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Universal Bioprocessor LDRD Final Report

Blake A. Simmons, Eric B. Cummings, Rafael V. Davalos, David Reichmuth, Karen L. Krafcik, Allen J. Salmi, James E. VandeVreugde, Pierre Ponce, Yusef Syed, Kevin Luongo, and Poorya Sabounchi

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Abstract

Microsystems pose unparalleled opportunity in the realm of real-time sample analysis for multiple applications,, including Homeland Security monitoring devices, environmental monitoring, and biomedical diagnostics. The need for a universal means of processing, separating, and delivering a sample within these devices is a critical need if these systems are to receive widespread implementation in the industry and government sectors. Efficient particle separation and enrichment techniques are critical for a range of analytical functions including pathogen detection, sample preparation, high-throughput particle sorting, and biomedical diagnostics. Previously, using insulator-based dielectrophoresis (iDEP) in microfluidic glass devices, we demonstrated simultaneous particle separation and concentration. As an alternative to glass, we evaluate the performance of similar iDEP structures produced in polymer-based microdevices and their enhancement through dynamic surface coatings. There are numerous processing and operational advantages that motivate our transition to polymers such as the availability of numerous innate chemical compositions for tailoring performance, mechanical robustness, economy of scale, and ease of thermoforming and mass manufacturing. The polymer chips we have evaluated are fabricated through an injection molding process of the commercially available cyclic olefin copolymer Zeonor®. We demonstrate that the polymer devices achieve the same performance metrics as glass devices. Additionally, we show that the nonionic block copolymer surfactant Pluronic F127 has a strong interaction with the cyclic olefin copolymer at very low concentrations, positively impacting performance by decreasing the magnitude of the applied electric field necessary to achieve particle trapping. The presence of these dynamic surface coatings, therefore, lowers the power required to operate such devices and minimizes Joule heating. The results of this study demonstrate that polymeric microfluidic devices with surfactant coatings for insulator-based dielectrophoresis provide an affordable engineering strategy for selective particle enrichment and sorting.

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1. Introduction

1.1 Background and Theory of Dielectrophoresis

Recent national and global events have drawn attention to the need for the rapid and accurate monitoring of water distribution networks. To detect particles or pathogens at low concentrations in raw liquid samples, it is vital to develop selective techniques to collect, concentrate, and deliver particles of interest for testing and identification. Dielectrophoresis (DEP) has been shown to be an effective means to manipulate such particles. DEP is the motion of particles driven by conduction effects in a nonuniform electric field[1]. It has been shown that DEP can be used to transport suspended particles utilizing either oscillating (AC) or steady (DC) electric fields[2]. DEP is suitable for differentiating biological particles (e.g., cells, spores, viruses, DNA) because it can collect specific types of particles rapidly and reversibly based on intrinsic properties such as size, shape, conductivity and polarizability.

Many device architectures and configurations have been developed to sort a wide range of biological particles by DEP. For example, early DEP experiments carried out by Pohl *et al.*, utilized pin-plate and pin-pin electrodes to differentiate between live and dead yeast cells and collected them at the surface of the electrode[3, 4]. Currently, the typical dielectrophoretic device generates a non-uniform electric field using an array of thin-film interdigitated electrodes within a flow channel that interacts with particles near the surface of the electrode array[5]. The nonuniform electric fields are typically generated by a single-phase AC source, and, in addition, multiple-phase sources can trap and sequentially transport particles in a technique called traveling-wave dielectrophoresis[6]. These electrode-based DEP devices are effective for separating and concentrating cells[7], proteins[8], DNA[9], and viruses[10].

Another approach is insulator-based dielectrophoresis (iDEP), which uses insulating obstacles - instead of electrodes - to produce spatial nonuniformities in an electric field that is applied through the suspending liquid. This iDEP technique was first presented by Masuda *et al.* [11] and subsequently developed further as a means of separating particles by Lee *et al.* [12]. It has been demonstrated utilizing insulating glass structures and AC electric fields that iDEP can separate DNA molecules, *Escherichia coli* cells, and red blood cells[13]. Similarly, Zhou *et al.*[14] and Suehiro *et al.*[15] used channels filled with insulating glass beads and applied AC electric fields to separate and concentrate yeast cells in water. We expanded these experimental findings to microfluidic glass-based devices using iDEP with a DC electric field applied across an array of insulating posts inside the microfluidic channel. Examples include trapping of polystyrene particles[16], separating live from dead bacteria[17], differentiating live species of bacterial prokaryotic cells[18], and the trapping and concentration of viruses[19]

While glass-based iDEP microdevices perform well, sample throughput is low because of the geometrical limitations of isotropically etched devices[20]. Typical sample flow rates for glass-based devices are in the range of ten microliters per hour. In contrast, polymer-based devices can be easily scaled to handle larger sample volumes using commercially available and inexpensive techniques[21, 22]. Polymer-based microfluidic devices have been developed for many lab-on-a-chip applications. Among them are liquid/liquid sorting, particle separation,[23] capillary electrophoresis, miniaturized polymerase chain reaction (PCR) chambers, nucleic acid analysis, protein analysis, and fluidic mixers[24, 25]. The main appeal

of these polymeric devices is that they are relatively inexpensive, employing standard mass fabrication techniques such as injection molding and hot embossing instead of microlithography[26]. We have demonstrated that these polymeric iDEP elements can be made from a cyclic olefin copolymer, Zeonor[®][20]. Cyclic olefin copolymers have received a significant amount of interest in microfluidics due to their low auto-fluorescence and high chemical resistance to a wide range of polar solvents[27].

