Evaluation of Electrokinetic Flow Mobility Using Isotacho-Electrophoresis Techniques

J. H. An, Y. H. Joo, C. Y. Lee, Y. J. Lee, and C. W. Park*

School of Mechanical Engineering, Kyungpook National University, Daegu 702-701, Korea

In the present study, we separated the marker particles from the suspending particle mixture solution using iso-tacho-electrophoresis technique, a novel quantitative ionic particle separation method, in the microchannel. A multiple stacking zone of the suspending particle was visualized with variations in electric field strength, pH value and concentration of the ionic solution. In particular, the electrophoretic mobility of ionic particle (fluorescein) was estimated based on the electrophoretic velocity value measured by the particle image velocimetry. As a result, isotacho-electrophoresis zones were clearly visualized as going downstream in the electric field. The particle migration velocity increased proportional to the applied voltage increase; it was also affected by the pH value variations in the ionic solution.

Keywords : isotacho-electrophoresis, particle separation, ionic solution, mobility

1. Introduction

With the advancement in bio-technology and optics, cellular value micro-environment analysis has recently become an important research issue in the fields of bioengineering and fluid dynamics branch [1]. Based on the development of the potential platform of micro-fluidics, more cellular array investigation and precise cell separation methods have attracted much attention. Particularly, the separation technique is applied to recognize a particular cell from the suspension solution by specifying the fluorescence light on a target material [2]. The interrogation domain size and channel diameter decrease using the MEMS technology, but, supplying the working fluid into the small microchannel using a pressure-driven method is very difficult. To overcome the disadvantages of this kind of transportation, the electrokinetic force has been used in various protein and DNA chips with many microchannels [3, 4].

As an effective electrokinetic flowing technology, electroosmosis is used to develop the flow momentum because it can induce driving pressure relatively easily at a small scale system by applying electric potential to the entire flow field. Electroosmotic flow is the motion of liquid itself induced by an applied electric potential across

*Corresponding author: Tel: +82-53-950-7569
Fax: +82-53-950-6550, e-mail: chwoopark@knu.ac.kr

a tube, that is, the transport process of an ionic buffer solution. The flow rate increment is proportional to the increase in electric potential in electroosmotic flow [5]. Another electrokinetic flow is electrophoresis that induces the direct maneuver of ions in a buffer solution in an electric field. This kind of electrophoresis method is generally used to separate dissolved biological samples from the suspending solution such as protein mixture or DNA fragments. It is the main mechanism of chromatography.

For decades, numerous theoretical and experimental studies have been performed on electrophoresis [6]. Measurement of electrical characteristics has many merits in precise data acquisition, but it can be considered as an indirect method in terms of observation. Therefore, optical visualization based on physical property evaluation could be an advantageous approach method with a direct viewing.

We employ the capillary electrophoretic particle separation technique under hydrodynamic condition in a microchannel in this study. Through the influence of a uniformly applied electrical field, the charged particles can migrate toward specific electrodes in an ionic solution [7, 8]. Based on the difference in the mobility of particle due to the gravitational weight difference of various particles, the particle moving distance can change going downstream. This kind of electrokinetic mobile phenomenon can be defined as isotacho-electrophoresis. Therefore, in

the present study, we carry out a visualization experiment of isotacho-electrophoresis zone stacking qualitatively. Structural information acquire from flowing and stacking zones based on visualization could be used as preliminary data for determining the exact location of downstream separating branches for encapsulated drugs in micro-channels [9]. The electrophoretic particle mobility is estimated using particle image velocimetry algorithm, which is known as a quantitative flow visualization technique in fluid dynamics.

2. Experimental Apparatus and Methods

In the present study, we separated the marker particles from the mixture using the isotacho-electrophoresis technique in a microchannel. Fig. 1 shows the experimental setup for the visualization of a multiple stacking layer of particles. The isotacho-electrophoresis measurement system consists of an inverted-type fluorescent microscope, CCD cameras (color, cooled), mercury lamp, frame grabber, delay generator and source meter (KEITHLEY). The cooled-CCD camera (SensiCam) was used to acquire images for particle image velocimetry (PIV) calculation. The microchannel is made of PDMS material and has a square cross-section (180 μm width [W] × 180 μm height [H] × 1 cm length [L]).

