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Proposal and experimental validation of the electrophoretic Coulter method for analyzing microparticles and biological cells

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ABSTRACT

Zeta potential measurement is one of the simplest methods for analyzing the electrical surface properties of microparticles and biological cells in a solution. The authors propose a new methodology for simultaneous measurement of the number, size and zeta potential of different specimens in a microchannel, referred to as the electrophoretic Coulter (EC) method. First, a microchannel is fabricated using soft lithography, a small amount of a specimen is injected into it, and ion current modulation through the microchannel is measured while a DC electric field is applied. The results are then compared with those obtained using the conventional methods involving dynamic light scattering (DLS) method and microscopy. The results of the EC method show good agreement with those of the conventional approaches. Accordingly, the technique enables high-throughput analysis of different specimens including nano materials and biomolecules using a micro/nanochannel, thereby significantly contributing to the field of bio-nano fusion research.

1. Introduction

Microparticles or biological cells dispersed in solutions have many interesting properties, and their analysis has been quite important in the fields of analytical chemistry, pharmacy and clinical medicine [1,2]. The most prevalent validations of their properties come from measurement of the zeta potential, size distribution and mass of these particles. Various techniques for each type of validation have been developed, and the range of application for each approach has been broadened.

Zeta potential is defined as the electric potential at the slipping plane of a charged particle; the slipping plane is that between a moving particle and a static solution, and zeta potential is utilized for estimation of the surface conditions and dispersion stability of particles [3,4]. In order to induce electrophoresis, a constant electric field is applied to the solution containing the particles in question, and their electrophoretic mobility is measured by means of Laser Doppler Velocimetry (LDV) or moving image analysis [5,6]. From the electrophoretic mobility ascertained, the zeta potential is estimated using the Smoluchowski [7] or Hückel equations [8]. Measuring the zeta potential of biological cells is essential in the field of cellular biology and biotechnology because electrophoresis does not cause cell damage or require molecular labeling with enzymes, fluorescent dyes or radioisotopes to investigate cell surface properties [9,10].

Size distribution is measured using the Coulter method [11,12] or dynamic light scattering (DLS) [13]. In particular, the Coulter method allows accurate size distribution measurement as it can be used to detect individual specimens while avoiding the influence of their shape, refractive index and density. In addition, the approach uses simple electrical signals instead of the optical signals used in DLS, and is much more favorable for terms of measurement system integration. A typical piece of equipment used for the Coulter method consists of two separated chambers connected by a pore; the chambers are filled with a solution containing particles. When a particle passes through the pore, the electrical resistance of the solution is changed, and this change is detected by measuring the voltage across the pore using electrodes immersed in the solution in each chamber. Each time a particle passes through the pore, a voltage pulse is detected; the amplitude of each pulse indicates the size of each particle, and the number of pulses corresponds to the number of particles. Recently, the Coulter method has been utilized in the field of bio-nano fusion science [14–17].

The mass of specimens is estimated using static light scattering (SLS) [18], and the intensity of scattered light is analyzed using the Rayleigh equation.

To deepen understanding of the behavior of microparticles or biological cells, it is important to acquire information on factors such as the zeta potential, size and mass of each particle. However, the sequence of measurement for these factors is time-consuming in terms of analysis, and the equipment required is large, complex and expensive. Accordingly, several research groups have reported new techniques for simultaneous measurement of particles; a carbon nanotube-based Coulter counter (CNCC) has been used to

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estimate the zeta potential and size of a single nanoparticle [19], and a suspended microchannel resonator (SMR) has been adopted to estimate the zeta potential and mass of a single microparticle [20]. However, these techniques require sophisticated device fabrication processes or complicated signal analysis.

In this paper, we propose the electrophoretic Coulter (EC) method, which enables simultaneous measurement for the zeta potential and size of single particles using a simplified device fabrication process and signal analysis. We validate the accuracy of the EC method in comparison with the conventional methods of LDV and microscopy.

In Section 2, we will describe the principle of the EC method, in which the electrophoresis method and the Coulter method are incorporated into a microchannel. In Section 3, we will explain the experimental details, the fabrication of the microchannel and the procedure for the electrophoresis experiments. In Section 4, we will show the experimental results and discuss them. First, we will evaluate the fundamental ability of the EC method to characterize zeta potential and size using results from standard polystyrene particles. Next, we will characterize zeta potential and the size of sheep's red blood cells (RBCs) as a first step for application to biological cells. In Section 5, we will summarize the results and outline future prospects for the EC method.

