Dielectrophoretic Cell Separation: Some Hints and Kinks

W. Michael Arnold
MacDiarmid Institute at Industrial Research Limited
Lower Hutt, New Zealand
phone: +64 (4) 931-3233
e-mail: m.arnold@irl.cri.nz

***Abstract*—In order to predict the motion of biological cells in response to non-uniform electric fields (dielectrophoresis or DEP), the dielectric properties of live and permeabilised cells are assessed on the basis of multi-shell models. The predictions are compared with experimental results where separation of such cells was achieved at either of two widely-separated field frequencies. After description of permittivity-enhanced media and also of a thermo-convective device for cell separation, some results from combinations of these techniques to DEP-mediated cell and particle separations are presented.**

I. Introduction

The use of dielectrophoresis (DEP) to distinguish, and to attempt to separate, living from dead cells is not new [1]. However, significant improvements to the technique were made when negative dielectrophoresis was introduced for biological work [2,3]: to a large degree this was only possible by the design and use of microfabricated electrodes [4,5]. Arrays of such electrodes are usually produced lithographically and are therefore planar: used alone in a static fluid they are capable only of micro-scale, *in situ*, separations. In order to separate larger numbers of cells and deliver them as a product stream, it is necessary that the DEP can be combined with some form of fluid motion, e.g. as in free-flow DEP [6]. The present work describes some factors that can contribute to the successful operation of a DEP-based cell separator.

II. Dielectrophoresis (DEP)

A. General Theory

The time-averaged dielectrophoretic (DEP) force [7] exerted by a non-uniform dipolar field of r.m.s. strength E on a particle of radius a is given by:

$$\overline{F} = 2\pi a^3 \varepsilon_m \varepsilon_0 \; U'_{(\omega)} \; \nabla E^2 \tag{1}$$

The divergence of E^2 indicates that DEP can only operate in a non-uniform field. $U'_{(\omega)}$, the *real* part of the complex and frequency-dependent Clausius-Mossotti factor $U^*_{(\omega)}$ determines the sign of the DEP force. Positive or negative values of force imply attraction

or repulsion of particles to/from the regions of higher field. The direction reflects the relative polarizabilities of the particle and medium:

$$U^*_{(\omega)} = \frac{\varepsilon^*_p - \varepsilon^*_m}{\varepsilon^*_p + 2\varepsilon^*_m} \tag{2}$$

where for both particles (p) and medium (m), relative permittivities (ε) and conductivities (σ) are related by:

$$\varepsilon^* = \varepsilon' - j\sigma/\omega\varepsilon_0 \tag{3}$$

where ε_0 is the absolute permittivity of vacuum, $j=\sqrt{(-1)}$ and $\omega=2\pi f$ where f is the cyclic field frequency. Results using electrorotation [3, 7-9] are mentioned below: this single-particle technique is the "complement" of dielectrophoresis in that the torque induced by a rotating electric field is proportional to the *imaginary* part of $U^*_{(\omega)}$.

When comparing and contrasting DEP under different conditions, Eq. 1 makes it clear that the effects of varying the medium or the field frequency can be taken into account by considering the product $\varepsilon_m U'_{(\omega)}$. Equation (2) leads to the prediction that $U'_{(\omega)}$ will be in the range 1.0 (+ve DEP) to -0.5 (-ve DEP). Hence, in water, the product $\varepsilon_m U'_{(\omega)}$ can be expected to be in the range 78 to -39. However, aqueous solutions of highly polar solutes with permittivity values of above 300 are possible [10,11] in which case $\varepsilon_m U'_{(\omega)}$ may range from 300 to -150. Further, when operating in the high frequency region where conductivities have little influence, if ε_p is slightly less than ε_m (giving weakly negative DEP), then a doubling of ε_m will have the advantage of increasing the magnitude of $U'_{(\omega)}$ many times over.

B. Frequency Regions of DEP Change

In practice it is found that biological cells show strongly frequency-dependent DEP, often changing from negative to positive and back again as the frequency is raised from 1 kHz to 100 MHz. These changes are a consequence of:

1) the strong frequency-dependence of the dielectric properties of intact cells [12]: this dependence is largely a consequence of the structure of the cells, in particular the isolation of regions of high conductivity from each other by well-insulating membranes, and;

2) the change, for typical salt solutions, of the properties of the suspension from predominantly conductive at low frequencies to predominantly capacitive as the frequency is raised. For non-dispersive media where σ_m and ε_m are both frequency-independent, the dividing line between these regions is the frequency f_0 that gives conductive and capacitive currents of equal magnitude:

$$\sigma = 2\pi f_0 \varepsilon_m \varepsilon_0 \tag{4}.$$

Typical laboratory single-distilled water (conductivity 0.1 mS/m) has f_0 = 23 kHz; a 0.8 mM solution of KCl in water (conductivity of 13 mS/m) has f_0 = 2.9 MHz. The latter is closely related to the change-over frequency from positive to negative DEP for the permeabilised cells in Fig. 2. This is the same relationship as for the electrorotation maximum (Eq. 10 of Ref. [8]) of a conductive particle in a conductive dielectric medium.

