Dielectrophoretic Separation of Single Microparticles with Quadrupole Microelectrode

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In a microcell with a two-dimensional quadrupole microelectrode, polystyrene particles showed positive and negative dielectrophoresis (DEP) in lower (1 k–18 kHz) and higher (32 k–1 MHz) ac frequency regions, respectively, whereas only positive DEP was observed for carbon particles in a whole region of 1 k–1 MHz frequency. These findings were quantitatively explained by the difference in the conductivity of both particles. A DEP separation between the two kinds of particles was attained at a 1 MHz frequency.

A number of separation and characterization methods for molecules, *e.g.*, high performance liquid chromatography, capillary zone electrophoresis and solvent extraction, have been widely employed in various practical analyses. On the other hand, environmental chemists, chemical engineers, and biologists are also interested in relatively large particles (0.1–10 μm in diameter), *e.g.*, microcapsules, colloids, liposomes or biological cells, but there are a few methods effective for their separation and characterization, *i.e.*, density-gradient centrifugation, field-flow fractionation (FFF)[1] and electrophoresis. These methods can distinguish only the difference in electric charge, density or size of particle, and thus new techniques that can discriminate the differences in other physical properties of particles are highly required.

Dielectrophoresis (DEP) is the motion of a particle that possesses dielectric permittivity different from that of a surrounding liquid medium in a nonuniform electric field.[2] Strength and direction of a DEP force for a particle depend on dielectric properties of both the particle and medium. "Positive DEP" implies that a particle migrates to a region of stronger electric field, and "negative DEP" is the opposite phenomenon.[2] Using DEP forces, it has been demonstrated that ill and healthy cells[3] can be distinguished, and that a molybdenum tip is coated with diamond powders.[4] DEP techniques are applicable to all kinds of particles whether charged or not, but only few reports on fundamental studies and quantitative analyses of DEP behaviors appeared. We will now propose a quantitative analysis of DEP migration with a two-dimensional hyperbolic quadrupole microelectrode, and demonstrate the DEP separation between carbon and polystyrene particles by a novel sepation mode.

An aqueous suspension containing 0.05% (w/w) spherical carbon particles (4.4±0.6 μm in diameter), donated from Biotech Research Co. (Saitama), and 0.001% Triton X-100® was prepared. 0.005% (w/w) (3.5×10^6 particles per cm^3) of polystyrene particles (POLYBEAD® CARBOXYLATE, 3.001 μm in diameter), purchased from Funakoshi Co. (Tokyo), was suspended in an aqueous solution containing Rhodamine B of 2.6×10^{-7} mol/dm^3, which was added as a fluorescent dye to aid in observing particle behaviors clearly. A DEP cell with the microelectrode was made by an ordinary photolithographic technique on a glass plate. An electrode and its opposite one were wired to be the same polarity of alternating current (ac), and the remaining pair was the opposite polarity of ac. Excitation and emission wavelengths of a fluorescence microscope were set to observe Rhodamine B fluorescence. The migration of single particles was measured as previously.[5] Briefly, an aliquot (3 mm^3) of the sample solution was dropped in the working area of the DEP cell, and an ac voltage was applied (root mean square voltage, U_{rms}=3.54 V; frequency, f=1 k–1 MHz). The working area of the DEP cell was observed under the fluorescence microscope with a CCD camera and the images were recorded on a video tape. The distance (R) from the center of the quadrupole electrode to a migrating particle was obtained from the images as a function of time (t). Experiments were done in a thermostatted room at 25 ± 1 °C.

Behaviors of electrorotation for cells have been studied with four flat electrodes,[6,7] but a uniform electric field is formed by the electrodes.[7] An ideal and nonuniform electric field is generated by the hyperbolic quadrupole microelectrode,[8] and no quantitative analyses on DEP migration of single particles in the electric field appear except for ours.[5] The theoretical expression of the migration of a single particle in the microelectrode was described in detail before.[5] The R can be expressed as $\ln R = \alpha \cdot t + \ln R_0$, where α and R_0 are the DEP mobility coefficient and the initial R value, respectively. α is expressed as:

$$\alpha = \beta \frac{2 r_e^2 \varepsilon_m U_{rms}^2}{3 \eta d^4} \cdot \frac{(\sigma_p - \sigma_m)}{(\sigma_p + 2\sigma_m)} \qquad (1),$$

where β is the efficiency of the electric field (=0.34, see followings), r_e the radius of the spherical particle, ε_m the medium permittivity, η the viscosity of the medium, d the radius of the inscribed circle in the working area of the microelectrode (=65 μm), and σ the conductivity. The subscripts p and m refer to particle and medium, respectively. Plots of ln R vs. t showed linear relationships, from which α values were obtained.