The present study demonstrates the capabilities and increased flexibility of polymer-based iDEP devices and explores the possibility of performance enhancement through dynamic coatings to separate and concentrate water-borne bacteria, spores and inert particles. The DEP behavior of the particles (both biological and inert) was observed to be a function of the magnitude of the applied DC electric field and the characteristics of the particle such as size, shape, and conductivity. Additionally, we demonstrate that by using surfactants we discover a new flexibility to meet a wide range of operational requirements. The utilization of surfactants as dynamic coatings has been widely investigated in capillary electrophoresisⁱ and other microfluidic applications.¹ We present a quantitative determination of the interaction between the hydrophobic Zeonor 1060r polymer surface and the nonionic block copolymer surfactant, Pluronic F127, as a function of concentration through Quartz Crystal Microbalance (QCM) studies. The presence of the nonionic surfactant at very low concentrations in the solution produces a fully saturated surfactant coating on the polymer as well as a dramatic reduction in the magnitude of the applied electric field necessary to trap inert beads. This lowers the power required to operate such devices and minimizes Joule heating term present and increases the operational stability of the iDEP device. In summary, the polymeric devices exhibit the same characteristic DEP behavior of glass devices, but with higher throughput (milliliters/hour), ease of mass fabrication, the ability to support a variety of scalable physical formats, and the flexibility of surfactants as dynamic coatings to meet a wide range of operational requirements.

Dielectrophoresis is defined as the motion of a particle due to its polarization induced by the presence of a non-uniform electric field. The DEP force acting on a spherical particle can be described by the following[28, 29]:

$$F_{DEP} = 2\pi\epsilon_0\epsilon_m r^3 \operatorname{Re}\{f(\tilde{\sigma}_p, \tilde{\sigma}_m)\}\nabla E^2 \quad , \quad (1)$$

where ϵ_0 is the permittivity of free space, ϵ_m is the relative permittivity of the suspending medium, r is the radius of the particle, ∇E^2 defines the local gradient of the electric field, $\tilde{\sigma}_p$ and $\tilde{\sigma}_m$ are the complex conductivities of the particle and the medium respectively, and $f(\tilde{\sigma}_p, \tilde{\sigma}_m)$ is defined as the Clausius-Mossotti factor:

$$f(\tilde{\sigma}_p, \tilde{\sigma}_m) = \left[\frac{\tilde{\sigma}_p - \tilde{\sigma}_m}{\tilde{\sigma}_p + 2\tilde{\sigma}_m} \right] , \quad (2)$$

where the complex conductivity terms are defined as:

$$\tilde{\sigma} = \sigma + i\omega\epsilon , \quad (3)$$

where $i = \sqrt{-1}$, and ω is the radian frequency of the applied electric field. Equations (1-2) indicate that the dielectrophoretic force acting on a particle can be positive or negative in magnitude. For frequencies below 100 kHz, the real currents typically dominate displacement currents and the Clausius-Mossotti factor can be approximated in terms of the real conductivities:

$$f(\tilde{\sigma}_p, \tilde{\sigma}_m) \approx \left[\frac{\sigma_p - \sigma_m}{\sigma_p + 2\sigma_m} \right] \quad (4)$$

In this scenario, if the conductivity of the particle is greater than the conductivity of the medium, then the particle will exhibit positive DEP behavior and move toward regions of high electric field. If, as is typical for biological particles, the particle is less conductive than the suspending medium, the particle exhibits negative DEP and moves away from regions of high electric field. We utilize DC electric fields exclusively in our devices, and therefore the dielectrophoretic force exerted on the particles for a given electric field gradient depends only on the conductivity of the particle, the conductivity of the medium, and the size of the particle.

2. Experimental and Fabrication Details

2.1 Experimental Details

2.1.1 Chemicals

Deionized water from a reverse osmosis filter was titrated with KOH and HCl to a pH of approximately 8. Conductivity was then adjusted by titration with KCl to an endpoint of 1-2 $\mu\text{S}/\text{cm}$. Carboxylate (green) and/or rhodamine (red) surface-modified polystyrene microspheres, FluoSpheresTM, (Molecular Probes, Eugene, OR) having a density of 1.05 mg/mm^3 and various sizes were diluted 1:1,000,000 in the background solution from a 2 wt.% stock suspension. Bead suspensions were stored in a refrigerator and sonicated between steps of serial dilution and before use. The bead suspensions were filtered using an appropriate pore size syringe filter to remove larger bead aggregates before use.

