and biophysical chemists because it is a convenient technique for congregating different types of cells in a suspension for electrofusion (2). Dielectrophoresis of a cell reflects the electric properties of the cell and mechanisms of electric field induced motions have been of considerable theoretical interest (3–7). Marszalek et al. (8, 9) have described a novel experimental design which enabled them to measure the complete dielectrophoresis spectrum of single cells and test different models of dielectrophoresis (10). Specifically they have compared the models of Sher (4) and Sauer (5) and have shown that the Sauer's model which treats a living cell as a particle of lossy dielectric is a better model for describing the dielectrophoresis of cells. They have also studied dielectrophoresis of yeast, protoplast *cucumis anguria varlongipes,* and *Neurospora crassa* slime cells and have obtained certain electric parameters of *N. crassa* slime by the analysis of its dielectrophoretic spectrum.

In another study, Kaler and Joines (11) have measured dielectrophoretic levitation spectra of Canola protoplasts and ligament fibroblast cells. From the so called breakpoint frequency of the spectrum of single cells, the capacitance of the cell membrane can be determined. This method is an elegant demonstration of how the dielectrophoresis of a cell may be used to derive electric parameters of the cell membrane. However, the method requires rather sophisticated instrumentation and its use is also limited to the positive range of the dielectrophoresis (a cell moves towards high-field intensity). This latter limitation makes it unsuitable for determining the critical frequency, F_0, of a cell. At F_0, the dielectrophoresis changes its sign either from the negative (a cell moves away from high-field intensity) to the positive, or vice versa (9, 10). At this frequency, the effective polarizability of a cell is zero and so is the net force experienced by the cell. As will be shown, F_0 of a cell contains quantitative information on the electric parameters of the membrane and these parameters can be extracted by regression analysis of the data according to the theory of Sauer (5).

In this paper we report F_0 of murine myeloma cell line Tib9 and *Neurospora crassa* slime (*N. crassa*) in media of different conductivities. Electric parameters of these cells were determined and those of *N. crassa* are compared with data obtained earlier using the dielectric spectroscopic method (9, 10).

Address correspondence to Dr. Tsong.

0006-3495/91/05/982/06 $2.00

THEORETICAL CONSIDERATION

F_o is sensitive to changes in certain electric parameters of a cell but not to others. Experiments must be designed to exploit this sensitivity. For this purpose we have examined some properties of F_o. The equation describing the dielectrophoretic force acting on a spherical cell assumes the form (5, 10),

$$F = \tfrac{1}{4} V \cdot \chi_{eff} \cdot \nabla |E|^2, \quad (1)$$

where

$$\chi_{eff} = 3 \text{ Re } (\epsilon_1) \cdot Re[(\epsilon_{eff} - \epsilon_1)/(\epsilon_{eff} + 2\epsilon_1)]. \quad (2)$$

In Eqs. 1 and 2, E and V are the amplitude of the applied electric field and the volume of the cell, respectively. χ_{eff} is the effective polarizability of the cell. ϵ_1 is the complex dielectric permittivity of the medium, and $\epsilon_1 = \epsilon'_1 - i\sigma_1/(2\pi f)$, where ϵ'_1 is the dielectric permittivity of the medium, or water, and is equal to 80 ϵ_o ($\epsilon_o = 8.85 \times 10^{-12}$ *F/m*, the dielectric permittivity of the free space). σ_1 is the conductivity of the medium, and f is the frequency of the field. The symbol Re denotes the real part of a complex formula. ϵ_{eff} is the effective complex dielectric permittivity of a cell (12). When a cell and its environment are separated into three phases, namely, the internal, the membrane and the external phases, as shown in Fig. 1, ϵ_{eff} may be expressed in electric parameters of the first two phases as (12),

$$(\epsilon_{eff} - \epsilon_{membr})/(\epsilon_{eff} + 2\epsilon_{membr}) = [R/(R + d)]^3(\epsilon_{int} - \epsilon_{membr})/(\epsilon_{int} + 2\epsilon_{membr}), \quad (3)$$

