

ELECTROKINETIC VELOCITY CHARACTERIZATION OF MICROPARTICLES IN GLASS MICROCHANNELS

José I. Martínez-López, Blanca H. Lapizco-Encinas, Héctor Moncada-Hernández, Marco Rito-Palomares and Sergio O. Martínez-Chapa

Tecnológico de Monterrey, Monterrey, México

Abstract

Insulator-based dielectrophoresis (iDEP) is an efficient technique with great potential for miniaturization. It has been applied successfully for the manipulation and concentration of a wide array of particles, including bioparticles such as macromolecules and microorganisms. When iDEP is applied employing DC electric fields, other electrokinetic transport mechanisms are present: electrophoresis and electroosmotic flow. In order to achieve dielectrophoretic trapping of bioparticles, dielectrophoresis has to overcome electrokinetics (electroosmosis and electrophoresis). Therefore, to improve and optimize iDEP-based separations, it is necessary to characterize these electrokinetic mechanisms under the operating conditions employed for dielectrophoretic separations. The main objective of this work was to identify the operating conditions that will benefit dielectrophoretic trapping and concentration of particles when electrokinetics is present.

This study presents the estimation of the electrokinetic mobility of microparticles suspended inside a microchannel. Micro Particle Image Velocimetry (μPIV) was employed to measure the velocity of 1- μm -diameter inert polystyrene particles suspended in a 3-cm-long, 10- μm -deep, 1-mm-wide, straight glass microchannel. A parametric study was carried out by varying the properties of the suspending medium (conductivity and pH) as well as the magnitude of the applied DC electric field. The results obtained using μPIV allowed to identify the conditions under which the electrokinetic force (i.e. particle velocity) is lowest, i.e., optimal conditions for dielectrophoretic trapping. It was shown that high conductivity and low pH values for the suspending medium produce lower electrokinetic mobilities, i.e., low electrokinetic force, thus benefiting dielectrophoretic trapping. These findings were proved by carrying out dielectrophoretic trapping of microparticles employing a glass microchannel that contained cylindrical insulating structures. The results obtained in this study will provide with guidelines for the optimization of iDEP-based separations.

Introduction

Dielectrophoresis (DEP) is an electrokinetic transport mechanism in which a force is exerted on a particle when it is subjected to a non-uniform electric field. DEP has been used successfully for the manipulation and concentration of a wide array for bioparticles, from macromolecules to parasites (Ozuna-Chacon *et al.*, 2008). Majority of these studies have used electrode-based DEP, where nonuniform electric field is generated employing an array of electrodes and AC fields (Markx *et al.*, 1994; Washizu, 1995; Rousselet *et al.*, 1998). However, there are some drawbacks with this approach: high cost of electrode construction, complex fabrication processes and decrease of functionality due to fouling effects. The use of insulators rather than electrode arrays to produce non-uniform electric field has some advantages: they retain their functionality despite surface fouling; in addition insulators can be made from a

variety of materials, including plastics, which facilitates the fabrication of equipment to handle higher flow rates. Additionally, a DC field can be used for solution and particle flow through the device by electroosmotic flow, which eliminates the need of a micro-pump (Lapizco-Encinas *et al.*, 2004a). There is growing number of studies that employ insulator-based DEP (iDEP). In iDEP the nonuniform electric field is obtained by employing arrays of insulating structures and only two electrodes. The electric field is applied along an array of micro-insulating structures creating zones of higher and lower electric field intensity throughout the array, creating dielectrophoretic traps of several biological particles like DNA (Chou *et al.* 2002), protein (Lapizco-Encinas *et al.*, 2008), yeast cells (Zhou *et al.*, 2002; Suehiro *et al.* ,2003) and microorganisms (Lapizco- Encinas *et al.*,2004a; Lapizco-Encinas *et al.*, 2004b;). When iDEP is applied through DC voltage other transport mechanisms must be considered: electrophoresis and electroosmotic flow. Electrokinesis is the superposition of electrophoresis and electroosmosis. In order to achieve dielectrophoretic trapping and concentration of particles, DEP must overcome electrokinesis and pressure-driven flow, if present. Suspending medium properties have a strong influence on the magnitude of electrokinetic force (Kirby and Hasselbrink, 2004).

