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Correcting off-axis effects in an on-chip resistive-pulse analyzer

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A resistive-pulse analyzer is a device that utilizes measurements of the electrical resistance of a solution-filled pore to determine the size of particles that pass through the pore. The relation between particle size and changes in the pore's resistance is complicated by particles that travel off the central axis of the pore. Here, we present data taken using a microfabricated pore and latex colloids that illustrates the effects of off-axis particles, and propose an algorithm for removing those effects from the data. We show that the ability to remove off-axis effects increases the precision of devices that transport particles through the pore with a pressure-driven flow relative to those that use electrophoretic flow. © *2002 American Institute of Physics.* [DOI: 10.1063/1.1519932]

Resistive-pulse analysis is a method for measuring the size of particles in a solution: A particle passing through a solution-filled pore displaces conducting fluid which in turn causes a transient increase in the electrical resistance of the pore that is quantitatively related to the size of the particle. This technique has been used to measure the size and concentration of a variety of particles, such as cells,[1] viruses,[2] and colloids.[3] In those cases, the pore was made using relatively complicated methods, such as etching the tracks of radioactive particles after they have passed through a material.

Recently,[4] we utilized relatively simple microfabrication techniques to develop the first chip-based microfluidic device capable of sizing submicron particles in a solution through the resistive pulse technique. In that initial work, we drove the particles through the pore electrophoretically, thus requiring the particles to carry a relatively high electrostatic charge for effective electric-field-driven motion. Motivated by the desire to measure particles that are not highly charged, such as viruses or protein-coated colloids, we have developed a second version of the device that utilizes hydrostatic pressure to drive the particles through the pore.

The analysis of the pulses produced while utilizing a pressure-driven flow is complicated by the effects of particles that travel off the central axis of the pore. Relative to particles of identical size that travel on axis, off-axis particles take longer to transit the pore (causing wider pulses) and produce larger electrical resistance changes. The former effect, which we refer to as the hydrodynamic off-axis effect, is simply due to the parabolic distribution of fluid velocity within the pore. The latter effect, which we refer to as the electrical off-axis effect, occurs because off-axis particles enhance the nonuniformity in the distribution of electrical current density and, consequently, further increase the electrical resistance.[5] In this Note, we present two main results. First, we show how off-axis particles affect data taken on populations of colloidal particles and propose a method to remove these effects. Second, we point out that a device utilizing a pressure-driven flow will have an increased resolution over one using an electrophoretic flow, since the algorithm we have developed to remove off-axis effects can only be performed for a pressure-driven flow. As we will demonstrate, both results should increase the precision of future applications of the resistive-pulse technique.

To quantitatively describe our data, we follow the work of Berge *et al.*,[6] who formulated phenomenological equations to describe the two aforementioned off-axis effects. For the hydrodynamic effect, they found that previous experimental data[7] on the time τ for a particle to pass through the pore are well described by

$$\tau = \frac{\tau_0}{(1-x^2)(c_1 - c_2 x^5)}, \tag{1}$$

where $\tau_0 = 16\eta(L/D)^2/\Delta P$ is the on-axis transit time for an infinitely small particle, η is the fluid viscosity, L is the pore length, D is the pore diameter, ΔP is the pressure drop across the pore, $x = 2b/D$ is the fractional radial position for a particle centered a distance b off of the pore axis, $c_1 = 1 - (2/3)(d/D)^2$, $c_2 = 23.36(1-c_1)$, and d is the particle diameter (see Fig. 1). Berge *et al.*[6] then utilized Eq. (1) to empirically describe the variation in the change in electrical resistance ΔR with an off-axis coordinate x as

$$\Delta R = \Delta R_0 \left[1 + \alpha \left(\frac{xd}{D} \right)^3 \right], \tag{2}$$

where $\Delta R_0(d,D,L)$ is the change in resistance for the on-axis particle (see Ref. 1 for its functional form) and α is a constant whose value varies between 4.2 and 7.5.

In Fig. 2, we plot the values we measured of the normalized change in electrical resistance $\Delta R/R$ versus τ for pulses produced by two populations of latex colloids: one population with a mean diameter of 470 nm, and one with a mean diameter of 514 nm. The data were taken using a pore that is 9.4 μm in length and 1.16 μm in diameter. For both types of colloids, there is a clear positive and nearly linear correlation between $\Delta R/R$ and τ as qualitatively expected from Eqs. (1) and (2). One interpretation of this positive correlation is that

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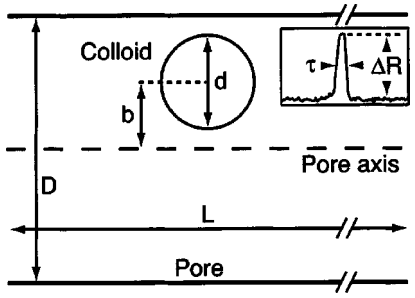

FIG. 1. Schematic of the measurement geometry for a pore of diameter D and length L containing a colloid of diameter d that travels a distance b off the pore axis. Inset: Typical trace of the measured resistance vs time showing the passage of a single colloid that produces a resistance pulse of width τ and height ΔR.

it is due to deviations in the size of individual colloids within each population, since it is clear that relatively larger colloids will both move slower and produce larger pulse amplitudes. The 470 nm diameter colloid population shown in the lower portion of the data plotted in Fig. 2 has a standard deviation of 12 nm as measured by the manufacturer. Equation (1) predicts that the expected variation in τ of on-axis particles, due solely to differences in particle size within the population, will be ~2%. As seen in Fig. 2, the measured values for τ vary by much more than that (~80%). We thus conclude that the measured variations in τ can be attributed almost entirely to off-axis effects and not to differences in particle size.

