As microfluidic technologies mature, increasingly complex solutions are employed, and accurate methods for the measurement of electroosmotic flow rates are becoming increasingly important. The methodologies of both a direct method and an indirect method of flow rate measurement are presented here. The direct method involves flow visualization using trace amounts of a caged fluorescent dye. The indirect method is based on the change in current that occurs when one solution in the microchannel is replaced by another. The results of concurrent and independent measurements of electroosmotic velocities of Tris–aceta with EDTA (TAE) and Tris–borate with EDTA (TBE) at $1 \times$ concentration in fused silica capillaries are presented. Although these buffers are commonly used in biological chemistry, these mobilities have not previously been reported. Strong agreement among data collected with both methods establishes confidence in the electroosmotic mobility values obtained and indicates that the current-based method, which requires less infrastructure than the direct method, can provide accurate flow rate measurements under these conditions. Constant electroosmotic mobilities of $4.90 \times 10^{-8}$ m$^2$ V$^{-1}$ s$^{-1}$ for TAE and $3.10 \times 10^{-8}$ m$^2$ V$^{-1}$ s$^{-1}$ for TBE were determined by tests in a range of electrical field strengths from 5 to 20 kV/m. A linear flow rate increase with applied field strength indicated that constant mobility and negligible Joule heating effects were present. Applicability and limitations of both the measurement methods and these buffers are discussed in the context of microfluidic applications.

**Key Words:** electroosmotic flow; microchannel; flow rate; velocity; flow visualization; TAE; TBE.

### 1. INTRODUCTION

Recent advances in manufacturing have made possible the fabrication of microsized fluidic devices. Microsized chemical synthesis and analysis systems offer many advantages over their traditional, macro-sized counterparts due to the increase in surface area-to-volume ratio accompanying miniaturization. Most often, advantages stem from a reduction in sample volume and processing time. Electroosmotic flow, where an applied electric potential difference induces fluid motion, is becoming more and more common as a method of fluid transport in such devices (1–5). As these technologies mature, there is an increased need for accurate and compact methods for the determination of volumetric flow rate, a key parameter. The measurement of extremely low flow rates (e.g., $\sim 500$ pL/min), characteristic of microfluidics, is generally not feasible with traditional, macro-sized techniques.

An indirect method for measuring the average electroosmotic velocity in microchannels has been developed (6–8). In this method, a capillary is filled with an electrolyte solution and then brought into contact with another solution of the same electrolyte but with a slightly different ionic concentration. Once the two solutions are in contact, an electrical field is applied along the capillary in such a way that the second solution is pumped into the capillary, replacing the first solution. As the replacement occurs, the overall liquid conductivity in the capillary is changed, resulting in a change in the electrical current draw. When the second solution has replaced the first one completely, the current through the capillary reaches a plateau. The concentration difference must be small to ensure uniform and constant zeta potential and ionic distributions, resulting in spatially and temporally constant electroosmotic flow. Under these conditions, the average electroosmotic flow velocity can be determined by

$$\bar{u}_{\text{ave}} = \frac{L_{\text{total}}}{\Delta t},$$

where $\bar{u}_{\text{ave}}$ is the average electroosmotic velocity, $L_{\text{total}}$ is the total length of the capillary tube, and $\Delta t$ is the time required for completion of the replacement (determined from the current record). The resolution of this technique is determined by the sharpness of the current–time response, which is determined by the nature of the solutions employed. In some cases, a linear relationship may be readily apparent, but it may be difficult to determine $\Delta t$ due to nonlinear current fluctuations at the beginning and end of the replacement. For such situations, a slope-based method for determining the average velocity has been proposed (8). In all cases presented here, however, the end points of the replacement processes were well defined and the straightforward calculation in Eq. [1] was applied.