where $\epsilon_k = \epsilon'_k - i\,\epsilon''_k$. ϵ'_k is the dielectric permittivity of phase k, and $\epsilon''_k = \sigma_k/(2\pi f)$, where σ_k is the electric conductivity of phase k when the frequency of the field is low. ϵ_{eff}, ϵ_1, and χ_{eff} all depend on frequency but χ_{eff} can also change sign. Fig. 2 shows the theoretical relationship between χ_{eff} and the frequency of the applied field when a cell is suspended in media of different conductivities. For frequencies at which $\chi_{eff} > 0$, the cell undergoes positive dielectrophoresis. In contrast, the cell undergoes negative dielectrophoresis for frequencies where $\chi_{eff} < 0$. By definition, the critical frequency, F_o, is the frequency at which,

$$\chi_{eff}[\epsilon_{eff}(F_o), \epsilon_1(F_o)] = 0. \quad (4)$$

As seen in Fig. 2, for each dielectrophoresis spectrum, there are two frequencies at which χ_{eff} would be zero. The F_o which occurs at lower frequencies is remarkably sensitive to the conductivity of the suspending medium, but the one that occurs ~100 MHz is not. For this

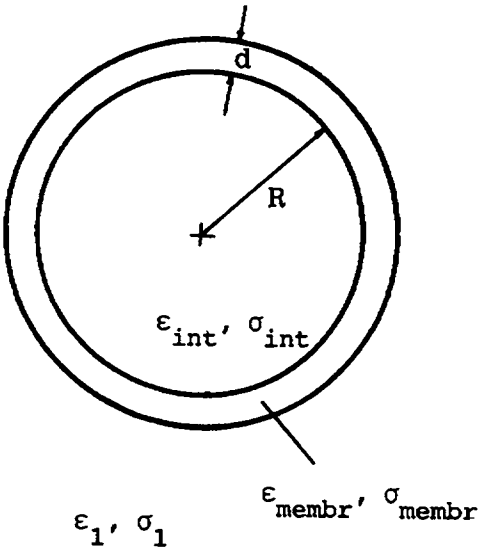

FIGURE 1 One-shell electrical model of a spherical cell. The dielectric permittivity, ϵ, and electric conductivity, σ, in different phases are specified. The subscripts, int, membr, and 1 denote, respectively, cell interior, cell membrane, and suspending medium. The radius of the cell and the membrane thickness are given, respectively, in R and d.

communication, attention will be directed only to the first critical frequency. Curve 5 (Fig. 2) exemplifies a case of low internal conductivity (0.05 S m^{-1}) which is close to the conductivity of the external medium (0.045 S m^{-1}). Curves 1–4 (Fig. 2) give theoretical dielectrophoretic spectra of a cell of σ_{int} 0.5 S m^{-1} with varying σ_1. It is clear that F_o is sensitive to the conductivity of the

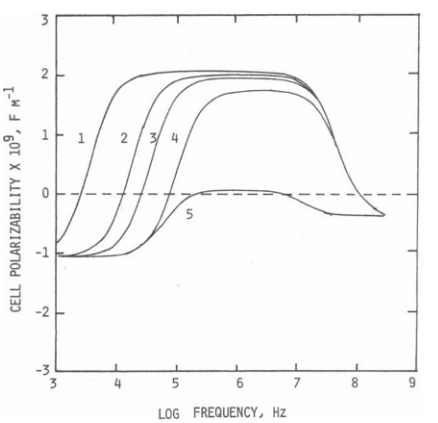

FIGURE 2 Dielectrophoretic spectra of the model cell shown in Fig. 1. Cell electric parameters were $\epsilon_{int} = 45\ \epsilon_o$, $\sigma_{int} = 0.5$ S m^{-1}, $\epsilon_{membr} = 8\ \epsilon_o$, $\sigma_{membr} = 1 \times 10^{-8}$ S m^{-1}, $R = 10$ μm, $d = 8$ nm. For curves 1–4, the medium conductivity, σ_1, was 0.001, 0.005, 0.01, and 0.03 S m^{-1}, respectively. For curve 5, σ_{int} was 0.05 S m^{-1}, and σ_1 was 0.045 S m^{-1}.

external medium. Although all these spectra converge to plateau values at low and high frequencies.

