Accuracy evaluation of zeta-potential measurement using current monitoring technique and closed electrokinetic cell technique

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Abstract
The present study was performed to evaluate and to improve the measurement accuracies for zeta-potentials of particle and wall. Electric potentials near colloid particle surfaces and channel wall surfaces are defined as the zeta-potential of particle and wall, respectively. Their accurate measurements are important because both zeta-potentials are the key factors to control many microfluidic applications. Electroosmotic flow is used as a means of liquid transport, and its rate is determined by the zeta-potential of wall. In addition, the zeta-potential of particle is also an important parameter to control many properties of colloid particles. The previous studies developed the measurement techniques of zeta-potentials, which are called the current monitoring technique and the closed electrokinetic cell technique. However, the measurement accuracies of both techniques have not been well discussed, and thus, the present study compared the error ratios based on the measurement uncertainties of both techniques. For the measurements of negative zeta-potentials of wall using negatively charged particles, their error ratios of the current monitoring technique and the closed electrokinetic cell technique were 14.2% and 9.6%, respectively. However, when using positively charged particles, it was difficult to measure zeta-potentials due to the adsorption of positively charged particles on negatively charged channel wall surfaces. In order to reduce the adsorption, the surface modification technique was used to alter the electric charge on the channel wall surface. Then, for the measurements of positive zeta-potentials of particle and wall, their error ratios using the current monitoring technique were 31.9% and 31.6%, respectively, and those using the closed electrokinetic cell technique were 13.0% and 17.7%, respectively. It was revealed that the measurement uncertainties of the closed electrokinetic cell technique were superior to those of the current monitoring technique, even if negatively and positively charged particles were used.

Keywords: Electroosmotic flow, Electrophoresis, Zeta-potential, Micro-PIV, Closed electrokinetic cell technique, Current monitoring technique, Surface modification

1. Introduction
The electric potentials near channel wall surfaces and colloid particle surfaces, which are known as zeta-potentials of wall and particle, respectively, need to be accurately measured, because a lot of microfluidic applications were controlled by both zeta-potentials. Zeta-potentials of wall determined the rate of electroosmotic flow, which were mainly used as the driving force of the electroosmotic pumps in the microfluidic devices, such as lab-on-a-chip, micro-TAS, and capillary electrophoresis (Shoji 1997; Reyes et al. 2002; Auroux et al. 2002; Tsukahara et al. 2010), instead of mechanical pumps. This is caused by having numerous advantages; no need for moving parts, ease of fabrication, controllability, and so forth. Additionally, the decentralized stabilization of colloid particles is dependent on the zeta-potentials of particle, whose absolute values are the indicators to keep colloid particles in dispersion, to control the fluidity and the cohesiveness,
and to maintain the conservation of colloid particles (Adamczyk and Weronski 1999). For further improvement of the controllability of electrokinetic pumps and colloidal particles, it is required to measure zeta-potentials of particle and wall accurately.

The previous studies developed the measurement techniques of zeta-potentials, which are called the current monitoring technique (Ocvirk et al. 2002; Devasenathipathy et al. 2002; Sze et al. 2003) and the closed electrokinetic cell technique (Mori and Okamoto 1980; Ichiyanagi et al. 2005; Ichiyanagi et al. 2014a, 2014b). The current monitoring technique used two aqueous solutions with different ion molar concentrations and could obtain the electroosmotic flow velocity by monitoring the time-series electric current inside the channel. The electrophoretic velocity was evaluated by subtracting the electroosmotic flow velocity from the observed particle velocity. On the other hand, the closed electrokinetic cell technique was able to measure the electrophoretic velocity by using micron-resolution particle image velocimetry (micro-PIV), and the electroosmotic flow velocity was also evaluated by subtracting the electrophoretic velocity from the observed particle velocity. In summary, the electroosmotic flow velocity and the electrophoretic velocity were evaluated by both the current monitoring technique and the closed electrokinetic cell technique. Then, zeta-potentials of particle and wall were simply evaluated from the electrophoretic and the electroosmotic flow velocities, respectively. The required error range of zeta-potential measurements is about 10% because it includes the statistical fluctuation of zeta-potential itself (Oddy and Santiago 2004), and the measurement accuracies of both techniques have not been well discussed under the same experimental conditions.

