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Concentrating Bacterial Spores from Milk and Juices Using Dielectrophoresis Based Microfluidic Capture Systems

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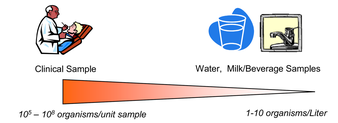
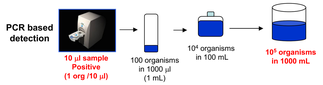
Motivations

- For food defense purposes, there is a critical need to develop technologies that can rapidly and efficiently separate and concentrate bio-threat agents from food matrices, thereby rendering them available for detection.
- A key limitation of current bio-threat agent detection and characterization technologies is linked to the small sample volumes that can be analyzed by the current methods.



Hypothetical scenario of intentional contamination of milk by 10 g of *botulinum toxin* has predicted casualty level of 100,000 people (Wein & Liu, *Proc. Natl. Acad. Sci.*, 2005)

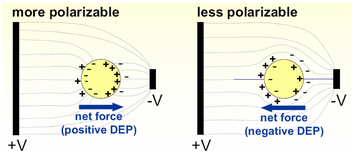


Objectives

- To design and develop a prototype device that can be ultimately scaled up for concentrating bacterial spores from large volumes of milk and apple juice.
- Utilization of dielectrophoresis (DEP) to concentrate *Clostridium sporogenes* (as surrogates of *B. anthracis* and *Clostridium botulinum* spores) from milk and apple juice.

Dielectrophoresis (DEP)

DEP is the motion of polarizable particles that are suspended in an ionic solution and subjected to a spatially non-uniform electric field. The particle motion is produced by the dipole moments induced on the particle and the suspending fluid due to the non-uniform electric field. Polarizability of particle relative to the suspending medium determines the basic direction of DEP force (positive/ negative DEP), which also strongly depends on the frequency of the applied electric field.



Methods

- Designing of a microscale device using photo-lithographic techniques (Fig 1) and the associated experimental setup (Fig 2) for demonstration of spore concentration from milk and apple juice.
- Observation of spore motion under the influence of applied AC electric field and electrode configurations using fluorescence microscopy.

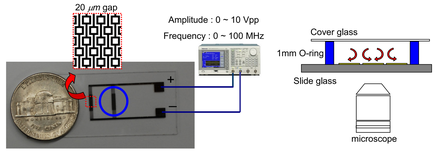


Fig. 1 Schematic of the microfluidic device. A Fluid chamber was created by placing a 1 mm thick O-ring spacer on the electrode, and a microscope cover glass was used to close the chamber.

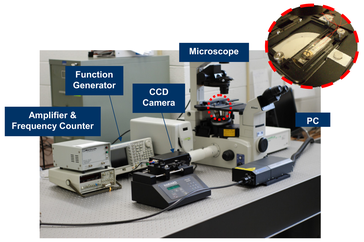


Fig. 2 Photograph of the experimental setup

Major Achievements

Scaling analysis

Performed a scaling analysis that considered relative magnitudes of Brownian motion, electrophoresis (EP), AC-electroosmosis (AC-EO), dielectrophoresis (DEP), and gravitational forces experienced by the particles.

Positive DEP capture in low conductivity media

Parametric experimental study of the DEP force and correlation with the phase diagram as a function of the electric field frequency, amplitude and the conductivity of the ionic solution.

Negative DEP capture in high conductivity media

Capturing spores in the electric potential well created by the novel planar electrode design for high conductivity media (Apple juice – 0.22 S/m, milk – 0.53 S/m).

Results

Theory

- DEP force

$$F_{DEP} = 2\pi\varepsilon_0 \varepsilon a^3 \operatorname{Re}\{K\} \nabla |\vec{E}|^2$$

- Clausius-Mossotti factor

$$K(\varepsilon_p^*, \varepsilon^*) = \frac{\varepsilon_p^* - \varepsilon^*}{\varepsilon_p^* + 2\varepsilon^*}$$

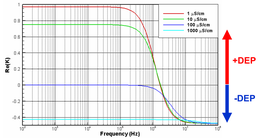


Fig. 3 Plots of the real part of the Clausius-Mossotti factor as a function of the electric field frequency and conductivity of the suspending medium.

Scaling Analysis

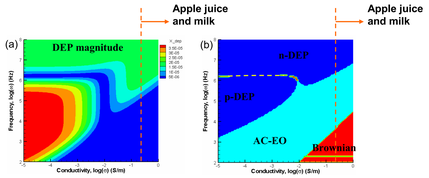


Fig. 4 Plots of (a) the magnitude of DEP displacement and (b) frequency-conductivity phase diagram

Initial experiments were conducted utilizing interdigitated electrode geometry that amplified positive DEP. We have shown excellent capture for low ionic strength solutions, typically corresponding to that of bottled water (Fig. 5). However, the capture efficiency of positive DEP quickly diminished with increased conductivity, as predicted by the phase diagram (Fig. 4).