III. Modelling of the DEP Response of Cells

For this work, yeast cells were modelled in a medium having a conductivity similar to that of 1 mM KCl solution in order to enable comparison with available data. Calculations were done for two different medium permittivities and two sorts of cells. Cells were modelled as either healthy controls having functional membranes and high internal conductivities, or else as permeabilised such that their internal conductivities reduced to values close to that of the medium. In a typical yeast culture, some permeabilised cells will always be present, and such cells show themselves to be permeable by taking up stains such as methylene blue.

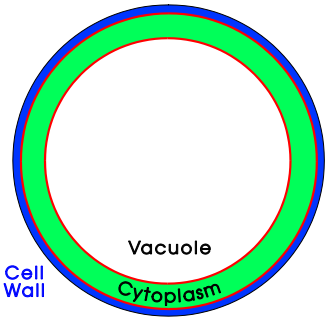

Fig. 1. The 5-phase concentric-shell model used to simulate control and permeabilised yeast cells. The nucleus and other smaller organelles are neglected in the presence of the large vacuole. Both the vacuole and cytoplasm are surrounded by relatively insulating but very thin membranes (red): these loose their isolating properties when permeabilised, causing the previously highly salt-bearing interior to drop in conductivity. The outermost shell represents the cell wall, which is porous and has properties close to those of the surrounding medium.
Parameters used to model control yeast cells: Vacuole: radius 2.5 µm, $\sigma = 0.8$ S/m, $\varepsilon = 75$;
Cytoplasm: thickness 0.5 µm, $\sigma = 0.4$ S/m, $\varepsilon = 50$; Wall: thickness 0.16 µm, $\sigma = 0.02$ S/m, $\varepsilon = 0.75\ \varepsilon_m$;
Membranes: thickness 3.5 nm, $\varepsilon = 3$ (0.008 mF/m^2), $\sigma = 10$ nS/m (plasmamembrane), 10 µS/m (vacuole).
Permeabilised cells are assigned membrane $\sigma = 0.01$ S/m, and both vacuole and cytoplasm $\sigma = 0.04$ S/m.

The effective dielectric properties of a concentric-shelled particle, usually taken as a fair approximation even for non-spherical cells, can be calculated using successive applications of the effective homogenous sphere method [13]. In the case of yeast, the 5 shells shown in Fig. 1 (vacuole, vacuole membrane, cytoplasm, plasmamembrane, cell wall) were included. The complexity beyond that of a single-membrane model is required because of the large size of the vacuole, and also because of the significant contribution that the cell wall of micro-organisms can make to the dielectric response. In cases where the cell wall contains high concentrations of charged groups and counter-ions, as is the case with many gram-positive bacteria [14], it can act as a conductive screen and effectively dominate the dielectric properties. In yeast cell walls there is less charge, but electrorotation measurements have shown that the wall still makes a significant difference to the electrokinetic properties of living yeasts in low-conductivity media [15], and determines the response of cells permeabilised with detergents or broken open mechanically [16].

The legend to Fig. 1 gives the parameters used in the models and the calculated spectra are given in Fig. 2. The data for live cells (intact membranes, high internal conductivity)

are shown by continuous lines, whereas the data for cells with degraded membranes (and therefore low internal conductivity) are shown by interrupted lines. Blue traces show the response predicted for unmodified aqueous media (permittivity of 78), whereas red traces indicate the responses in a medium of increased permittivity (permittivity of 155).
The unbroken traces between 0.1 – 10 MHz indicate the strong positive DEP response typical of cells having a highly conductive interior surrounded by an insulating membrane. The asymmetry of these peaks reflects the influence [17] of a large internal structure - the vacuole - in these cells. It is apparent that in normal aqueous media (relative permittivity of 78) both live and dead cells exhibit considerable but opposite responses at 10 kHz, so that DEP-mediated separation should be very easy. At 100 MHz and above the responses are reversed although that of the permeabilised cells is weak, so that clean separation may not be possible. However, the situation changes if the medium permittivity is approximately doubled to 155. This causes, besides a general increase in the DEP force, a much more significant increase in the negative DEP force so that a frequency (10 MHz) can be found that gives substantial but opposite responses for the two types of cells. It is also important to note that negative DEP can be selected for either cell sort by choice of frequency.