The objective of the present study was to evaluate and to improve the measurement accuracies for zeta-potentials of particle and wall obtained by the current monitoring technique and the closed electrokinetic cell technique. Negatively or positively charged particles and channels were used to measure zeta-potentials, and the measurement uncertainties in each condition were compared. In order to inhibit the adsorption of positively charged particles to silica glass microchannels (negatively charged channels), the surface modification technique was utilized, which could change the electric charge on the channel wall from the negative to the positive. We compared the number densities of positively charged particles when using the silica glass microchannel (negatively charged channel) and the surface modified microchannel (positively charged channel), and it was evident that the number densities for adsorbed positively charged particles on walls were significantly decreased by using the positively charged channel. In addition, the zeta-potentials of positively charged particles and walls were evaluated by using five kinds of aqueous solutions at different pH values, which changes zeta-potentials of both particles and walls.

2. Experimental Setup

2.1 Design of microchannel

The present study used negatively and positively charged channels. Two kinds of negatively charged channels were prepared, whose geometries are shown in Figures 1 and 2. Figure 1 shows a schematic of the microchannel, which was comprised of a silica glass microchannel (VitroCom Inc., Vitrotubes Synthetic Fused Silica 5005S) and cylindrical fluid reservoirs. Its width and depth was 500 µm and 50 µm, respectively. This channel was used in the experiments with both
the current monitoring technique and the closed electrokinetic cell technique in Sections 4.1-4.2 (referred to as “the non-modified channel”). Figure 2 illustrates a schematic of the closed electrokinetic cell, which was comprised of a silica glass microchannel (VitroCom Inc., Vitrotubes Synthetic Fused Silica W5005), silicone tubes as fluid reservoirs, and tube clamps. Its width and depth was 1.0 mm and 50 µm, respectively (the aspect ratio of width/depth is 20). This cell was used in the experiments with only the closed electrokinetic cell technique in Sections 4.1-4.3 (referred to as “the non-modified cell”). The differences between Figures 1 and 2 were their widths and electrodes setup.

On the other hand, this study also prepared two kinds of positively charged channels, which were made by modifying the amino group on wall surfaces of the microchannel as shown in Figure 1 (referred to as “the modified channel”) and the closed electrokinetic cell as shown in Figure 2 (referred to as “the modified cell”). The modified channel was used in Section 4.4, and the modified cell was used in Sections 4.3-4.4. The procedure of the surface modification (Ichiyanagi et al. 2009) was as follows. Firstly, for cleaning and exposing the hydroxyl group on silica glass channel wall surfaces, the piranha solution, which was prepared by mixing the 30% hydrogen peroxide (Wako Pure Chemical Industries, Ltd) and the sulfuric acid (Wako Pure Chemical Industries, Ltd) at a ratio of one to four, was injected into both the non-modified channel and the non-modified cell, and after leaving for one hour, the channel and cell were washed by flowing distilled water. Secondly, the cleaned non-modified channel and cell were modified using silane-coupling solution (Shin-Etsu Chemical Co., Ltd, KBE-903), which was diluted with distilled water at a volume fraction of 5.0%. The silane-coupling solution was injected into both the channel and cell, and these were left for two hours. Then the channel and cell were washed by using distilled water and dried sufficiently. These successive operations induce to modify the amino group on wall surfaces of the non-modified channel and cell, and make the positively charged channels (Gorl and Humsche 1998). Table 1 summarizes the microchannel and the closed electrokinetic cell used in the experiments in Section 4. A DC electric field of $2.0 \times 10^3$ V/m was applied to all the channel and cell as listed in Table 1, and the electrokinetic flow was generated. The detail of experimental procedure based on the measurement principle will be explained in Section 3.

### 2.2 Properties of working fluids and particles

Table 2 depicts the properties of aqueous solutions, which were used as working fluids and changed the zeta-potentials of particle and channel wall. The aqueous solutions with almost the same pH value and the different molarity listed in Table 2 (a) were used in Sections 4.1-4.4, and Table 2 (b) shows the aqueous solutions with the different pH values and almost the same molarity used in Section 4.4. They were made to work as the buffer solutions consisting of a weak acid and its conjugate base, and the chemical components of each buffer solution were different. Note that the potassium chloride (KCl) with the high dissociation constant was included in all aqueous solutions, and it controls the ion molarity that affects the zeta-potentials (Probstain 2003). Table 3 depicts the properties of florescent particles, whose surfaces were modified by the carboxyl group (-COOH), the sulfate group (-SH) or the amino group (-NH$_2$). In the aqueous solutions, the carboxy- and sulfate-modified particles were charged negatively, and the amino-modified particles were charged positively. The particle diameter was determined from the following two points. Firstly, since it must be small enough to follow the flow field, it is required that the Stokes number is less than unity. Secondly, since it must be large enough to be seen, it is necessary that the particle diameter is larger than the axial resolution of the measurement system based on the CCD camera resolution and the lens magnification, whose detail was described in Section 2.3. Additionally, the particle material was determined by considering the low sedimentation velocity of particles based on the Stokes’ law, which means that the density of particle should be almost the same as that of working fluid.