The main disadvantages of the indirect, current-based technique are (a) the requirement that one solution replace another...
similar solution and (b) the lack of spatial and temporal resolution. The main advantage of the technique is its simplicity. The current-based experiments described here can be run quickly and with relatively little infrastructure. In the broader context of microfluidic applications, however, the simplicity of current-based measurements has far greater implications. As noted in a recent review paper (9), infrastructure surrounding microfluidics remains, for the most part, macrosized (examples include microscopes, CCD cameras, lasers, and power supplies). Although this infrastructure is often required for research purposes, it mitigates the commercial viability of microfluidic technologies. Thus, there is an increasing need for microscale control and detection technologies to augment microscale fluid networks. Although current monitoring may be an indirect method of determining flow rate, it is directly applicable to microfluidic technologies, in that it requires minimal addition infrastructure. In the experiments reported here, the change in current is used to determine the average electroosmotic velocity during a replacement. Alternatively, however, if these parameters were known, instantaneous flow rates could be derived from individual current measurements. In a microfluidic network, real-time current records from each reservoir could be used to determine how much fluid has flowed from each reservoir. Using these current feeds as input to a computer, parameters such as sample size, sample dispersion, and degrees of mixing could be determined through appropriate modeling.

Various methods of microflow visualization have evolved for the direct measurement of fluid velocities and flow rates. The method employed here requires a specialized, caged fluorescent dye to be present in trace amounts throughout the fluid. “Photoinjection” of free fluorescent dye is accomplished by exposing the initially nonfluorescent solution to intensely focused ultraviolet light. Where the fluid has been exposed, caging groups are broken and fluorescent dye molecules are released. The free fluorescent dye may then be imaged using classical fluorescence techniques. Velocity data are then calculated from the images of the dye transport, using digital image processing and numerical analysis techniques. The use of caged dyes is one variety of molecular tagging velocimetry (MTV) techniques that has been applied to both macroscale (10) and, more recently, microscale (11–13) applications.

The main disadvantages of the direct, flow visualization-based method are (a) the required use of specialized caged dyes and (b) the size and extent of the hardware required. In these experiments, however, the small concentration of caged dye employed was found to have a negligible effect on the electrokinetic properties of the solutions. The amount of hardware and expenditure required is essentially the cost of the spatial and temporal resolution this method affords. Resolved velocity measurements up to 2.5 μm from the wall have been previously demonstrated (13). Employing microfluidics in chemical and biological applications requires the use of solutions other than simple and dilute electrolytes. During electrophoresis in various lab-on-a-chip processes, such as DNA sequencing, gene expression, and genotyping, water can be electrolyzed, generating protons at the anode and hydroxyl ions at the cathode. The cathode end of the electrophoresis chamber then becomes basic and the anodal end acidic. Buffer solutions are used to overcome this problem. Two commonly used buffers are Tris–acetate with EDTA (TAE) and Tris–borate with EDTA (TBE) (14). For controlling various on-chip microfluidic processes, it is desirable to know the electroosmotic mobilities and general electrokinetic properties of TAE and TBE buffers. Unfortunately, this information is not available in the published literature. Here, both the direct and indirect velocity measurement techniques were applied (concurrently and independently), and electroosmotic velocities of both TAE and TBE at 1× concentrations in fused silica capillaries at a range of electrical field strengths are presented.

### 2. EXPERIMENTS AND METHODOLOGY

A schematic diagram showing the flow module and the supporting hardware for both the indirect and direct measurements is given in Fig. 1. During the experiments, a 10-cm length of 100-μm-i.d. polyimide-coated fused silica capillary joined the two small reservoirs. Platinum electrodes were embedded into each reservoir. Current data for the indirect method were recorded from the power source by a personal computer-based data acquisition system. Direct, flow visualization measurements were taken through a viewing window at the midpoint between the two reservoirs.

#### 2.1. Indirect, Current-Based Method

Initially, the upstream reservoir (reservoir 1) was filled with buffer. The capillary and the downstream reservoir (reservoir 2) were connected and filled with the same buffer as reservoir 1, but at a 5% lower concentration. Reservoir 2 was connected to a ground, and reservoir 1 was connected to a high voltage power supply.

![FIG. 1. Schematic illustration of experimental setup.](image-url)
supply via platinum electrodes. Then, the capillary (already connected to reservoir 2) was connected to reservoir 1, the current jumped to a non-zero value, and electroosmotic flow was initiated. The higher concentration buffer from reservoir 1 gradually filled the capillary, replacing the lower concentration solution. The data acquisition computer recorded the voltage (in kilovolts) and the current (in microamperes) as a function of time (s). When the replacement was complete, the current reached a plateau value. As an example, Fig. 2 shows the current–time record obtained during a TAE solution replacement process. In the figure, the sloped, replacement region and the plateau are well defined. There is a small curvature at the start of the process corresponding to the initiation of flow and settling of the electrical instruments. After the replacement, the average velocity, \( \bar{u}_{ave} \), was calculated directly from Eq. [1].