In this work an examination of the optimal conditions of the suspending medium (pH and conductivity) for the dielectrophoretic trapping is made through by measuring the electrokinetic mobility of microparticles suspended inside a microchannel. Microparticle image velocimetry (μPIV) was used to determinate the velocity of 1- μm -diameter polystyrene carboxylated microspheres suspended inside in a 3-cm-long, 1-mm-wide and 10- μm -deep microchannel made from glass. From this measurement was possible to determine the operating conditions at which electrokinetic force is the lowest, thus, benefiting dielectrophoretic trapping. These results were confirmed by carrying out dielectrophoretic trapping of the same microparticles employing a glass microchannel containing an array of cylindrical insulating posts.

Theory

Electrokinetic phenomena are related to the formation of an electrical double layer (EDL) as result of the interaction of an aqueous solution with the static charges in dielectric surfaces. There is a conventionally introduced slipping plane that separates mobile fluid from fluid that remains attached to the surface. Electric potential at this plane is called electrokinetic potential or ζ zeta potential. Usually this potential is used to estimate the degree of charge of the EDL and it is related to the thickness of the Debye Length, Equation 1 shows the relation between the zeta potential and the electroosmotic mobility (Kuhn and Hoffstetter-Kuhn, 1993).

$$\zeta = -\frac{\mu_{eo} \eta}{\varepsilon_0 \varepsilon_m} \quad (1)$$

where η is the medium viscosity, ε_0 is permittivity of free space and ε_m is the relative permittivity of the suspending medium. The electrokinetic velocity is directly related to the electric field E and stands for the effects of the electroosmotic mobility μ_{eo} and electrophoretic mobility μ_{ep} as shown below:

$$V_{ek} = \mu_{ek} E \quad (2)$$

$$\mu_{ek} = \mu_{eo} + \mu_{ep} \quad (3)$$

When relative large particles are employed (diameter > 1 μm) with a low surface charge, it is possible to neglect the effect of the electrophoretic mobility (Equation 4), thus we can consider electroosmotic mobility and electrokinetic mobility as equal.

$$\mu_{ek} \approx \mu_{eo} \quad (4)$$

Electroosmotic flow can be defined as the motion of liquid over a charged solid surface as a response of an applied voltage (Kirby and Hasselbrink, 2004). The source of the charge comes from the solid-electrolyte interaction. In glass, a negatively charged surface is produced by the deprotonation of the silanol groups forming an EDL. Equation (5) shows the linearity (first order equation) of the electroosmotic flow with the applied electric field E .

$$V_{eo} = \mu_{eo}E \quad (5)$$

Particle image velocimetry (*PIV*) is a well known technique employed for the measuring of particle's velocity by capturing images (Adrian, 1984). This theory applied to traditional photography was modified by Westerweel (Westerweel, 1997) to include digital images. *PIV* has evolved from macroscopic world to the microscale preserving its principles of operation. Images of particles in motion are obtained with known space delays that allow the calculation of velocity vectors from measured distances (Raffel *et al.*, 1998). However, illumination and other noise sources could interfere with particle tracing. With this limitation, *PIV* allows quantifying the average velocity of a group of particles in regions identified as interrogation windows, which generates displacement vectors per interrogation window (Prasad, 2000).

Materials and Methods

A set of experiments was carried out to measure the electrokinetic velocity of 1- μm -diameter carboxylated polystyrene microspheres by varying the suspending medium properties and the magnitude of the applied DC electric field. From this data, the particles' electrokinetic mobility and the zeta potential of glass microchannel were determined. Figure 1 show the procedure followed to obtain the electrokinetic mobility from the electrokinetic velocity using μPIV .

