Detection of Plant Cell Compartments and Changes in Cell Dielectric due to Arsenic Absorption via Traveling Wave Dielectrophoresis

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ABSTRACT

Changes in cell dielectric properties caused by arsenic absorption were detected by means of cell velocity and a frequency causing the cell being repelled from an octa-pair interdigitated electrode. This study found that the velocity spectrum was affected by solution conductivity of which the cells were suspending during the experimentation. An abrupt change in the velocity pattern explained non homogeneous phase, cell wall and the plasmalemma, only if the solution conductivity was small. There was a slightly shift of velocity spectrum towards a lower frequency value with respect to the pretreated arsenic levels. Utilizing our previous Laplace and RC models, curve-fittings with the experimental data revealed that the membrane conductivity was increased with the arsenic levels. Although, arsenic up to 100 ppm prevented cell growth but the velocity spectrum remained similarly to the living cell.

Keywords: cell dielectric property, traveling wave dielectrophoresis, velocity, critical frequency, arsenic

1 INTRODUCTION

Traveling wave dielectrophoresis has shown potential applications in medical diagnostics, drug delivery and cell therapeutics in terms of selectivity, isolation, concentration, purification and separation of bio-particles mixtures [1, 2]. Previous studies were reported using a planar linear interdigitated electrode of one array [1, 3, 4] or two parallel arrays [2, 5, 6], and the driven electric field was generated by sinusoidal quadrature-phase voltages. A phase sequence addressing to the electrodes had been described in details elsewhere [1, 3, 4, 5, 6]. For two parallel arrays interdigitated electrode (TPI), the Clasius-Mossotti factor (CMF) composed of real [Re(CMF)] and imaginary [Im(CMF)] function (see Fig. 1) is governed by complex conductivity and permittivity of the cell related with cell medium. These functions are frequency dependent and hence might affect the cell by either collecting it at the TPI or pushing it -

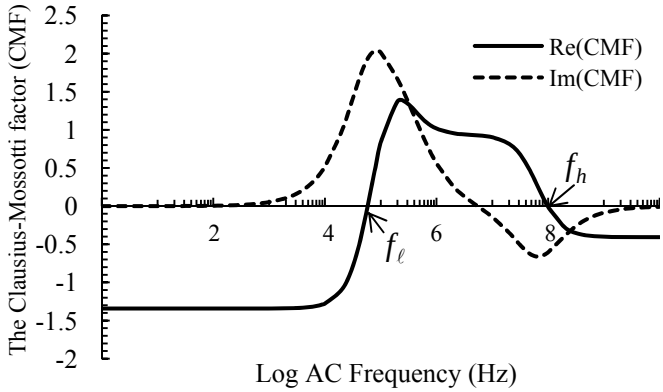


Figure 1: Theoretical plots for real [Re(CMF)] and imaginary [Im(CMF)] part of the CMF.

through electrode central channel, respectively. The negative value of the real function results in cell being repelled from the electrode, representing by a negative velocity, and vice versa. This allows cells with different properties could be separated by means of an appropriate frequency range. However, when dielectric values were predicted, it was time consuming and the model used was questionable, particularly when an ellipsoidal cell was tested. Therefore, our laboratory has investigated for a simpler method, suitable for cell technologists.

Mathematical model has further extended using Laplace method to explain cell velocity and RC methods to express two critical frequencies (Sakshin et. al., to be published). With the RC model, increasing the conductivity of cell suspending medium causes the two frequencies to converge and, finally, join at a critical conductivity, of which the cytoplasmic conductivity is revealed. This work observes cell velocity over a spectrum of field frequency and detects the two cross over values (f_ℓ, f_h). Phytoplankton with several arsenic pretreatment was used as a test model.