2.1.2 Labeling of Biological Organisms

Bacillus thuringiensis and *Bacillus subtilis* spore suspensions were obtained from Raven Biological Laboratories Inc. (Omaha, NE). The unmodified spore samples were then labeled with Syto[®] 11 dye (Molecular Probes, Inc., Eugene, OR) following the same protocol used with the live bacterial cells. The final concentration of the labeled spores was 1×10^9 spores/ml. The labeled spore solutions were then diluted between 1:20 and 1:100 by volume in pH and conductivity controlled deionized water. Approximately 20 μL of diluted sample was added to the inlet reservoir of the flow manifold via pipette.

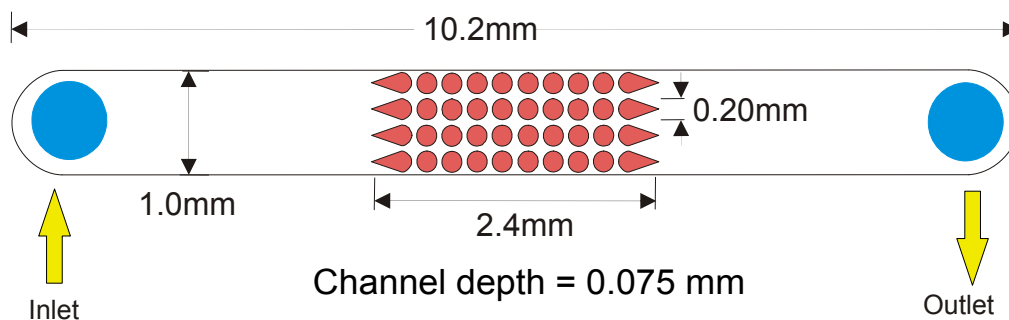


Figure 1. Schematic depiction of typical experimental device configuration used in iDEP studies showing the arrays of insulating posts in the middle of the channel and the fluidic reservoirs in which the electrodes are placed.

2.1.3 iDEP Microfluidic Design

A schematic of a microchannel can be seen in Figure 1. Each microchannel is 1-mm wide, and 10.2-mm end to end, with a nominal depth of 75- μm . Towards the middle of the microchannel are etched an array of insulating posts that are arranged in 10 sequential columns of 4. These circular posts are 200- μm in diameter, are spaced 250- μm center-to-

center, and traverse the entire depth of the channel. The posts of the front and back columns have the same width and spacing, but taper outward to reduce fouling.

2.1.4 Replication Tool

A custom stamp with a negative of the microchannel troughs and posts on its surface was used to injection mold the polymeric microfluidic devices. The stamp was fabricated using a silicon master with features for eight independent microchannels photolithographically etched into its surface. A deep reactive ion etch “Bosch” process was employed to produce features for eight independent microchannels with straight sidewalls with a depth of 75- μm on the Si master (Figure 2a). After patterning, the masters were sputter coated with an electroplating base material, in this case 500 Å of chrome (for adhesion promotion) and 1500 Å of copper. The masters were then placed into a Digital Matrix commercial DM3M electroplating machine. The bath chemistry utilized was a standard nickel sulfamate with controlled pH, typically around 4, to minimize stresses accordingly. Electroplating occurred at 48 °C for a total of 40 amp-hours and produced nickel films with thickness typically on the order of 1-mm. The electroplated nickel stamp was then planarized and machined to the set dimensions for use in our custom in-house fabrication facilities and released from the master. The nickel stamp was then thoroughly characterized through metrology, visual inspection, and electron microscopy (Figure 2b). Since the stamp is a negative of the master, the master and the final polymer disc contain the same features.

2.1.5 Polymer Replication

Polymeric discs were injection molded from Zeonor[®] 1060 resin (Zeon Corp., Tokyo, Japan) using the custom stamp containing the negative of the eight independent microchannels (Figure 2b). Injection molding was carried out utilizing a 60-ton Nissei[®] TH-60 vertical injection molding machine (Nissei[®] America, Los Angeles, CA). Pellets of Zeonor[®] 1060r resin (Zeon Chemicals, Louisville, KY) were dried at 40 °C for at least 24 hours before use. The resin was then fed to the machine through a gravity-assisted hopper connected externally to the injection molding barrel. Device operators empirically optimized the operational conditions using a starting point recommended by the resin supplier. Cross-polarized optical interrogation of the replicated substrates was employed to assess and minimize residual stresses in the injection molded parts. The resulting Zeonor[®] microchannel is shown in Figure 2c.

