Dielectrophoretic spectra of single cells determined by feedback-controlled levitation

K. V. I. S. Kaler* and T. B. Jones‡

*Department of Electrical Engineering, University of Calgary, Calgary, Alberta T2N 1N4 Canada; and ‡Department of Electrical Engineering, University of Rochester, Rochester, New York 14627

ABSTRACT In this paper we have utilized the principle of dielectrophoresis (DEP) to develop an apparatus to stably levitate single biological cells using a digital feedback control scheme. Using this apparatus, the positive DEP spectra of both *Canola* plant protoplast and ligament fibroblast cells have been measured over a wide range of frequencies (1 kHz to 50 MHz) and suspending medium conductivities (11–800 μS/cm). The experimental data thus obtained have been interpreted in terms of a simple spherical cell model. Furthermore, utilizing such a model, we have shown that various cellular parameters of interest can be readily obtained from the measured DEP levitation spectrum. Specifically, the effective membrane capacitance of single cells has been determined. Values of 0.47 ± 0.03 μF/cm^2 for *Canola* protoplasts and 1.52 ± 0.26 μF/cm^2 for ligament fibroblasts thus obtained are consistent with those determined by other existing electrical methods.

INTRODUCTION

In the past, a number of techniques have been developed to study the dielectric properties of biological cells. The most widely used of such available methods is the "suspension" technique, and it has been used quite extensively to study the frequency-dependent dielectric properties of biological particles and cells in suspension (Schwan, 1957, 1963; Hanai et al., 1979). In this technique, the cell suspension forms a heterogeneous dielectric material which is introduced into a capacitance chamber. Both conductance and capacitance are measured over a wide frequency range using sensitive impedance bridge techniques. From such measurements the dielectric properties of the cells may then be extracted by invocation of the appropriate mixture formulae (Fricke, 1955; Carstensen, 1967; Hanai et al., 1979).

Such measurements on cell suspensions have provided valuable insights into the various frequency-sensitive polarization response mechanisms of intact cells and tissue samples. Furthermore, they have proven to be particularly useful in measuring the membrane capacitance of a variety of cell types. On the other hand, the suspension method does not have the inherent sensitivity to measure cell membrane conductance (Takashima et al., 1988). Furthermore, because the measurements are necessarily performed on large numbers of cells, the electrical properties derived from the measurements are averages of large cell populations. An alternative method for probing the electrical properties of cells, particularly single cells and membrane patches, is the micropipette technique (Sakmann and Neher, 1983). This technique facilitates measurement of the membrane bulk conductance as well as the capacitance, however, these measurements are generally restricted to low frequencies due to the large parasitic impedance of the micropipette used (typically 2–3 pF in parallel with 10–20 MΩ). Recently, Takashima et al. (1988) reported such measurements on erythrocytes in the frequency range from ~4 Hz to 10 kHz.

A noninvasive technique for performing measurements on single cells is the electrorotation method (Arnold and Zimmermann, 1982). This method has the advantage of simplicity, although data collection has to be carried out manually and can be rather tedious and, furthermore, cells are exposed to moderate electric field intensities. In this paper, we report on a new method of studying the electrical properties of lone intact biological cells which provides a useful complement to electrorotation. The new method, based upon the principle of feedback-controlled DEP levitation, enables study of the frequency-dependent dielectric properties of single cells suspended in media of varied conductivity over a wide range of frequencies (from ~100 Hz to ~50 MHz). The frequency-dependent polarization spectra of a number of cell types may be determined; however, in this paper attention is restricted to levitation data obtained for rapeseed (*Canola*) plant protoplasts and mammalian (rabbit) ligament fibroblasts. The data are used to determine membrane capacitance as a means of verifying the proper function of the DEP levitator.

THEORY

Dielectrophoresis is the motional response of a neutral but polarizable particle when under the influence of a nonuni-

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form (ac or dc) electric field. Comprehensive treatments of dielectrophoresis have been provided by Pohl (1978) and Pethig (1979). The net time-average DEP force acting on a particle depends on its volume, permittivity relative to that of the surrounding medium, and the gradient of the electric field intensity squared, that is, ∇E^2. In the case of a spherical particle of radius r and complex permittivity $\underline{\epsilon}_{eff}$ surrounded by a medium of complex permittivity $\underline{\epsilon}_1$, the force can be expressed as (Sauer, 1985)

$$\overline{F}_{DEP} = 2\pi r^3 \epsilon_1 \mathrm{Re}[\underline{K}_e]\nabla E^2, \quad (1)$$

with the frequency-dependent polarization

$$\underline{K}_e(\underline{\epsilon}_{eff}, \underline{\epsilon}_1) = \frac{\underline{\epsilon}_{eff} - \underline{\epsilon}_1}{\underline{\epsilon}_{eff} + 2\underline{\epsilon}_1}. \quad (2)$$

Here, E is the externally applied electric field, ω is the angular frequency, $\underline{\epsilon}_1 \equiv \epsilon_1 - \sigma_1/j\omega$ is the complex permittivity of the suspension medium, and ϵ_1 and σ_1 are the permittivity and electrical conductivity of the suspension medium, respectively. It may be noted here in passing that, whereas the DEP spectra of interest in this paper are sensitive to $\mathrm{Re}[\underline{K}_e]$, the rotational spectra are related to $\mathrm{Im}[\underline{K}_e]$ through the time-average torque expression. Eq. 1 ignores higher-order multipolar contributions to the net ponderomotive force (Jones, 1986). These terms are generally insignificant when the size of a particle is small compared to the characteristic dimensions of the electrode structure and if the particle is not located near either electrode.

In Appendix A, a simple plant protoplast model is introduced and approximate expressions for $\underline{\epsilon}_{eff}(\omega)$ and $\mathrm{Re}[\underline{K}_e]$ are presented. These expressions facilitate a comparison of the model to the experimentally obtained DEP levitation spectra. It is apparent that for $\mathrm{Re}[\underline{K}_e] > 0$ the DEP force is positive, i.e., directed so as to impel the particle into regions of field intensity maxima. This is the case of positive DEP. If, on the other hand, $\mathrm{Re}[\underline{K}_e] < 0$, then the particle is pushed into a region of electric field minimum. This is referred to as negative DEP. The complex polarization $\underline{K}_e$ is frequency dependent and can change sign as a function of the frequency. Thus, a particle or cell suspended in a liquid can exhibit positive and negative DEP in different regions of the frequency spectrum.