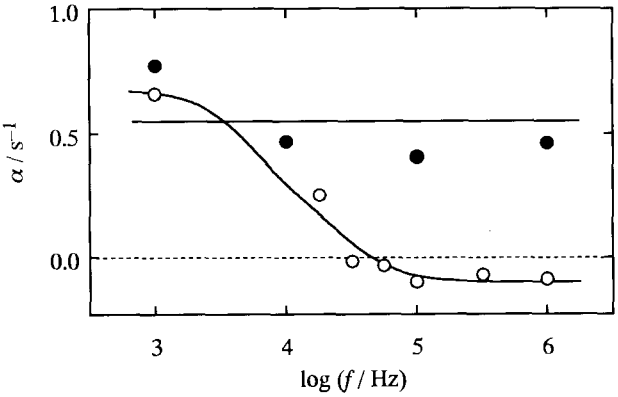

Figure 1. Effect of frequency (f) of ac electric field on the DEP mobility coefficients (α) of carbon and polystyrene particles. U_{rms}, 3.54 V. ●, carbon, $\sigma_m=4.6\times10^{-4}$ S/m, pH=5.89; ○, polystyrene, $\sigma_m=3.1\times10^{-4}$ S/m, pH=6.57.

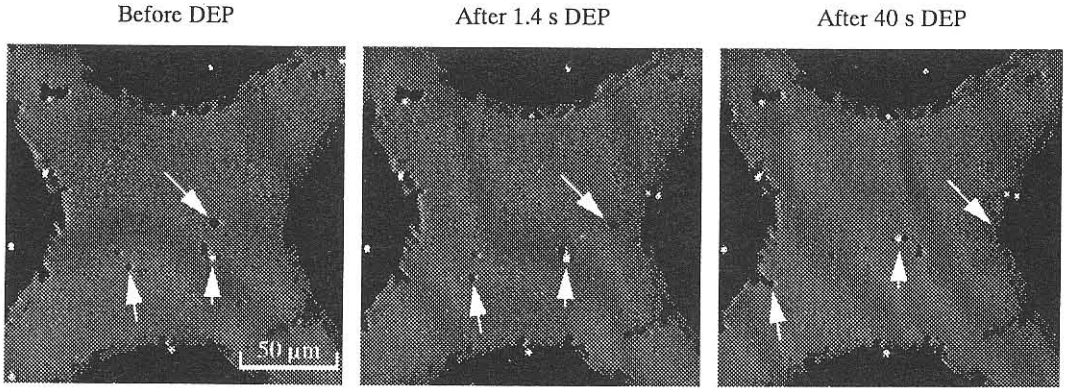

Figure 2. Successive pictures demonstrating the DEP separation between carbon (black points, positive DEP) and polystyrene particles (white point, negative DEP). Black regions are electrodes made of chromium and gold. f=1.0 MHz; σ_m=9.04×10^{-4} S/m; U_{rms}=3.54 V.

The observed α values of the carbon particles were positive and almost constant (0.55±0.17 s^{-1}) at the whole frequency region as shown in Figure 1. Glassy or amorphous carbon has a much higher conductivity (about 2×10^4 S/m) than the medium, and therefore the positive α was explained by eq 1. Quantitatively, the α value was predicted to be 1.4 s^{-1} independent of the ac frequency, if the β value was unity. The observed α values were less than the predicted α value, suggesting that the electric field did not ideally operate on the particles. This difference in efficiency was attributable to the fact that the thickness of the metal plate of the microelectrode (0.15 μm)[5] was less than the particle size. The efficiency of the ac electric field, β, was estimated as 0.39 from the ratio of 0.55/1.4. This efficiency was defined in the present report for the first time, and is a characteristic property in the two-dimensional electrode system. We have measured DEP behaviors of one kind of conductive particle and have found that they are explained by a common theory for the first time.

The α values observed for single polystyrene particles were positive at a lower (1 k–18 kHz) frequency region, and were negative at a higher (32 k–1 MHz) frequency region as shown in Figure 1. Since the conductivity of the insulating polystyrene (σ_p) is less than that of medium (σ_m), the α value should be negative at the whole frequency region from eq 1. There are carboxylate groups on the surface of the polystyrene particle, and thus cations can exist near the particle surface to form an electric double layer. Many researchers have proposed theories that this anomalous positive DEP is caused by the high conductivity of the double layer at a lower frequency region where the ions can move adequate distances.[9] However, studies on the positive DEP are carried out even now[10] because the theories based on the double layer can not explain the positive DEP. Recently we have examined this anomalous positive DEP in detail by changing ac voltage, radius of particle, kind of particle, electrolyte, and medium conductivity, and have found that the anomalous DEP is caused by a conductive diffusion cloud of cations around the particle, which is induced by ac electric field.[11] Negative DEP observed for f≥32 kHz were caused by the lower conductivity of neat particles than the medium. The solid line drawn for the polystyrene particles in Figure 1 is the calculated α values by the diffusion cloud model.

The carbon particle showed positive DEP at the whole frequency region, whereas the polystyrene particle showed negative DEP in the higher frequency region. Therefore, these two kinds of particles must be separated in the higher frequency region. Applying an ac of 1 MHz to a mixture of carbon and polystyrene particles resulted in the photographic pictures shown in Figure 2; the black and white points were the carbon and polystyrene particles, respectively. The carbon particles fast migrated toward the electrode (positive DEP), whereas the polystyrene particle slightly moved after 1.1 s DEP. After 40 s DEP, the polystyrene particle moved to the cell center (negative DEP). They were separated by the difference in the particle conductivity. Such a definite separation mode in DEP does not appear in the other separation techniques, *e.g.*, electrophoresis and FFF. Furthermore, the DEP technique has a high potential to collect conductive particles, *e.g.*, metal particles or metal colloids, from other insulating particles.

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