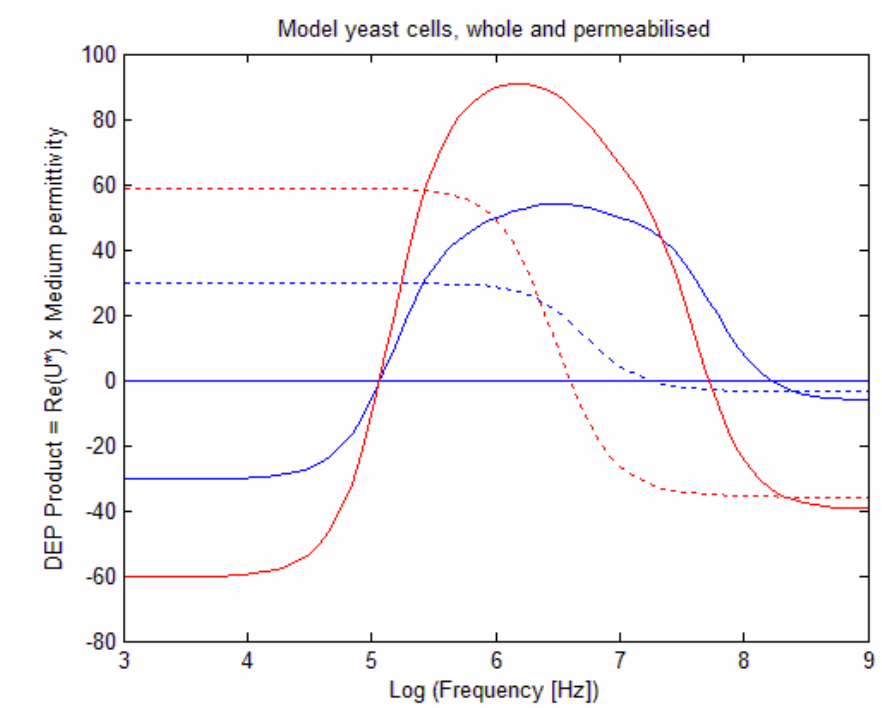

Fig. 2. Computed frequency spectra of the the product $\varepsilon_m U^{/}{}_{(\omega)}$ of whole yeast cells (solid lines), and of cells with non-functional (permeabilised) membranes (interrupted lines). Blue traces (close to zero response above 100 MHz) were calculated for $\varepsilon_m = 78$, whereas red traces (having a response of -40 units above 100MHz) were calculated for $\varepsilon_m = 155$. Medium conductivity assigned as 13 mS/m.

IV. ENHANCED-PERMITTIVITY MEDIA

Values of permittivity above about 80 are not usual in models of aqueous systems, however high concentrations of zwitterionic (doubly, but oppositely, charged) molecules can achieve values of 300 or more. Some are useable in biological DEP if their side effects of increased viscosity, osmotic strength and electrical losses at higher frequencies [18,19] are not detrimental. The low-frequency conductivity of such solutions can be very low, if the opposite charges are exactly balanced. Some representative data is given in Fig. 3, including also values for the viscosity of these solutions because these values determine the drag on particles held in a DEP trap when they are subjected to a fluid flow. The increase in permittivity is almost linear with zwitterion concentration, even up to values of 3 M (mole/liter) or more in some cases. However, viscosity increases at a quadratic or higher rate, so that the viscosity-normalised permittivity increase exhibits a maximum, usually at a concentration of 1 M or below. These normalizations are of direct relevance to positive DEP which usually scales almost linearly with the medium permittivity: Section IIA explained why negative DEP scales more rapidly with permittivity.