In the past, most DEP measurements on biological cells and other particles have been conducted on suspensions of particles. The batch collection and continuous flow separation data obtained for these suspensions have provided only averaged DEP spectra for large cell populations. Due to cell-to-cell variability, such techniques cannot provide the resolution in measurement needed to advance our understanding of cell structure as revealed via dielectric response. On the other hand, dielectrophoretic levitation of individual particles offers the means to study the polarization response of single cells. This technique is based upon a balance of the gravitational or buoyant force and the DEP force to suspend a particle stably in a fluid of known properties.

A clear distinction must be drawn between the conditions for levitation of particles exhibiting negative and positive dielectrophoresis. For $\mathrm{Re}[\underline{K}_e] < 0$, i.e., negative DEP, the requirement can be met by a local minimum in the electric field magnitude. Such minima are allowed in divergence- and curl-free electrostatic fields (Jones and Bliss, 1977; Lin and Jones, 1984). Thus, passive levitation of particles, droplets, and bubbles exhibiting negative dielectrophoresis is readily achieved with a cusped axisymmetric electric field (Veas and Schaffer, 1969; Parmer and Jalaluddin, 1974; Jones and Bliss, 1977; Bahaj and Bailey, 1979). Passive levitation has been exploited as a means for precision measurement of the dielectric constant of homogeneous insulating particles by Kallio and Jones (1980).

To levitate particles exhibiting positive DEP ($\mathrm{Re}[\underline{K}_e] > 0$) in a similar fashion would require that a field intensity maximum exist at an isolated point in space detached from electrode surfaces, but such maxima are not allowed in divergence- and curl-free electrostatic fields (Jones and Bliss, 1977). Because most biological cells exhibit positive dielectrophoresis at frequencies of biophysical interest, passive levitation has only limited application. Instead, stable levitation is achieved by resorting to dynamic feedback-control of cell position. A simple implementation of this scheme was used by Kaler and Pohl (1979) who performed measurements on yeast cells. Individual cells were observed through a microscope and the voltage adjusted manually to maintain them at fixed position. Jones (1981) proposed a feedback-controlled levitation system using proportional control and described the requirements on the cusped electric field to achieve stable levitation. The first computer-controlled implementation of such a system (Jones and Kraybill, 1986) was used to levitate 50–125 micron particles including metallic spheres and Rochelle salt particles in insulating oils.

Feedback controlled levitation

The conditions required for the stable levitation of a particle experiencing a positive DEP force have been discussed at length by Jones and Kraybill (1986). The particle, assumed to be in equilibrium on the axis in an axisymmetric electric field, must be stable with respect to radial and axial motions. Radial stabilization is achieved using a focused electric field with intensity decreasing as the particle moves away from the axis. Refer to Fig. 1. In

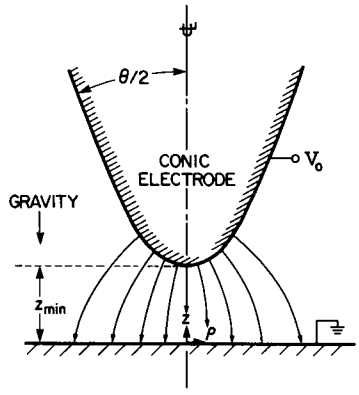

FIGURE 1 Cone-plate electrode structure which produces focused electrostatic field suitable for feedback-controlled DEP levitation when $\mathrm{Re}[\underline{K}_e(\omega)] > 0$.

such a structure, the particle will unavoidably be unstable with respect to axial motions. To overcome the instability in the axial direction, vertical particle displacements from equilibrium must be sensed and an error signal generated to adjust the levitation voltage.

For a spherical particle of radius r and mass density γ_2 suspended in a medium of density γ_1, the net gravitation force F_g is given by:

$$F_g = 4\pi r^3(\gamma_2 - \gamma_1)g/3, \tag{3}$$

where $g = 9.81$ m/s^2 is the acceleration due to gravity. Imagine a buoyant particle with $\mathrm{Re}[\underline{K}_e] > 0$ to be introduced along the axis into the cusped field depicted in Fig. 1. The particle is automatically centered upon the axis and rises into stronger electric field intensity until the DEP force balances the buoyant force of gravity. For a particle located on axis and stably levitated at some point z by feedback control of the voltage applied to the electrodes, the DEP force can be equated to the gravitational force.

$$F_{DEP} = F_g \tag{4}$$

Using Eqs. 1, 3, and 4,

$$\mathrm{Re}[\underline{K}_e(\omega)] = 2|\gamma_2 - \gamma_1|g/3\epsilon_1|\partial E^2/\partial z|. \tag{5}$$

The gradient term $|\partial E^2/\partial z|$, which depends on voltage squared and must be known to determine $\mathrm{Re}[\underline{K}_e(\omega)]$, is ordinarily obtained in a calibration step, where a conducting particle (with $\underline{K}_e[\omega] = 1$) of known density is levitated in an insulating liquid of known density and dielectric permittivity ϵ_1. However, the electrostatic field solution along the axis $E_z(0, z)$ is analytically well approximated for the cone-plate electrode configuration of Fig. 1 (Jones and Kraybill, 1986).

$$E(0, \underline{z}) = 2V_o/h(1 - \underline{z}^2) \ln [(1 + \underline{z}_{min})/(1 - \underline{z}_{min})], \tag{6}$$

where $\underline{z}_{min}$ is the electrode spacing, θ is the asymptotic cone angle, $\underline{z} = z/h$, $h = z_{min}/\cos(\theta/2)$, $\underline{z}_{min} = z_{min}/h$ and V_o is the voltage applied between the conic electrode and the ground plane. For the above axial electric field profile, the expression for $|\partial E^2/\partial z|$ is hence given by

$$\partial E^2/\partial z|_{0,z} = -1\,6V_o^2\,\underline{z}/h^3(1 - \underline{z}^2)^3 \cdot \ln [(1 + \underline{z}_{min})/(1 - \underline{z}_{min})]. \tag{7}$$

The feedback controller is designed to maintain the particle at fixed position and all DEP spectra in this paper are represented by plots of voltage squared versus frequency with particle position fixed. The frequency dependence of the levitated cell is then inferred from the measured spectra using the proportionality $V_o^2 \propto 1/\mathrm{Re}[\underline{K}_e]$.