2 EXPERIMANTAL AND METHODS

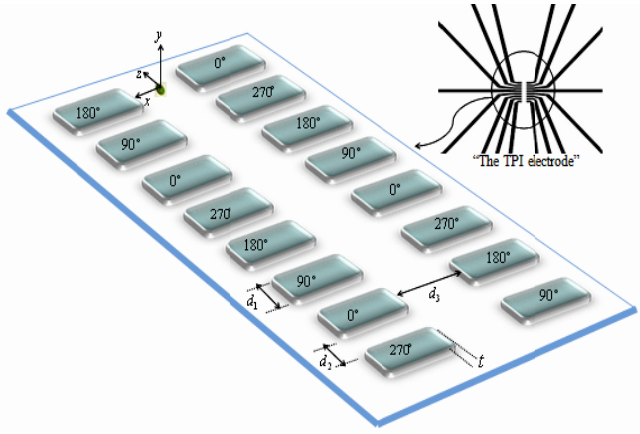
Tetraselmis sp., a spheroid (10.0 ± 0.7 μm × 8.0 ± 0.5 μm), was obtained from the National Institute for Coastal Aqua-culture (NICA), Songkhla, Thailand. The cells were centrifuged and suspended twice in 0.5 M sorbitol using 7,000 rpm for 2 min. Conductivity of the solution (σ_s) was adjusted using 0.1 M KCℓ solution, and the conductivity was measured (Tetracon 325, LF318). An $NaAsO_2$ (Sigma-Aldrich, 99%) arsenic stock solution in distilled water was diluted to a desired concentration from 1-150 ppm for cell pretreatment. After 24 hr, cells were centrifuged and washed as described above. Cell density was 9.2×10^6 and 4.0×10^5 cell/mℓ during experimentation to avoid cell-to-cell interactions. A cell sample was dropped in the middle of a TPI electrode (see Fig. 2a). A commercial available glass slides of 80 × 30 × 1 mm dimension (Marienfeld, Germany) was photo masked and designed using a layout software (AutoCAD). Each gold bar was 200 μm long, 100 μm wide (d_1), and 0.2 μm thick (t). The separation of the adjacent bars on the same array (d_2) is 100 μm and that for the central channel (d_3) is 300 μm. The electrode was energized with four sinusoidal signals (a quadrature phase) of 1.4, 2.8 and 7.1 V (rms), in phase sequence as described by Wang *et al*. (1995). A function generator (Standford Research Systems, Model DS345, and California) was connected to a phase shift unit (PSU), sending 4 signals to an inter-junction unit (IJU) to control the phase sequence (Fig. 2b). During cells undergo dielectrophoresis, velocities ($\vec{v}_{DEP}$) were recorded using a CCD camera (Sony SLV-Japan) which was connected to a microcomputer (Acer, Aspire 4310). The Winfast PVR™ program was employed to store and display the recorded files. A digital stop-watch function of the Winfast program was used to determine cell velocities. Each experiment took place within 5 min., and the f_ℓ, f_h was simultaneously recorded. Electric field derived from an applied signal was numerical calculated through the Quick Field™ program version 5.5.

3 RESULTS AND DISCUSSION

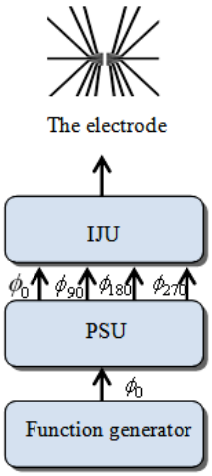
From the Winfast program, all experiments described below perform cell translational velocity towards the TPI electrode in accord with the frequency used under selected σ_s. In all cases, only f_ℓ was detected, due to the limitation of our function generator and it was plotted against the solution conductivity. A series of experimental results was grouped as following.

3.1 Characteristics of Control Cells

When 9.2×10^6 cell/mℓ *Tetraselmis* sp. was subjected



(a)



(b)

Figure 2: A configuration of the octa-pair interdigitated electrode and the quadrature phase sequence. (a) Three dimensional view of the electrode on a glass slide (not to scale) (b) Diagram of electrical set up.

to 28 kV.m^{-1} field strength (1.4 V signal), they underwent the positive velocity at frequencies between 50 kHz to 30 MHz. This was observed when varying the medium conductivity (σ_s) from 0.01 to 0.10 S.m^{-1}. Fig. 3a shows that an increasing in the σ_s reduced the velocity magnitude of the spectra and the observed f_ℓ was shifted to a higher value. As is seen, only when 0.01 S.m^{-1} is used it appears a sharp velocity peak at $9.4\,\mu$m.s^{-1}. This could be due to the field experiences a non homogeneous phase at about 200 kHz. In this case, it could be the outer shell of the cell (i.e. cell wall and the plasmalemma), which is normally much smaller in its conductivity compared to the cell interior. Evidences showing the finding of non homogeneous particle by electric field were previously reported [7, 8].When the σ_s is increased, the peak disappears and the

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spectra shows a bell shape with a plateau value of 5.2, 4.4, and 3.5 μm.s^{-1}, respectively. The frequency dependence velocity extends from 500 kHz to 30 MHz or more, since close to zero velocity at the higher critical frequency f_h is not found in all cases. The lower critical frequency f_ℓ is shifted to a higher value at 200, 300, and 500 kHz with respect to the increased σ_s. Fig. 3b shows that the velocity is proportional to the electric field while the f_ℓ is 300 kHz, independent of the increased field. This is also true for the higher σ_s used. Increasing the field strengths further increases the peak velocity to 29 μm.s^{-1}. This study found that minimum field should not be less than 28 kV.m^{-1} otherwise the velocity would be uncertain and rather difficult to measure. Fig. 3c shows that changing the cell density from 4.0×10^5 cell/mℓ to 9.2×10^6 cell/mℓ have no effect on the velocity spectrum. This confirms that electric field strength during experimentation was not affected by the presence of neighboring cells.

