ELSEVIER

Biochimica et Biophysica Acta 1195 (1994) 39–44

BBA Biochimica et Biophysica Acta

Dielectrophoresis of cell-size liposomes

N.G. Stoicheva [1], S.W. Hui *

Department of Biophysics, Roswell Park Cancer Institute, 666 Elm Street, Buffalo, NY 14206, USA

Received 13 December 1993; revised 19 April 1994

Abstract

The dielectrophoresis (DEP) behavior of cell size liposomes were studied in the frequency range from 20 kHz to 3 MHz. Liposomes in the size of about 10 μm in diameter were made from egg phosphatidylcholine (PC), egg phosphatidylethanolamine (PE), egg phosphatidylglycerol (PG) and brain phosphatidylserine (PS). These liposomes, having an internal conductivity of 58 μS/cm, were suspended in either PEG or Ficoll solutions of conductivities 9 and 20 μS/cm, respectively. The liposomes were induced to form pearl chains in an electric field gradient in specially designed chambers with either coaxial or flat electrodes. Liposome rotation and convection (mixed positive and negative dielectrophoresis) were observed just beyond the experimental frequency range, within which positive dielectrophoresis leading to pearl chain formation were found. The threshold voltages that caused pearl chain formation were recorded within the experimental frequency range. The threshold voltages remained more or less constant within the positive DEP frequency range, and increased at both ends of the range. Charged liposomes (PS, PG) were found to have lower threshold than uncharged ones (PC, PE). Theoretical DEP spectra were calculated using a model proposed by Kaler and Jones ((1990) Biophys. J. 57, 173–182), for uncharged liposomes. The surface current effect in charged liposomes was accounted for, using an approximation proposed by Schwartz ((1962) J. Phys. Chem. 66, 2636–42). The experimental data agreed with theoretical prediction in general. The higher cutoff frequencies observed experimentally were thought to represent a slight lowering of internal conductivity due to leakage. The higher than predicted ratio between threshold voltages of charged and uncharged liposomes was interpreted to be due to the slight difference in size of these two types of liposomes. The agreement between theory and experiment showed that the available theory was adequate, and that liposomes provide a good model to study the nature of dielectrophoretic forces.

Keywords: Liposome; Phospholipid; Electrofusion; Dielectrophoresis; Dipole moment

1. Introduction

Dielectrophoresis (DEP) is the motion of neutral or charged particles in a non uniform electric field [1]. This definition applies to both the amplitude (i.e., translational motion) and the phase (rotational motion) of dielectrophoretic motion. The dielectrophoreses effect has been applied to characterize cells, facilitate cell fusion, and to study cell surface structures. In recent years, an increasing number of studies have been concerned with qualitative and quantitative dielectrophoretic investigations of living organisms [2–9]. Their purpose was to investigate the physical mechanism of this phenomenon, to analyzed the effect of an electric field on cells and the dependence of the dielectrophoretic force on the field frequency. Recent experimental works on dielectrophoresis of biological objects include culture and blood cells, membrane ghosts, protoplasts, yeast, etc.

In order to understand precisely the dielectrophoresis of cells, we undertook a study of a well defined object – liposomes. Liposomes are simple systems with given shape, known and controllable surface characteristics, and uniform and adjustable interior content. The use of liposomes would enable us to compare theory and experimental data more directly. No dielectrophoresis study of liposomes has been reported, though there are a few papers about electrofusion of cell-size liposomes, where the contact between liposomes was established by the application of an alternating electric

* Corresponding author. Fax: + 1 (716) 8458683. E-mail: roswhui@ubvms.cc.buffalo.edu

[1] Permanent address: Central Laboratory of Biophysics, Bulgarian Academy of Sciences, Acad. G. Bouchev Str. 21, 113 Sofia, Bulgaria.

SSDI 0005-2736(94)00178-2

field, resulting in dielectrophoresis and pearl-chain formation ([10], accompanying paper).

2. Materials and methods

2.1. Chemicals

Egg phosphatidylcholine (PC), transacylated egg phosphatidylethanolamine (PE), and bovine brain phosphatidylserine (PS), were obtained from Avanti Polar Lipids (Alabaster, AL); egg-phosphatidylglycerol, Ficoll Type 400 (M_r 400 000), polyethylene glycol (PEG) (M_r 8000) were obtained from Sigma (St. Louis, MO). All chemicals were of analytical grade. All water used was double-distilled in an all-glass apparatus.