### 2.3 Measurement system

The measurement system was composed of an inverted microscope (Nikon Corp., TE2000), a continuous mercury lamp, an oil immersion 40× magnification objective lens (Nikon Corp., CFI Plan Fluor, NA 1.3), a 12-bit cooled CCD

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Table 1. Summary of microchannel and closed electrokinetic cell used in Section 4

| Section | Microchannel and closed electrokinetic cell | $U_{EOF}$ [µm/s] |
|---------|--------------------------------------------|-----------------|
| 4.1     | Non-modified channel (Figure 1), Non-modified cell (Figure 2) | 60 - 115 |
| 4.2     | Non-modified channel (Figure 1), Non-modified cell (Figure 2) | 40 - 90 |
| 4.3     | Non-modified cell (Figure 2), Modified cell (Figure 2) | - |
| 4.4     | Modified channel (Figure 1), Modified cell (Figure 2) | –90 - –10 |
camera (Hamamatsu Photonics K. K., C4880-80, 656 × 494 pixels), a power source, and a digital multi-meter. This setup corresponded to a measurement area of 271 × 204 µm. The mercury lamp lighted the fluorescent particles in the aqueous solutions by passing through an excitation filter (=450-490 nm) and a dichroic mirror was reflecting wavelengths below 505 nm. The CCD camera captured the fluorescence emitted from the particles by passing through an objective lens and a barrier filter transmitting wavelengths longer than 520 nm. The exposure time and frame interval of the CCD camera were set properly in each condition. The power source applied a DC electric field to all the channel and cell. The digital multi-meter was connected to the power source with the non-modified and the modified channels to measure the electric current inside the channels. The axial resolution of the measurement system was 0.2 µm, so that the experiments were carried out using the particles of 0.5 µm and 1.0 µm diameter. The depth resolutions of this measurement system using particles of 0.5 µm and 1.0 µm in diameter were 2.5 µm and 3.7 µm, respectively (Meinhart et al. 2000).

### 2.4 Experimental procedure

In the current monitoring technique, two aqueous solutions at different molar concentrations were used. One is the solution with a concentration of C that is equal to the molarity shown in Table 2. The other is the solution with a concentration of 0.95C, which is made by adding the deionized water to the solution with a concentration of C. The fluorescent particles with different functional groups listed in Table 3 were added to both the aqueous solutions. Then, the solution with a concentration of 0.95C was injected into one reservoir and filled up the non-modified or the modified channel as shown in Figure 1, and the solution with a concentration of C was injected into the other side of the reservoir. When a DC electric field of 2.0 × 10^3 V/m was applied to the channels, the aqueous solution with a concentration of C was moved to the opposite side as the electroosmotic flow, because of the different conductivities and molarity. The electroosmotic flow velocity was directly obtained by using the digital multi-meter and measuring the period during the migration of the solution, and the zeta-potential of wall was easily calculated from the electroosmotic flow velocity. While the migration period was being monitored, colloid particle images were captured to measure the observed particle diameter.
velocity by micro-PIV, and the electrophoretic velocity, which is related to the zeta-potential of particle, was evaluated by subtracting the electroosmotic flow velocity from the observed particle velocity.

On the other hand, in the closed electrokinetic cell technique, only the aqueous solution with a concentration of $C$ was injected into the non-modified cell or modified cell as shown in Figure 2 and sealed by the tube clamps. When a DC electric field of $2.0 \times 10^3$ V/m was applied to the cells, a circulating flow was generated in the cells, which denied the effects of the electroosmotic flow. By measuring the velocity distributions inside the cells by micro-PIV, the electrophoretic velocity was evaluated, which caused the zeta-potential of particle. In addition, using the non-modified or the modified channel as shown in Figure 1 under the identical condition with the measurements in the cells, the observed particle velocity was measured by micro-PIV. Thus, the electroosmotic flow velocity was evaluated by subtracting the electrophoretic velocity from the observed particle velocity, and the zeta-potential of wall was calculated by the electroosmotic flow velocity. The details of principles of measurement techniques will be explained in Section 3.