In Fig. 3, the dependence of F_o on different electric parameters of the system are shown. σ_{int} was varied in Fig. 3 *A*, ϵ_{membr} in Fig. 3 *B*, and σ_{membr} in Fig. 3 *C*. Fig. 3 *A* indicates that for the $\sigma_1 < 0.05$ S m^{-1}, F_o was insensitive to the variation of σ_{int} in the range 0.1–1 S m^{-1} (Curves 2 and 3). F_o became sensitive to σ_{int} when σ_{int} was close to σ_1 (Curve 1). However, as shown in Fig. 2, Curve 5, F_o cannot be determined accurately in this case. Because of Joule heating, dielectrophoresis experiments were generally done in a medium of low conductivity, i.e., $\sigma_1 <$ 0.05 S m^{-1}. In contrast, F_o was quite sensitive to ϵ_{membr}, indicating that dielectrophoresis can be used to determine this parameter. Fig. 3 *C* indicates that F_o was fairly sensitive to the membrane conductivity, σ_{membr}, when it was $> 2 \times 10^{-6}$ S m^{-1} but was less sensitive for its value below 1×10^{-7} S m^{-1}. The membrane conductivity of cells in an isotonic KCl medium is $\sim 1 \times 10^{-8}$ S m^{-1} by the micropipette technique (13) and the present method would not be adequate. However, the membrane conductivity of certain cells, or cells suspended in a low ionic medium (e.g., isotonic sucrose), is often found to be $> 2 \times 10^{-6}$ S m^{-1} and the present method may be applied (Gimsa, J., P. Marszalek, U. Loewe, and T. Y. Tsong, unpublished results).

Regression analysis of the data was done to minimize the function S using an optimized parameter variation technique. The parameters to be optimized are ϵ_{membr} and σ_{membr}.

$$S = \sum_i [F_{o,i}^{exp}(\sigma_{1,i}) - F_{o,i}^{theor}(\sigma_{1,i})]/\Delta F_{o,i}^{exp}(\sigma_{1,i})]^2, \qquad (5)$$

where $F_o^{theor}(\sigma_1)$ is the theoretical function based on Eqs. 2 and 4, and $F_0^{exp}(\sigma_1)$ is from the experimental data. $\Delta F_{o,i}^{exp}(\sigma_{1,i})$ are errors of $F_{o,i}^{exp}(\sigma_{1,i})$ for the ith data point.

MATERIALS AND METHODS

Dielectrophoresis of a sedimenting cell

The method developed and described previously by Marszalek et al. (8, 10) was employed. This method observes the trajectory of a single cell sedimenting by gravitation in an applied, horizontal, nonuniform, alternating electric field (ac). A schematic of the chamber used for these experiments is shown in Fig. 4 *A*. A square shaped enclosure of plexiglass, 3 mm high, was mounted on a glass slide to hold a cell suspension. In the plexiglass enclosure a platinum sheet (3 × 0.1 × 15 mm) and a platinum wire (diameter 0.2 mm, length 15 mm) were positioned 0.2 mm apart to serve as electrodes. A cover slip was placed on top of the enclosure to hold the cell suspension. The chamber was then placed on the microscope table in such a way that the two electrodes were oriented in the vertical direction. The chamber was connected to a model 148A waveform generator (Wavetek San Diego, Inc., San Diego, CA) and a model 7704A oscilloscope (Tektronix, Inc., Beaverton, OR) for dielectrophoresis experiments.