2.2. Cell-size liposome preparation

Cell-size liposomes were prepared following a method of Hub et al. [11] with some modifications. Briefly, 3 mg lipid was dissolved in 2 ml chloroform. The solvent is then evaporated under vacuum. 20 ml of 0.5 mM NaCl solution was added to swell the dried lipid for 4 h in 70°C water bath. Gentle shaking of the glass flask for a few seconds resulted in the formation of liposomes of diameters up to 50 μm. It should be noted, however, that the average diameter of liposomes formed from different lipids is slightly different. There are higher percentage of large 'unilamellar' liposomes made from charged lipids compared to uncharged lipids. In order to create conductivity difference outside and inside the liposomes, they were washed and centrifuged at $12000 \times g$ for 20 min and resuspended in 1 mM sucrose to maintain equi-osmolarity.

2.3. Conductivity measurements

Conductivity measurements were performed on an Electro-Mark Analyzer (Markson, Del Mar, CA). All external conductivities were measured between 0.86 μS/cm, and 20 μS/cm. The conductivity inside the liposomes was 58 μS/cm, i.e., the conductivity of 0.5 mM NaCl solution. According to conductivity measurements, the liposomes are stable (against leakage) within 30 min, i.e., there are no noticeable changes in outside conductivity.

2.4. Dielectrophoresis measurement

The dielectrophoretic measurements were performed in 2.5% PEG 8000 (conductivity 9 μS/cm) or 2.5% Ficoll 400 000 solutions (conductivity 20 μS/cm). The solution osmolarity did not change appreciably. Some DEP measurements were done in a dielectrophoretic chamber with cylindrical symmetry. Briefly, a stainless steel wire of radius 0.25 mm was placed in a hollow metal cylinder (stainless steel) with an inner radius of 1 mm and a height of 5 mm. It was found later that this chamber had a resonance frequency centering at 600 kHz. This resonance superimposed on all DEP spectra measured including that of calibrating latex spheres. The spectra were remeasured in a chamber consisting of interdigitated planar metal electrodes evaporated on glass [12]. The geometric field gradient of this chamber was different from that of the coaxial chamber, but the principle was the same. The liposome 'pearl chain' formation was observed with a phase contrast microscope.

The application of a non-uniform electrical field on a polarized liposome produces a net force that induced a translational motion towards the region of highest field intensity. The liposomes were attracted to each other because of the induced dipoles, in the so-called mutual dielectrophoresis. This led to the formation of 'pearl chains' of liposomes. The rate the liposomes approached the central electrode and toward each other depended on the dielectrophoretic force exerted on the liposomes and the dynamic and geometric parameters of the particles and the surrounding fluid.

We measured the threshold voltage V_0 necessary to obtain 'pearl chains' at a length of 150–200 μm after 1 min of a.c. field application, and used this voltage to plot the DEP spectrum. The DEP force counteracted the Brownian motion of the liposomes which randomized their relative positions in the absence of electric field gradient. Since the liposome size, mass and the media viscosity for all samples were approximately the same, and since the same geometric factor applied to measurements from the same chamber, the square of the threshold voltage, V_0^2, was proportional to the reciprocal of the real part of the complex permittivity (see Eq. (1) of the next section). We determined the dielectrophoretic spectrum from 20 kHz to 3 MHz. Below 20 kHz we observed additional effects such as thermal convection and rotation, and above 3 MHz, rotation and negative DEP which caused repulsion of liposomes from the electrode. Only positive DEP frequency leading to good 'pearl chain' formation was quantitated.

2.5. Theoretical consideration

Theoretical treatments of dielectrophoresis have been provided by Pohl [1] and Pethig [13]. DEP force acting on a particle depends on its volume, complex permittivity, and the gradient of the electric field intensity squared. For a spherical particle of radius r, the force is:

$$F_D = 2\pi r^3 \epsilon_e Re(K(\omega)) \Delta E^2 \quad (1)$$

where ϵ_e is the medium permittivity and

$$K(\omega) = (\varepsilon_i - \varepsilon_e)/(\varepsilon_i + 2\varepsilon_e) \quad (2)$$

is the frequency-dependent polarization, ω is the angular frequency; $\varepsilon = \epsilon - j\sigma/\omega$ is the complex permittivity of the suspension medium; σ and ϵ are, respectively, the electrical conductivity and dielectric constant of the internal (subscript i) and external (subscript e) media. To calculate the DEP force of cells or liposomes that are bound by a relatively low conductance wall, a model has been proposed [8] in which the internal medium permittivity ε_i is replaced by an effective permittivity of the vesicle ε_{eff}, which is a function of the membrane capacitance C_m.