3. Principles of the Measurement Techniques

3.1 Evaluation of zeta-potentials

Figure 3 illustrates the flowchart of the leading processes to measure the zeta-potentials of wall and particle by using both the current monitoring technique and the closed electrokinetic cell technique. The zeta-potential of wall, $\zeta_w$, was given by the following equation:

$$\zeta_w = -\frac{\mu U_{\text{EOF}}}{\varepsilon E}$$ (1)

where $\varepsilon$ was the permittivity of aqueous solutions, $U_{\text{EOF}}$ was the electroosmotic flow velocity, $\mu$ was the viscosity, and $E$ was the magnitude of the DC electric field. Furthermore, the zeta-potential of particle, $\zeta_p$, was given as:

$$\zeta_p = \frac{\mu U_{\text{EP}}}{\varepsilon E}$$ (2)

where $U_{\text{EP}}$ was the electrophoretic velocity. It was observed in Eqs. (1) and (2) that zeta-potentials of wall and particle were calculated by $U_{\text{EOF}}$ and $U_{\text{EP}}$, respectively. The following sections explain the principles of the two techniques to evaluate the electrophoretic and electroosmotic flow velocities.

3.2 Principle of the current monitoring technique

When a DC electric field was applied to a microchannel, the aqueous solution was moved as the electroosmotic flow to the cathode. Since the wall surface of silica glass channel was charged negatively, the positive charges in the aqueous
solution were collected near the wall surface, which generated the electroosmotic flow. Simultaneously, negatively or positively charged particles were driven to the anode or the cathode as the electrophoresis, respectively. Thus, the observed particle velocity by micro-PIV, $U_{\text{obs}}$, was the summation of $U_{\text{EOF}}$ and $U_{\text{EP}}$. Figure 3 (a) shows the leading process of the current monitoring technique (Devasenathipathy et al. 2002). Two aqueous solutions with different molar concentrations were injected into both ends of the non-modified or the modified channel. When applying the DC electric field, the higher concentration solution was moved towards the other side, the electric current was decreased, and the lower concentration solution was replaced by the higher one, which reached the minimum constant value of electric current. Thus, $U_{\text{EOF}}$ was evaluated by measuring the electric current and given as:

$$U_{\text{EOF}} = \frac{L}{\Delta t}$$

where $L$ was the total length of microchannel and $\Delta t$ was the period during the migration of the solution. $\zeta_w$ was calculated by substituting $U_{\text{EOF}}$ in Eq. (1). Furthermore, since the electroosmotic flow and the electrophoresis were simultaneously occurred, $U_{\text{EP}}$ was expressed by the following equation, and $\zeta_p$ was calculated by substituting $U_{\text{EP}}$ in Eq. (2):

$$U_{\text{EP}} = U_{\text{obs}} - U_{\text{EOF}}$$

### 3.3 Principle of the closed electrokinetic cell technique

Figure 3 (b) shows the leading process of the closed electrokinetic cell technique (Ichiyanagi et al. 2005; Ichiyanagi et al. 2014a, 2014b). When the aspect ratio (width/depth, $k$) of the cell is larger than 20, an application of a DC electric field to the cell generated the electroosmotic flow and caused the aqueous solution near the wall surface to move toward the cathode, while the aqueous solution at the depth-width midpoint moved to the anode due to the law of mass conservation, and a circulating flow was formed in the cell. In contrast, due to a uniform motion of the electrophoresis, $U_{\text{EP}}$ was estimated by eliminating the effect of $U_{\text{EOF}}$. Mori and Okamoto (1980) analyzed $U_{\text{EP}}$ in the closed electrokinetic cell and derived the following equations to approximate the particle velocity, $U_{\text{cell}}$, and to obtain $U_{\text{EP}}$:

$$U_{\text{EP}} = \left(\frac{K_0 + (1/3 + 0.420/k) K_1}{K_0} \right) (U_1 + U_2)$$

$$U' = K_2 z'^2 + K_1 z' + K_0, \quad U'^* = \frac{U_{\text{obs}}}{U_1 + U_2}, \quad z'^* = \frac{z - b}{2b}$$