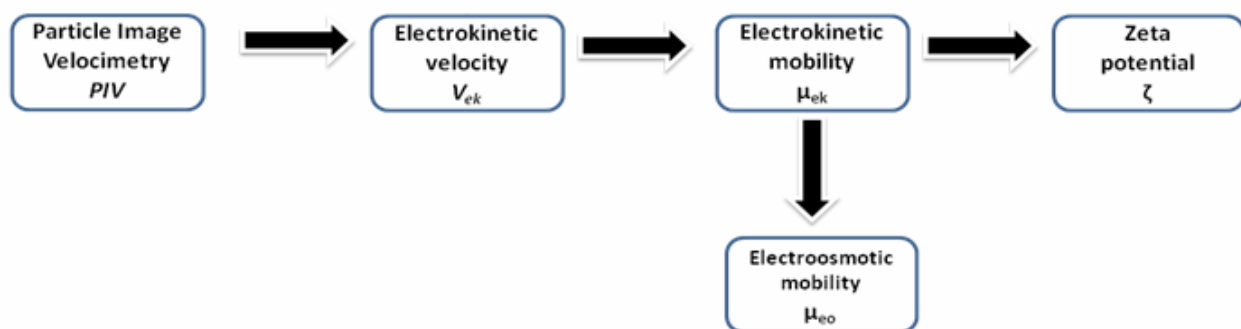


Figure 1: Methodology for obtaining electrokinetic mobility from μPIV measurements.

Figure 2 is a schematic representation of the microdevice utilized for the electrokinetic mobility determination. This device was a glass microchannel 30 mm x 1 mm x 10 μm (l x w x d), made from two Schott 263D glass wafers (Howard Glass, Worcester, MA, USA). The particles employed were fluorescent carboxylated green-yellow 1- μm -diameter microspheres (Invitrogen, Carlsbad, CA, USA), with a concentration of 7.639×10^{10} microspheres/ml. These particles contain a fluorescent dye with excitation/emission of 505/515 nm.

Twelve suspending mediums were prepared adjusting pH to 6, 7, 8 and 9, and conductivity of 25, 50, 100 $\mu\text{S}/\text{cm}$, they were prepared from bidistilled water by adding NaOH and KH_2PO_4 to adjust pH and conductivity to desired value using a pH and conductivity meter, HI255 from Hanna Instruments (Ann Arbor, MI, USA). The image and video capture device utilized for *PIV* was an inverted microfluidic microscope SVM 340 by LabSmith (Livermore, CA, USA). The microscope's control and *PIV* measurements were made with μScope software, which was provided with the microscope. A High power supply HVS448 (Livermore, CA, USA) was also used to apply DC electric fields.

Before each experiment the microchannel was cleaned with bidistilled water and then one of the 12 solutions was introduced into the channel. Pipette tips were placed at the channel inlet, to work as reservoirs, 80 μl of sample was introduced at the inlet reservoir and flow was stabilized to eliminate pressure-driven flow. Four different electric fields were applied with each suspending medium: 50, 100, 200 and 300 V/cm, each experiment was repeated twice, *i.e.* 12 videos of 30 fps were recorded for each one of the 12 suspending mediums employed. The *PIV* analysis was performed at the central region of the microchannel with a 256 x 256 pixels interrogation region using a cross correlation algorithm. The analysis of videos with *PIV* produced the values of particles velocities as a function of the suspending medium and applied electric field.

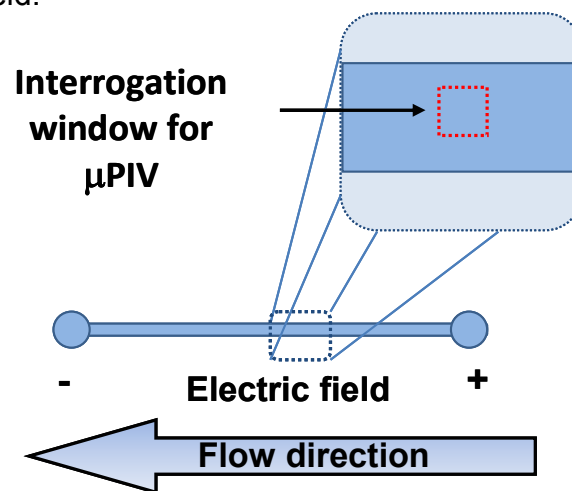


Figure 2: Experimental setup for μPIV measurements.