Cell levitation apparatus

A sectional view of the experimental levitation chamber and a block diagram of the control system are shown in Fig. 2. The cone-plate electrode assembly (with $z_{min} = 450$ μm and $\theta = 60°$ as defined in Fig. 1) is housed in a Plexiglas chamber fitted with cover glass windows to aid optical monitoring of the chamber contents. One of the optical windows is removable to facilitate cleaning of the chamber and introduction of fresh dilute cell suspension each time a new experiment is to be conducted. The window is secured in place and sealed with vacuum grease to eliminate evaporation and the influence of air currents. The assembled chamber is mounted and held in place with spring-loaded clips on a vertical microscope stage with the electrodes connected to a programmable wideband signal generator (model No. 60 Wavetek, San Diego, CA). Levitation voltages for cells in the suspending media never exceed ~7 V (rms).

A conventional light source fitted with an infrared filter is used to illuminate the chamber and the cell image is focused into a video camera (model #WV1550, Panasonic Co., Secaucus, NJ). The video camera, linked to a monitor and a video recorder, serves the obvious functions of permitting observation of cell position in the chamber and facilitating examination of cells before spectra are obtained. However, the video system is also used to detect cell position for the feedback control loop. Video camera images are interfaced to a real-time image acquisition system (model MVP-AT, Matrox Electronic Systems Ltd., Dorval, Quebec, Canada) which is linked directly to a laboratory digital computer (286-based IBM-AT) via the internal bus. The computer is interfaced to the signal generator via the GPIB-488 bus to facilitate control of both the amplitude and frequency of the ac voltages applied to the chamber electrodes.

An assembly language program runs the control algorithm for stable levitation while permitting operator

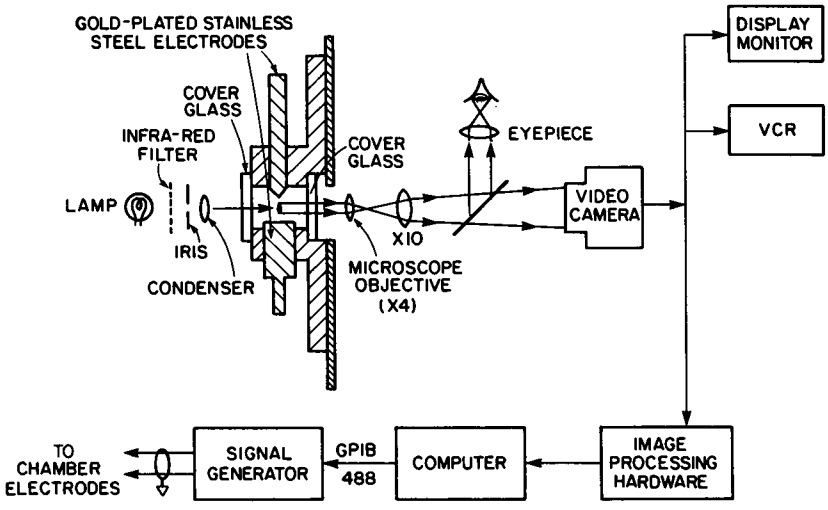

FIGURE 2 Sectional view of levitation chamber and block diagram of the video camera–based computer-controlled levitation system.

control of the frequency of the voltage applied to the levitation chamber in real time. This capability makes it possible to select and hold a suitable cell while the rest settle to the bottom of the chamber. The selected cell is then windowed by the imaging hardware while a thresholding scheme is employed to locate its position. The video threshold is adjusted to detect the cell edges in both the x and y image planes. From this data, the location of even irregularly shaped cells can be unambiguously determined. With the present system we detect cell position at a sampling rate of 4 Hz. Coded cell location data is provided to the feedback control software, which adjusts the voltage applied to the chamber electrodes. Voltage control is based on a simple linear proportional-integral (PI) control algorithm, where the voltage $V(nT)$ applied to the electrodes is adjusted as follows:

$$V(nT) = G_p e(nT) + G_i \sum_{k=1}^{k=n-1} e(nT), \qquad (8)$$

where G_p and G_i are the proportional and integral gain constants, respectively, n is a time index, k is the sample number, T is the sampling interval, and $e(nT)$ is the position error from the video image acquisition system. Both G_p and G_i can be adjusted during execution of the control program.

The control software is designed such that once the cell is stably levitated at a fixed location for a prescribed time period (typically 10 s), the frequency of the applied voltage is changed automatically. Preprogrammed frequency values are chosen so that when plotted on a logarithmic scale, the data points are evenly spaced. In such a manner the frequency-dependent levitation spectra can be obtained with minimal operator intervention. This operational feature is especially useful for cells, such as heat-sensitive or anchorage-dependent mammalian types, not tolerant to prolonged field exposure or suspension.

Sample preparation

Protoplasts

The plant protoplasts used in this investigation were extracted from *Canola* leaves that had been enzymatically treated to digest the glycan and pectin linkages in the cell wall. To minimize starch content, the *Canola* leaves were obtained from 3-wk-old plants kept in the dark for ~8 h before harvesting. The leaves, sterilized before dissection, were immersed in a digestion mixture consisting of 0.4% cellulase Onozuka RS, 0.025% Pectolyase Y23 (supplied by Kanematsu-Gosho, Tokyo, Japan), 8% sorbitol, and 100 mg/ml $CaCl_2$. The pH of the digestion mixture was adjusted to 5.8. The *Canola* leaves immersed in the digestion mixture were incubated for at least 5 h at room temperature, or until sufficient protoplast density was attained. The protoplasts were then separated from the protoplasting solution by centrifuging the mixture for 4 min at 40 g. The supernatant was discarded and the protoplast pellet resuspended in 8% sorbitol. This procedure was repeated three more times before the cells were finally diluted, ready for the levitation experiments. In experiments where the conductivity of the suspending medium was varied, KCl was added and the conductivity measured by a conductivity meter (model No. 1710, Bio-Rad Laboratories, Richmond, CA).