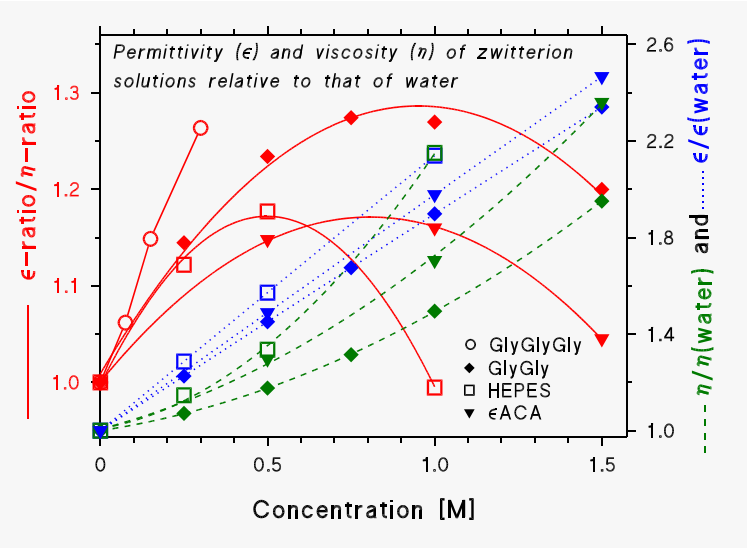

Fig. 3. Permittivity (ε) and viscosity (η) data of a series of solutions made from four different zwitterions. The data are normalized as follows: (dotted blue lines) permittivity data divided by that of water; (dashed green lines) viscosity data divided by that of water; solid red lines) permittivity ratio divided by the viscosity ratio. Abbreviations: εACA, ε-aminocaproic acid; HEPES, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; GlyGly, glycylglycine; GlyGlyGly, glycylglycylglycine. The useful range of the last peptide is restricted by its limited solubility; by contrast, HEPES has a much higher solubility, but its solutions have higher viscosity.

V. DEP-MEDIATED CELL SEPARATION

Experimental verification of the above modeling is shown in Fig. 4, which shows the results when a mixture of control and permeabilised yeast cells is allowed to sediment from a dilute suspension onto an array of structured (modified "castellated"), parallel micro-electrodes on a glass substrate.

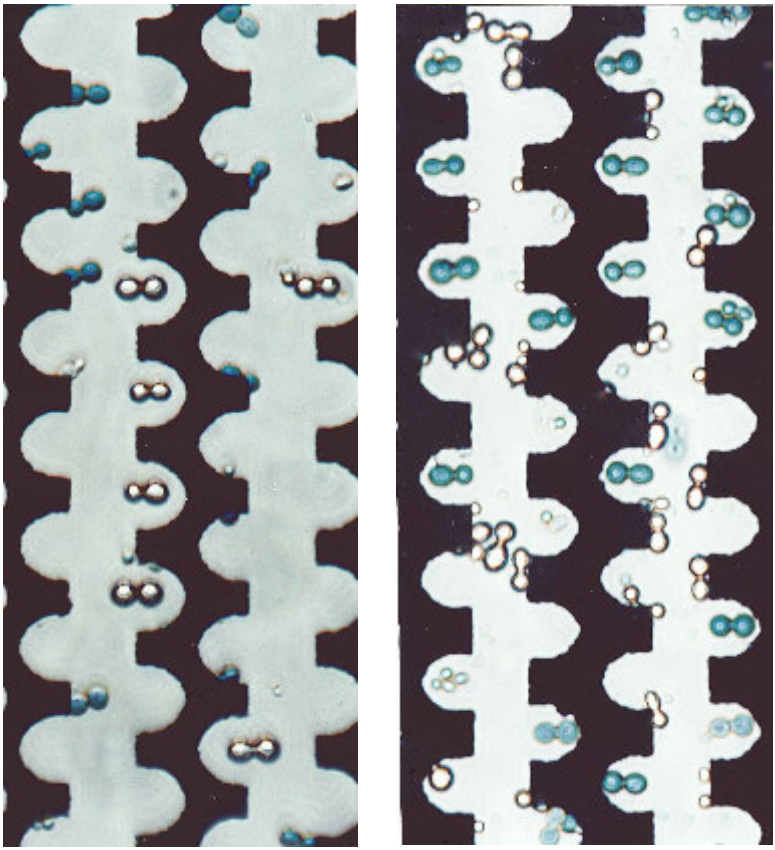

Fig.4. Demonstration of the frequency-mediated reversal of DEP behaviors of yeast cells which had not taken up methylene-blue and those that had, the latter indicating that their membranes had become permeable. Most cells are doublets, i.e. cells just before division: these were harvested from a rapidly-growing culture. The aqueous medium, which had a permittivity of 155, contained 1M ε-amino caproic acid, 2.4 μg/ml methylene blue and a pH-buffer (2.5mM HEPES, 50% as Na salt), total conductivity 19 mS/m. The electrodes (dark) were of planar interdigitated design and had a feature size of 20 μm (electrode pitch 60 μm). Voltage: 0.6 V pk between adjacent electrodes. Left) using a field frequency of 10 kHz. Right) using 10 MHz.

Between and just above such structured electrodes, the field is highly non-uniform, giving rise to pronounced field maxima and minima: the latter can, in combination with gravity, form trapping regions for cells under negative DEP. Dye uptake gives intense staining in many cases, although the dimensions of the cells appear unchanged. As expected (methylene blue is used as an indicator for permeabilised cells), the dyed cells follow the predictions made in Fig. 2 for permeabilised cells. At 10 kHz they exhibit positive DEP and consequently are attracted to the electrode extremities where the field is strongest, but at 10 MHz they are repelled by negative DEP into the field minima in the electrode recesses. This latter effect was definite only in the enhanced-permittivity medium: in unmodified aqueous media negative DEP in this higher-frequency region was

either absent or else too weak to be useable. The control cells show the opposite behavior to that of the permeabilised ones at both frequencies, again as predicted by the modeled spectra.