The electrodes were embedded inside a microchannel on top of the slide-glass as shown in Fig. 2. To supply the suspending solutions, a circular well with 1 mm diameter was located in front of the channel inlet. In this study, we employed five buffer particle suspending solutions. The leading electrolyte (LE) was tri-HCl (350 mM, pH 4.6) and the trailing electrolyte (TE) was sodium tetraphenyl-borate (50 mM). Tri-serine (50 mM) was used as a basis
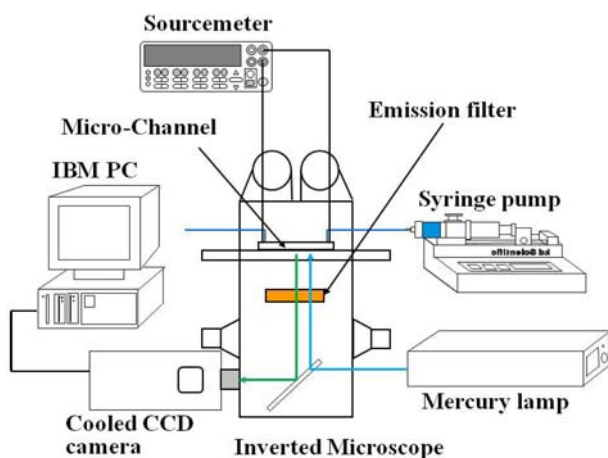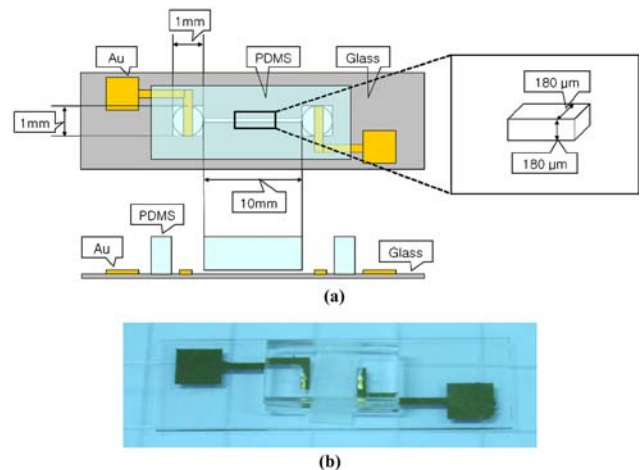

Fig. 2. (Color online) Arrangement of the microchannel structure. (a) Dimension of the microchannel and field of view; (b) Photograph of the PDMS microchannel.

sample ionic solution. Both OGCA (oregon green 488 carboxylic Acid, 10 μM, green) and FITC (fluorescein-5-isothiocyanate, 10 μM, green) were used to detect fluorescence. A uniform electric field was applied in the range of 15-60 V to induce ionic particle movement. We also evaluated the particle moving speed using PIV with variations in applied voltage intensities, pH value, and concentration of the ionic solution. The PIV technique is a reliable velocity field measurement method in the fluid dynamics research field implementing optical images of scattering particle [10].

3. Results and Discussion

Isotacho-electrophoresis is one of the electrokinetic maneuver techniques for analyte preconcentration and separation where ions a form discrete and continuous regime between a fast leading electrolyte and a slow trailing electrolyte [11]. This kind of isotacho-electrophoresis can become a more advanced separation technique than conventional electrophoresis, which is commonly used in a separation procedure of a charged particle from the mixing solution in most chemistry research fields. When an electrical field is applied to the sample constituents of various weighing in an ionic solution, the resultant velocity and acceleration levels can be different depending on the particle weight. Therefore isotacho-electrophoretic particles can be gathered together and distinguished from the ionic solution with linear characteristics [12].