Premanufactured 1.6-mm thick discs of Zeonor[®] 1060r from Zeon Chemicals (Louisville, KY) were used as lids to seal the channels. 1-mm diameter vias were drilled through the discs using a Uniline-2000 drill (Excellon Automation Co., Rancho Dominguez, CA) to provide a fluidic interface at each end of the 8 microchannels. The holes are located at either end of the channel an approximately 2.9-mm from the array of posts.

The discs containing the microfluidic channels were then thermally bonded to discs containing the vias using a Carver press (Carver, Inc., Wabash, IN). Bonding conditions were held constant at the following: the press was heated to 190 °F with a constant applied load of 750 psig and a corresponding cycle time at temperature of 60 minutes. The bonded assembly was then cooled to 75 °F under constant load and then removed from the press. All bonded assemblies were checked for flow and channel blockage before use.

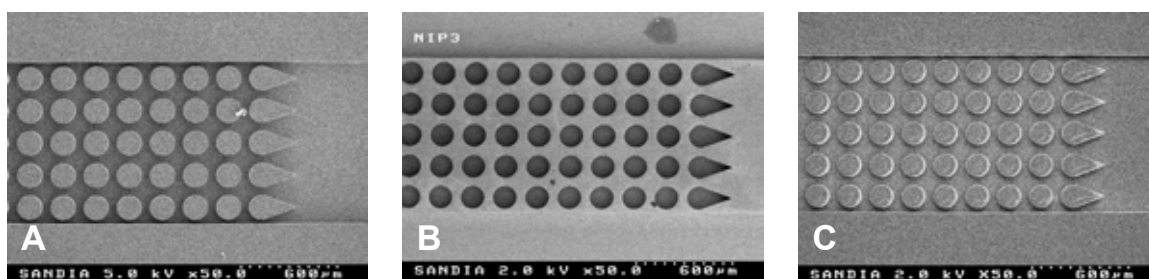


Figure 2. Images depicting the fabrication route of a polymer-based iDEP device from the (a) initial silicon features to (b) electroformed nickel stamp and finally to (c) injection molded polymer replicate.

2.1.6 Experimental Setup

The bonded disc was reversibly sealed to the base of a custom PDMS manifold using a vacuum chuck. The manifold is ported with 16 openings spanning its thickness that coincide with the inlet and outlet vias of each channel. Each opening can accept a slip tip syringe and forms a watertight seal with the syringe and a drilled via in the lid. The channels were primed by gently forcing background solution through the channel. A programmable high voltage sequencer, Labsmith HVS 448 (Livermore, CA) was used to apply voltages of up to 1500V. A manually controlled power supply, Bertran ARB 30 (Valhalla, NY) was used for higher voltages. Data was collected with an inverted epifluorescence microscope, Olympus model IX-70 (Olympus, Napa, CA), equipped with a Sony digital camera (Sony, San Diego, CA) and an appropriate fluorescence filter set.

2.1.7 Quartz Crystal Microbalance

Interactions of Zeonor™ 1060R films with surfactant solutions were studied using a modified commercially available compensated phase-locked oscillator (CPLO)-equipped quartz crystal microbalance (QCM) (RQCM, Maxtek Corporation, Cypress, CA). The CPLO is used along with extensions of standard QCM physical models²⁶ to help resolve difficulties associated with glossy films and to facilitate analysis of processes occurring during swelling and dissolution. Zeonor® films were prepared by dissolving injection-molded Zeonor™ 1060R stock in decalin, cineole, or mixtures of the two. Thin films were spin cast on gold-electrode coated AT-cut quartz crystals with nominal fundamental resonant frequencies of 5 MHz. Following coating, the Zeonor® films ($0.325 \pm 0.005 \mu\text{m}$ thickness) were baked in air at $180 \text{ }^\circ\text{C}$ for 10 min. Film thickness characterization of witness pieces by profilometry and interferometry indicated that highly reproducible, striation-free films are produced under the spin-casting conditions employed. Coated wafers were mounted in immersion holders. Covered glass containers filled with solutions of interest were placed in a thermostatted bath at $20 \pm 0.1 \text{ }^\circ\text{C}$ and allowed to equilibrate for several hours. Surfactant samples were introduced and measurements were immediately initiated. Solutions were stirred using a teflon-coated stir bar. The concentration range studied ranged from 1×10^{-7} to 1×10^{-1} moles/liter over a time interval of 450 seconds.

2.2 Safety Considerations

The use of high voltage is a hazard that requires specific training and safety measures, such as interlocks and current-limiting features. All institutional requirements and safeguards were followed. The Syto® series labels were handled with care to prevent uncontrolled release and/or contamination by using containment protocols and appropriate personal protective equipment. All organisms used are considered Risk Group 1 and therefore pose no risk to healthy adult humans. Care was taken to handle all materials and dispose of the waste according to the US Center for Disease Control and Prevention (CDC) guidelines and Sandia National Laboratories' policies.