where $U_1$ and $U_2$ were the velocities at the top side ($z = 1 \mu m$) and bottom side ($z = 49 \mu m$) in the cell, respectively, $K_0$, $K_1$ and $K_2$ were the coefficients of Eq. (6), $U'$ was the normalized velocity, $z$ was the position coordinate in the depth-width direction, $z'^*$ was the normalized position coordinate, $2b$ was the depth of the cell. As shown in Figure 4, $U_{\text{cell}}$ was obtained at $z = 1, 5, 9, 13, 17, 21, 25, 29, 33, 37, 41, 45, \text{ and } 49 \mu m$ in the cell by using micro-PIV. $U_{\text{EP}}$ was calculated using Eqs. (5) and (6), and $\zeta_p$ was calculated by substituting $U_{\text{EP}}$ in Eq. (2). Furthermore, $U_{\text{EOF}}$ was evaluated by using micro-PIV and expressed by the following equation, and $\zeta_w$ was calculated by substituting $U_{\text{EOF}}$ in Eq. (1):

$$U_{\text{EOF}} = U_{\text{obs}} - U_{\text{EP}}$$

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Fig. 4. Example of particle velocities in closed electrokinetic cell, $U_{\text{cell}}$, in terms of depth-wise position, which were obtained at 13 measurement planes in non-modified cell by micro-PIV when using aqueous solution with $6.04 \times 10^{-3}$ mol/l with 1.0 \mu m diameter particles on application of 2000V/m.
4. Results and Discussion

4.1 Evaluation of current monitoring technique and closed electrokinetic cell technique when using negatively charged particle with negatively charged channel

Electroosmotic flow velocity and electrophoretic velocity were measured using the negatively charged particles with the negatively charged channels. The florescent particles with different functional groups were added to each aqueous solution at different molar concentrations which was shown in Table 1. In the current monitoring technique, the aqueous solutions were injected into both ends of the non-modified channel. When the DC electric field was applied to the channel, \( U_{\text{EOF}} \) and \( \zeta_w \) were measured, and subsequently, \( U_{\text{EP}} \) and \( \zeta_p \) were evaluated using Eqs. (4) and (2), respectively.

The measurement uncertainties at 95% confidence intervals were evaluated using the bias errors and the standard deviations, whose detail was described in Section 4.5. For evaluating the standard deviations, we obtained each experimental result based on average 4 measurements. Figure 5 shows \( U_{\text{EP}} \) and \( \zeta_p \) in terms of the molar concentration, and Figure 6 plots \( U_{\text{EOF}} \) and \( \zeta_w \) in terms of the molar concentration. The error bars of Figures 5-19 indicate the measurement uncertainties at 95% confidence intervals, and the dotted line in Figures 6, 8 and 10 denotes the zeta-potential of silica glass (the non-modified channel) calculated by the following empirical equation (Scales et al. 1992).

\[
\zeta_{\text{silica}} = (-0.058 \log_{10} (pH) + 0.026)(-\log_{10} (C))^{1/2}
\]

where \( pH \) and \( C \) were the pH value and the molarity of the aqueous solutions, respectively. The average error ratios between the measurement uncertainties and the experimental results in Figures 5 (particle) and 6 (wall) were evaluated to be 14.4% and 14.2%, respectively. It was found that the absolute values of zeta-potential of wall using the negatively charged particles were reasonably agreed with the empirical equation, and the current monitoring technique could measure both the negative zeta-potentials of particle and wall.

On the other hand, Figure 7 plots \( U_{\text{EP}} \) and \( \zeta_p \) in terms of molar concentration, which were measured in the non-modified cell by using the closed electrokinetic cell technique. Then, \( U_{\text{obs}} \) were measured in the non-modified channel, and \( U_{\text{EOF}} \) and \( \zeta_w \) were evaluated using Eqs. (7) and (1), respectively. Figure 8 shows \( U_{\text{EOF}} \) and \( \zeta_w \) in terms of molar concentration. It was obvious that the closed electrokinetic cell technique could also measure both the negative zeta-potentials of particle and wall. The average error ratios between the measurement uncertainties and the experimental results in Figures 7 (particle) and 8 (wall) were evaluated to be 7.4% and 9.6%, respectively. The error ratios of the closed electrokinetic cell technique were smaller than those of the current monitoring technique, and thus, it was revealed that the measurement accuracy was improved by the closed electrokinetic cell technique when using the negatively charged particles and the negatively charged channels.
4.2 Evaluation of current monitoring technique and closed electrokinetic cell technique when using positively charged particle with negatively charged channel