3.2 Arsenic Pretreatment

Velocity spectra of arsenic pretreated cells in Fig. 4a shows that greater arsenic content in the cells shifts f_ℓ towards a lower value while the peak velocity is not affected. Those lines are drawn to fit the experimental data, using the appropriate values of dielectric parameters, to verify our previous Laplace model. It appears that the frequency shifting to the lower value is caused by the arsenic and possibly causes pore blockage and, hence, increasing its electric permittivity. This result is in consistency with the f_ℓ *vs.* σ_s plots, using our RC model in Fig. 4b, since the same parameters were utilized fro curve fittings. Theoretically, it shows the same single f_h line, regardless of arsenic content. Noted that at 0.25 S.m^{-1}, the cell velocity was 4 μm.s^{-1} and further reduced towards a negligible value when 0.35 S.m^{-1} solution was used. This implied that critical conductivity of *Tetraselmis* sp. is 0.35 S.m^{-1}, indicating the conductivity of cytoplasm. Our previous results [9] showed that arsenic pretreatment also increased the membrane conductance similar to what found in dead cells, and cell proliferation ceased when the arsenic reached 100 ppm level (see Fig. 5). An experiment using dead cells showed that the f_ℓ diminished from the velocity spectrum. It is, therefore, ascertained that these arsenic pretreated cells are still alive, and the cessation of cell growth could be due to some difficulties in taking up nutrients from its environment. Normally, dead cells possess large membrane conductance, while the increased membrane conductance in this case is caused by an arsenic accumulation in the membrane.