Going downstream, the two electrolytes are separated by the influence of a uniformly applied local electric field. The tracer particle migrates through multiple iso-tachophoresis zones toward specific electrodes and the

**Fig. 1.** (Color online) Schematic diagram of the isotacho-elec-trophoresis measurement system.
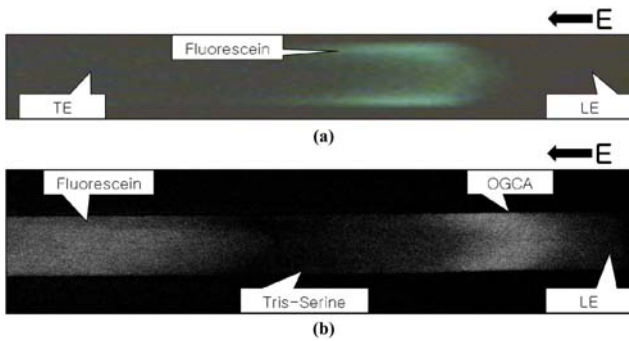
Fig. 3. (Color online) Visualized image of isotacho-electrophoresis. (a) Stacking of one suspension particle; (b) Detection of three different ionic particle species mobility (cooled-CCD).

corresponding intensity level increases in these layers as shown in Fig. 3. However, in this study, the linearity of the isotachophoresis zone is not guaranteed for the relatively fast particle velocity. This means that the viscous friction effect around the channel wall regime is still dominant. To secure the linearity, the electrophoretic velocity should slow down relatively.

Several qualitatively visualized isotacho-electrophoresis color images with variations in the applied electrical potential are shown in Fig. 4. As the applied voltages

increase, the mobility of the suspending particle increases with more random maneuvers of the particles. To evaluate the movement speed of the front and rear edges of the stacking zone, the intensified light emission image of FITC were obtained using a cooled CCD camera, as shown in Fig. 5. The camera cannot detect individual movement because the fluorescein particles are tens of nanometers in size, but it can acquire the bulk interfered emission wavelengths when the intensity is strong, such as in the stacking zone. On both sides of a channel wall region, the intensities are relatively strong due to accumulation of FITC particles [10]. This is attributed to the unstable surface charge distribution and slightly increased viscosity caused by the wall roughness. Fig. 5(b) shows the velocity profile distributions of the front and rear edges of the stacking zone after a time interval (Δt) of 200 ms calculated with the PIV algorithm by assuming Fig. 5(a) is the initial state image. The average moving speed of front edge of the stacking zone is about 67 μm/s.

To evaluate the electrophoretic mobility, we measured the particle electrophoretic velocity using the PIV technique at the central regime of the microchannel using cross-correlation algorithm [10]. Fig. 6 shows the measured migration velocity of the particle (i.e., fluorescein) with electric potential increments based on the evaluated mean
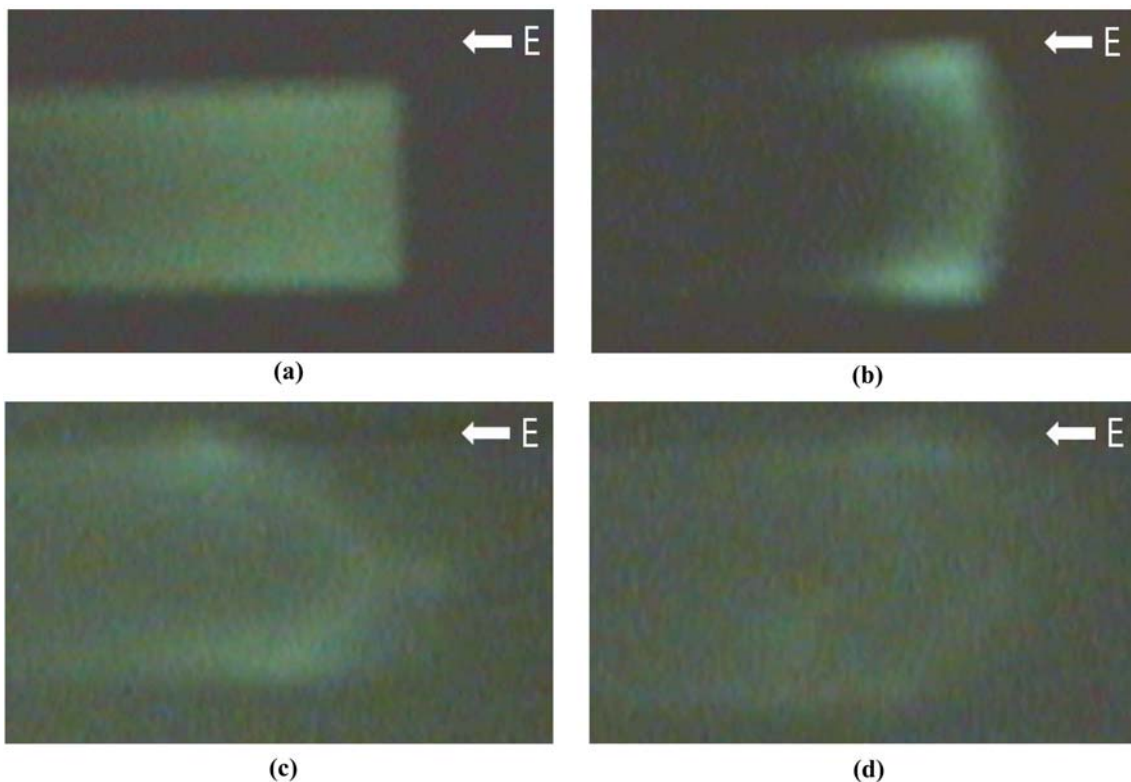
Fig. 4. (Color online) Movement of the ionic suspending particle (fluorescein) with variations in applied electric field strength. (a) 15 V; (b) 30 V; (c) 45 V; (d) 60 V.