2. Principle of the electrophoretic Coulter method

Fig. 1a shows a schematic diagram of our experimental system, and Fig. 1b shows the shape of the microchannel and its equivalent circuit. As illustrated in the latter figure, the microchannel is configured into five regions – one aperture, two spreads and two flow channels. The electrical resistances of the solution in each region, R_a, R_s and R_f are calculated using the following equations, respectively:

$$R_a = \frac{l_a}{\sigma h_a w_a} \tag{1}$$

$$R_s = \frac{l_s}{\sigma h (w_f - w_a)} \ln\left|\frac{w_f}{w_a}\right| \tag{2}$$

$$R_f = \frac{l_f}{\sigma h_f w_f} \tag{3}$$

where l is the length, h is the height and w is the width of each region, and σ is the electric conductivity of the solution in the microchannel. The total resistance of the solution in the microchannel R_t is the sum of the electrical resistance for the five regions:

$$R_t = R_a + 2R_s + 2R_f \tag{4}$$

In this calculation, the resistances of the solution in the insert holes can be ignored because they are negligible compared to that of the solution in the microchannel. To induce electrophoresis, a DC voltage is applied to the solution in the microchannel, and the resultant ion current is continuously measured. As each measurement particle passes through the aperture, the electrical resistance of the solution in it increases from R_a to R_a':

$$R_a' = \frac{2 \arctan\left((d/2)/\sqrt{(hw_a/\pi) - (d/2)^2}\right)}{\sigma\pi\sqrt{(hw_a/\pi) - (d/2)^2}} + \frac{l_a - d}{\sigma h w_a} \tag{5}$$

where d is the diameter of the particle [21]. Accordingly, the total resistance of the solution in the microchannel with the particle in the aperture R_t' is given by:

$$R_t' = R_a' + 2R_s + 2R_f \tag{6}$$

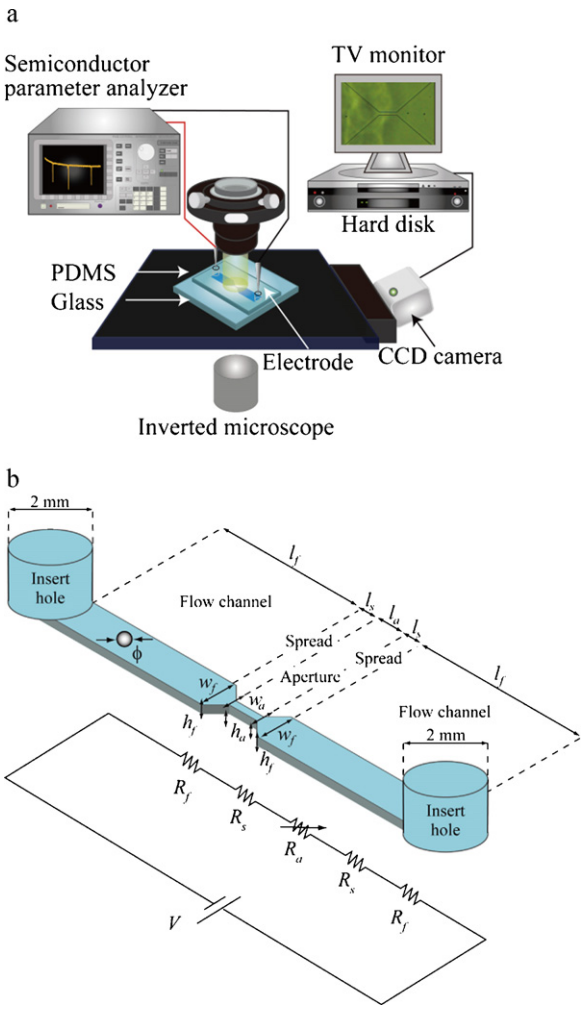

Fig. 1. Schematic diagram of the experimental system. (a) Configuration of measurement instruments. The measurement system consists of a microchannel, an inverted microscope, a semiconductor parameter analyzer and a CCD camera. The movement of the specimens is observed and recorded digitally. (b) Shape of the microchannel and its equivalent circuit. The narrow part at the centre of the microchannel is called the aperture, and the electrical resistance of a solution in the aperture increases as each specimen passes through it.

The ion current without the particle in the aperture I and that with the particle in the aperture I' are calculated using

$$I = \frac{V}{R_t} \tag{7}$$

and

$$I' = \frac{V}{R_t'} \tag{8}$$

respectively, where V is the applied voltage. Each time a particle runs into the aperture, the ion current through the aperture decreases and a pulse is generated. The ion current modulation is therefore described as

$$\frac{\Delta I}{I} = 1 - \frac{R_t}{R_t'} \tag{9}$$

where ΔI is the amplitude of the pulse, described as

$$\Delta I = I - I' \tag{10}$$

Thus, the number of pulses for the ion current corresponds to the number of particles passing through the aperture, and the amplitude of each pulse corresponds to the size of each particle.