INSULATOR-BASED DIELECTROPHORESIS OF PROTEIN PARTICLES USING DIRECT CURRENT ELECTRIC FIELDS

Sandra Ozuna-Chacón, Blanca H. Lapizco-Encinas and Marco Rito-Palomares
Tecnológico de Monterrey, Monterrey, NL, México

Abstract

Dielectrophoresis is a method of rapid response with sufficient selectivity for manipulation and separation of bioparticles, such as: microorganisms and biomolecules. Due to the great importance of proteins in the biotechnological and pharmaceutical processes, the present study demonstrates the potential of the insulator-based dielectrophoresis (iDEP) and DC electric fields to manipulate and concentrate protein solutions, using bovine serum albumin as a model. Samples containing fluorescently labeled bovine serum albumin protein were manipulated inside a microchannel made from glass that contained an array of cylindrical insulating structures. DC electric fields were applied and the dielectrophoretic response of the particles was observed. It was shown that the magnitude of the applied electric field and the conductivity and pH of the suspending medium have a strong effect on the dielectrophoretic response of the protein particles.

Introduction

Miniaturization has brought important advantages to bioseparation technology. Numerous fields, including environmental, pharmaceutical and biochemical, have beneficiated from the advances of microanalytical systems. There is a growing interest on the development of separation techniques that can applied in microscale. Dielectrophoresis (DEP) is a non-destructive electrokinetic transport mechanism, widely employed in microfluidics to manipulate bioparticles. DEP is the movement of particles in a nonuniform electric field, due to polarizations effects, and it can occur in alternating current (AC) or direct current (DC) electric fields. This technique was described for the first time by Pohl in 1951 [1]. The majority of the studies reported in literature, have been carried out employing electrodes, where non-uniform electric fields are generated using an array of electrodes and AC fields [2-3]. However, it is also possible to generate non-uniforms electric fields by means of electric insulator materials. Insulator-based DEP (iDEP) is an alternative to the traditional electrode-based-DEP. In this technique, only two electrodes are needed since the nonuniformity of the electric field is generated with insulating structures [4]. There are a number of advantages offered by iDEP: fabrication microdevices for iDEP are economical and simpler, leading to more robust devices that can conserve their functionality in despite of fouling.

The manipulation of protein molecules has been demonstrated with electrode-based DEP. Washizu *et al*. in 1994 [5], reported the dielectrophoretic manipulation of the proteins: avidin, concanavalin, chymotripsinogen, and ribonuclease A, employing a set of corrugated electrodes and AC electric fields. In 1998 Bakewell and collaborators [6] demonstrated the dielectrophoretic manipulation of the avidin, using polynomial electrodes. In 2004, Zheng and col. [7], reported the dielectrophoretic manipulation of protein of bovine serum albumin (BSA), using quadrupole electrodes. The manipulation of proteins molecules has not been demonstrated with iDEP.

The present study reports the manipulation and concentration of BSA employing insulator-based-DEP and DC electric fields. The protein BSA was concentrated inside a glass microchannel containing an array of cylindrical insulating posts. These results showed that the magnitude of the applied electric field and suspending medium properties (conductivity and pH) have a strong effect on the dielectrophoretic response of the protein particles. The results presented here are the first demonstration on manipulation and concentration of protein employing DC-iDEP.

Theory of dielectrophoresis

The magnitude of the dielectrophoretic force depends on the intensity of the applied electric field, particle size, and on the dielectric properties of particles and suspending medium [8, 9]. The dielectrophoretic force acting on a spherical particle is defined as:

$$F_{DEF} = 2\pi\varepsilon_0\varepsilon_m r^3 \,\mathrm{Re}\lfloor f_{CM} \rfloor \nabla(E \cdot E) \quad (1)$$

where ε_0 is the permittivity of free space, ε_m is the relative permittivity of suspending medium, r is the particle radio and $\mathrm{Re}\lfloor f_{CM} \rfloor$ it is the real part of the Clausius-Mossotti (CM) factor. According to Hughes [10] when low frequency electric field are used (frequency ≤ 100 kHz), the CM factor can be written as:

$$f_{CM} = \left[\frac{\sigma_p - \sigma_m}{\sigma_p + 2\sigma_m} \right] \quad (2)$$