VI. CELL SEPARATION USING DEP AND CONVECTION

A. The Lev-vection concentrator

When voltage is applied between adjacent electrodes in an array such as shown in Fig. 3 in a conductive medium, then heat will be generated. Even though the temperature rise may be just a few degrees, a convection cell can easily be generated above it if the geometry encourages this: a large electrode array (3-10 mm diameter) with a comparable depth of liquid is required [19, 20]. Such an arrangement is shown in Fig. 5.

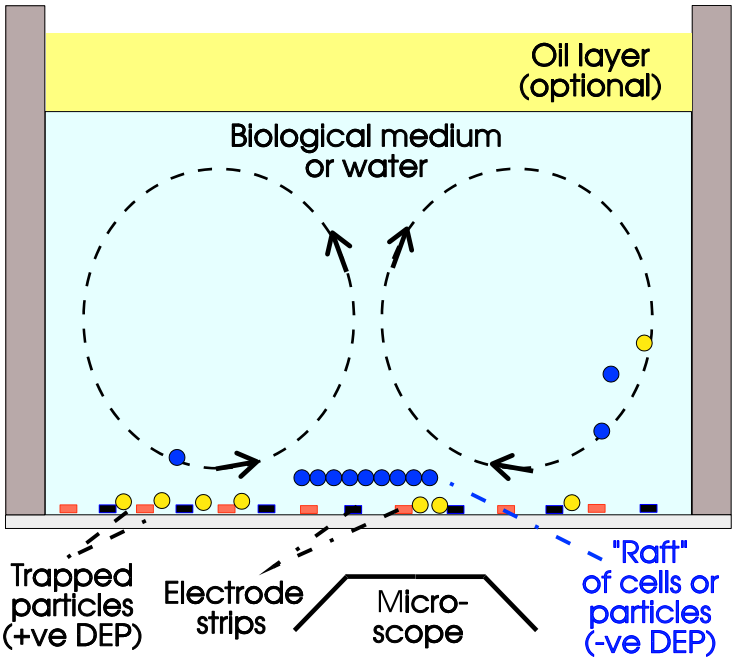

Fig. 5. The production and use of a convection cell by the heat developed just above an electrically energised array of planar microelectrodes. The circulation concentrates suspended (subsequently levitated) microparticles to a predictable region just above the centre of the electrode array. Cells follow the combined forces due to the circulation and gravity, except close to the array, where either negative DEP causes them to form a thin "raft" or positive DEP will entrain them in the inter-electrode space. The liquid paraffin is required to keep the liquid free of contaminating cells, and also to prevent evaporation in long runs. Typical voltages, for the apparatus and media discussed here, are 1.5 – 3.0V peak if a cell raft is required. Higher voltages, up to 10V peak, will give much faster circulation [20] which will re-disperse the rafted cells.

B. Example of a cell separation using lev-vection

The above apparatus can be used to concentrate a number – be it small or large - of cells to a pre-determined central position, and then hold them in stable levitation. The levita-

tion height, for cells showing negative DEP, can be controlled by adjusting frequency (Fig. 2) and/or voltage (Fig 6), whilst the convection speed can be adjusted with the voltage (at a given conductivity) [20]. Heat dissipation by convection is efficient, so the medium may be more conductive than is usual for DEP work. Therefore a full culture broth may be used, hence growth and division of cells can be observed whilst they are slightly levitated and held in single-cell traps such as in Fig 4 (left) [21]. This technique allows small colonies (clones) of cells to be grown in isolation from each other.

Using higher fields than applied in Fig. 4 or shown in Fig. 6, it was possible to levitate the methylene-blue stained cells out of the field traps whilst trapping the non-stained control cells on the electrodes. The result, using a large number of stained and non-stained cells, is shown in Fig. 7. Good separation of the stained cells (the blue "raft", containing thousands of cells, prominent in the upper panel) from the unstained controls is apparent. The latter are trapped in zig-zag chains directly between the electrodes, visible in the further magnified and contrast-enhanced lower panel.