Fibroblasts

Rabbit ligament fibroblast cells derived from the medial collateral knee ligament were cultured in Dulbecco's

modified Eagle medium (DMEM) supplemented with 15% fetal bovine serum (FBS, lot No. 28k6180, GIBCO, Grand Island, NY) and 250 μM L-ascorbic acid. The cells used in this investigation were harvested from a culture grown to confluency at 37.5°C in a 5% CO_2 atmosphere. Because the fibroblast cells in culture are attached to the bottom of the culture flask, they were enzymatically detached using 0.05% Trypsin and 0.01% EDTA and incubated for 3 min at 37.5°C. Once the cells were detached, the enzyme solution was deactivated by the addition of 15% FBS in DMEM. The cell suspension was then centrifuged at 200 *g* for 4 min, after which the supernatant was discarded and the cells resuspended in 0.3 M mannitol solution of the appropriate conductivity. The fibroblasts suspended in mannitol solution were subjected to three wash cycles before the suspension was appropriately diluted for use in the levitation experiments.

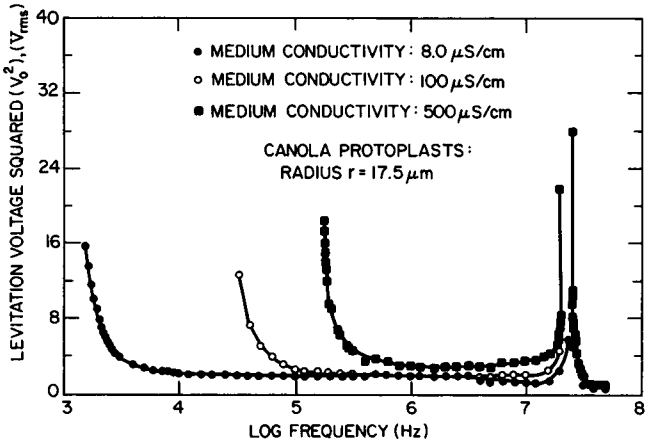

FIGURE 3 Positive DEP levitation spectra ($V_o^2 \propto 1/\mathrm{Re}[\underline{K}_e(\omega)]$) of 35.0-$\mu$m-diam *Canola* plant protoplasts suspended in sorbitol solutions of varied electrical conductivity σ_1.

RESULTS

Frequency-dependent positive DEP levitation spectra were obtained for a number of *Canola* plant protoplasts, suspended in 8% sorbitol solutions with conductivities in the range 11–700 μS/cm, over the frequency range of ~1 kHz to ~50 MHz. All measurements were carried out at 22°C. Typical examples of the spectra, plotted here as levitation voltage squared (V_o^2) versus the logarithm of the frequency ($f = \omega/2\pi$), are shown in Fig. 3. As expected, the low-frequency breakpoint exhibits strong sensitivity to the conductivity of the suspending medium σ_1, while, in contrast, the high frequency behavior is virtually unaffected by σ_1. These results are qualitatively consistent with the expressions for f_{low} and f_{high} from Appendix B, viz. Eqs. B1 and B2.

The high frequency behavior is more complex than predicted by the protoplast model of Appendix A. Evidence of the predicted breakpoint in the positive DEP response, as illustrated by Fig. A3 to occur near 38 MHz, has been seen in some DEP spectra, but is not apparent in Fig. 3. Frequency limitations in the signal generator prevented an extension of the investigation to higher frequencies. Some spectra obtained with the *Canola* protoplasts (and ligament fibroblasts) show a sharp peak in the DEP response at ~20 MHz. This peak is sensitive to the suspension pH, suggesting that conformation of protein molecules in the membrane is influential. The cause of the anomalous peak near 20 MHz is unknown at present.

As a preliminary test of the levitator's proper function, *Canola* protoplast data in the flat portion of the +DEP spectrum (where levitation voltage is minimized and essentially constant) were used for a direct determination of $\mathrm{Re}[\underline{K}_e]$. In addition to the voltage $(V_o)_{min}$ plus the analytical expression of the electric field, Eq. 7, the density difference between the particle and the suspending medium $(\gamma_2 - \gamma_1)$ was required. Rather than measuring γ_1 and γ_2 separately, we clocked the settling of single cells over a distance of ~200 μm in the levitation chamber. With measured values of sedimentation velocity (~7 μm/s) and protoplast radius (~17.5 μm) plus the medium viscosity (0.012 poise), we used the well-known Stokes drag equation to calulate the density difference: $(\gamma_1 - \gamma_2) = 12.7$ kg/m^3. Eq. 5 was used then to calculate the experimental value of $\mathrm{Re}[\underline{K}_e]_{exp'l} = 0.94$ (± 0.04) in the flat portion of the positive DEP spectrum. This value compares favorably with a theoretical calculation using Eq. A11 to obtain $\mathrm{Re}[\underline{K}_e]_{theory} = 0.97$, where $c_m = 0.47$ μF/cm^2, $r = 17.5$ μm, $\epsilon_1 = 80\epsilon_o$, and ϵ_o, the permittivity of free space, is equal to $8.854 \cdot 10^{-12}$ F/m.

In the case of biological cells, the density difference $(\gamma_1 - \gamma_2)$ can be expected to vary from cell to cell in a given population, hence, undue reliance must not be placed on determination of the absolute magnitude of $\mathrm{Re}[\underline{K}_e]$. Instead, for quantitative investigation of cells using DEP levitation spectra, frequency breakpoints (as defined in Appendix B) provide more precise information about cell structure because they are insensitive to $(\gamma_1 - \gamma_2)$. To determine accurately the low frequency breakpoint, we carried out a linear regression analysis of the data points near the 3 db frequency to provide estimates for f_{low} at various values of suspension conductivity. See Appendix B. The results of this analysis are shown in Fig. 4, where the product $r \cdot (f_{3db})_{est}$ is plotted vs. σ_1. In Fig. 4, each point represents at least 10 data points taken near the 3 db point. For the *Canola* protoplast data, the coefficient of linear regression is 0.98 and,