The experiments described above were performed in order to identify the best operating conditions for dielectrophoretic trapping of particles, *i.e.*, lowest electrokinetic force which benefits dielectrophoretic concentration of particles. To corroborate the results found with *PIV*, a set of iDEP experiments was carried out using a microchannel containing an array of cylindrical insulating posts. The microchannel used in this work was 1-cm-long, 1-mm-wide, 10- μm deep, and had an array of 8 columns x 4 rows of cylindrical insulating posts 200- μm in diameter and arranged 250- μm center-to-center (Figure 3).

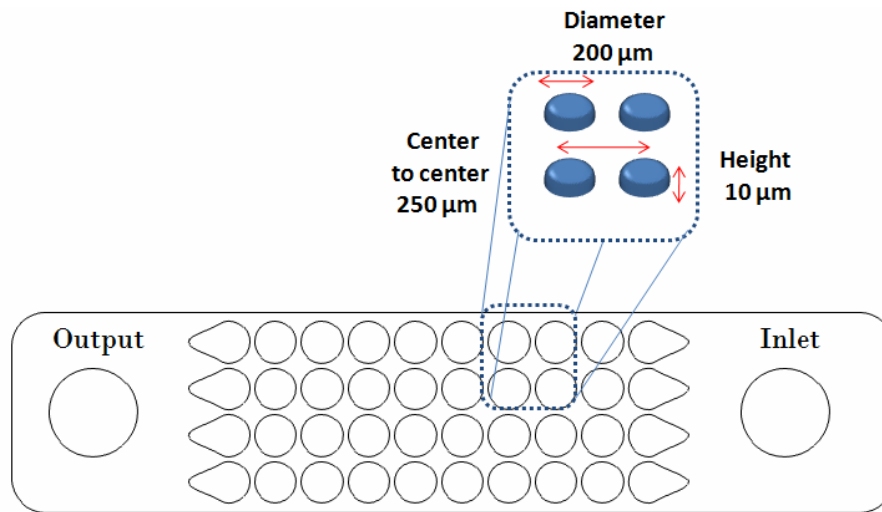


Figure 3: Microchannel for iDEP trapping.

Prior to experimentation, the microdevice was cleaned with bidistilled water and filled with the suspending medium of interest. A sample of 80 μl of the microspheres was introduced at the inlet reservoir. Care was taken in eliminating any pressure driven flow present. The experimental setup is shown in Figure 4.

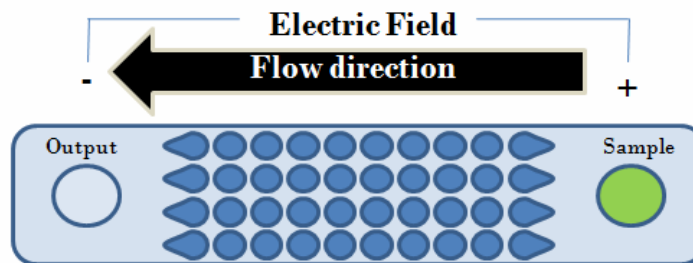


Figure 4: Experimental setup for iDEP trapping.

Results and discussion

Figures 5 and 6 show the results obtained for microparticle velocity and electrokinetic mobility, respectively, as a function of the electric field and suspending medium pH and conductivity. Electrokinetic mobility values were obtained employing Equation 4. The values employed in these calculations were: $\epsilon_m = 78$, $\epsilon_0 = 8.85419 \times 10^{-12} \text{ F/m}$ y $\eta = 0.001 \text{ (N}\cdot\text{s/m}^2\text{)}$. As mentioned, each experiment was repeated twice, the maximum standard deviation obtained with these experimental data was 15.86 %.

From Figures 5 and 6 one can observe that increasing the conductivity of the suspending medium decreases the magnitude of the EOF (lower velocity and mobility). The relation between conductivity and electroosmotic mobility can be explained considering the existence of the EDL (electrical double layer). When the conductivity of the suspending medium increases the thickness of the EDL decreases, isolating the charge on the channel surface, by a shielding effect, resulting in electroosmotic flow being diminished (Kirby *et al.*, 2005). Therefore, increasing conductivity decrease electroosmotic flow and promotes dielectrophoretic trapping of particles at lower electric field values.