It should be noted that the translational DEP spectrum is sensitive to $Re(K)$ and the rotational spectrum is sensitive to $Im(K)$. To calculate the complex permittivity of cell-size liposomes in various suspension media, we used the approximation formula given by Kaler and Jones (1991):

$$K(\omega) = \frac{\omega^2(\tau_e\tau_{mi} - \tau_i\tau_{me}) - 1 + j\omega(\tau_{me} - \tau_e - \tau_{mi})}{2 - \omega^2(\tau_i\tau_{me} + \tau_e\tau_{mi}) + j\omega(\tau_{me} + 2\tau_e + 2\tau_{mi})} \quad (3)$$

where relaxation times are defined as $\tau_i = \epsilon_i/\sigma_i$, $\tau_{mi} = C_m r/\sigma_i$, $\tau_e = \epsilon_e/\sigma_e$ and $\tau_{me} = C_m r/\sigma_e$. For a simple spherical cell-size liposome, we take C_m, the liposome membrane capacitance per unit area, to be 7 mF/m^2, $\epsilon_i = \epsilon_e = 80\epsilon_0$, and the measured conductivities σ_i and σ_e for numerical calculation. Fig. 1 is a plot of $Re(K)$ vs. frequency for cell-size liposomes at two values of σ_e, namely, 9 μS/cm for PEG solution and 20 μS/cm for Ficoll solution (the case of charged vesicles is considered later). Our pearl chaining measurement is concerned with $Re(K)$ only, therefore the $Im(K)$ curves are not shown.

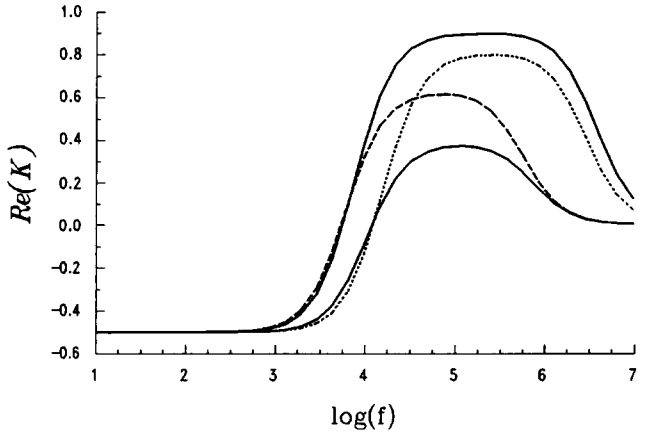

Fig. 1. The real part of the complex polarization K calculated from Eq. (3), for neutral vesicles, with internal conductivity of 58 μS/cm, and external medium conductivity of 9 μS/cm (dashed curve) or 20 μS/cm (lower solid curve); also for charged vesicles in media conductivity of 9 μS/cm (upper solid curve) or 20 μS/cm (dotted curve).

The real part of K is negative at low frequencies, positive at mid frequencies and decrease asymptotically to zero at high frequency. The two 3 dB breakpoint frequencies marking the transitions between three regions are given by Kaler and Jones (1991) to be:

$$F_{low} = (2)^{1/2}/\pi\tau_{me} \quad (4a)$$

$$F_{high} = \sigma_i/2\pi((2\epsilon_e + \epsilon_i)(4\epsilon_e - \epsilon_i))^{1/2} \quad (4b)$$

The calculated F_{low} for PC liposomes in 2.5% PEG and 2.5% Ficoll solutions are 11.6 and 25.7 kHz, respectively. The value for F_{high} is 440 kHz.