3. Device Evaluation

3.1 Differential Trapping of Various Particle Types

The typical experimental apparatus utilized in our experiments is diagrammed in Figure 1. The first step in comparing the performance of the polymer devices to that of glass is to evaluate how well the system performs at selectively separating and removing fluorescent polystyrene beads, which we employ as a surrogate background. The iDEP behavior of fluorescently labeled beads was quantified to compare the polymeric device performance to that of glass microfluidic devices reported previously. As indicated by zeta potential measurements of Zeonor[®] [27], stable electrokinesis of particles toward the negative electrode is observed at low field strengths. As the field strength is increased, dielectrophoresis increases as the square of the field according to Equation (1). Electrokinetic flow, which only increases linearly with the field, is overcome and the polystyrene beads are observed to trap between the posts as shown in the image in Figure 3a. The value of the applied electric field when the dielectrophoretic force dominates the electrokinetic force is, therefore, termed the trapping threshold. This trapping of particles is reversible, and the beads are released from the traps when the electric field is decreased or removed. In addition to being able to trap a certain size of bead, differential trapping, based on bead size, was recorded as shown in Figure 3b and 3c. In Figure 3b, two sizes of fluorescently labeled polystyrene beads were mixed together to form a suspension of green (2 μm diameter) and red (0.5 μm diameter) beads. The applied field was then increased in magnitude until differential trapping was observed, as indicated by the formation of localized regions of red and green beads between the posts. As the field is increased, we can achieve trapping of both beads as well as a banding and separation effect within the trapping region, as shown in Figure 3c. In Figure 3c, differential trapping of green (1 μm diameter) and red (2 μm diameter) beads is shown. These results demonstrate that the capability of the polymer-based iDEP devices to trap and differentiate particles based on size in a manner equivalent to that observed in the glass microdevices, but are also capable of operating at higher flow rates up to 7 mL/h [20] due to the inherent cross-sectional area limitation of the glass isotropic etch as compared to the straight-walled reactive ion etch used to create the silicon master.

The next area of research was to assess whether polymeric iDEP microdevices are capable of separating and concentrating suspensions of mixed biological and inert particles. Figure 3d is an example of particle separation using the iDEP platform. Since the beads continue to travel between the posts as the spores are collected, the result shows how particles of different dielectrophoretic mobilities can be separated. These results agree with the theory of iDEP and underscore the effectiveness of these devices in manipulating and separating water-borne biological particles as compared to the glass devices. In the case of *Bacillus subtilis* vegetative cells, for example, the trapping threshold in the glass devices is 48.1 ± 2.3 V/mm, whereas for the Zeonor[®] devices it is 40.8 ± 1.4 V/mm. The higher trapping threshold in glass is expected as the zeta potential of the glass is higher than that of Zeonor[®] [27] and produces a higher electrokinetic force on the particle that must be overcome before trapping. A summary of the trapping thresholds observed for a variety of biological and inert particle types for the Zeonor[®] iDEP devices is presented in Figure 4. We observe the typical dependence on both particle size and type as that reported previously for glass iDEP devices. These results clearly demonstrate that for a given DEP mobility, the operation of the polymer

iDEP devices can be tailored to effectively separate and enrich a given particle against a diverse background.