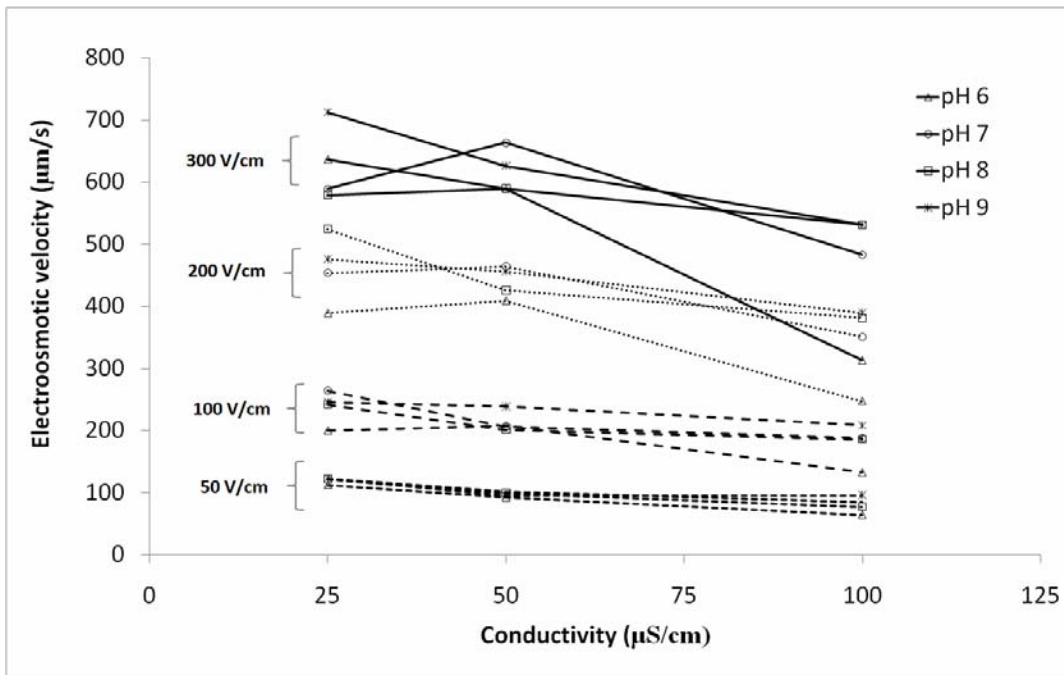


Figure 5: Microparticle velocity as a function of the electric field, pH and conductivity of the suspending medium.