Electroosmotic flow velocity and electrophoretic velocity were measured using the positively charged particles with the negatively charged channels. The amino-modified florescent particles were added to each aqueous solution at different molar concentrations which was shown in Table 1 (a). \( U_{\text{EOF}} \) and \( \zeta_w \) were measured in the non-modified channel by using the current monitoring technique, and \( U_{\text{EP}} \) and \( \zeta_p \) was also evaluated. Figure 9 shows \( U_{\text{EP}} \) and \( \zeta_p \) in terms of molar concentration, and Figure 10 plots \( U_{\text{EOF}} \) and \( \zeta_w \) in comparison to empirical equation versus molarity. The average error ratios between the measurement uncertainties and the experimental results in Figures 9 (particle) and 10 (wall) were evaluated to be 35.5% and 24.5%, respectively. As shown in Figures 9 and 10, although the current monitoring technique could measure both the zeta-potentials of positively charged particles and negatively charged walls, their error ratios were larger than the measurement error ratios as described in Section 4.1. Note that, since the adsorption of particles to the non-modified channels occurs during measurements as shown in Section 4.3, it is required to complete all the measurements in a short time by being a specialist. On the other hand, Figure 11 plots the \( U_{\text{EP}} \) and \( \zeta_p \) in terms of molar concentration, which was measured in the non-modified channel by using the closed electrokinetic cell technique. It was concluded from Figure 11 that the closed electrokinetic cell technique could not measure the zeta-potentials of positively charged particles, because the particle images could not be captured due to the adsorption of positively charged particles to the non-modified channels and cells (negatively charged channels).
4.3 Effect of adsorption of negatively and positively charged particles on negatively and positively charged channel surface

For the quantitative evaluation of the adsorption of particles on channel walls, the number densities of particles were evaluated by capturing the particle images and are shown in Figures 12 and 13. The experiments were conducted by flowing the aqueous solution with $60.0 \times 10^{-3}$ mol/l with the negatively and the positively charged particles into the non-modified and the modified cells on an application of $2.0 \times 10^3$ V/m, and particle images were captured at $z = 30 \mu m$.
Images at \( z = 30 \) µm and 50 µm were used to investigate the number densities of particle in aqueous solution and the number densities of adsorbed particle on the channel wall surfaces, respectively. In addition, since we confirmed that the difference of channel geometry between Figures 1 and 2 does not affect the surface modification of channel and the adsorption of particles, Figures 12 and 13 shows only the results using the non-modified and the modified cells whose geometry is shown in Figure 2. Figure 12 plots the time evolution of number densities of (a) the adsorbed particles on the wall surface and (b) the particles existing in aqueous solution when using the non-modified cell (negatively charged channel). It was revealed that the number density of adsorbed positively charged particles was larger than that of negatively charged particles, and in contrast, the number density of positively charged particles in aqueous solution was smaller than that of negatively charged particles. From Figure 12, it was noted that the positively charged particles were adsorbed on the negatively charged channel wall surfaces and the number of positively charged particles in aqueous solution was reduced, which caused the zeta-potential of wall to be changed and micro-PIV to be hardly conducted. Continuously, the number density of particles was also measured using the modified cell (positively charged channel). Figure 13 shows the time evolution of number densities of (a) the adsorbed particles on the wall surface and (b) the particles existing in aqueous solution when using the modified cell. The number density of the adsorbed positively charged particles was smaller than that of the negatively charged particles, and the number density of the positively charged particles in aqueous solution was larger than that of negatively charged particles. From these results, it was confirmed that the use of the modified channel and the modified cell gives to reduce the adsorption of positively charged particles on the channel wall surfaces and to maintain the number density of particles in aqueous solution.