The electric charges on the surface of PS and PG liposomes create an electric double layer on the bilayer surface. The effect of the counter-ion cloud was theoretically investigated by Schwan et al. [14] and Schwarz [15]. This effect is most pronounced at low frequencies. These authors developed their theory for colloidal particles in electrolyte solution. They proposed that the existence of surface conductance is responsible for dielectric dispersion phenomena in colloidal suspensions at low frequencies. They considered so call 'shell' model.

$$\frac{Y - K_s}{Y - 2K_s} = \frac{(R - d/R)^3(K_i - K_s)}{K_i + 2K_s} \quad (5)$$

where K is the complex conductivity and the subscripts i and s indicate internal and surface respectively; Y is the specific admittance of an equivalent homogeneous particle, which can replace the shell-surrounded particle without changing its field perturbation in the suspending medium. Providing the following conditions are met: (1) the particle conductivity is small compared to that of the surface layers, and (2) the thickness of the surface layer is much smaller than the particle radius, Eq. (5) simplifies to:

$$Y = K_i + 2(d/R)K_s \quad (6)$$

We estimated the surface conductance at each suspending medium from the ion densities calculated from the Boltzmann equation, using a surface potential of 250 mV for liposomes as deduced from the assumption that each singly charged PS or PG molecule occupies 0.6 nm^2 of surface [16]. The surface conductivity is calculated to be 25–50 mS/cm, and the surface conductance thus deduced is about 10^{-8} S, therefore condition (1) is satisfied. The Debye length for liposomes in solutions of equivalent ionic conductivity is 24 and 36 nm respectively for Ficoll and PEG solutions, so that condition (2) is also satisfied. The equivalent internal conductivity for PS and PG liposomes may now be calculated from Eq. (6) to be about 300–500 μS/cm, considerably higher than the case for neutral liposomes (58 μS/cm).

Table 1
Theoretical and experimental values of high and low frequency break points (f_{hi} and f_{lo}, respectively) of the dielectrophoresis spectra, for charged (PS) and neutral (PC) liposomes in respective suspension media

	f_{lo} theory (kHz)	f_{lo} exp (kHz)	f_{hi} theory (MHz)	f_{hi} exp (MHz)
PS/PEG	11.6	15±4	3.58	4.8±0.3
PS/Ficoll	25.7	23±3	2.53	5.0±0.5
PC/PEG	11.6	12±4	0.44	2.6±0.2
PC/Ficoll	25.7	25±3	0.44	3.3±0.2

Break point frequencies were taken at 3 dB points of the average basin values.
Theory: theoretical values calculated from Eq. (4a,b).
Exp: Experimental values estimated from Figs. 4 and 5.

Fig. 1. shows also the calculated $Re(K)$ spectra for charged PS and PG vesicles with their equivalent internal conductivity in PEG and Ficoll solutions. The $Re(K)$ values for charged liposomes are higher and the positive range broader than those of uncharged vesicles. The high frequency break points are considerably higher (Table 1). Since our experimental measurements are proportional to $1/Re(K)$, the reciprocal permittivity values are plotted for comparisons between neutral (PC,PE) and charged (PS,PG) liposomes, as well as for liposomes suspended in PEG ($\sigma_e = 9$ μS/cm) and Ficoll ($\sigma_e = 20$ μS/cm) solutions, at the experimental frequency range (Fig. 2).

3. Results

The 'pearl chains' of vesicles are formed as a result of the DEP force against the Brownian motion, which randomizes the positions of vesicles in suspension, in the absence of other forces. This randomization force

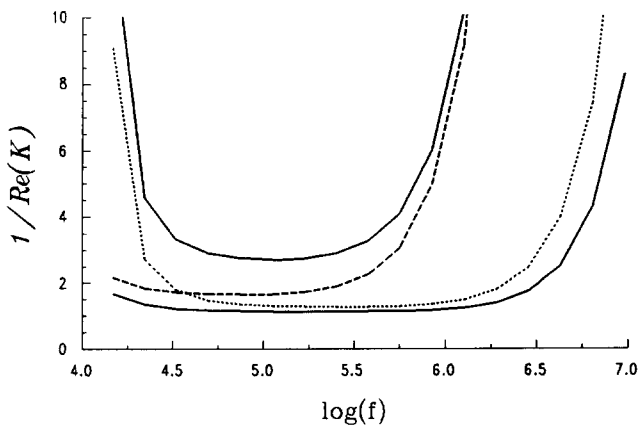

Fig. 2. The reciprocal plot the real part of the complex polarization K within the experimental frequency range. The upper solid curve is for neutral liposome in 20 μS/cm conductivity medium. The lower solid curve is for charged liposomes in 9 μS/cm conductivity medium. Other symbols are indicated in the Fig. 1 legend.