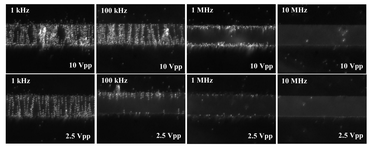


Fig. 5 Images of *C. sporogenes* stained spores suspended in DI water after applying AC electric fields for 1 minute at various frequency and amplitude. Gold electrodes and glass are observed as dark and light gray, respectively, while the stained spores are white. Characteristic electrode separation is 30 μm.

Only negative DEP can be utilized for concentration of spores suspended in high ionic strength media such as milk, apple juice and other physiological buffers.

	Apple Juice	2% Milk	PBS	Blood
Conductivity	0.22 S/m (measured)	0.53 S/m (measured)	~0.8 S/m	~1.5 S/m

Electrode design and numerical analysis

Using the correlation map as an efficient designing tool, we designed the electrodes that amplify the negative DEP motion by creating a low electric potential well. In order to predict the resultant DEP force acting on the spores, we utilized numerical simulations (Fig. 6).

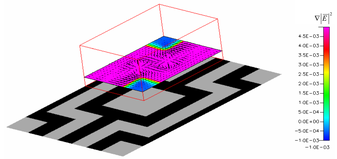


Fig. 6 Electric field strength contour and DEP force vector on z=11 μm plane.

Capture of spores in apple juice and milk

The designed electrodes were fabricated and tested. Capture and concentration of the spores at designed electric potential wells were successfully demonstrated using apple juice and 2% milk (Fig. 7).

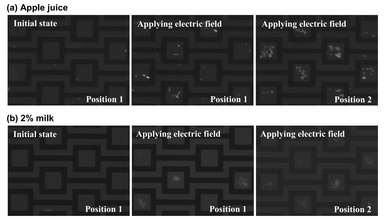


Fig. 7 Images of C. sporogenes stained spores suspended in (a) apple juice and (b) 2% milk before (initial state) and after applying AC electric fields for 5 minutes at 10 V amplitude and 50 MHz frequency. Characteristic electrode separation is 20 μm.

Conclusions

- Explained the limitations of positive DEP in high conductivity food sample matrices.
- Successfully demonstrated the utilization of negative DEP for capture and concentration of spores from milk and apple juice.
- Based on the DEP capture device, a continuous flow microfluidic system using a combination of ultrasonic standing wave (USW) , flow field fractionation (FFF), and negative DEP is currently under development.
- Key results enable new device designs and technologies that will potentially allow microfluidic systems, which can concentrate bacterial spores from large volumes of high conductivity media, such as apple juice and milk.

Ongoing Research

We are designing a *continuous flow microfluidic system* to separate bacterial spores from milk and apple juice using a combination of *ultrasonic standing waves* (*USW*), and *pressure driven flow (PDF)* to induce *flow field fractionation* (*FFF*). The negative dielectrophoresis technique will be utilized for concentrating bacterial spores at specific regions of the device.

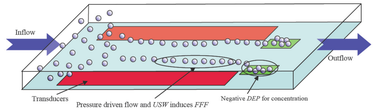


Fig. 8 A schematic of continuous microfluidic concentrator

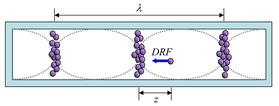


Fig. 9 An illustration of particle behavior under the influence of ultrasonic standing wave (USW)

- Direct Radiation Force, $DRF = -\pi P^2 V \beta / (2\lambda) \cdot \phi(\beta, \rho) \cdot \sin(4\pi z / \lambda)$

where P is the sound pressure amplitude, V is the particle volume, λ is the wavelength of the driving frequency, z is the distance from the pressure node, β is the compressibility, ρ is the density, ϕ is the contrast factor, given by $\phi = (5\rho_p - 2\rho)/(2\rho_p + \rho) - \beta_p/\beta$.

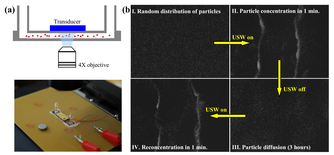


Fig. 10 (a) Schematics and a photograph of the device used for preliminary *USW* tests. Ultrasonic transducer was attached on the top of the microchannel to create *USW*, and a PCB board was used to hold the channel and make electrical connections. (b) Fluorescent images of 1 µm polystyrene particles suspended in DI water – I. Random distribution of particles; II. Particle concentration within 1 minute of applying 3.32 MHz *USW*; III. Particle dispersion via Brownian motion (*USW* is off); IV. Re-concentration within 1 minute.

Acknowledgement

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