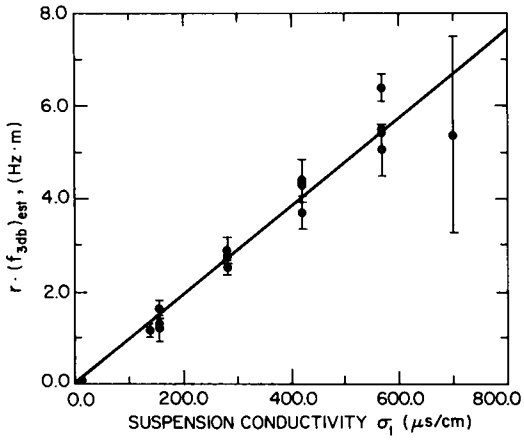

FIGURE 4 Conductivity dependence of the product of cell radius (r) and estimated low-frequency 3-db breakpoint ($f_{3db})_{est}$ from DEP spectra of *Canola* protoplasts. Range of cell radii for these data: $\sim 17 < r < \sim 35$ μm. Representative error brackets for 99% confidence limits are shown for some of the plotted points. The estimate for membrane capacitance of these protoplasts ($c_m = 0.47 \pm 0.03$ μF/cm^2) is obtained from slope of linear regression line.

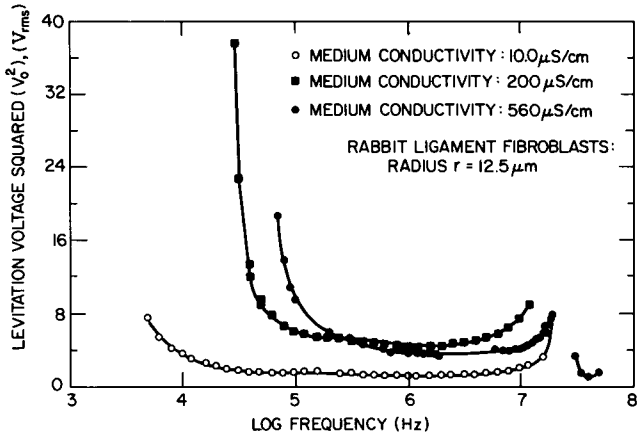

FIGURE 5 Positive DEP levitation spectra ($V_0^2 \propto 1/\mathrm{Re}[\underline{K}_e(\omega)]$) of 25.0-$\mu$m-diam rabbit ligament fibroblasts in sorbitol solutions of varied electrical conductivity σ_1.

using Eq. B1, the slope of the regression line yields an estimate of $c_m = 0.47 \pm 0.03$ μF/cm^2 at a confidence level of 99%. A departure from linear dependence, only evident at large suspension conductivities ($\sigma_1 > 700$ μS/cm), is probably attributable to joule heating of the suspension medium. The membrane capacitance result correlates well with values obtained for a variety of plant protoplasts using the electrorotation method: e.g., 0.48 μF/cm^2 (Arnold and Zimmermann, 1982), 0.24–0.40 μF/cm^2 (Glaser et al., 1983), and 0.56 ± 0.08 μF/cm^2 (Lovelace et al., 1984). The fact that all these values for c_m are approximately one-half of the generally accepted value of 1.0 μF/cm^2 for lipid membranes may be due to an effective series connection of the plasma and vacuole (or chloroplast) membranes of plant cells (Arnold and Zimmermann, 1988).

The DEP levitation method has also been used to estimate the membrane capacitance of rabbit ligament fibroblasts. Typical DEP spectra for these mammalian cells are shown in Fig. 5. Again using the corner frequency analysis technique described in Appendix B, estimates for the 3 db frequencies of a number of individual fibroblasts in suspension conductivities ranging from 14 to 800 μS/cm have been obtained. These frequency values, multiplied by their associated measured cell radii, are plotted vs. σ_1 in Fig. 6. The coefficient of linear regression is 0.84. The poorer correlation of the fibroblasts may be attributed to several factors. The trypsin treatment used to detach the cells may have damaged the cell membranes. Furthermore, these cells are irregular in shape, a factor influencing the precision of the DEP levitation data. Using Eq. B1, the regression line slope yields an estimate of $c_m = 1.52 \pm 0.26$ μF/cm^2 at a confidence level of 99%.

CONCLUSION

In this paper we have successfully applied dielectrophoretic levitation to measure the dielectric responses of

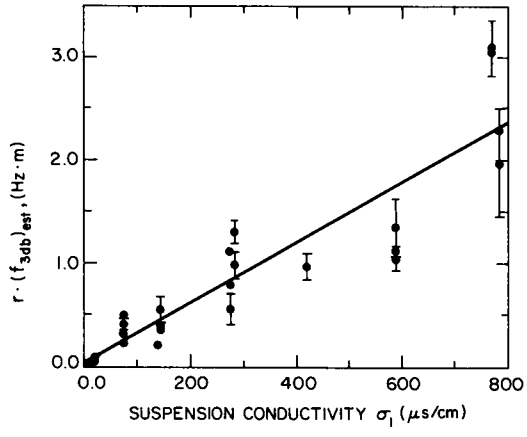

FIGURE 6 Conductivity dependence of the product of cell radius (r) and estimated low-frequency 3-db breakpoint ($f_{3db})_{est}$ from the DEP spectra of rabbit ligament fibroblasts. Range of cell radii for these data: $\sim 9 < r < \sim 15$ μm. Representative error brackets for 99% confidence limits are shown for some of the plotted points. The estimate for membrane capacitance of these fibroblasts ($c_m = 1.52 \pm 0.26$ μF/cm^2) is obtained from slope of linear regression line.

individual plant protoplasts and ligament cells. A variety of different cell types have now been levitated and the +DEP spectra over frequencies from ~1 kHz to ~20 MHz have been obtained. A simple spherical model consisting of conductive cytoplasmic fluid contained within an insulating capacitive membrane has been used to interpret the results and to validate the experimental data. A technique has been developed and used to analyze the corner or 3 db frequency breakpoints to obtain estimates for the membrane capacitance. The capacitance values so obtained (0.47 ± 0.03 μF/cm^2 for *Canola* plant protoplasts and 1.52 ± 0.26 μF/cm^2 for ligament cells) fall within the generally accepted values for this parameter as determined by other methods.