2.2. Direct, Flow Visualization-Based Method

The microfluidic flow visualization system interfaces with the flow module as shown in Fig. 1. The focused pulse of ultraviolet light enters the capillary from below, and both the excitation light and resulting fluorescent emission are transmitted through the objective. To facilitate direct flow visualization, all solutions involved were seeded with caged fluorescent dye. During the experiments, ultraviolet light was focused into a sheet crossing the capillary (perpendicular to the flow direction). The resulting uncaged fluorescent dye was continuously excited and its fluorescent emission was collected with a laser-powered epifluorescent microscope. Full frame images of the dye transport were recorded by a progressive scan CCD camera and saved automatically on the image acquisition computer. Details of the image analysis employed and the degree of near-wall resolution achieved with this visualization method were documented in a recent paper (13), and only a concise overview is given here.

The ultraviolet light was provided by a 300-\( \mu \)J, \( \lambda = 337 \text{ nm} \), pulsed nitrogen laser. A single-line, 200-mW, \( \lambda = 488 \text{ nm} \), argon laser provided a continuous flood of excitation light. The argon laser beam passed through a 10 \( \times \) beam expander before entering the epifluorescent microscope head. Inside the microscope, the beam is reflected off a dichroic mirror (\( \lambda = 510 \text{ nm} \) long pass) directly into the 25X, oil immersion objective with numerical aperture, NA = 0.75, as illustrated in Fig. 1. A single drop of optical oil surrounded the windowed portion of the capillary and connected directly to both the objective and the blank base optic.

Figure 3 shows a sequence of images (at 267-ms intervals) of dye uncaged in the electroosmotic flow of TAE with 7.5 kV/m. The camera was run in open video mode and a delay generator fired the laser at convenient intervals (the laser was pulsed three times at 30 Hz at each uncaging event). The camera was run at 15 Hz with individual exposure times of 1/125 s. The acquired images had a resolution of 640 \( \times \) 484 pixels. This corresponded to a 346-\( \mu \)m visible length of capillary, with each pixel representing a 0.54-\( \mu \)m square in the object plane. To remove any nonuniformity present in the imaging system, dark-field subtraction and bright-field normalization were performed (15).

Although the mass-weighted average velocity of the fluid, \( \bar{u} \), is of interest, only the transport of the uncaged dye ions was observed. The numerical analysis technique must infer the mass-weighted average velocity, \( \bar{u} \), from images of ion transport which can differ from the advective transport due to forced and ordinary diffusion. In locations where the dye concentration gradient is zero, the observed ionic transport is only the addition of a known electrophoretic velocity and the desired, mass-weighted average velocity. In this method, each of these locations of zero axial dye concentration gradient were chosen as marker points, such that the cross-sectional velocity profile could be calculated directly from the observed velocity of these points as follows:

\[
\bar{u} = \bar{u}_{ob} - \bar{u}_{ph},
\]

where \( \bar{u} \) is the mass-averaged velocity of the fluid, \( \bar{u}_{ob} \) is the observed velocity of the dye, and \( \bar{u}_{ph} \) is the electrophoretic velocity of the dye.

In the numerical image analysis, a zero gradient marker point was located along each axial line of pixels, using a weighted average of the highest intensity values on that line. The set of

![Image 1](image1.png)

**FIG. 2.** A typical current–time relationship measured for 1\( \times \) TAE buffer solution in a 10-cm length of 100-\( \mu \)m-i.d. capillary, under an applied electrical field of 7.5 kV/m.

![Image 2](image2.png)

**FIG. 3.** Images of dye uncaged in an electroosmotic flow of 1\( \times \) TAE buffer, though a 100-\( \mu \)m-i.d. capillary at 267-ms intervals with an applied electric field strength of 7.5 kV/m.
markers from each image formed a cross-stream, concentration maxima profile. Figures 4a–4c each show concentration maxima profiles calculated from each of eight images, in sequence, obtained with applied electric field strengths of 5, 7.5, and 10 kV/m respectively. In a given sequence, any pair of profiles could provide a velocity profile by dividing the distance between them, Δx, by the corresponding timestep, Δt. With 8 images, 28 velocity profiles could be constructed. Here, all 28 were calculated and an error-weighted averaging technique was applied. Velocity profiles of the fluid (calculated from the maximum dye concentration profiles in Fig. 4) are shown in Fig. 5.