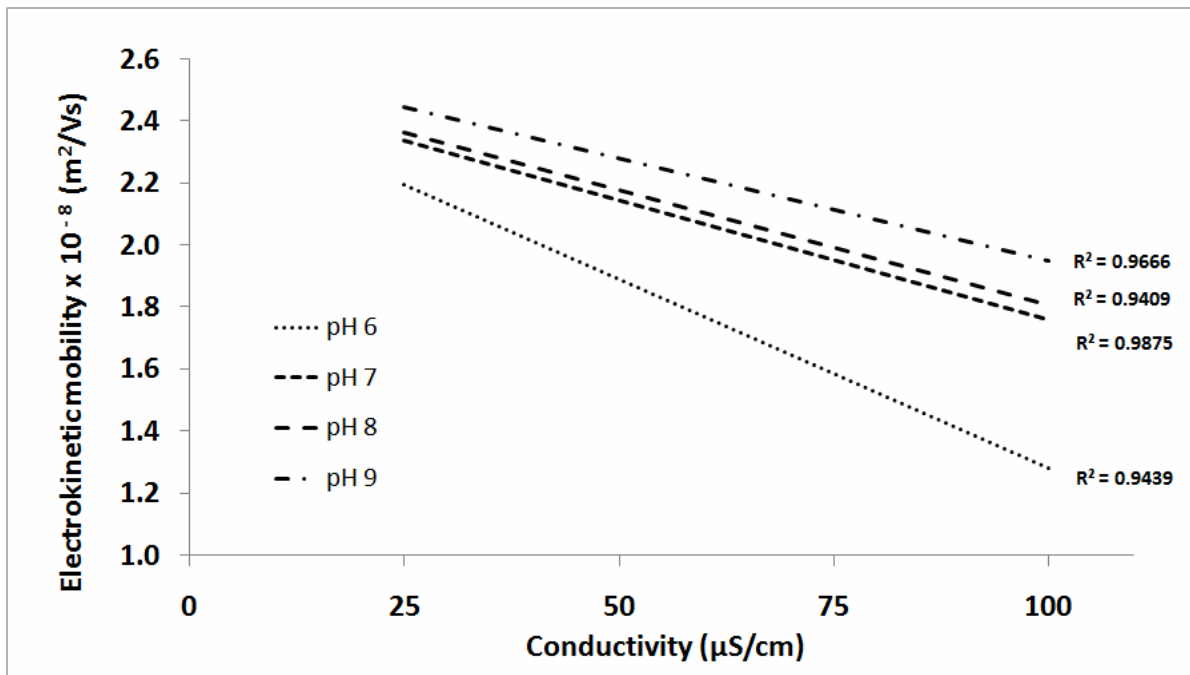


Figure 6: Electrokinetic mobility as function of pH and conductivity of the suspending medium.

In contrast, pH and electrokinetic mobility are directly related, an increasing in the pH value reflects the raise in the OH⁻ groups in the medium; as a result, there is an increase in the number surface silanol groups on the glass channel surface, increasing the surface charge, the zeta potential and therefore the magnitude of the electroosmotic flow (Hayes *et al.*, 1993). As the electroosmotic flow increases, the energy needed to trap particles in iDEP microdevice will also rise; therefore the lower pH values will offer lower electrokinetic mobility and better dielectrophoretic trapping conditions.

These results were corroborated by carrying out iDEP experiments and are shown in Figure 7. As it can be observed, under the same electric field ($E= 600$ V/cm), the strongest dielectrophoretic trapping of the microparticles was obtained with a suspending medium of low pH and high conductivity (Figure 7a, pH=6 $\sigma=100$ μ S/cm). Dielectrophoretic trapping decreased by increasing the pH to a value of 9 (Figure 7b: pH 9 $\sigma=100$ μ S/cm) or by decreasing the conductivity (Figure 7c. pH 6 $\sigma=25$ μ S/cm). The results shown in Figure 6a confirm that dielectrophoretic trapping is improved when the electrokinetic force is decreased by manipulating the suspending medium properties. These results demonstrate that operating conditions can be varied in order to enhance dielectrophoretic concentration of particles.

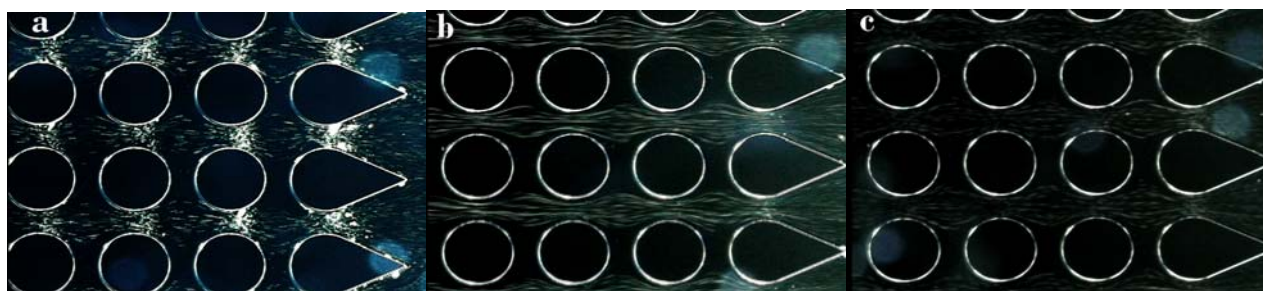


Figure 7: Effect of pH and conductivity in the dielectrophoretic trapping of microparticles at $E=600$ V/cm. Flow direction is from right to left, post diameter is 200 μ m.