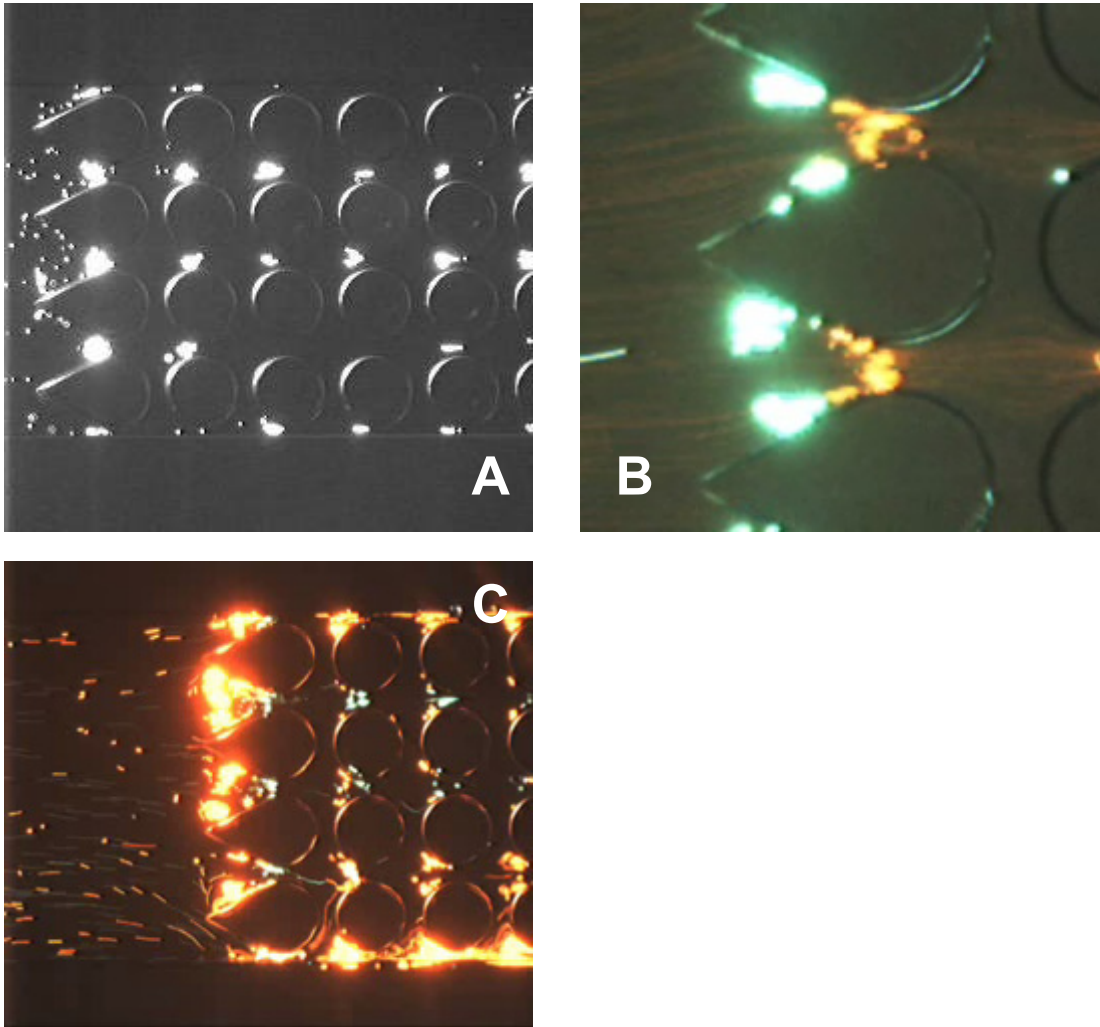


Figure 3. Images obtained from digital movies taken of (a) trapping of 2 μm fluorescent polystyrene beads using an applied voltage of 300 V, (b) differential trapping of 0.5 (red) and 2 (green) μm beads at 1250 V, (c) differential trapping of 1 (green) and 2 (red) μm fluorescent polystyrene beads at 1000 V. The net particle motion in all images is from left to right. Posts are 200-μm in diameter with a 250-μm center-to-center spacing.

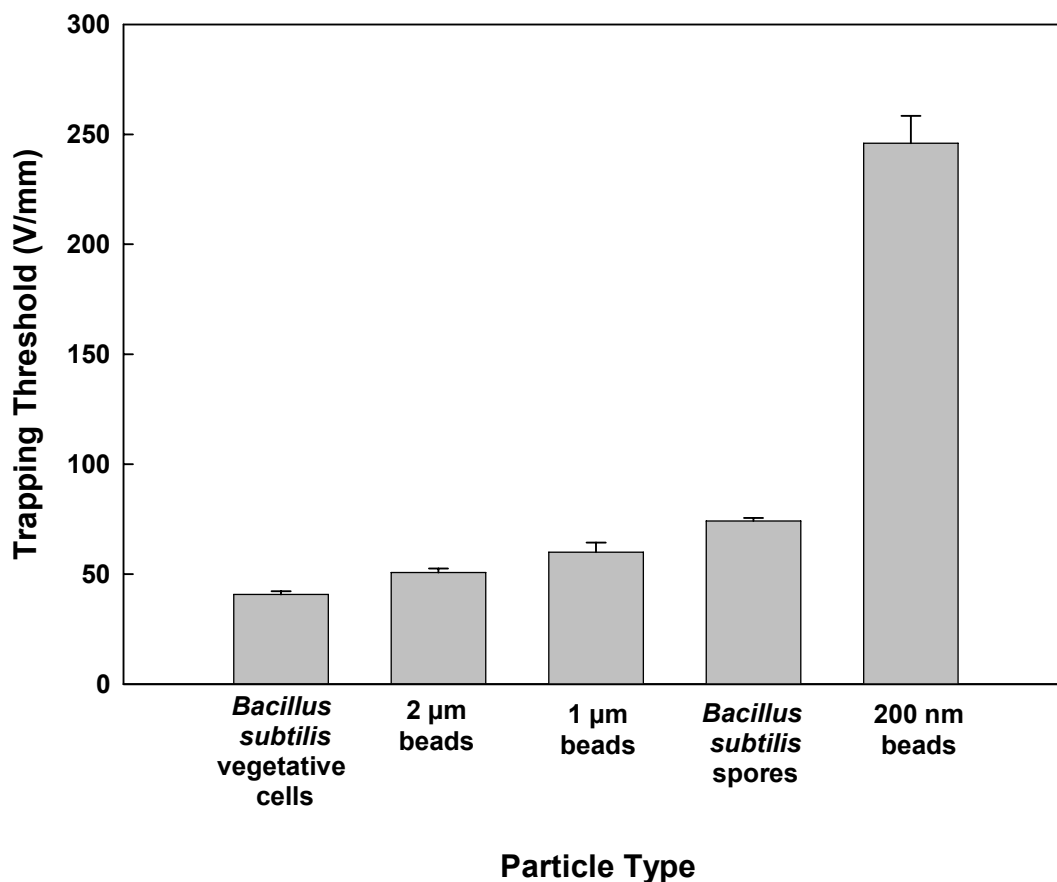


Figure 4. Summary of the different magnitudes of the applied DC electric fields required to trap, defined here as the trapping threshold (y-axis), a variety of different inert and biological particle types (n=5).