2.3. Chemicals

TAE buffer 1× contained 40 mM Tris base, 40 mM glacial acetic acid, and 1 mM EDTA in demineralized and distilled water, resulting in a pH of 8. The TBE buffer 1× contained 89 mM Tris base, 89 mM boric acid, and 2 mM EDTA in demineralized and distilled water, resulting in a pH of 8.3. Although termed “1×,” these buffers differ substantially in ionic concentrations. Although it is difficult to calculate the exact concentration of dissociated ions, the initial concentrations would indicate a bulk counterion concentration on the order of 10⁻² M for TAE and 10⁻¹ M for TBE at minimum. When the direct, flow visualization method was applied, all solutions included a 1.5 × 10⁻³ M concentration of caged dye. The caged fluorescent dye employed here was 5-carboxymethoxy-2-nitrobenzyl (CMNB) caged fluorescein (826.81 MW). Using the Nernst–Einstein relation (16), a measured value of the diffusion coefficient of the uncaged dye was used to determine the electrophoretic mobility to be 3.3 × 10⁻⁸ m² V⁻¹ s⁻¹. Immediately before use, all solutions were filtered using 0.2-μm pore size syringe filters, and capillaries were rinsed with pure water and then with the appropriate buffer.

3. RESULTS AND DISCUSSION

To ensure that the addition of caged dye was not having a significant effect on the electrokinetic properties of the buffers, initial replacement experiments were run with and without dissolved caged dye (all other parameters were kept constant). The resulting velocity values were indistinguishable within the accuracy of the technique.

Then, concurrent (direct and indirect) experiments were performed for each buffer. During the experiments, direct velocimetry measurements were taken by firing the laser at 10-s intervals with the delay generator. Figure 3 shows a sequence of images of the resulting uncaged dye at 267-ms intervals. The plug-like motion, characteristic of electroosmotic flow, is apparent from the one-dimensional transport of the dye. Other image sequences taken during the same replacement displayed similar behavior and similar electroosmotic velocity. Maximum concentration profiles and calculated velocity profiles corresponding to these images are given in Figs. 4b and 5b, respectively. The velocity profiles remain fairly flat right to the wall. This is to be expected as the relatively high ionic concentration results in a compact electrical double layer (EDL). A conservative estimate of the
Debye length from the classical theory (16) gives $1/\kappa = 3$ nm, which corresponds to an EDL thickness on the order of 10 nm. This EDL thickness is well below the spatial resolution of light microscopy. Thus, the small degree of curvature at the edges of the velocity profiles is an artifact of the vanishing signal-to-noise ratio near the wall.

For TAE, the indirect method gave a velocity of 0.374 mm/s, as compared to 0.375 mm/s given by the direct method. For TBE, the indirect method gave a velocity of 0.204 mm/s as compared to 0.213 mm/s given by the direct method. The results of these and multiple independent measurements (from each of the direct and indirect methods) for TAE and TBE are presented in Figs. 6 and 7 respectively.

Several aspects of the results presented in Figs. 6 and 7 are noteworthy. In general, the agreement between the two methods is strong, and the methods themselves are mutually validated in this sense. For TBE, the direct and indirect measurements differ by less than 5% in most cases (five out of seven) and by 10% or less in all cases. For TAE, values obtained using the two techniques differed by less than 9% in all cases with the exception of the lowest velocity value at 5 kV/m. The difference between methods in this one case (over 50%) is believed to be due to errors encountered using the indirect, current-based method. This measurement involved the lowest electric field strength, corresponding to the lowest current values and the lowest velocities measured. As the velocity is decreased, the replacement process takes longer to complete and the once sharp concentration front between the two solutions becomes progressively blurred. This mixing causes the sloped and the plateau region of the current–time relationship to be smoothed together, which can lead to erroneous measurements, either by estimation of the end time or through the use of the slope method (8). Although many factors affect the resolution of the current-based measurements, 5 kV/m was found here to be a lower limit of applicability for this particular solution–capillary system. One possible way to measure velocities at lower electric field strengths would be to decrease the capillary length and hence decrease the replacement time.