Conclusions

Dielectrophoresis is a proved technique with great potential for concentrating a wide array of bioparticles. In this work it has been demonstrated that the optimal values for dielectrophoretic trapping are low pH and high conductivity. Conductivity and pH of the suspending medium are directly related to the overall performance of the insulator-based dielectrophoretic trapping. Increasing the conductivity will produce the reduction of the electrokinetic force that prevents dielectrophoresis to occur. On the contrary, higher pH will produce stronger electroosmotic flow, resulting in a decrease of dielectrophoretic trapping of particles. These results provide with guidelines for the operation and optimization of microdevices for insulator-based dielectrophoresis.

Acknowledgments

The authors wish to thank the financial support given by the CONACYT GRANT No. CONACYT-CB-2006-53603, and *Cátedras de Investigación* of *Tecnológico de Monterrey* (CAT161 and CAT041). The authors are thankful M.Sc. Sandra Ozuna Chacón for providing support with experimental work.

References

1. Adrian R.J. (1984), "Scattering particle characteristics and their effect on pulsed laser measurements of fluid flow: speckle velocimetry vs. particle image velocimetry", *Appl. Opt.*, 23, 1690-91.
2. Hayes, M.A., Kheterpal, I., and Ewing, A. (1993), "Effects of Buffer pH on Electroosmotic Flow Control by an Applied Radial Voltage for Capillary Zone Electrophoresis," *Anal. Chem.*, 65:27-31.
3. Kirby, B.J, and Hasselbrink, E.F. (2004), "Zeta potential of microfluidic substrates: 1. Theory, experimental techniques, and effects on separations," *Electrophoresis*, 25:187-202.
4. Kuhn, R. and Hoffstetter-Kuhn (1993), Capillary Electrophoresis: principles and practice. 5-33.
5. Lapizco-Encinas, B.H., Simmons, B.A., Cummings, E.B., Fintschenko, Y. (2004b), "Insulator-Based Dielectrophoresis for the Selective Concentration and Separation of Live Bacteria in Water," *Electrophoresis*, 25:1695-1704.
6. Lapizco-Encinas, B.H., Ozuna-Chacón S., Rito-Palomares, M., (2008) "Protein manipulation with insulator-based dielectrophoresis and direct current electric fields. In press, *J. Chrom. A.*, 1206:45-51.
7. Lapizco-Encinas, B.H., Simmons, B.A., Cummings, E.B. and Fintschenko, Y. (2004a), "Dielectrophoretic Concentration and Separation of Live and Dead Bacteria in an Array of Insulators," *Anal.Chem.*, 76:1571-1579.
8. Li, H., and Bashir, R. (2002),"Dielectrophoretic separation and manipulation of live and heat-treated cells of *Listeria* on microfabricated devices with interdigitated electrodes", *Sens. Actuators, B*, 86(2-3): 215-221.
9. Markx, G.H., Y. Huang, X.F. Zhou, and Pethig R. (1994), "Dielectrophoretic characterization and separation of micro-organisms", *Microbiology*, 32:29-37.
10. Ozuna-Chacon, S., Lapizco-Encinas, B.H., Rito-Palomares, M., and Martínez-Chapa., S.O. (2008), "Performance characterization of an insulator-based dielectrophoretic microdevice", *Electrophoresis*, 29:3115-3122.
11. Prasad A.K., (2000) "Particle image velocimetry", *Curr. Sci.* 79:51-60.
12. Raffel, M., Willert, C. and Kompenhans J., Particle Image velocimetry: A Practical Guide. Berlin: Springer, 1998.
13. Rousselet, J. Markx, G. and Pethig, R. (1998),"Separation of erythrocytes and latex beads by dielectrophoretic levitation and hyperlayer field – flow fractionation.", *Colloids Surf., A*, 140: 209-216
14. Washizu, M., Kurosawa, O., Arai, I., Suzuki, S., and Shimamoto, N. (1995), "Applications of electrostatic stretch-and-positioning of DNA," *IEEE Trans. Ind. Appl.*, 31:447-456.
15. Westerweel, J. (1997), "Fundamentals of digital particle image velocimetry", *Meas. Sci. Technol.*, 8:1379-1392.