(a)

(b)
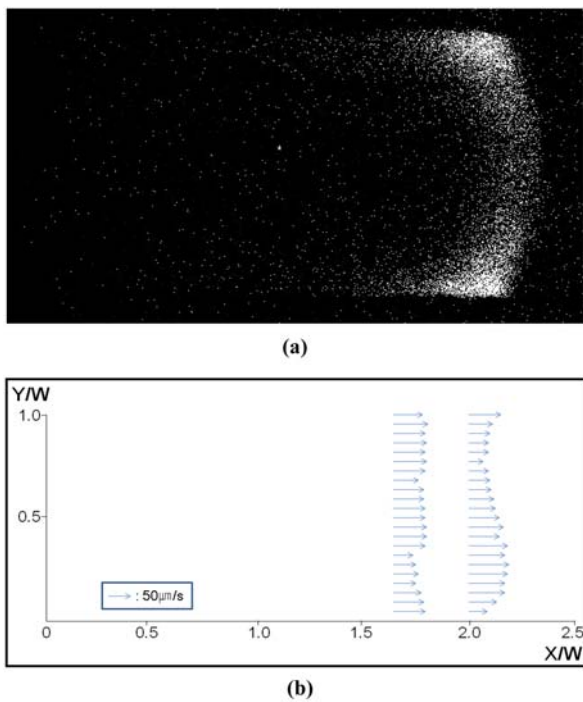
Fig. 5. (Color online) Velocity profile distribution of the front and rear edges of the stacking zone. (a) Intensified emission image of FITC (E = 40 V); (b) Instantaneous velocity profile of the stacking zone (Δt = 200 ms).

velocity of the FITC leading edge. The electrophoretic velocity is increased in proportion to the applied voltage increase. The electrophoretic mobility (μ_{ep}) is expressed as follows:

$$\mu_{ep} = \frac{v_{ep}}{E} \tag{1}$$

where, v_{ep} is electrophoretic velocity of species (μm/s), and E is the electrical field strength (V/μm) [12]. Based on the electrophoretic velocity measurement result of the
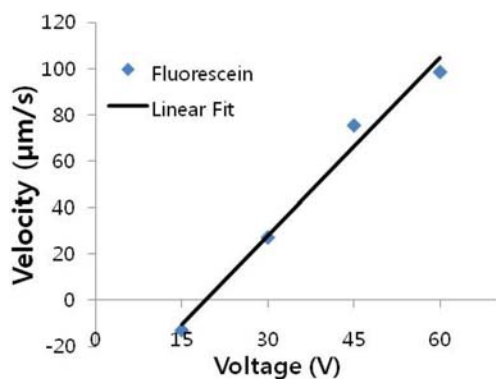
Fig. 6. (Color online) Effect of the applied electric potential on the electrophoretic velocity of a particle.
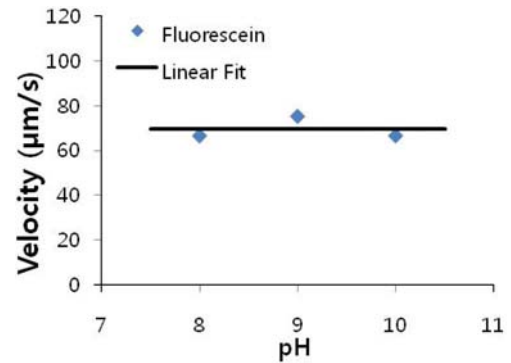
Fig. 7. (Color online) Effect of pH variation on the electro-phoretic velocity of a particle.