The electroosmotic velocity of both buffers was shown to be directly proportional to the applied electrical field strength. Thus, the electroosmotic behavior of these buffers, under these circumstances, may be characterized by single electroosmotic mobility values. Mobilities were calculated in both cases from the slope of a least-squares fit to the data obtained with the direct method. Electroosmotic mobilities of $4.90 \times 10^{-8}$ and $3.10 \times 10^{-8}$ m$^2$ V$^{-1}$ s$^{-1}$ were determined for TAE and TBE, respectively. Applying classical theory, these mobilities indicate zeta potentials of $\zeta_{\text{TAE}} = -63$ mV and $\zeta_{\text{TBE}} = -40$ mV. The difference in zeta potentials, corresponds to the difference in ionic concentrations between 1× TAE and 1× TBE. This is in keeping with data reported by Hunter (16) for vitreous silica surfaces in contact with solutions at high pH.

The trend of the data presented in Figs. 6 and 7 is linear and extends through the origin. Although this is to be expected from the classical theory, it is often not found to be the case due to Joule heating effects. If the fluid temperature were raised above the ambient temperature, the electroosmotic mobility would increase. An overall increase in the mobility would be the combined effect of viscosity decreasing (increasing mobility) and decreasing relative permittivity (slightly decreasing mobility). Unfortunately, specific Joule heating effects cannot be studied in isolation from the heat transfer characteristics of the channel and the surroundings. This is left for another study. Here, it is important to note that using a fused silica capillary in open air (which is a relatively well insulated channel in the context of most microfluidic applications), Joule heating effects were shown to be negligible for both buffers with applied electrical fields up to 20 kV/m in the short period of time required.

**FIG. 6.** Plot of average electroosmotic velocities versus applied electric field strength for 1× TAE buffer. Results from both the indirect, current-based method and the direct, flow visualization based method are shown.

**FIG. 7.** Plot of average electroosmotic velocities versus applied electric field strength for 1× TBE buffer. Results from both the indirect, current-based method and the direct, flow visualization-based method are shown.
Electroosmotic flow velocity measurements in microchannels

(typically, a measurement by the indirect method is complete in less than 200 s).

Concerning applicability to microfluidic applications, the TAE buffer was found to behave very well. The significantly higher ionic concentration in the TBE buffer, however, was found to present some practical problems. First, the electroosmotic velocity was quite slow. Second, and more importantly, some bubbles were detected at the electrodes when even moderate electric fields were applied. Although the pH in the reservoir would be maintained by the action of the buffer, bubbles themselves are highly undesirable in most microfluidic applications. One way to avoid this may be to use 0.5× or 0.25× TBE buffer strength as opposed to the more common 1× concentration.

4. SUMMARY

Both a direct method and an indirect method for measuring electroosmotic flow velocity and flow rate in microchannels were presented. Advantages and disadvantages of both methods, especially in the context of microfluidic technologies, were discussed. Most notably, the simplicity of current-based methods was noted as a great advantage with respect to the commercial viability of microfluidic technologies. Through concurrent and independent tests, the two methods were mutually validated. These methods were applied to study the electroosmotic characteristics of two buffers commonly employed in chemical and biological work, TAE and TBE. Strong agreement in velocity values obtained for each buffer established confidence in the results, and linear relationships between electroosmotic velocity and applied electric field strength were obtained. Under these conditions, constant mobility values of \(4.90 \times 10^{-8}\) and \(3.10 \times 10^{-8} \text{ m}^2 \text{ V}^{-1} \text{ s}^{-1}\) were determined for 1× TAE and 1× TBE buffers, respectively. In light of the results, Joule heating effects and applicability of these buffers to microfluidic applications were discussed.

ACKNOWLEDGMENTS

The authors thank Dr. S. Davies (Bio-informatics Lab., University of Toronto) for generously providing the TAE and TBE buffer solutions used in this study. The authors also thank the support of a Research Grant of the Natural Sciences and Engineering Research Council (NSERC) of Canada to D. Li, a NSERC scholarship to D. Sinton, a CONACYT scholarship to C. Escobedo-Canseco, and an Ontario Graduate Scholarship to L. Ren.

REFERENCES