We envision using DEP levitation for quantitative experimental investigations of living cells and other biological particles in situations where it is advantageous or necessary to study cells individually. For example, it should be possible now to monitor in time the characteristics of single cells to study their response to various environments. Among the possible measurements envisioned are time-dependent measurements of membrane conductance and low frequency surface conductivity associated with the counter-ion cloud in the electrolyte surrounding the single cells. Low frequency DEP levitation measurements will require the development of a precision passive (negative DEP) levitator and, even then, will present several challenges, principally joule heating and electrophoresis. In the present (positive DEP) levitator, joule heating becomes pronounced for $\sigma_i > 10^3$ μS/cm. Furthermore, it is observed that the electrode geometry (viz., cone angle θ and spacing z_{min}) strongly influences the onset of thermal convection. Still further, the influence of electrophoresis (and electroconvection) upon low-frequency DEP levitation measurements is unknown at this time.

One significant improvement to the present active-feedback levitator might be an automated cell injection system to facilitate data collection and to permit investigations of the temporal response of cells to changes in suspension environment (pH, etc.) and cell-to-cell variability. The DEP levitation technique, not restricted to levitation of single cells, may be useful in studying cell chains. Levitation spectra of such chains could provide valuable insight into the nature of dipole–dipole interaction forces developed between contacting cells. Such measurements may prove invaluable in probing the underlying mechanism(s) operative in electric field mediated cell fusion (electrofusion). In conclusion, the DEP levitation technique shows promise as an experimental tool for basic investigations of the electrical characteristics of biological cells and membranes which should complement existing methods (suspension, electrorotation, patch clamp) presently in use.

APPENDIX A: DEP SPECTRA OF PROTOPLASTS

Elements of a simple model for the dielectrophoretic response of a protoplast cell are summarized in this appendix. The effect of the counter-ion cloud (Schwarz, 1962), most pronounced at low frequencies (<1 kHz) where negative dielectrophoresis reigns, are ignored. Membrane conductance is also ignored. The DEP levitation technique should facilitate studies of both these influences on the low-frequency dielectric response of cells; however, they are not addressed in the present work. The cells are assumed to be spherical. This is an excellent assumption for plant protoplasts but not as accurate in the case of fibroblasts. The cell interior (cytoplasm) and the suspension medium are modeled as linear ohmic dielectrics with no dielectric loss mechanisms. The analysis presented below may be modified to take dielectric loss into account, but, as the purpose here is to establish the baseline performance of the feedback DEP levitator, the simpler case of ohmic fluid media is assumed.

Refer to Fig. A1 showing a spherical particle consisting of an ohmic dielectric with permittivity ϵ_3, conductivity σ_3, and radius r enclosed by an insulating layer of permittivity ϵ_2, and thickness δ. The permittivity and conductivity of the external medium are ϵ_1 and σ_1, respectively. The particle is assumed to be located in a slightly nonuniform electric field which varies sinusoidally in time, that is,

$$E(\bar{r}, t) = \mathrm{Re}[\underline{\bar{E}}(\bar{r}) \exp(j\omega t)], \tag{A1}$$

where $j = \sqrt{-1}$ and $\omega = 2\pi f$ is the radian frequency. The sinusoidal steady-state dielectric response of the particle can be represented by an equivalent homogeneous sphere of radius $r + \delta$ and complex permittivity $\underline{\epsilon}_{eff}$ (Pauly and Schwan, 1959), where

$$\underline{\epsilon}_{eff} = \epsilon_2 \left[\frac{a^3 + 2\underline{K}(\underline{\epsilon}_3, \underline{\epsilon}_2)}{a^3 - \underline{K}(\underline{\epsilon}_3, \underline{\epsilon}_2)} \right]. \tag{A2}$$

Here, $\underline{\epsilon}_3 = \epsilon_3 - j\sigma_3/\omega$, $a = (r + \delta)/r$, and $\underline{K}$ is the same complex polarizability function defined in Eq. 2.

For present purposes, the shell is intended to represent the cell membrane in which case $\delta/r \ll 1$. Then, $a^3 \approx 1 + 3(\delta/r)$ and Eq. A2 becomes

$$\underline{\epsilon}_{eff} \approx c_m r \left[\frac{j\omega\tau_3 + 1}{j\omega(\tau_m + \tau_3) + 1} \right]. \tag{A3}$$

Here, $c_m = \epsilon_2/\delta$ is the cell membrane capacitance per unit area, while $\tau_3 = \epsilon_3/\sigma_3$ and $\tau_m = c_m r/\sigma_3$ are time constants.

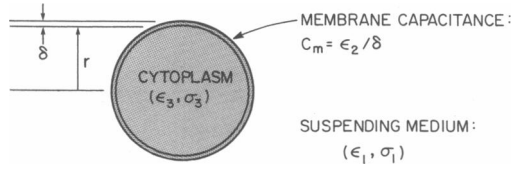

FIGURE A1 Simple spherical model for protoplast with membrane and homogeneous ohmic cytoplasmic interior.

Debye representation

The particle with its insulating membrane represents a classic Debye relaxation system, a fact evident if $\underline{\epsilon}_{eff}(\omega)$ is expressed in terms of its real and imaginary components.

$$\underline{\epsilon}_{eff} = \epsilon'_{eff}(\omega) - j\epsilon''_{eff}(\omega), \tag{A4}$$

where

$$\epsilon'_{eff} = \epsilon_\infty + \frac{\epsilon_s - \epsilon_\infty}{\omega^2\tau_m^2 + 1} \tag{A5}$$

and

$$\epsilon''_{eff} = \frac{(\epsilon_s - \epsilon_\infty)\omega\tau_m}{\omega^2\tau_m^2 + 1}, \tag{A6}$$

with $\epsilon_s = c_m r$ and $\epsilon_\infty = \epsilon_3$. Eqs. A5 and A6 are based on the approximation, ordinarily valid for cells, that $\tau_3 \ll \tau_m$. The real and imaginary parts of $\underline{\epsilon}_{eff}(\omega)$ are plotted in Fig. A2 for parameters typical of a plant protoplast. Note the loss peak that occurs at frequency $f_m = 1/2\pi\tau_m$.