Fig. 4 *B* illustrates how an experiment was done. The microscope was focused near the center of the two electrodes. A cell sedimenting at a constant speed came into view and was arbitrarily selected for observation. An ac field of intensity 200 V cm^{-1} and of frequency 12 kHz was applied. At this frequency, the cell was deflected toward the wire electrode indicating a positive dielectrophoresis. When the frequency was changed to 8 kHz, the cell changed its trajectory toward the sheet electrode indicating a negative dielectrophoresis. After several frequency adjustments, the cell finally began to sediment in a straight line with gravitation, at a constant speed. This last frequency was assigned F_o. Each determination took a few minutes to complete. The intensity of the ac field can influence the speed of the cell movement toward either electrode but it is not critical for the determination of F_o.

A light microscope (Carl Zeiss, Inc., Thornwood, NY) was used in these experiments. Most experiments were done by visual observation. However, some data were obtained using a video recording system,

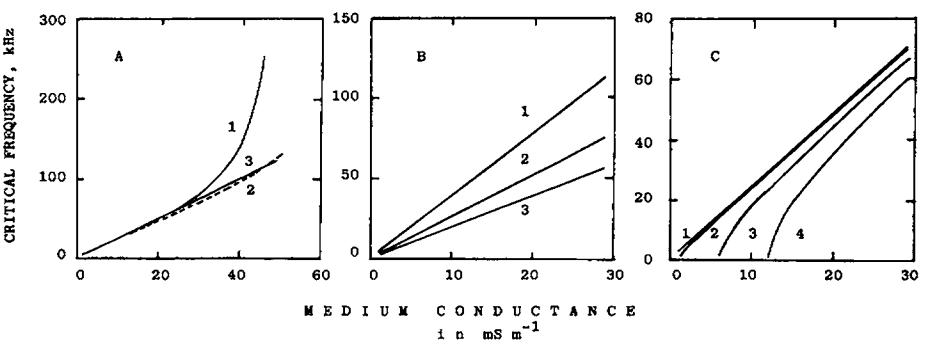

FIGURE 3 Different sensitivities of the critical frequency of dielectrophoresis (F_o) on electric parameters of a cell. (*A*) Influence of the cell interior conductivity, σ_{int}. Its values were 0.05, 0.10, and 1.0 S m^{-1} for curves 1, 2, and 3, respectively. Other electric parameters are given in Fig. 1. (*B*) Influence of the dielectric permittivity, ϵ_{membr}. Its values were 5 ϵ_o, 7.5 ϵ_o, and 10 ϵ_o for curves 1, 2, and 3, respectively. (*C*) Influence of the membrane conductivity, σ_{membr}. Its values were $\leq 1 \times 10^{-7}$, 1×10^{-6}, 5×10^{-6}, and 1×10^{-5} S m^{-1} for curves 1, 2, 3, and 4, respectively.