### 4.4 Evaluation of current monitoring technique and closed electrokinetic cell technique when using positively charged particle with positively charged channel

Electroosmotic flow velocity and electrophoretic velocity were measured using the positively charged particles with the positively charged channels. The amino-modified particles were added to each aqueous solution at different pH values which was shown in Table 1 (b). \( U_{\text{EOF}} \) and \( \zeta_w \) measured in the modified channel by using the current monitoring technique, and continuously, \( U_{\text{EP}} \) and \( \zeta_p \) were evaluated using Eqs. (4) and (2), respectively. For the current monitoring technique, Figure 14 shows \( U_{\text{EP}} \) and \( \zeta_p \) in terms of the pH value, and Figure 15 plots \( U_{\text{EOF}} \) and \( \zeta_w \) in terms of the pH value. The diamond-shaped gray plots of Figures 14-17 show the reference data, which were measured using the commercial measurement equipment of zeta-potential, whose uncertainty was about ±2% (Moleon Baca et al. 1991). The average error ratios between the measurement uncertainties and the experimental results in Figures 14 (particle) and 15 (wall) were evaluated to be 31.9% and 31.6%, respectively. It was revealed that the current monitoring technique using the modified channel could measure the positive zeta-potentials of particle and wall. Furthermore, for the closed electrokinetic cell technique, Figure 16 shows \( U_{\text{EP}} \) and \( \zeta_p \) in terms of the pH values, which were measured in the modified cell, and Figure 17 represents \( U_{\text{EOF}} \) and \( \zeta_w \) in terms of the pH values, which were measured in the modified channel and evaluated using Eqs. (7) and (1), respectively. The average error ratios between the measurement uncertainties and the
experimental results in Figures 16 (particle) and 17 (wall) were evaluated to be 13.0% and 17.7%, respectively. Thus, for the positive zeta-potential measurements with the surface modification, the closed electrokinetic cell technique was also superior to the current monitoring technique.

In addition, it is generally known that the zeta-potentials are changed with both the pH values and the molar concentrations. When changing the pH values listed in Table 1 (b), it was shown from Figures 16 and 17 that the closed electrokinetic cell technique was useful to measure the positive zeta-potentials of particle and wall. Thus, we applied the technique to the different molar concentration conditions listed in Table 1 (a). Figure 18 shows the electrophoretic velocity and zeta-potential of particle obtained by the closed electrokinetic cell technique using positively charged particle with modified cell with 95% confidence interval versus molarity. Figure 19 shows the electroosmotic flow velocity and zeta-potential of wall obtained by the closed electrokinetic cell technique using positively charged particle with modified channel with 95% confidence interval versus molarity.

4.5 Evaluation of measurement uncertainty

The measurement uncertainties at 95% confidence intervals, $U_{RSS}$, of the electrophoretic velocity and the electroosmotic flow velocity for both the current monitoring technique (Figures 5, 6, 14 and 15) and the closed electrokinetic cell technique (Figures 7, 8, 16 and 17) were evaluated using the following equations (ASME ed. 1986):

$$U_{RSS} = \left( B_m^2 + t_s S_m^2 \right)^{1/2}, \quad B_m = \left( \sum B_i^2 \right)^{1/2}, \quad S_m = \left( \sum S_i^2 \right)^{1/2}$$

(9)

where $B_i$ and $S_i$ are the bias and precision elemental errors, respectively, $B_m$ and $S_m$ are the bias and precision indexes, respectively, and $t_s$ is a value based on the Student’s t-test which was 2.7 under the present experimental conditions. The uncertainty due to the total measurement errors, $U_{RSS}$, is given by the summation of the bias index, $B_m$, and the precision errors.
For the accurate measurement of the zeta-potentials, we compared the zeta-potentials of particle and wall obtained by the current monitoring technique and those by the closed electrokinetic cell technique. The experiments were performed using the negatively and positively charged particles and the negatively and positively charged channels. The important conclusions obtained from this study are summarized below.

For the measurements of negative zeta-potentials of particle and wall, their error ratios using the current monitoring technique were 14.4% and 14.2%, respectively, and those using the closed electrokinetic cell technique were 7.4% and 9.6%, respectively. Thus, it was found that the accuracy of the negative zeta-potential measurement was improved by the closed electrokinetic cell technique.

When using the positively charged particles and the negatively charged channels, the measurement error ratios using the current monitoring technique were 35.5% for particles and 24.5% for walls. On the other hand, for the closed