DEP spectrum of protoplast

Eq. 1 indicates that the dielectrophoretic effect is related to ϵ_{eff} through K_e as defined by Eq. 2. Using Eq. A2 with 2, we obtain

$$\underline{K}_e(\omega) = \frac{\omega^2(\tau_1\tau_m - \tau_3\tau'_m) - 1 + j\omega(\tau'_m - \tau_1 - \tau_m)}{2 - \omega^2(\tau_3\tau'_m + 2\tau_1\tau_m) + j\omega(\tau'_m + 2\tau_1 + 2\tau_m)}, \tag{A7}$$

where $\tau'_m = c_m r/\sigma_1$ and $\tau_1 = \epsilon_1/\sigma_1$. In general, $\mathrm{Re}[\underline{K}_e(\omega)]$ has the form shown in Fig. A3. Note the distinct differences between $\mathrm{Re}[\underline{K}_e(\omega)]$ and $\mathrm{Re}[\underline{\epsilon}_{eff}(\omega)]$. While $\underline{\epsilon}_{eff}(\omega)$ has a single relaxation frequency f_m, the polarizability $\underline{K}_e(\omega)$ has two critical frequency transitions. It is easy to show that

$$\underline{K}_e(\omega \to 0) = -0.5 \tag{A8}$$

$$\underline{K}_e(\omega \to \infty) = \frac{\epsilon_3 - \epsilon_1}{\epsilon_3 + 2\epsilon_1}. \tag{A9}$$

For parameter values typical of protoplasts, the model cell exhibits

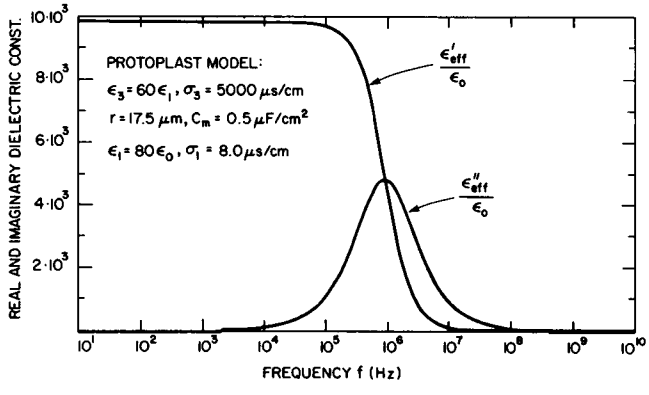

FIGURE A2 Plots of $\epsilon'_{eff}/\epsilon_0$ and $\epsilon''_{eff}/\epsilon_0$ vs. frequency using generally accepted plant protoplast parameters: $\epsilon_3/\epsilon_0 = 60.0$, $\sigma_3 = 5000.0$ μS/cm, $c_m = 0.5$ μF/cm², $\epsilon_1/\epsilon_0 = 80.0$, $\sigma_1 = 8.0$ μS/cm, and $r = 17.5$ μm.

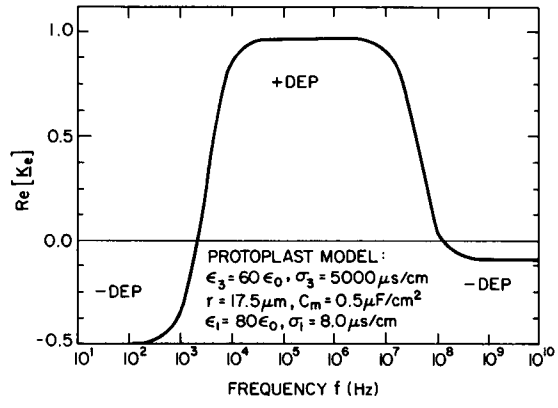

FIGURE A3 Plot of $\mathrm{Re}[\underline{K}_e]$ vs. frequency using generally accepted plant protoplast parameters (same as Fig. A2): $\epsilon_3/\epsilon_0 = 60.0$, $\sigma_3 = 5{,}000.0$ μS/cm, $c_m = 0.5$ μF/cm², $\epsilon_1/\epsilon_0 = 80.0$, $\sigma_1 = 8.0$ μS/cm, and $r = 17.5$ μm.

negative DEP for $\omega \to \infty$ and $\omega \to 0$, and positive DEP for an intermediate frequency band. Within the flat-band frequency range where positive dielectrophoresis reigns and where the cell can be levitated using feedback, we have

$$\mathrm{Re}\,[\underline{K}_e(\omega)] \approx \frac{\tau'_m - (\tau_1 - \tau_m)}{\tau'_m + 2(\tau_1 + \tau_m)}. \tag{A10}$$

Two limiting cases for Eq. A10 may be identified, based on the relative values of τ_m and τ_1.

$$\tau_1 \gg \tau_m: \quad \mathrm{Re}[\underline{K}_e(\omega)] \approx \frac{c_m r - \epsilon_1}{c_m r + 2\epsilon_1} \tag{A11}$$

$$\tau_1 \ll \tau_m: \quad \mathrm{Re}[\underline{K}_e(\omega)] \approx \frac{\sigma_3 - \sigma_1}{\sigma_3 + 2\sigma_1}. \tag{A12}$$

Note that usually, $c_m r \gg \epsilon_1$ and $\sigma_3 \gg \sigma_1$, so that $\mathrm{Re}[\underline{K}_e]_{max} \approx 1.0$ in the flat portion of the spectrum, irrespective of the relative values of τ_1 and τ_m.

APPENDIX B: BREAKPOINT ANALYSIS OF DEP SPECTRA

Experimental DEP spectra are conveniently represented in terms of voltage squared (V_o^2) vs. frequency ($f = \omega/2\pi$). From Eq. 5, note that $V_o^2 \propto 1/\mathrm{Re}[\underline{K}_e(\omega)]$. The predicted positive DEP spectrum for a protoplast with the same parameters used to obtain Fig. A3 is shown in Fig. B1. Corner or breakpoint frequency analysis of such spectra, yielding quantitative information about cell structure, is facilitated by definition of the 3 db points for V_o^2. Then $(V_o)_{3db} \equiv \sqrt{2}(V_o)_{min}$, or $\mathrm{Re}[\underline{K}_e]_{3db} \equiv 0.5 \cdot \mathrm{Re}[\underline{K}_e]_{max}$. Expressions for the high and low corner frequencies for the protoplast model may be obtained from the above definitions for the 3-db points using Eq. A7.