present study (Fig. 5), we can infer that the electro-phoretic mobility value is in the range between μ_{ep} = 0.60 and 1.65 μm²/V·s.

To test the pH value dependence of electrophoretic mobility, the solution pH of the leading electrolyte at different values from 8.0 to 10.0 were considered (Fig. 7). The electrophoretic velocity slightly changed but it was almost constant at the present pH level. Electrophoretic mobility is also a function of solution pH and the acid dissociation constant (pK_a) [11]. In the present study, we employed a synthetic FITC (Sigma-Aldrich, emission at 521 nm, USA) as a fluorescent tracer. FITC has a pK_a of 6.4. Therefore, pK_a could be slightly dependent within the range of the present study. At constant pK_a, the electro-phoretic mobility abruptly decreases as the pH value increases at the range between 2.0 and 4.0 [13]. However, there was a slight decrease in electrophoretic mobility above pH 5.0. Thus, our result is well matched with the theoretical expectation.

A visualization experiment was also performed using ionic suspending solution concentrations ranging from 10 to 100 μM. However, the ionic solution concentration had little effect on the speed of isotacho-electrophoretic stack-ing zone within the range of this study, i.e., the increase in the size of the stacking zone results from the incremental accumulation of particles [11]. The zone length increased linearly between 0.06 and 0.50 mm.

4. Conclusion

In the present study, we performed a basic isotacho-electrophoresis with separation of ionic particles accord-ing to the mobility difference. A multiple stacking zone of the suspending particle was visualized with variations in electric field strength, pH, and concentration of the ionic solution. To measure the isotacho-electrophoretic velocity of species, the PIV technique was employed using a high-

resolution cooled-CCD camera. The isotacho-electro-phoretic velocity of the species related to the mobility of ionic particle increased linearly as the applied voltage increased. The particle migration velocity was also affected by the pH value variations in the ionic solution as well. From these results, we identified the applicable potential of isotacho-electrophoresis to cell separation and its biological feasibility in future work.

Acknowledgements

References

[1] F. A. Gomez, Biological Applications of Microfluidics, John Wiley & Sons, New Jersey (2008) pp. 11-28.

[2] D. W. Inglis, R. Riehn, and J. C. Sturm, Appl. Phys. Lett. **85**, 5093 (2004).

[3] A. Brask, J. P. Kutter, and H. Bruus, Lab Chip **5**, 730 (2005).

[4] T. Glawdel and C. L. Ren, Mech. Res. Commun. **36**, 75 (2009).

[5] H. Lee, G. M. Kim, C. Y. Lee, C. W. Park, and D. J. Kim, Int. J. Mod. Phys. **B24**, 2627 (2010).

[6] J. A. Bietz and D. G. Simpson, J. Chrom. **624**, 53 (1992).

[7] D. Kaniansky, M Masar, R. Bodor, M. Zuborova, E. Olvecka, M. Johnck, and B. Stanislawski, Electropho. **24**, 2208 (2003).

[8] B. Preinerstorfer, M. Lammerhofer, and W. Linder, Electropho. **30**, 100 (2009).

[9] L. H. Hung and A. P. Lee, J. Med. Biol. Eng. **27**, 1 (2007).

[10] C. W. Park, S. J. Lee, and S. Shin, Int. J. Vas. Bio. Eng. **1**, 30 (2003).

[11] T. K. Khurana and J. G. Santiago, Anal. Chem. **80**, 279 (2008).

[12] R. D. Chambers and J. G. Santiago, Anal. Chem. **81**, 3022 (2009).

[13] M. Castagnola, D. V. Rossetti, M. Corda, M. Pellegrini, F. Misiti, A. Olianas, B. Giardina, and I. Messana, Electropho. **19**, 2273 (1998).