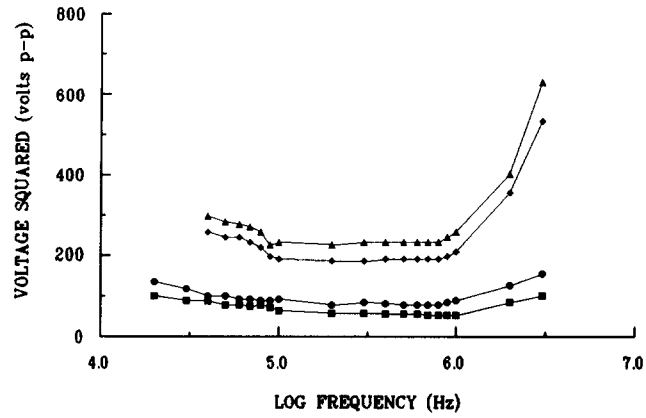

Fig. 3. Positive DEP spectra ($V_o^2 \propto 1/Re(K(\omega))$) of PS (■), PG (●), PE (♦), and PC (▲) cell-size liposomes in 2.5% Ficoll 400000 solution.

is approximately constant for our experiments under the same condition. Therefore, the threshold electric parameters to achieve 'pearl chaining' represent the parameters needed to generate a constant DEP force F_{D0} to overcome the randomization force. According to Eq. (1), this constant DEP force is proportional to $Re(K)$ times the square of the applied voltage, for the same vesicle and electrode geometry:

$$F_{D0} = A\epsilon_i Re(K) V_0^2$$

where A is a proportionality constant, and V_0 is the threshold voltage for 'pearl chain' formation. $\epsilon_i = 80\epsilon_0$ is the dielectric constant of water. For the same experiment, V_0^2 is proportional to $1/Re(K)$ at all 'pearl chain' forming frequencies.

Fig. 3 shows positive DEP spectra (V_o^2 vs. log f) of uncharged liposomes (egg PE, egg PC) and charged liposomes (egg PG and brain PS) in 2.5% Ficoll solution. The outside solution conductivity is 20 μS/cm. It is apparent from the figure that there is a significant difference between charged and uncharged liposomes in voltages necessary to obtain pearl chains; charged liposomes require a lower voltage to align. There are also a slight difference between the spectra of neutral PE and PC vesicles, and between those of charged PG and PS vesicles. All spectra show a most favorable frequency range of 100 kHz to 900 kHz for alignment. Below 20 kHz we observed positive DEP associated convection and rotation. Above 3 MHz we observed mixed positive and negative DEP, as well as rotation, and it is difficult to say which effect is dominant. Therefore, only positive DEP spectra between 20 kHz and 3 MHz were recorded.

Fig. 4 shows positive DEP spectra of cell-size liposomes (represented by uncharged egg PC and charged brain PS) in 2.5% PEG solution, as compared to those in 2.5% Ficoll solution. The outside PEG solution conductivity is 9 μS/cm. Similar shapes of spectra to

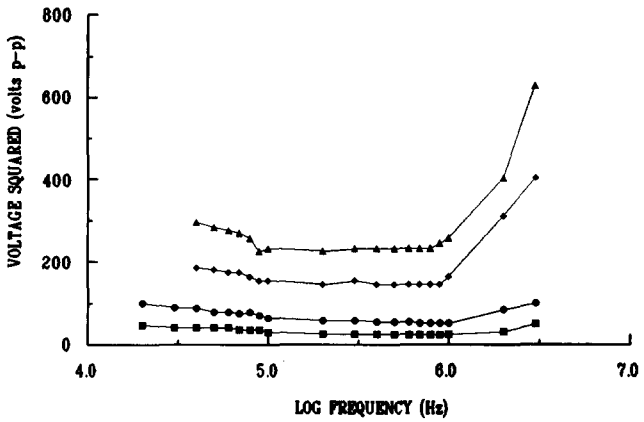

Fig. 4. Positive DEP spectra ($V_0^2 \propto 1/Re(K(\omega))$) of PS cell-size liposomes in 2.5% PEG 8000 solution (■) and in Ficoll 400000 solutions (●), compared to curves for PC (♦) and (▲), respectively.

Fig. 3 were observed. Because of the lower conductivity of 2.5% PEG solution, lower voltage of electric field is necessary to obtain positive DEP for both uncharged PC and charged PS. Adjusting the conductivity of 2.5% PEG solution to that of 2.5% Ficoll solution (20 μs/cm) resulted in producing almost identical spectra as those obtained in 2.5% Ficoll solution.