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**Table 4. Measurement uncertainties of electroosmotic flow velocity and zeta-potential of wall obtained by (a) current monitoring technique and (b) closed electrokinetic cell technique when using non-modified channel and aqueous solution of $9.7 \times 10^{-3}$ mol/l with carboxy-modified particles on application of 2000V/m.**

| Error factor     | Symbol  | Accuracy       | Error factor   | Symbol  | Accuracy       |
|------------------|---------|----------------|----------------|---------|----------------|
| pH meter         | $B_1$   | $\pm 9.74 \times 10^{-2}$ | pH meter       | $B_1$   | $\pm 9.74 \times 10^{-2}$ |
| Power supply     | $B_2$   | $\pm 3.89 \times 10^{-1}$ | Power supply   | $B_2$   | $\pm 3.35 \times 10^{-1}$ |
| Digital multi-meter | $B_3$ | $\pm 1.56 \times 10^{-2}$ | PIV            | $B_3$   | $\pm 5.00 \times 10^{-2}$ |
| Bias             | $B_4$   | $\pm 7.28$     | Bias index     | $B_5$   | $\pm 7.29$     |
| Bias index       | $B_m$   | $\pm 6.19$     |
| Standard deviation | $S_1$ | $\pm 1.95$     | Standard deviation | $S_1$ | $\pm 1.47$     |
| Precision index  | $S_m$   | $\pm 1.95$     | Precision index | $S_m$   | $\pm 1.47$     |
| Measurement uncertainty of $U_{EOF}$ | $U_{RSS}$ | $\pm 8.71$ | Measurement uncertainty of $U_{EOF}$ | $U_{RSS}$ | $\pm 6.67$ |
| Measurement uncertainty of $\zeta_w$ | $U_{RSS}$ | $\pm 6.19$ | Measurement Uncertainty of $\zeta_w$ | $U_{RSS}$ | $\pm 4.75$ |

index, $S_m$. The bias index was obtained from the accuracy of the used instruments (the pH meter, the power supply, the digital multi-meter, and so forth), the image processing (PIV), the bias error due to the difference between the measured velocities and the previous studies (Scales et al. 1992, Moleon Baca et al. 1991), and the error propagation. The precision index was calculated using the standard deviation of the measured velocities. Table 4 lists the elemental errors, the bias and precision indexes, and the measurement uncertainties for the current monitoring technique, and Table 5 compiles those for the closed electrokinetic cell technique. The errors of the used instruments were referred from the literature published by their manufacturers, and the errors of the pH meter, the power supply and the digital multi-meter were ±pH 0.01, ±0.5%, and ±0.02%, respectively. The present PIV technique was in-house developed, which has the hierarchical processing based on the cross-correlation and the sub-pixel analysis using the correlation coefficients fitted to a Gaussian distribution. Its error was estimated to be ±0.002 pixels which corresponded to ±5.0 × 10⁻³ μm/s. Table 6 summarizes the average error ratios of both techniques, which were based on Tables 4 and 5. These results indicate that the closed electrokinetic cell technique was significantly improved over the current monitoring technique, regardless of the electric charge characteristics of particles and channels. When using the closed electrokinetic cell technique, the negative zeta-potential measurements fulfills the required error range of about 10% (Oddy and Santiago 2004), and however, the positive zeta-potential measurements does not satisfy the requirement. This was caused by increasing the standard deviation due to the low success rate of the surface modification, so that we believe that the improvement of the surface modification technique will contribute to the error reduction of the positive zeta-potential measurements.

5. Conclusions

For the accurate measurement of the zeta-potentials, we compared the zeta-potentials of particle and wall obtained by the current monitoring technique and those by the closed electrokinetic cell technique. The experiments were performed using the negatively and positively charged particles and the negatively and positively charged channels. The important conclusions obtained from this study are summarized below.

For the measurements of negative zeta-potentials of particle and wall, their error ratios using the current monitoring technique were 14.4% and 14.2%, respectively, and those using the closed electrokinetic cell technique were 7.4% and 9.6%, respectively. Thus, it was found that the accuracy of the negative zeta-potential measurement was improved by the closed electrokinetic cell technique.

When using the positively charged particles and the negatively charged channels, the measurement error ratios using the current monitoring technique were 35.5% for particles and 24.5% for walls. On the other hand, for the closed
electrokinetic cell technique, the zeta-potential could not be measured, because the particle images could not be captured due to the adsorption of positively charged particles on the negatively charged channel surfaces.

In order to prevent the adsorption of positively charged particles on channel walls, we made the positively charged channels by using the surface modification technique. For the measurements of positive zeta-potentials of particle and wall, their error ratios using the current monitoring technique were 31.9% and 31.6%, respectively, and those using the closed electrokinetic cell technique were 13.0% and 17.7%, respectively. It was revealed that the measurement accuracies of both the positive and negative zeta-potentials of particle and wall were improved by using the closed electrokinetic cell technique.

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