From Eqns. 1 and 2 it is possible to observe that the dielectrophoretic force exerted on a particle can be positive or negative depending on the sign of the CM factor. Additionally, the dielectrophoretic force depends on the intensity of the electric field, the particle size and dielectric properties of the particle and suspending medium. These operating conditions can be varied to manipulate the DEP force order to achieve separation and concentration of particles. The dielectrophoretic force is the second order with respect to the applied electric fields (Eqn. 1); at low applied electric fields, low electric fields gradients are produced, leading to negligible dielectrophoretic force. Therefore, the applied electric field has to be high enough for DEP to become significant and immobilize particles. The two regimes of iDEP are *streaming* and *trapping* DEP. *Streaming DEP* occurs when the dielectrophoretic force overcomes diffusion but does not overcome the electrokinetic flow. *Trapping DEP* is the reversible immobilization of particles in dielectrophoretic traps and it occurs when the DEP force overcomes diffusion and electrokinetic flow (as well as pressure-driven flow if present) [13-15]. The dielectrophoretic velocity is of second order effect of the electric field, can be expressed as:

$$v_{DEP} = -\mu_{DEP} \nabla E^2 \quad (3)$$

The electrokinetic flow is proportional to the electric field (Eqn. 4). The electrokinetic flow comprises the effects of electroosmosis and electrophoresis on particle motion relative to a fixed channel. For a glass system the electrokinetic velocity is obtained employing the electrokinetic mobility as follows:

$$v_{EK} = \mu_{EK} E = (\mu_{EO} - \mu_{EP}) E \quad (4)$$

where v_{EK} is the electrokinetic velocity, μ_{EK} the electrokinetic mobility and E is the applied electric field. This research work is focused on the demonstration of protein trapping employing iDEP. One of the objectives is to study the effect of bulk medium properties on the

dielectrophoretic response of protein particles. The bulk or suspending medium properties have an effect on the zeta potential of the microchannel wall, which affects the electroosmotic mobility:

$$\mu_{EO} = \frac{\zeta \varepsilon_m}{\eta} \tag{5}$$

where ζ is the zeta potential of the microchannel or capillary wall, ε_m and η are the permittivity and viscosity of the suspending medium respectively. In this work, suspending mediums with conductivities varying from 25 to 100 µS/cm were employed. The conductivity for BSA has been reported by Zheng *et al*. [7] as σ_p = 25 µS/cm. Employing Eqn. 2 it is possible to calculate the CM factor for the protein particles suspended in each one of the three different bulk medium employed, the values of the CM factors are reported in Table 1.

Table 1. Clausius-Mossotti factors of protein particles

Conductivity of the protein particles (µS/cm)	Conductivity of the suspending medium (µS/cm)	CM factor
25	25	0.00
25	50	-0.29
25	100	-0.33

Materials and Methods

Equipment description. A schematic representation of the experimental setup is shown in Figure 1. Experiments were conducted employing microchannel made from glass that was 10-µm deep, 10.16-mm long and 2-mm wide. An array of cylindrical insulating posts was etched inside the microchannel; the posts had a diameter of 440 µm, a height of 10 µm and a center-to-center separation of 520 µm. In order to prevent particles from crashing against the posts and plugging the system, “dove-tail” geometry was used for the first row of posts on either side (Fig. 1). A 3000 V high voltage sequencer, model HVS448 (LabSmith, Livermore CA) was used as power supply. Protein particles were dyed with fluorescein isothiocyanate (Sigma, Toluca, Mexico) following a standard protocol provided by the dye manufacturer. A sample of 40 µl of protein solution at a concentration of 30 mg/ml is added to the inlet reservoir of the microchannel, resulting in an approximate concentration of 15 mg/ml of protein in the microchannel. The dielectrophoretic behavior of protein particles was recorded in the form of videos by employing an inverted epifluorescence video microscope for microfluidics, model SVM340 (LabSmith, Livermore CA). A 4X microscope objective was used for all experiments. Prior to experimentation the microchannel was filled with an aqueous solution with adjusted pH and conductivity. The pH of this solution was adjusted to a value of 8 or 9 by adding NaOH and, the conductivity to values of: 25 µS/cm, 50 µS/cm and 100 µS/cm, by adding K_2HPO_4.