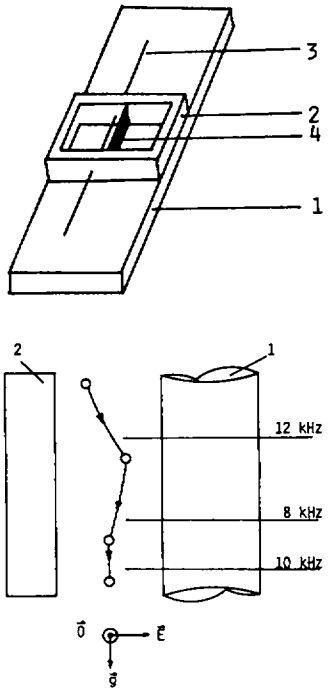

FIGURE 4 The dielectrophoresis experiment. (*A*) The cell chamber: 1, microscopic slide; 2, a vessel made of plexiglass, capacity ~0.6 cm^3; 3, platinum wire electrode 0.2 mm diam and 15 mm long; 4, platinum sheet electrode, 15 × 3 × 0.1 mm. The distance between the two electrodes was 0.2 mm. (*B*) The observations of cell movement in an ac field: 1, the Pt-wire electrode; 2, the Pt-sheet electrode. A cell is shown to enter at the top of the observation field by gravitation. When the ac field was tuned to 12 kHz, the cell moved toward the wire electrode (positive dielectrophoresis). When the ac field was tuned to 8 kHz, the cell moved toward the sheet electrode (negative dielectrophoresis). After several adjustments, the cell began to sediment in a straight line in the direction of the gravitational field. This frequency, 10 kHz, is the F_o. Electric field direction is denoted by *E*, gravitational field direction by *g*, and observation direction by *O*. ⊙ represents the vector normal to the plane of the page.

and motions of cells were displayed on a monitor. Results obtained by the two techniques were identical within experimental uncertainty.

Preparation of cells

For dielectrophoretic measurements *N. crassa* slime cells were prepared as described by Fikus et al. (14). Cells were suspended in 10% sorbitol. The conductivity of the suspension was adjusted by addition of NaCl, and was measured with a RLC E-316 bridge (Meratronik, Poland). The conductivity of each sample was monitored during the experiment by measuring the chamber current.

Murine myeloma cell line Tib9 was obtained from American Type Culture Collections. Cells were grown in RPMI 1640 medium (Sigma Chemical Co., St. Louis, MO) supplemented with 10% fetal bovine serum, 20% L-glutamine and 0.4% vol/vol gentamicin (10 mg/ml), at 37°C in 5% CO_2. Cells were collected by centrifugation at 800 g for 5 min, washed once with 0.3 M sucrose and resuspended in the same medium. The conductivity of the suspension was measured by a Yellow Springs Instruments, Inc. (Yellow Springs, CA) model 31A conductometer. Measurements of F_o were carried out at the room temperature (25 ± 1°C) in external media with different conductivities ranging from 1 to 30 mS m^{-1}.

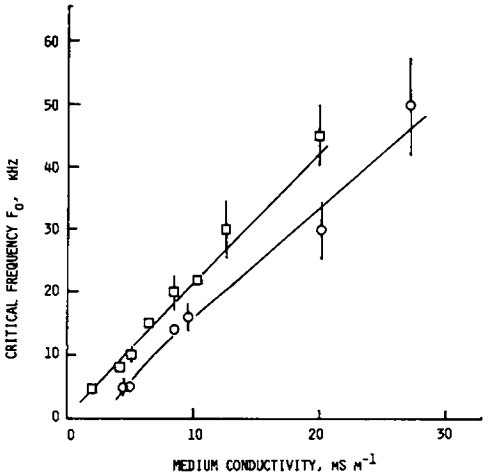

FIGURE 5 The critical frequency, F_o, of medium size (diameter 22 ± 2 μm, data in □) and large size (diameter 30 ± 3 μm, data in ○) *N. crassa* is plotted against the conductivity of the medium, σ_1. Solid lines represent the optimized theoretical function. The values of ϵ_{membr} and σ_{membr} were optimized. ϵ_{int} was assigned a value of 45 ϵ_o, and σ_{int} was assigned a value of 0.229 S m^{-1}. As shown in Fig. 3 *A*, F_o is not sensitive to these two parameters. Electric parameters of *N. crassa* are summarized in Table 1.

RESULTS AND DISCUSSIONS

The *N. crassa* slime culture was characterized by cells of relatively wide size distribution (diameter 8–40 μm).

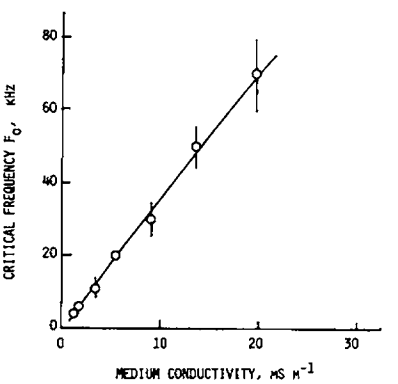

FIGURE 6 F_o of myeloma Tib9 (diameter 13 ± 1 nm) is plotted vs. the conductivity of the medium, σ_1. The solid line represents the optimized theoretical function. Electric parameters are summarized in Table 1. ϵ_{int} was assigned a value of 45 ϵ_o and σ_{int} was assigned a value of 0.2 S m^{-1}.