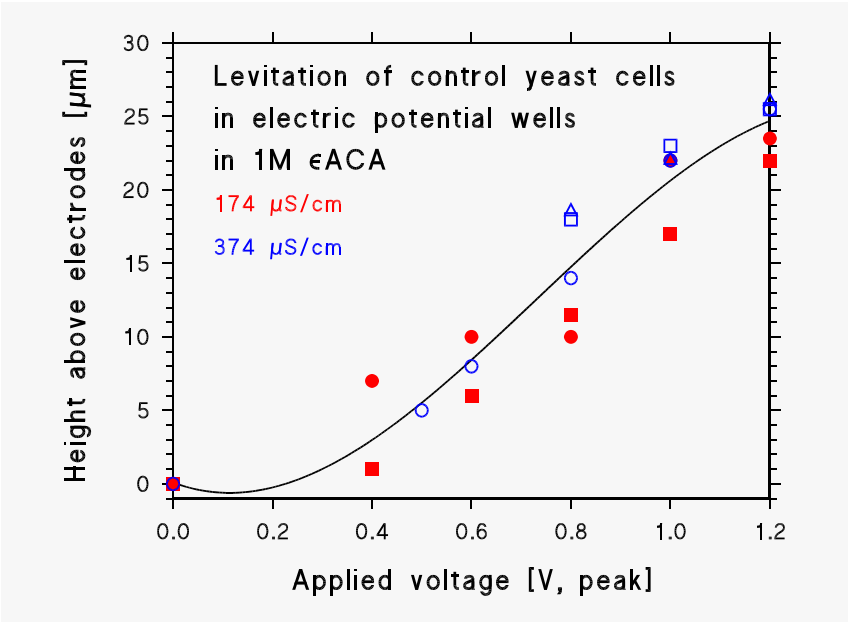

Fig. 6. Levitation heights for control (unstained) cells in field traps as in Fig. 4 (left panel). Two different conductivities were used: 17.4 mS/m (solid red symbols) and 37.4 mS/m (hollow blue symbols). Field frequencies of 9-36 kHz were applied. Each symbol represents data from a single cell, which was measured over the full range of voltage. No consistent differences between conductivities or frequencies were detected, so a cubic fit was made to the entire data set (black line). Individual cells could not be followed at voltages above 1.2V because they escaped from the traps to become subject to the convective cell (see Fig. 7). Abbreviation: εACA, ε-aminocaproic acid.

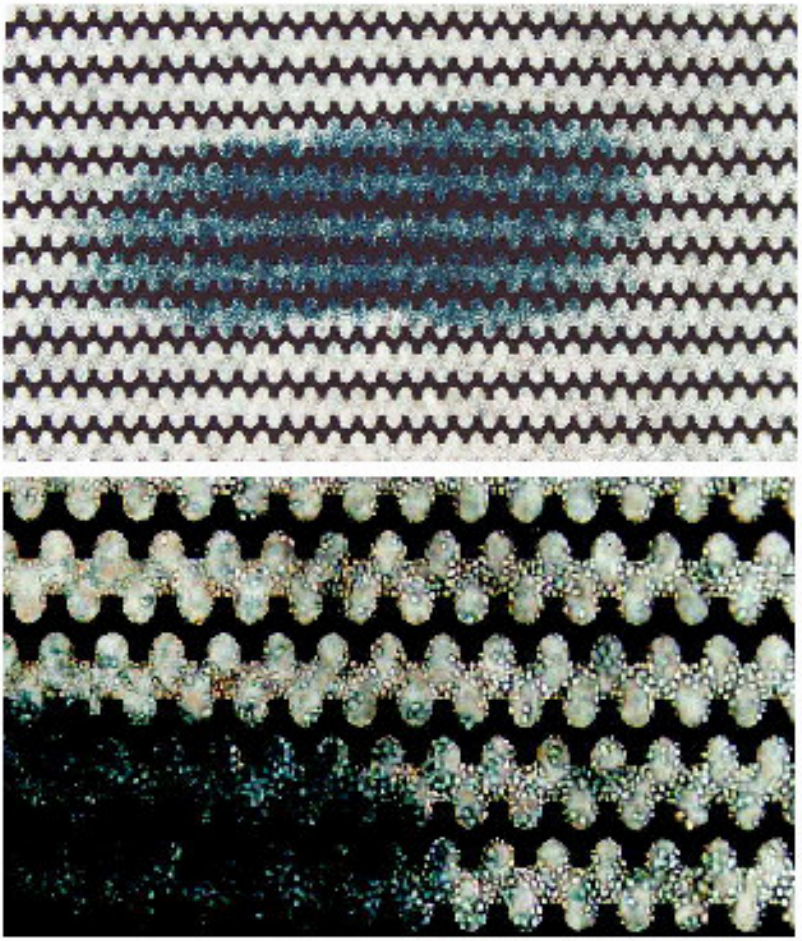

Fig. 7. Result of a lev-vection run using the apparatus of Fig. 5 to separate cells using a high permittivity medium and a 10 MHz field. Upper panel: the convection-cell centered, levitated raft of blue-dyed cells resulting from the uptake of methylene blue: this raft was formed at equilibrium between negative DEP and gravity some 50 μm above the electrodes (2.0V peak applied). Lower panel: magnified and contrast-enhanced section from the upper right part of the above image, showing zig-zag chains of control (undyed) cells held in place by positive DEP.