Results and Discussion

Figure 2 shows some of the results obtained using different pH and conductivity of the suspending medium. The effect of the magnitude of the applied field can be observed by comparing Figures 2a and 2b, where it is shown that by increasing the applied electric field to 900 and 1200 V/cm (employing a suspending medium pH=8 and σ=25 µS/cm), respectively, a

higher dielectrophoretic force is obtained. This dielectrophoretic overcomes the electrokinetic force, producing negative dielectrophoretic trapping. From Table 1, when a suspending medium with a conductivity of 25 µS/cm is employed, it is not expected that the protein particles will exhibit a dielectrophoretic response, since under these conditions CM factor is zero. However, the results shown in Figures 2a and 2b, demonstrate that the protein particles exhibited negative DEP behavior. This can be explained as follows: the conductivity value reported for the protein particles of 25 µS/cm [7] is an approximate value obtained employing AC electric fields. Under the operating conditions used in our work, where DC electric fields were applied, it is possible for the protein particles to behave less conductive, which would lead to a negative value for the CM factor.

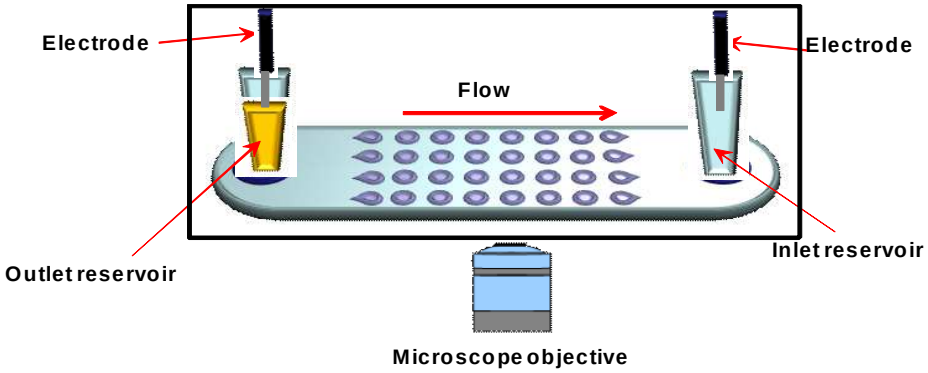
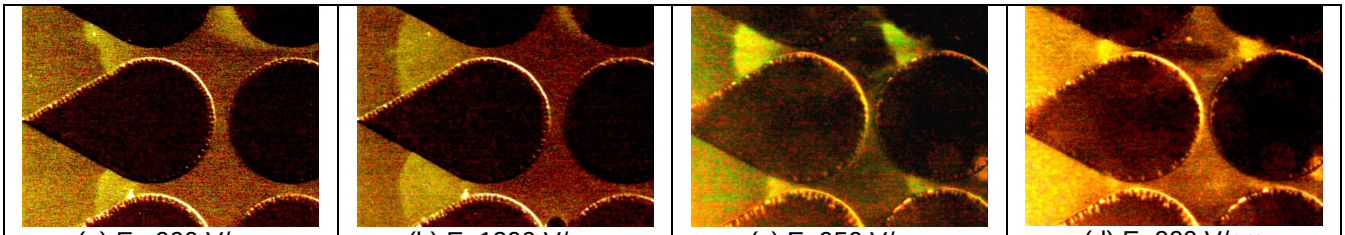


Figure 1. Experimental set-up showing the layout of a microchannel.

The effect of suspending medium conductivity can be observed by comparing Figures 2a and 2c. From the figures it can be seen that by increasing the conductivity of the suspending medium to 100 µS/cm (employing a pH of 8 and almost the same electric field), protein particles show stronger dielectrophoretic trapping, since protein bands appear brighter. These results show that by increasing the suspending medium conductivity, the magnitude of the negative CM factor increases (Eqn. 2 and Table 1), which in turn increases the dielectrophoretic force.