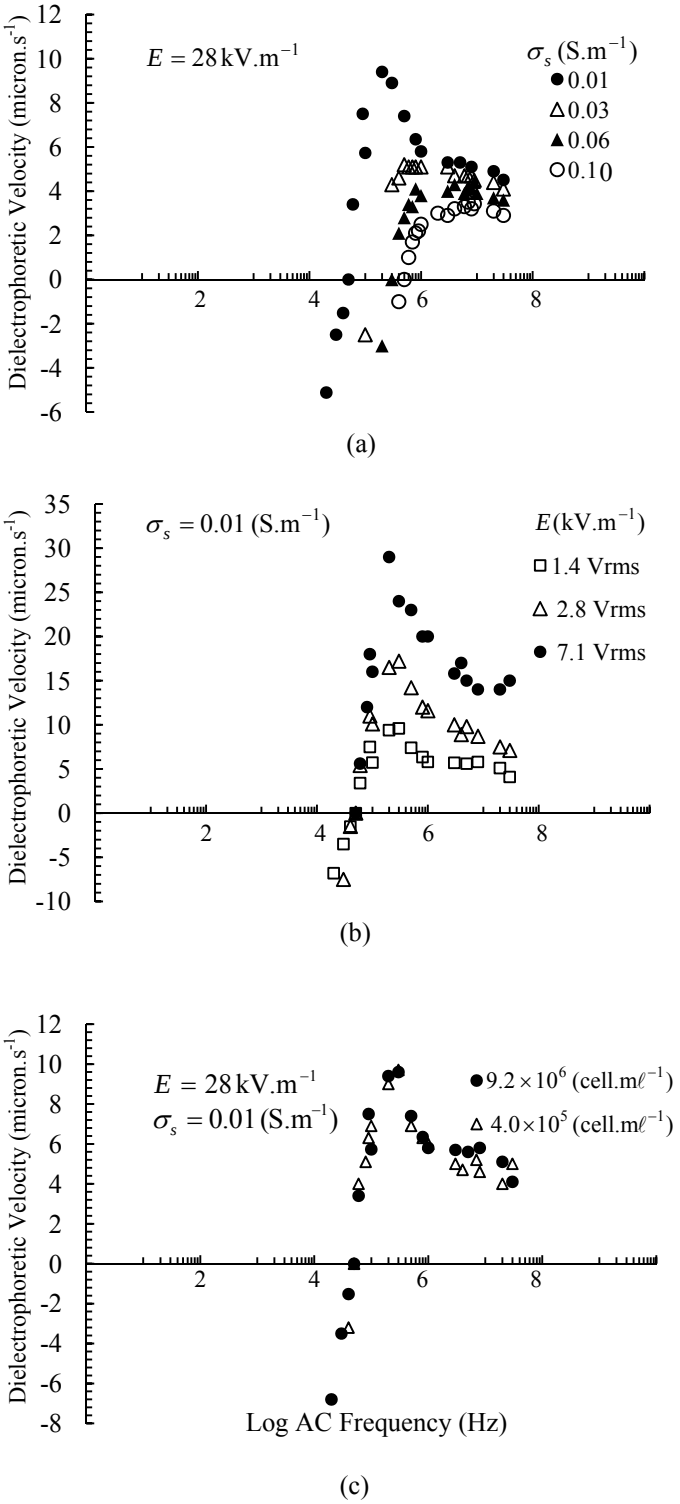

Figure 3: Velocity spectrum (a) effect of the conductivities of the suspending medium (b) effect of the electric field strengths and (c) effect of cell densities.

4 CONCLUSIONS

The home-made TPI electrode could detect the non - homoginuity of a test cell, i.e. cell wall and the plasmalemma of 10 nm thick, by showing a disruption of velocity spectrum only if 0.01 $S.m^{-1}$ sorbitol solution was used. The minimum field of 28 $kV.m^{-1}$ should be utilized for *Tetraselmic* cells so that the velocity was possible for the measurements. After arsenic absorption, the velocity spectrum remains in the similar manner as that of the control, except the membrane increases its conductivity due to arsenic blockage, followed by a cessation of growth if the arsenic level was as high as 100 ppm.

ACKNOWLEDGEMENT

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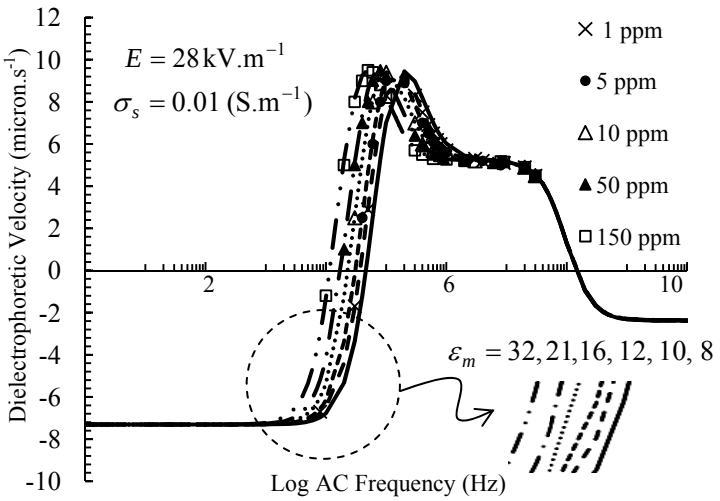

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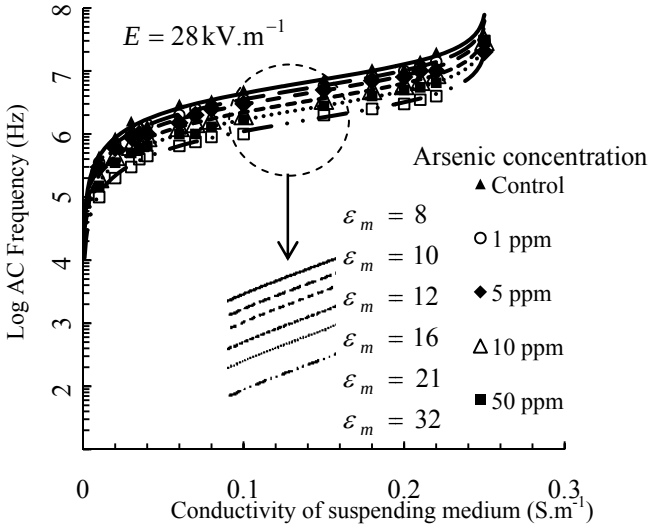

(b)

Figure 4: A comparison between control and arsenic pretreated cells (a) cell velocity (b) the lower critical frequency.

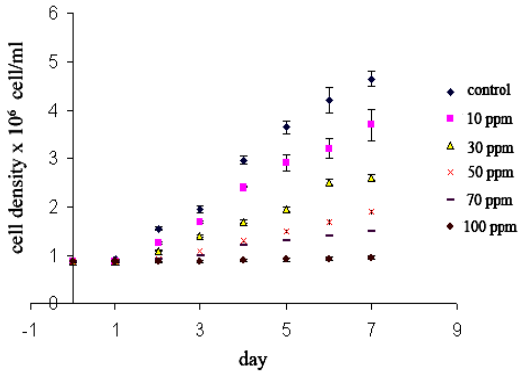

Figure 5: Test for viability by means of cell growth in accord with the arsenic levels after 24 hrs arsenic pretreatment.