C. Other characterization, levitation and separation work using high permittivities

There are reports of the use of enhanced permittivity media to enhance particle levitation heights and the elution performance of a DEP-based flow-through separator [3, 22], as well as to characterise the DEP behavior of ferroelectric particles (barium titanate) [23]. Enhanced negative DEP was observed both with polymer microparticles and yeast cells. The flow-through separators used both castellated and plain interdigitated electrodes (negative DEP traps can also be formed above plain electrodes if the flow is across, not parallel to, the electrodes). As can be seen from Fig. 8, in the presence of 0.27 M or 0.67 M concentrations of zwitterions, the flow rate needed to dislodge the microparticles from

the traps formed by the electric field pattern between the interdigitated castellated electrodes was increased by 40% - 100% compared with the flow rate needed to achieve this in water. This is a significant result, because the higher density and the higher viscosity (see Fig. 3) of these relatively concentrated solutions would have tended to decrease the flow rate required. Hence the dielectrophoretic effect of these materials was dominant in this case, and indicates that they can be used to increase the utility of this separation technique. For example, the lower fields required to give particle retention at a given flow rate will permit the use of higher salt concentrations than otherwise possible before thermal disturbance becomes too great.

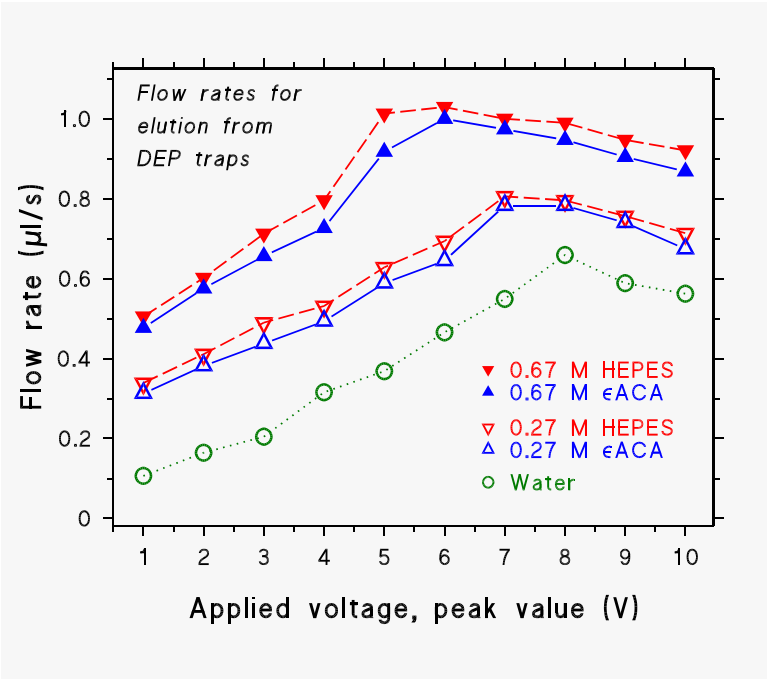

Fig. 8. Illustration of the ability of enhanced-permittivity media to increase the performance of a DEP-flow particle separation device (data redrawn from Ref. [22]). Abbreviations: εACA, ε-aminocaproic acid; HEPES, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid.

VII. Conclusion

The dielectrophoretic behavior of the yeast cells used here appears to be adequately predicted by a spherical shell model. Cell separations based on such predictions can use both positive and negative dielectrophoresis to make the separation as distinct as possible. Media with enhanced permittivity can help by increasing the DEP force available, especially in the case of negative DEP carried out at the higher frequencies (above the conductivity-dominated regime). The balance between DEP trapping forces and liquid drag in a flow separation system can also be favorably affected by the use of increased permittivity media.

REFERENCES

[1] H.A. Pohl, *Dielectrophoresis* , Cambridge University Press, Cambridge, 1978.

[2] Y. Huang and R. Pethig, "Electrode design for negative dielectrophoresis," *Meas. Sci. Technol.* vol. 2, pp. 1142-1146, 1991.

[3] G. Fuhr, W. M. Arnold, R. Hagedorn, T. Müller, W. Benecke, B. Wagner and U. Zimmermann, "Levitation, holding, and rotation of cells within traps made by high-frequency fields," *Biochim. Biophys. Acta* vol. 1108, pp. 215-223, 1992.

[4] E. Förster, C. C. Emeis, "Quantitative studies on the viability of yeast protoplasts following dielectrophoresis," *FEMS Microbiol. Lett*., vol. 26 (1985) 65-69.