For breakpoint analysis, the F_{low} and F_{high} are determined by the 3dB points of the spectra, i.e., at frequencies at which $V^2 = 2V_{min}^2$. The low frequency breakpoints were found to be about 23 kHz and 25 kHz for charged and uncharged liposomes in Ficoll, and 15 kHz and 12 kHz for these liposomes in PEG, respectively. The high frequency breakpoints for charged and uncharged liposomes in both Ficoll and PEG are listed in Table 1.

4. Discussion

The dielectric behavior of colloids is a well studied subject, and DEP theory has been known for more than a decade. Because of the application of electrofusion, electroporation, electromanipulation and electrorotation in biology, there have been recent interest in refining the theory for cells [8,17,18]. However, the physical properties of the internal and the surface of cells are not precisely known and cannot easily be changed, therefore the comparison between experiment and theory is limited to a narrow range of experimental conditions. Liposomes offer a unique advantage in DEP and related studies because the internal and the external solutions can be precisely controlled, and the wall conductance and resistance are well known and adjustable. They provide a precise test of the existing theories on the DEP of cells.

In this study, we successfully measured the frequency-dependent dielectrophoretic responses, as positive DEP spectra, of cell-size liposomes of different compositions – egg PC, egg PE, egg PG and brain PS, at two suspension media of different electric conductivity. Part of the reason for the absence of liposome electrofusion and DEP data in literature is that these giant unilamellar liposomes are difficult to form in media containing ions (higher conductivity inside than outside) and are fragile to handle. Ficoll solution was chosen from a technical point of view. Ficoll provides a slight refractive index difference between inside and outside of liposomes for easy viewing under the phase contrast microscope. PEG solution was chosen to investigate the polymer influence on the positive DEP spectra of cell-size liposomes. PEG solution has a low conductivity and causes a lower threshold voltage for DEP alignment (Fig. 4). When the conductivity of PEG solution was adjusted to that of the Ficoll solution, by adding a minute amount of NaCl, the resultant DEP spectra was identical to that of Ficoll solution (data not shown). Therefore the nature of polymer at 2.5 wt% concentration has no significant influence on the DEP spectra of cell-size liposomes.

To account for the charged surface in PS and PG liposomes, we considered previous theoretical treatments of charged colloidal particles. According to the model of Schwan and Schwarz [14,15] for low conductivity suspension media, the relaxation time is determined by the surface conductivity of the counter ion layer. For the case of very high electrolyte conductivities, the effect of bulk diffusion on the counterion polarization has to be taken into account, according to the model of Grosse and Foster [17,18]. In this case the relaxation parameters do not depend on particle radius and surface conductivity but rather on a combination of these variables. Since we used very low conductivity suspension media, we believe the Schwan and Schwarz model is appropriate for our experiment.

The DEP characteristics of liposomes follow in general the theoretical prediction. The main features of the theoretical curves (Figs. 1 and 2) are (1) the frequencies range for positive DEP of liposomes used is in the range of 20 kHz to 3 MHz, (2) liposomes in the medium with lower external conductivity have lower DEP threshold voltages, and (3) charged liposomes have lower threshold voltages than neutral ones. All these predictions are shown to uphold. It shows that the theories are essentially correct.

Quantitative comparison between theoretical and experimental curves, however, reveals some discrepancies. First, although the experimental low frequency break points agree quite well with theoretical prediction, the experimental high frequency break points are considerable higher than that given by theory (Table 1). The discrepancy is more pronounced for neutral liposomes. Since the high frequency break points are limited by the internal conductivity of liposomes (Eq.

(4b)), the higher break points indicate a lower internal conductivity than expected. It could be due to a slight leakage of ions from the liposomes in the low conductivity medium. The small leakage will not affect the external conductivity significantly, but may reduce the internal conductivity more, hence the high frequency behavior of liposomes. The discrepancy is less severe in charged liposomes because of their high equivalent internal conductivity.