3.2 Impact of Nonionic Dynamic Surface Coatings

The results from the QCM experiments monitoring surfactant adsorption are presented in Figure 5 and demonstrate the temporal and concentration dependence of the Pluronic F127 adsorption onto the Zeonor[®] 1060r thin film. As the surfactant is adsorbed onto the Zeonor[®] thin film it produces a negative frequency shift on the QCM crystal. This frequency response is therefore directly proportional to the amount of surfactant that adsorbs onto the surface of the polymer. It can be clearly seen from the data that the surfactant rapidly forms a saturated layer onto the Zeonor[®] 1060r surface at low concentrations of approximately 10 micromolar. This concentration is much lower than the commonly reported values of the critical micelle concentration of this surfactant (4-8 mM). Using the data obtained from the frequency shift plots and empirical correlations of Zeonor[®] dissolution, we have calculated that the F127 coating has a mass of about 8.4×10^{-12} ng/nm² and represents 0.4 F127 molecules per nm² on the polymer surface.

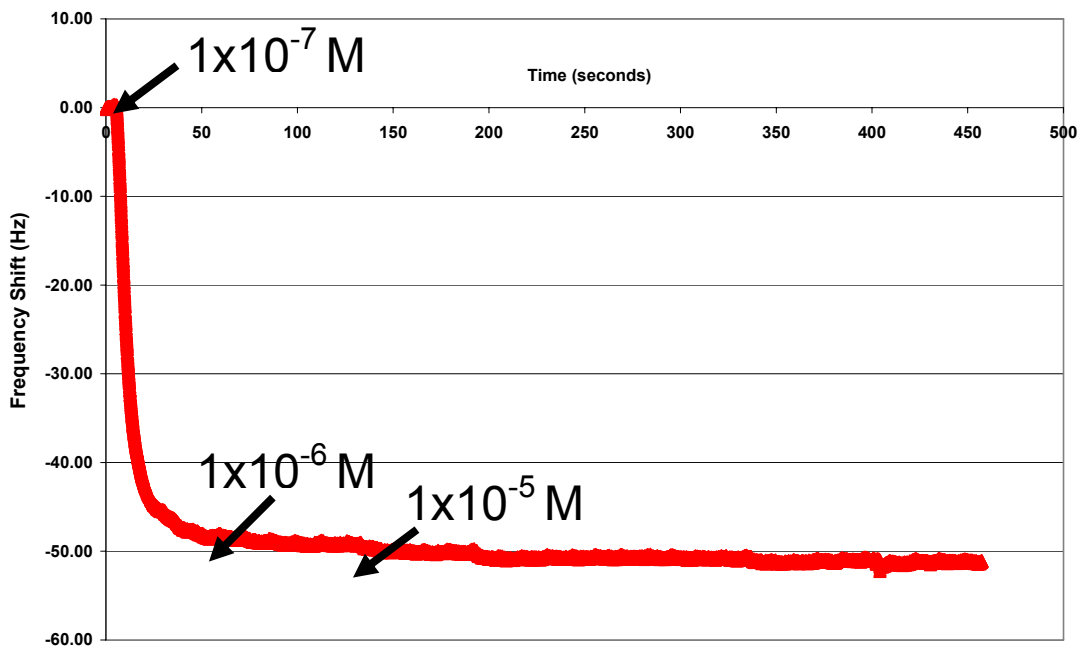


Figure 5. Plot of frequency shift of the QCM device as a function of time and Pluronic F127 concentration on a Zeonor[®] 1060r film.

These results are in good agreement with previously published data for this class of surfactant. It is known that the presence of this adsorbed surfactant layer on a polymer surface can effectively disrupt the electroosmotic flow present for a given solution by mitigating the surface charge migration that occurs in the Debye layer. Figure 6 depicts the trapping voltages for 2- μm carboxylate modified latex beads in the presence of an electroosmotic flow as a function of surfactant concentration. Although we did not measure the zeta potential to determine the impact of the surfactant on the electrophoretic mobility of the beads themselves, other published reports in the scientific literature indicate that this effect is relatively small when compared to the disruption to the electroosmotic component at the solid-liquid interface. Our results show that the trapping threshold decreases with increasing surfactant concentration, reinforcing our hypothesis that the presence of the surfactant is disrupting the electroosmotic flow velocity and thereby lowering the effective electrokinetic force of the system. This lowers the magnitude of the applied electric field that must be applied before the dielectrophoretic force term becomes dominant in the overall force balance felt on the particle and becomes trapped. The use of these surfactants lowers the power required to operate such devices, which minimizes the Joule heating present and increases the operational stability of the iDEP device. These results suggest that the nonionic block copolymers have the advantage that they can be employed at very low concentrations in order to greatly improve the performance of the polymer iDEP device while simultaneously maintaining good solution and sample characteristics. We will conduct further work into other types of ionic and nonionic surfactants, as well as photografting techniques, to develop a robust matrix of potential surface modifications and their impact on iDEP performance.