Given particle and pore dimensions, we can use Eqs. (1) and (2) to find the predicted dependence of ΔR on τ due to the off-axis effects. In Fig. 2, we plot this result and compare it to the measured data. For both types of colloids, we find good agreement between the predicted dependence and the measurements when $\alpha=6$ in Eq. (2); this value of α falls well within the range found by Berge *et al.*[6] The nearly linear measured correlation between ΔR and τ is then explained by the fact that variations in ΔR (caused by both electrical noise and the intrinsic size distribution of the colloid population) obscure the slight nonlinearity in the predicted dependence. Based on this, we propose that off-axis effects can be

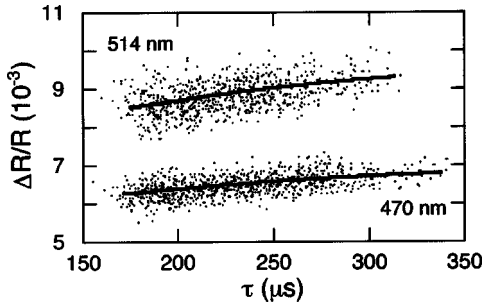

FIG. 2. Comparison of measured normalized pulse heights ($\Delta R/R$) and pulse widths (τ) and the predictions of Eqs. (1) and (2). Each point represents the measured pulse height vs pulse width for the passage of a 470 nm diameter latex colloid (lower group of points) or a 514 nm diameter latex colloid (upper group of points) through a pore of length 9.4 μm and diameter 1.16 μm. For each type of colloid, the correlation between the measured heights and widths of the pulses is a result of the effect of colloids that travel off the pore axis. The measured data agree well with the predictions of Eqs. (1) and (2) for each colloid size, shown here as the solid lines.

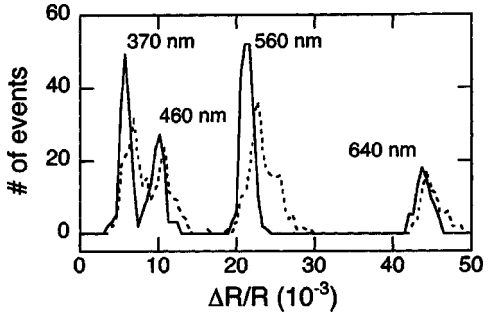

FIG. 3. Histogram of the normalized pulse heights ($\Delta R/R$) measured for a solution containing four different sizes of latex colloids (of diameters 370, 460, 560, and 640 nm as labeled); each peak corresponds to the colloids of a given size. The dotted line represents the raw data while the solid line shows the same data after correcting for the effects of off-axis particles, as described by Eq. (3). The distribution of measured pulse heights for each type of colloid is both sharpened and more symmetric after applying the correction. For example, the application of the adjustment caused a decrease in the coefficient of variation of the pulses measured from the 560 nm colloids from 7.1% to 3.5%.

effectively removed in the data analysis of a given population by first fitting a line $f(\tau)$ to the plot of ΔR versus τ, and then calculating an adjusted value ΔR_{adj} for each event of height ΔR and width τ:

$$\Delta R_{\mathrm{adj}}=\Delta R-[f(\tau)-f(\tau_{\mathrm{min}})], \tag{3}$$

where $\tau_{\mathrm{min}}=\tau_0/c_1$ is the minimum transit time measured. We thus use Eq. (3) as an algorithm to calculate the pulse height each colloid would have caused had it traveled on the central axis of the pore.

To illustrate the increase in resolution that results from employing Eq. (3), we have measured a polydisperse solution containing four different sizes of latex colloids (of diameters 370 nm, 460 nm, 560 nm, and 640 nm). In Fig. 3, we plot the distribution of measured ΔR values both before and after applying Eq. (3). As shown, the correction clearly sharpens the distribution for each type of colloid. For example, the coefficient of variation (standard deviation divided by mean) for pulses produced by 560 nm diameter colloids is reduced from 7.1% to 3.5%.

Previously,[4] we utilized an electrophoretic driving force and found relatively little correlation between the measured pulse heights and widths. In that data, we measured linear correlation coefficients R ranging from 0.1 and 0.2 between the pulse heights and widths; this is in contrast to typical values of $R\sim0.5$ for data obtained using a pressure-driven flow. Since we expect that the electrical off-axis effect must have been present in the electrophoretically driven data, we conclude that the electrophoretic velocity of the measured colloids does not vary significantly with the off-axis coordinate. This agrees with the fact that, in the absence of a colloid, the electric field across the pore is constant. It is possible that an off-axis effect on the velocity of a particle subjected to only an electrophoretic force would be caused by either inhomogenieties in the electric field due to the presence of the particle, or hydrodynamic interactions between the particle and pore wall. We can only conclude that these

possible effects are insignificant compared to the noise in our measurement.

The absence of an observable hydrodynamic off-axis effect while using an electrophoretic flow means that we are unable to apply an algorithm similar to Eq. (3) to remove the electrical off-axis effect for electrophoretic data. Distributions of pulse heights of a given colloid population measured using an electrophoretic driving force are, therefore, reduced in accuracy since they contain an intractable systematic source of error: the electrical off-axis effect. Devices using a pressure driven flow, where we are able to apply the correction described in Eq. (3), are thus more accurate than those that use electrophoretic flow.

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