[5] G. Fuhr, T. Müller, T. Schnelle, R. Hagedorn, A. Voigt, S. Fiedler, W. M. Arnold, B. Wagner, A. Heuberger, U. Zimmermann, "Radio-frequency Micro-tools for Particle and Live Cell Manipulation", *Naturwissenschaften*, vol. 81, pp. 528-535, 1994.

[6] X.-B.Wang, J. Vykoukal, F. F. Becker and P. R. C. Gascoyne, "Separation of polystyrene microbeads using dielectrophoretic/gravitational field-flow-fractionation" *Biophys. J.* vol 74, pp. 2689-2701, 1998.

[7] T. B. Jones, *Electromechanics of Particles*, Cambridge University Press, Cambridge, 1995.

[8] W. M. Arnold, H. P. Schwan, U. Zimmermann, "Surface conductance and other properties of latex particles measured by electro-rotation," *J. Phys. Chem.* vol. 91, 5093-5098, 1987.

[9] E. G. Cen, C. Dalton, Y. Li, S. Adamia, L. M. Pilarski, K. V. I. S Kaler, "A combined dielectrophoresis, traveling wave dielectrophoresis and electrorotation microchip for the manipulation and characterization of human malignant cells," *J. Microbiol. Meth.* Vol. 58, pp. 387-401, 2005.

[10] W. M. Arnold and U. Zimmermann, "Dielectric Properties of Zwitterion Solutions", *Biochem. Soc. London Trans*, vol. 21, p. 475S, 1993.

[11] W. M. Arnold, "Positioning and Levitation Media for the Separation of Biological Cells", *IEEE Trans. Ind. Appl.*, vol. 37, pp. 1468-1475, 2001.

[12] H.P. Schwan, "Dielectric Properties of Cells and Tissue" in: *Interactions Between Electromagnetic Fields and Cells*, A. Chiabrera, C. Nicolini and H.P. Schwan, Eds, New York: Plenum, 1985 pp. 75-97.

[13] A. Irimajiri, T. Hanai, A. Inouye, "A dielectric theory of 'multi-stratified shell' model with its application to a lymphoma cell," J. Theor. Biol. vol. 78, pp. 251-269, 1979.

[14] C. W. Einolf and E. L. Carstensen "Passive electrical properties of microorganisms. V. Low-frequency dielectric dispersion of bacteria," *Biophys. J.* vol. 13, pp. 8-13, 1973.

[15] W. M. Arnold, A. H. Jäger, U. Zimmermann, "The influence of yeast strain and of growth medium composition on the electro-rotation of yeast cells and of isolated walls," *DECHEMA Biotechnology Conferences* (VCH Verlag), vol. 3, pp. 653-656, 1989.

[16] W. M. Arnold, B. M. Geier, B. Wendt, U. Zimmermann, "The change in the electro-rotation of yeast cells effected by silver ions," *Biochim. Biophys. Acta* vol. 889, pp. 35-48, 1986.

[17] K. Asami and T. Yonezawa, "Dielectric Behavior of Wild-Type Yeast and Vacuole-Deficient Mutant Over a Frequency Range of 10 kHz to 10 GHz," *Biophys J*., vol. 71, pp. 2192-2200, 1996.

[18] W. M. Arnold, "Positioning, Levitation and Separation of Biological Cells" *Inst.Physics Conf. Series,* No. 163, pp. 63-68, 1999.

[19] W. M. Arnold, "Lev-vection of Highly Polar and of Dense Particles: Dispersion Effects in Polar Media", *Ann. Rep. Conf. Elec. Ins. Diel. Phenom.* IEEE DEIS, pp. 551-554, 2003.

[20] W. M. Arnold and B. Chapman, "The Lev-Vection Particle Concentrator: Some Operational Characteristics*," Ann. Rep. Conf. Elec. Ins. Diel. Phenom.* IEEE DEIS pp. 752-755, 2000.

[21] W. M. Arnold and N. R. Franich "Cell Isolation and Growth in Electric-field Defined Micro-wells," *Curr. Appl. Phys.*, vol. 6, 371-374, 2006.

[22] N. Flores-Rodriguez and G. H. Markx, "Improved Levitation and Trapping of Particles by Negative Dielectrophoresis by the Addition of Amphoteric Molecules" *J. Phys. D: Appl. Phys.* vol. 37, pp. 353-361, 2004.

[23] N. Flores-Rodriguez and G. H. Markx, **"**Anomalous dielectrophoretic behaviour of barium titanate microparticles in concentrated solutions of ampholytes," *J. Phys. D: Appl. Phys.,* vol. 39, pp. 3356-3361, 2006.