$$f_{low} = \frac{\sqrt{2}}{\pi\tau'_m} \tag{B1}$$

$$f_{high} = \frac{\sigma_3}{2\pi\sqrt{(2\epsilon_1 + \epsilon_3)(4\epsilon_1 - \epsilon_3)}}, \tag{B2}$$

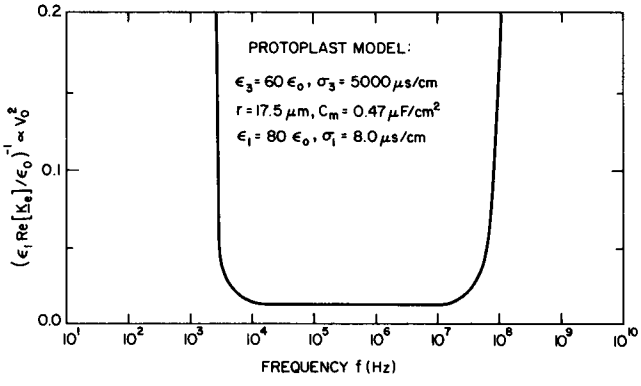

FIGURE B1 Computed positive DEP spectrum $(\epsilon_1 \mathrm{Re}[\underline{K}_e]/\epsilon_o)^{-1}$ using plant protoplast parameters (same as Fig. A2): $\epsilon_3/\epsilon_o = 60.0$, $\sigma_3 = 5{,}000.0$ μS/cm, $c_m = 0.5\,\mu$F/cm^2, $\epsilon_1/\epsilon_o = 80.0$, $\sigma_1 = 8.0\,\mu$S/cm, with cell radius $r = 17.5\,\mu$m.

where we have assumed that $\tau_1 \gg \tau_m$ and $c_m r \gg \epsilon_1$. From an examination of Fig. A3, it is clear that other 3-db frequencies might be defined and utilized if passive DEP levitation can be perfected for investigation of the dielectric response of cells.

In this paper, attention is restricted to the low-frequency breakpoint, in the vicinity of which $\underline{K}_e(\omega)$ may be approximated as follows.

$$\underline{K}_e \approx \frac{j\omega(\tau'_m - \tau_1 - \tau_m) - 1}{j\omega(\tau'_m + 2\tau_1 + 2\tau_m) + 2}, \tag{B3}$$

so that

$$\mathrm{Re}[\underline{K}_e] \approx \left(\frac{c_m r - \epsilon_1}{c_m r + 2\epsilon_1}\right)\left[\frac{\omega^2 - 2/\tau_a\tau_b}{\omega^2 + 4/\tau_b^2}\right], \tag{B4}$$

where $\tau_a \equiv \tau'_m - \tau_1 - \tau_m$ and $\tau_b \equiv \tau'_m + 2\tau_1 + 2\tau_m$. From the experimental standpoint, it is very inconvenient to measure the 3-db frequency directly because the flat-band value of the voltage is not known until the data have been recorded. Instead, a number of points around the corner frequency are measured and f_{3db} is estimated by least squares regression. Let x be the ratio of the voltage squared at some frequency $f_x = \omega_x/2\pi$ to the flat-band value so that $V_o(f_x) = \sqrt{x}(V_o)_{min}$. Then, from Eq. B4 we have

$$\left[\frac{\omega_x^2 - 2/\tau_a\tau_b}{\omega_x^2 + 4/\tau_b^2}\right] = \frac{1}{x}, \tag{B5}$$

or

$$f_x^2 = \frac{2x\tau_b + 4\tau_a}{4\pi^2\tau_a\tau_b^2(x-1)}. \tag{B6}$$

If $\epsilon_1 \ll c_m r$, then we may approximate

$$f_x \approx \frac{\sigma_1}{\pi c_m r}\, g(x), \tag{B7}$$

where

$$g(x) = \sqrt{\frac{1 + x/2}{x - 1}}. \tag{B8}$$

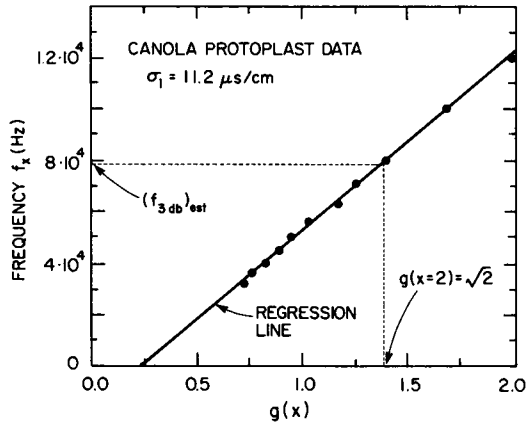

FIGURE B2 Measured values of frequency f_x vs. $g(x)$ near corner frequency, where $V_o/(V_o)_{min} \equiv \sqrt{x}$ and $g(x)$ is defined by Eq. B8, for *Canola* protoplast in sorbitol solution with suspension conductivity $\sigma_1 = 11.2\,\mu$S/cm. The flat-band value of voltage $(V_o)_{min}$ is determined by calculating the average levitation voltage for a set of frequencies well above the corner frequency.

Experimental data near the lower corner frequency were collected for *Canola* protoplasts and rabbit ligament fibroblasts suspended in media of varied conductivity. Refer to Fig. B2 plotting experimental values of f_x vs. $g(x)$ for $g(x) > 2.0$. For each levitated cell spectrum, a linear regression of f_x data vs. $g(x)$ was performed. The correlation coefficients of these regressions always exceeded 0.94 and were typically >0.98. From these regressions, estimates for the 3-db frequencies were calculated as shown in Fig. B2. These estimates $(f_{3db})_{est}$, multiplied by measured cell radii r, are plotted in Figs. 4 and 6, and used in a second linear regression to obtain the estimates for membrane capacitance c_m using Eq. B1.

Alan Tai prepared all cell and protoplast suspensions and collected much of the experimental data. The computer control software was developed by Ronald Platt. The data acquisition hardware was donated by Medtronics Canada Ltd. The DEP spectra and frequency plots of $\mathrm{Re}[\underline{K}_e(\omega)]$, etc., were obtained using a Pascal program written by Saulei Chan at the University of Rochester.

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