(a) E= 900 V/cm pH=8, σ=25 µS/cm	(b) E=1200 V/cm pH=8, σ=25 µS/cm	(c) E=950 V/cm pH=8, σ=100 µS/cm	(d) E=900 V/cm pH=9, σ=25 µS/cm

Figure 2. Dielectrophoretic response of protein of particles (shown green-yellow) inside a microchannel with cylindrical insulating structures, flow direction is from left to right, post diameter is 440 µm; a) negative dielectrophoretic trapping, b) strong dielectrophoretic trapping, c) strong dielectrophoretic trapping, d) Strong dielectrophoretic trapping.

The pH of suspending medium also affects the dielectrophoretic response, by comparing Figures 2a and 2d with a conductivity of 25 µS/cm and pH of 8 and 9, respectively,

and the same electric field) it is possible to observe that by increasing the pH of the suspending medium, the dielectrophoretic trapping is lower.

A series of experiments were carried out to observe the effect of conductivity and pH suspending medium on the magnitude of the minimum electric field required to achieve dielectrophoretic trapping. Six different suspending mediums with conductivity values of 25, 50, and 100 µS/cm and pH values of 8 and 9 were employed. The experiments were performed by slowly ramping up the applied field and observing the dielectrophoretic response of the protein particles, in order to identify the lowest required field to achieve dielectrophoretic trapping. The results are presented in Figure 3.

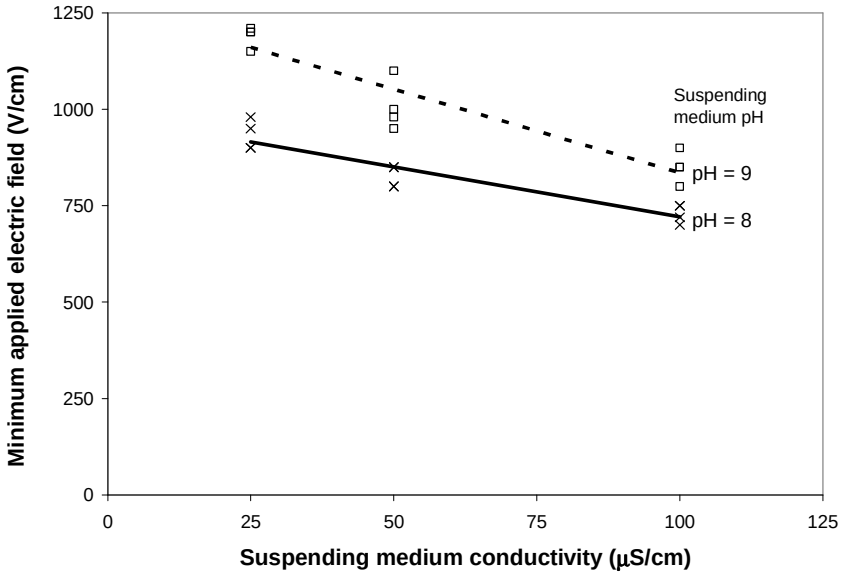


Figure 3. Minimum applied electric field required to achieve dielectrophoretic trapping of protein particles, results obtained by varying the conductivity and pH of the suspending medium.

Effect of suspending medium pH: As it can be observed in Figure 3, the minimum electric field necessary to achieve dielectrophoretic trapping increases with the pH of the suspending medium. These results can be explained as follows, a change in pH affects the zeta potential of the glass microchannels. The higher the pH of the suspending medium, the higher the desprotonization of the silanol groups on the glass surface, leading to a higher zeta potential that results in a stronger EOF (higher electroosmotic mobility Eqn. 5) [11, 12].

Effect of suspending medium conductivity: From Figure 3 it is possible to observe that increasing the conductivity of the suspending medium, decreases the magnitude of minimum electric required to achieve dielectrophoretic trapping. When the conductivity of the medium is increased, the magnitude of the negative CM factor also increases, leading to an increase on the magnitude of the dielectrophoretic force, improving dielectrophoretic trapping of protein particles. As shown in Table 1, the CM factor increases when the conductivity of the suspending medium increases.

These results demonstrate a way of manipulating dielectrophoretic separation of particles by varying the conductivity and pH of the suspending medium. The optimal operating conditions to reduce energy consumption are to use the lowest pH and highest conductivity possible for the suspending medium.