TABLE 1 **Electric parameters of *N. crassa* slime and myeloma Tib9**

Cell parameter	*Neurospora crassa*			
	Dielectrophoresis		Dielectric spectroscopy*	Myeloma Tib9 Dielectrophoresis
Diameter (μm)	22 ± 2	30 ± 3	Suspension of cells	13 ± 1
$\epsilon_{int}/\epsilon_o$	n.d.	n.d.	45.0	n.d.
σ_{int} ($S\ m^{-1}$)	n.d.	n.d.	0.23	n.d.
$\epsilon_{membr}/\epsilon_o$‡	9.4	8.3	7.9	9.4
C_{membr} ($\mu F\ cm^{-2}$)§	0.92	0.82	0.78	0.92
σ_{membr} ($S\ m^{-1}$)‡	$<1 \times 10^{-6}$	2.2×10^{-6}	n.d.	$<2 \times 10^{-6}$
G_{membr} ($S\ cm^{-2}$)§	$<1 \times 10^{-2}$	$2.4 \times 10\times^{-2}$	n.d.	$<2 \times 10^{-2}$

Notes: n.d. means data cannot be determined experimentally; *data from Fikus et al. (9) are listed for comparison; ‡ϵ_{membr} and σ_{membr} were optimized by using membrane thickness (d) of 9 nm; §electric parameter of the cell membrane may also be expressed in specific capacitance ($C_{membr} = \epsilon_{membr}\epsilon_o/d$) and specific conductance ($G_{membr} = \sigma_{membr}/d$) which are independent of membrane thickness.

Electrical parameters of slime cells were dependent on size. For this reason, we have arbitrarily selected two sizes of cells for the experiments, medium size cells (22 ± 2 μm) and large cells (30 ± 3 μm). The results are shown in Fig. 5. The solid lines in the figure were obtained by means of fitting the theoretical function, $F_o^{theor}(\sigma_1)$ to the experimental points, with the membrane dielectric permittivity, σ_{membr}, and membrane conductivity, σ_{membr}, as the parameters for optimization. F_o was insensitive to the conductivity of the cytoplasm, σ_{int} (Fig. 3*A*) and could not be determined by optimization of the experimental data. Instead we have used σ_{int} of 0.229 S m^{-1} reported in the literature (9) in our analysis. From Fig. 3*A*, it is clear that a slight variation in the value for σ_{int} would have little effect in our analysis. A similar procedure was applied for the analysis of results with myeloma cell line Tib9, and both experimental results and fitted theoretical curve are shown in Fig. 6.

The results of the nonlinear regression analysis of the above data, as well as previously obtained data using dielectric spectroscopy (9, 10), are summarized in Table 1. Of the four electric parameters (ϵ_{int}, σ_{int}, ϵ_{membr}, and σ_{membr}) describing a single shell model of a spherical living cell, only ϵ_{membr} can be compared with that obtained by the dielectric spectroscopy method. This latter method measures the dielectric relaxation spectrum of a cell "suspension" and can only measure the average value of a cell sample, as opposed to the dielectrophoresis method which measures electric parameters of single cells. Another difference between the two methods is that the dielectric spectroscopy method is not sensitive for the membrane conductivity, σ_{membr}, so it is usually assumed to be zero (9). Data in Table 1 show that the value of ϵ_{membr} for *N. crassa* determined by the dielectric spectroscopy was close to the values obtained by the dielectrophoresis method for the slime cells of two different sizes. This is indicative of the agreement between the two methods. However, by measuring F_o, σ_{membr} may also be determined. For *N. crassa* in 0.3 M sucrose, σ_{membr} appears to be in the range barely accessible by the present method and different for cells of different diameters.

