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Separating large microscale particles by exploiting charge differences with dielectrophoresis

Danielle V. Polniak, Eric Goodrich, Nicole Hill, Blanca H. Lapizco-Encinas*

Microscale Bioseparations Laboratory and Biomedical Engineering Department, Rochester Institute of Technology, Rochester NY, USA

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ABSTRACT

Dielectrophoresis (DEP), the migration of particles due to polarization effects under the influence of a nonuniform electric field, was employed for characterizing the behavior and achieving the separation of larger (diameter >5 μm) microparticles by exploiting differences in electrical charge. Usually, electrophoresis (EP) is the method of choice for separating particles based on differences in electrical charge; however, larger particles, which have low electrophoretic mobilities, cannot be easily separated with EP-based techniques. This study presents an alternative for the characterization, assessment, and separation of larger microparticles, where charge differences are exploited with DEP instead of EP. Polystyrene microparticles with sizes varying from 5 to 10 μm were characterized employing microdevices for insulator-based dielectrophoresis (iDEP). Particles within an iDEP microchannel were exposed simultaneously to DEP, EP, and electroosmotic (EO) forces. The electrokinetic behavior of four distinct types of microparticles was carefully characterized by means of velocimetry and dielectrophoretic capture assessments. As a final step, a *dielectropherogram* separation of two distinct types of 10 μm particles was devised by first characterizing the particles and then performing the separation. The two types of 10 μm particles were eluted from the iDEP device as two separate peaks of enriched particles in less than 80 s. It was demonstrated that particles with the same size, shape, surface functionalization, and made from the same bulk material can be separated with iDEP by exploiting slight differences in the magnitude of particle charge. The results from this study open the possibility for iDEP to be used as a technique for the assessment and separation of biological cells that have very similar characteristics (shape, size, similar make-up), but slight variance in surface electrical charge.

1. Introduction

There is an increasing demand for miniaturized systems that provide inexpensive, portable and easy-to-use avenues for biological sample analysis. Microfluidics is a rapidly growing field that can satisfy these demands and takes advantage of the benefits of working on the microscale, such as low sample and reagent consumption, rapid response times, and increased resolution and sensitivity. Important efforts are dedicated towards the continued development of analytical and separation techniques that are suitable for miniaturization.

Electrokinetics (EK), a family of phenomena that depend on the electrical double layer, is one of the main pillars of microfluidics, due to its simplicity and ease in application. Electric-field driven techniques, such as electroosmosis (EO), electrophoresis (EP) and dielectrophoresis (DEP), have been successfully used for the analysis, sorting, and separation of a wide array of bioparticles in microfluidic devices. These applications range from environmental assessments [1–3] to biomedical and clinical analyses [4–6]. In such systems, EO flow is usually employed to pump liquid and particles through the microchannels, eliminating the need for external pumping mechanisms or moving parts. Furthermore, in microscale EK systems many charged particles will also experience EP motion under the influence of electric fields [7,8]. Separation of particles employing electric fields can be achieved by the simultaneous control of EO and EP effects, as particles with distinct charge magnitude will migrate at different velocities. There are numerous successful reports in the literature on the separation of nano-bioparticles, such as proteins and DNA [9–11]. However, separating larger particles (diameter >1 μm) via electrophoresis can be challenging due to their low charge to mass ratio, which results in low electrophoretic mobilities, in many cases, much lower than the EO mobility.

* Corresponding author at: Micoscale Bioseparations Laboratory, Rochester Institute of Technology, Institute Hall (Bldg. 73), Room 3103 160 Lomb Memorial Drive, Rochester, NY, USA.
E-mail address: bhlbme@rit.edu (B.H. Lapizco-Encinas).

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Separation of larger particles, such as bacteria and other microbes, with EP techniques was proposed by Hjerten et al. in 1987 [12] and reported for the first time by Ebersole and McCormick in 1993 [13]. Two research groups in particular have made several significant contributions to this field. The first group, Armstrong and collaborators published a series of reports on the separation of microbes employing electrophoretic techniques. Specifically, the excellent article published in 1999 [14] demonstrated that intact biological cells could be efficiently separated by employing techniques usually limited to macromolecules. In this study, a combination of capillary electrophoresis (CE) and capillary isoelectric focusing (CIEF) were employed for the separation and identification of seven distinct species of microbes (six bacteria and one yeast species) with a wide array of sizes and shapes. To achieve successful separations, polyethylene oxide (PEO) was used as EO flow suppressant, since the EO mobility can be much greater than EP mobility for biological cells. All reported separation took less than 20 min. The resulting electropherograms had excellent resolutions (good peak shape) and efficiencies as high as 1,600,000 plates per meter were reached [14]. One significant advantage of these types of electrophoretic separations is that several parameters can be exploited: particle size, shape, and surface charge. This work was later extended for the identification of bacterial pathogens responsible for urinary tract infections, with analysis times below 10 min and efficiencies in the range of one million plates per meter [15]. Furthermore, by combining CE with laser-induced fluorescence, Armstrong and He [16] were able to successfully assess cell viability with automated UV detection, opening the opportunity for high throughput analysis. This approach was also extended to food analysis [17], where the rapid quantification of the total number of live cells in a food sample was demonstrated, highlighting the potential of these techniques for applications in regulatory agencies and food safety analysis. CE coupled with fluorescence was also used for monitoring migration behavior of microorganisms, allowing for observation of the separation process and identification of optimal operating conditions [18], as well as the dynamics behind microbe focusing effects and cell aggregation [19]. More recent applications reported by Armstrong's group include the detection of bacterial contamination, where CE offers a much quicker alternative to traditional methods (minutes vs. weeks) [20] and an enhanced sensitivity for sterility tests [21], including characterization of surfactants used to enhance bacterial detection [22]. Even bacterial phenotype has been assessed employing CE methodologies [23].