Conclusions

The present study reports the manipulation of protein particles employing insulator-based dielectrophoresis and DC electric fields. The results showed that the protein particles can be trapped with negative dielectrophoresis as a function of the magnitude of the applied electric field, and the pH and conductivity of the suspending medium. Higher applied electric fields produced strong negative dielectrophoretic trapping of BSA protein particles. Increasing the conductivity of the suspending medium, increases dielectrophoretic trapping, while increasing the pH of the suspending medium decreases dielectrophoretic trapping. The properties of the suspending medium are conditions that can be varied in order to manipulate dielectrophoretic separations of bioparticles. These results demonstrate the great potential of iDEP as technique for bioseparations.

Acknowledgements

The authors would like to acknowledge the financial support provided by CONACYT grant No. CONACYT-CB-2006-53603 and *Cátedras de Investigación* (CAT161 and CAT162) of *Tecnológico de Monterrey*.

References

1. Pohl H., (1951). “The motion and precipitation of suspensoids in divergent electric fields,” *Applied Physics,* 22; pp.869-871.
2. Li H. and Bashir R., (2002). “Dielectrophoretic separation and manipulation of live and heat-treated cells of Listeria on microfabricated devices with interdigitated electrodes,” *Sensors and Actuators B:Chemical*, 86 (2-3); pp.215-221.
3. Medoro G., Manaresi N., Leonardi A., Altomare L., Tartagni M. and Guerrieri R., (2002). “A lab-on-a-chip for cell detection and manipulation,” in *IEEE Sensors, Proc. IEEE*, Orlando, FL, USA, pp. 472-477.
4. Wang X., Huang Y., Gascoyne P.R.C. and Becker F., (1997). “Dielectrophoretic manipulation of particles,” *IEEE Transactions on Industry-Applications-Society*, Lake Buena Vista, FL, 33 (3); pp.660-669
5. Washizu M., Suzuki S., Kurosawa O., Nishizaka T. and Shinohara T., (1994). “Molecular dielectrophoresis of biopolymers,” *IEEE Transactions on Industry Applications* 30(4); pp. 835-843.
6. Bakewell D., Hughes M., Milner J.J. and Morgan H., (1998). “Dielectrophoretic manipulation of avidin and DNA,” in *20th International Conference of the IEEE in Medicine and Biology Society*, 20(2); pp.1079-1082.
7. Zheng L.J., Brody P. and Burke P., (2004). “Electronic manipulation of DNA, proteins, and nanoparticles for potential circuit assembly,” *Biosensors & Bioelectronics*, 20; pp.606-619
8. Pohl H., (1978). *Dielectrophoresis*. Cambridge: Cambridge University Press, pp.573.
9. Halaka F.G., (2003). “Dielectrophoretic dynamic light-scattering (DDLS) spectroscopy”, *Proceedings of the National Academy of Sciences of the United States of America*,

100(18); pp.10164–10169.
10. Hughes, M.P., Morgan, H., Rixon, F.J., (2001). “Dielectrophoretic manipulation and characterization of herpes simplex virus-1 Capsids,” *European Biophysics Journal* 30; pp. 268–272.
11. Kirby B.J. and E.F. Hasselbrink, (2004). “Zeta potential of microfluidic substrates: 1. Theory, experimental techniques, and effects on separations,” *Electrophoresis*, 25; pp.187-202.
12. Adamson A. and Gast A., (1997). *Physical Chemistry of Surface*s. 6a ed. United States of America; pp.808.
13. Cummings E.B. and Singh A.K., (2000). “Dielectrophoretic trapping without embedded electrodes,” *SPIE: Conference on Microfluidic Devices and Systems III*, 4177; pp.164-173.
14. Cummings E.B. (2002). “A Comparison of Theoretical and Experimental Electrokinetic and Dielectrophoretic Flow Fields,” in *32nd AIAA Fluid Dynamics Conference and Exhibit* 2002. St. Louis, Missouri; paper 2002-3193, pp.1-17.
15. Cummings E. and Singh A., (2003). “Dielectrophoresis in Microchips Containing Arrays of Insulating Posts: Theoretical and Experimental Results,” *Analytical Chemistry*, 75; pp.4724-4731.