The dielectric constant of the myeloma cell membrane was found to be comparable to that of *N. crassa*. Its membrane conductivity is closer to that of the larger size *N. crassa* slime.

In conclusion, we have shown that of the four electric parameters of a cell shown in Fig. 1, two may be determined by the dielectrophoresis method previously described (8, 10). This is a method for single cell measurements as compared to other classical methods, e.g., dielectric spectroscopy, which usually measures average values for a population of cells. Furthermore, the present method requires no sophisticated apparatus. The parameter ϵ_{membr} is an essential quantity for understanding electrostatic interactions of molecules within a cell membrane, and σ_{membr} is a quantity important for understanding membrane ionic transport activity.

We thank Dr. Jan Gimsa for many valuable discussions and C. J. Gross for help with the manuscript. This work was supported in part by a grant from the United States Office of Naval Research to T. Y. Tsong. P. Marszalek was a Junior Fulbright Fellow at the University of Minnesota.

REFERENCES

1. Pohl, H. A. 1978. Dielectrophoresis. Cambridge University Press, New York.
2. Neumann, E., A. E. Sowers, and C. A. Jordan, editors. 1989. Electroporation and Electrofusion in Cell Biology. Plenum Publishing Corp., New York.
3. Dimitrov, D. S., and D. V. Zhelev. 1987. Dielectrophoresis of individual cells. Experimental methods and results. *Bioelectrochem. Bioenerg.* 17:549–557.
4. Sher, L. D. 1968. Dielectrophoresis in lossy dielectric media. *Nature (Lond.)*. 220:695–696.

5. Sauer, F. A. 1985. Interaction forces between microscopic particles in an external electromagnetic field. *In* Interactions between Electromagnetic Field and Cells. A. Chiabrera, C. Nicolini, and H. P. Schwan, editors. Plenum Publishing Corp., New York. 181–202.
6. Chizmadzhev, Yu. A., P. I. Kuzmin, and V. P. Pastushenko. 1985. Theory of the dielectrophoresis of vesicles and cells. *Biol. Membr. (Russian)*. 2:1147–1164.
7. Schwan, H. P. 1989. Dielectrophoresis and rotation of cells. *In* Electroporation and Electrofusion in Cell Biology. Plenum Publishing Corp., New York. 3–21.
8. Marszalek, P., J. J. Zielinsky, and M. Fikus. 1989. A new method for the investigation of cellular dielectrophoresis. *Z. Naturforsch. Sect. C. Biosci.* 44c:92–95.
9. Fikus, M., P. Marszalek, S. Rozycki, and J. J. Zielinsky. 1987. Dielectrophoresis and electrofusion of Neurospora crassa slime. *Stud. Biophys.* 119:73–76.
10. Marszalek, P., J. J. Zielinski, and M. Fikus. 1989. Experimental verification of a theoretical treatment of the mechanism of dielectrophoresis. *Bioelectrochem. Bioenerg.* 22:289–298.
11. Kaler, K. V., and T. B. Jones. 1990. Dielectrophoretic spectra of single cells determined by feedback-controlled levitation. *Biophys. J.* 57:173–182.
12. Schwan, H. P. 1957. Electrical properties of tissue and cell suspensions. *In* Advance in Biology and Medical Physics. Lawrence, J. H. and C. A. Tobias, editors. Academic Press, New York. 147–209.
13. Asami, K., Y. Takahashi, and S. Takashima. 1990. Frequency domain analysis of membrane capacitance of cultured cells (HeLa and myeloma) using the micropipette technique. *Biophys. J.* 58:143–148.
14. Fikus, M., E. Grzesiuk, P. Marszalek, S. Rozycki, and J. J. Zielinsky. 1985. Electrofusion of Neurospora crassa slime cells. *FEMS (Fed. Eur. Microbiol. Soc.) Microbiol. Lett.* 27:123–127.