The second group that has led several significant advances on the use of CE for bacterial manipulation, enrichment, and separation is Buszewski and collaborators. In 2003, they reported the separation of bacteria by CE [24], much like Armstrong's findings [14]. In order to enhance the EP mobility of the cells, EO flow was suppressed by employing γ-(trimethoxysilyl)propyl methacrylate followed by acrylamide. They were able to determine the EP mobility for four distinct bacterial species as well as the analysis of peak shape for *E. coli* under a range of buffer pH and ionic strength. The separation of a mixture containing all four distinct types of bacteria was achieved in less than eight minutes employing an 8.5 cm long capillary with a 75 μm diameter and suppressed EO flow. This group continued this work with the successful separation of four bacterial species employing PEO and EO flow suppressant and capillaries modified with divinylbenzene or trimethylchlorosilane [25]. The separation time was shortened to 5 min for four bacterial species and eight minutes for five species in an 8.5 cm long capillary with high EP mobilities. This work was later extended to highly pathogenic species such as *E. coli* (urinary tract infections) and *Helicobacter pylori* (stomach colonization) [26,27]. They reported the successful enrichment and detection of *E. coli* for direct urine samples with capillary zone electrophoresis (CZE) in less than 14 min and identification of *H. pylori* aggregates in less than 35 min. These findings demonstrate the great potential of electromigration techniques for the diagnosis of microbe-based illnesses [28]. Further work on CZE illustrated that monolith beds could also be used for the separation of pathogenic bacteria [29]; and that CZE separations could be validated with molecular methods [30]. The option of using in line fluorescence detection with a stereomicroscope employing a short 2 mm long capillary further demonstrated the applicability of CE as an analytical technique for microbes [31]. An excellent report in 2009 summarized the distinct approaches of electromigration techniques for the separation of intact cells [32]. More recent reports by this group have studied the dynamics of bacterial aggregates, with impressive separation times under five minutes. Their findings included the effects of modification of bacterial surface with calcium ions, and the focusing of bacterial aggregates by performing the separation in an isotachophoretic mode. An excellent recent review on the use of electromigration techniques for microbiological and clinical applications is available in this reference [33].

The use of CE or CZE techniques for the rapid detection and assessment of intact cells keeps gaining momentum as EP-based techniques offer an efficient, robust, and much more rapid alternative for cell analysis compared to traditional techniques. CZE has been used for viability assessments with potential application in food analyses [34]. Other new reports have focused on high speed CE separations with short-end capillaries, where the identification of oral bacteria was achieved in 95 s [35]. These types of separations can also be carried out in capillaries etched with critical water, as demonstrated with the use of CZE to separate antibiotic resistant bacteria from prepared laboratory samples [36] and infected whole human blood [37].

However, in some cases, electrophoretic separations are not feasible for particles and cells that are weakly charged, neutral, or have very similar charge to others present in the sample. In addition, some of the EP-based cell separations mentioned above required the addition of chemicals to suppress EO flow, either by coating capillaries or by adding a reagent to the running buffer [14–19,24–29]; these reagents could alter cell characteristics, since PEO, for example, can be used to inhibit bacterial aggregation [38]. An alternative to EP-based separations is to employ DEP, which is the motion of polarizable particles (neutral or charged) toward or away from regions of high electric field gradients under the effects of a nonuniform electric field [39]. The net dielectrophoretic particle motion is the result of the interaction of the induced multipole experienced by the particles and the nonuniform electric field [40]. Furthermore, particles can exhibit positive or negative dielectrophoretic behavior. Positive DEP is when particles migrate towards strong electric field regions and negative DEP is when particles move away from these regions [41]. DEP also offers additional flexibility, since DEP is more strongly dependent on the field gradient rather than on field magnitude. Thus, it can be applied employing direct current (DC) and/or alternating current (AC) electric fields.

The two most popular methods of DEP are electrode-based DEP (eDEP) and insulator-based DEP (iDEP); the former is when microelectrodes are used to create the required nonuniform electric fields, and the latter is when insulating structures are used for the same purpose [42–44]. An advantage of iDEP over eDEP, is that insulating structures provide a truly 3-dimensional dielectrophoretic effect, since usually insulators transverse the entire height of the microchannel or chamber. Planar electrodes, which are common in eDEP, have the disadvantage that the dielectrophoretic force acting on the particles decreases rapidly as it moves away from the electrode surface. Therefore, planar electrodes are limited to low throughput applications [1]. The fabrication of 3-dimensional elec-

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