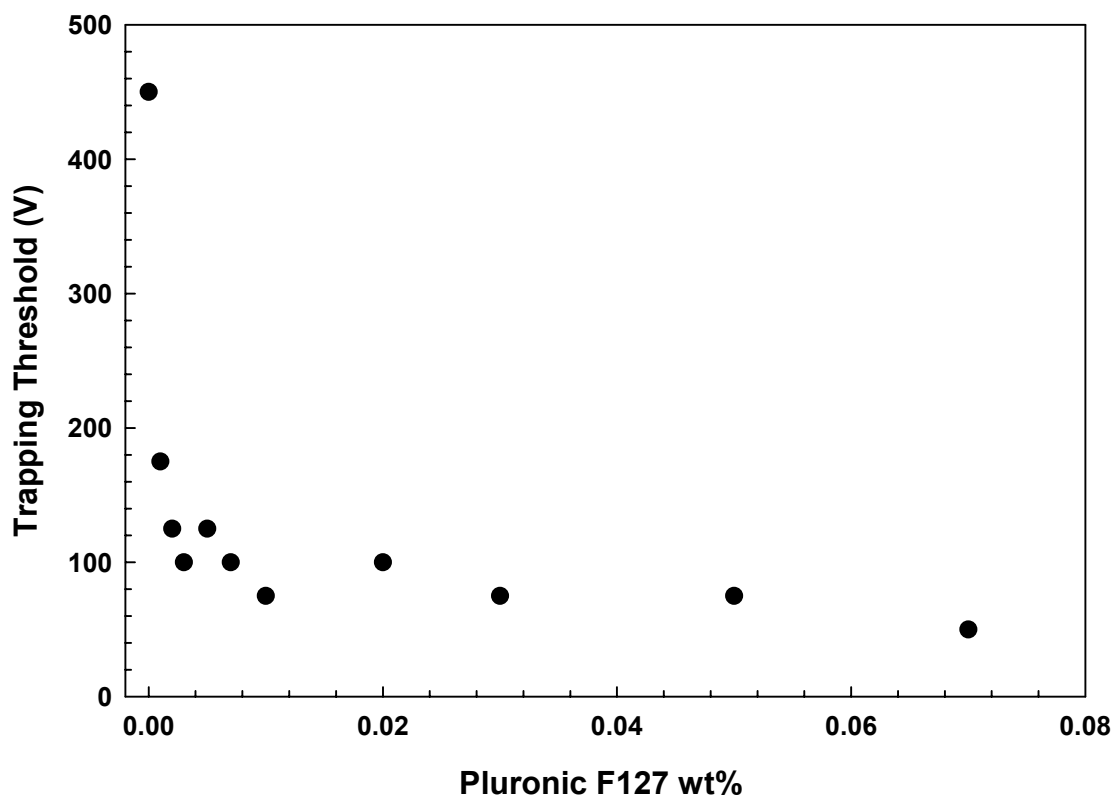


Figure 6. Plot of threshold trapping voltages for 2- μm carboxylate modified Fluospheres in the presence of an electroosmotic flow as a function of Pluronic F127 weight%.

4. Conclusions

We have demonstrated that polymer-based iDEP devices are effective for the selective trapping and concentration of a range of biological and inert particles in an aqueous sample. A nonuniform electric field was generated by applying a DC electric field across a polymeric microchannel containing insulating posts. Regions of high field intensity generated between these posts repelled insulating particles dielectrophoretically, producing selective and field-tunable particle traps for a wide range of particle types. The performance of the polymer-based iDEP microdevices at removing and concentrating particles selectively is similar to that obtained in the glass-based iDEP microdevices. We also found that the surfactant Pluronic F127 interacts strongly with the hydrophobic polymer surface and is believed to disrupt the electroosmotic flow in the devices. The presence of dilute amounts of these surfactants greatly reduces the required electric fields to trap particles. This discovery opens the door to a whole host of other surface modifications, both dynamic and static, in order to improve iDEP device performance. These results illustrate the great potential of polymer-based iDEP devices for the concentration and sorting of a wide variety of bacteria and particles. We envision a role for polymeric iDEP devices in front-end sample preparation to enhance bacterial analysis and detection.

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