Secondly, although the trends of influences of liposome surface charge density and media conductivity are as predicted, the numerical ratios between basin (inverse plateau) values of DEP forces under different conditions are in slight variance with theory. The ratio of theoretical basin values of $1/Re(K)$ between same liposomes in two media are 1.6 and 1.1 for neutral and charged liposomes respectively, whereas the corresponding ratio of $V^2_{0,\min}$ are 1.6 and 2.2, respectively. Furthermore, similar theoretical ratios between neutral and charged liposomes in the same medium are 1.5 and 2.2 for low and high conductivity medium respectively, whereas the corresponding experimental values are 3.0 and 4.0, respectively. While the theoretical and experimental ratios for neutral liposomes in two different media agree exactly, there are discrepancies in ratios involving charged liposomes. Experimental ratios are about twice the values of theoretical ones. Since both chambers produced similar results, the discrepancy is not likely due to the field distortion by the geometric factor of electrodes. One must bear in mind that charged liposomes are on the average larger than neutral ones. When a difference of 25% in liposome radius size is taken into account in Eq. (1), the $V^2_{0,\min}$ ratios between charged and neutral liposomes in either medium would agree exactly with theoretical values.

In theory, the basin values of charged liposomes should be rather insensitive to the changes of external conductivity because the equivalent internal conductivity of charged liposomes is much higher than that of neutral liposomes (ratio = 1.1). Yet there is an observable difference in the $V^2_{0,\min}$ measurement in two media (Fig. 4). It could be that the approximation in Eq. (6) is no longer accurate when the Debye length approaches 1% of the liposome size, or else that some electric field correction should be applied to the electrodes at the very low field strength limit. On the other hand, the measured threshold for field-induced liposome aggregation is found under conditions in which they are close together. According to Gast and Zukoski [19] the point dipole approximation begins to fail as the two particles are brought into close proximity, i.e., higher order terms become important and they have a greater effect for conductive particles. Therefore, the theoretical values are inaccurate.

The results of this study show that cell-size liposomes can be used successfully as a model system in quantitative investigation of various aspects of dielectrophoresis and electrofusion.

Acknowledgement

This work is supported by a grant GM-30969 from the National Institutes of Health. Travelling fellowship to NGS was supported from Foundation in 'Cyrill and Methodius' and grant N-189 from MCES of Bulgaria. We appreciate the discussion with Dr. D. Zhelev of Duke University.

References

[1] Pohl, H.A. (1978) Dielectrophoresis, Cambridge University Press, Cambridge.
[2] Dimitrov, D.S., Tsoneva, I., Stoicheva, N. and Zhelev, D. (1984) Biol. Phys. J. 12, 26–30.
[3] Stoicheva, N.,Tsoneva, I. and Dimitrov, D.S. (1985) Z. Naturforsch. 40c, 735–739.
[4] Stoicheva, N. and Dimitrov, D.S. (1986) Electrophoresis 7, 339–341.
[5] Jones, T.B. (1986) J. Electrostat. 18, 55–62.
[6] Jones, T.B. and Bliss, G.W. (1977) J. Appl. Phys. 48, 1412–1417.
[7] Gimsa, J., Marszalek, P., Loewe, U. and Tsong, T.Y. (1991) Biophys. J. 60, 749–760.
[8] Kaler, K.V.I.S. and Jones, T.B. (1990) Biophys. J. 57, 173–182.
[9] Miller, R.D. and Jones, T.B. (1993) Biophys. J. 84, 1588–1595.
[10] Buschl, R., Ringsdorf, H. and Zimmermann, U. (1982) FEBS Lett. 150, 38–42.
[11] Hub, H.H., Zimmermann, U. and Ringsdorf, H. (1982) FEBS Lett. 140, 254.
[12] Stenger, D.A., Kubiniec, R.T., Purucker, W.J., Liang, H. and Hui, S.W. (1988) Hybridoma 7, 505–517.
[13] Pethig, R. (1979) Dielectric and Electronic Properties of Biological Materials, pp. 186–206, John Wiley and Sons, Chichester.
[14] Schwan, H.P., Schwarz, G., Maczuk, J. and Pauly, H. (1962) J. Phys. Chem. 66, 2626–2635.
[15] Schwarz, G. (1962) J. Phys. Chem. 66, 2636–2642.
[16] McLaughlin, S. (1977) Curr. Topics Membr. Transport 9, 71–143.
[17] Grosse, C. and Schwan, H.P. (1992) Biophys. J. 63, 1632–1642.
[18] Foster, K.R., Sauer, F.A. and Schwan, H.P. (1992) Biophys. J. 63, 180–190.
[19] Gast, A.P. and Zukoski, C.F. (1989) Adv. Coll. Int. Sci. 30, 153–202.