A novel injection scheme is described in which ultrasmall samples in the attoliter (10⁻¹⁸ L) and low femtoliter (10⁻¹⁵ L) range, or even single molecules, are controllably introduced into a tapered capillary so that electrophoretic separation can be carried out. To match the dimensions of the capillary inlet with that of the sample, capillary tips are tapered to an inside diameter ranging from hundreds of nanometers to a few micrometers. To inject an ultrasmall sample, optical trapping is used to immobilize and manipulate the sample in order to place it inside or next to the capillary inlet. A small controlled suction results in the loading of the sample into the capillary.

The ability to manipulate single cells, subcellular organelles, and single macromolecules for the purpose of analyzing the content of such biological samples with a powerful separation technique will have far reaching implications in biochemistry, biology, and molecular medicine. In recent years, capillary electrophoresis (CE) has emerged as a powerful and versatile separation tool for the study of minute quantities of samples.1–10 This capability makes CE suitable for the separation and analysis of cellular and subcellular contents. Many challenges exist, however, in applying CE to the study of biochemical problems. These challenges can be broadly divided into three parts: (1) manipulation and introduction of ultrasmall samples into the CE column, (2) separation of the introduced sample, and (3) detection of the separated compounds. Intense research on the separations aspect of CE has led to a variety of methods.11–20 Examples include the use of capillary electrophoresis to separate polycyclic aromatic hydrocarbons and chiral compounds12,20 and capillary gel electrophoresis to sequence DNA18 and to separate DNA fragments.17 Much effort has also been spent to couple the separation power of CE with a multitude of detection schemes21–36 for the ultrasensitive and quantitative determination of compounds.
Examples include the use of mass spectrometry,\textsuperscript{21,22} NMR,\textsuperscript{23} electrochemistry,\textsuperscript{26} laser-induced fluorescence,\textsuperscript{29} and many others as detection schemes. By combining CE with laser-induced fluorescence, for example, the presence of only one or a few fluorescent molecules can be detected and analyzed.\textsuperscript{32–34} Recently, biosensors using whole cells\textsuperscript{35} and the patch clamp technique\textsuperscript{36} have also been developed as selective detectors for bioactive substances. Although many powerful separation and detection schemes are available, relatively few techniques have been developed to manipulate and introduce ultrasmall samples into the separation capillary.

The manipulation and introduction of samples into the separation column becomes particularly challenging for subcellular organelles such as vesicles, mitochondria, and single DNA molecules. Because these samples are small (tens of nanometers to a micrometer in diameter) with volumes in the attoliter range, they tend to move rapidly and randomly in solution. To inject controlled amount of samples into the CE column, it is necessary to match the dimension of the inlet (tip) of the column with that of the sample being introduced. When CE is used to sample the extracellular environment, the spatial resolution is directly related to the tip dimension of the CE column. In this article, we describe a novel technique of using an optical trap\textsuperscript{37–40} to load ultrasmall samples, or even single molecules, into a tapered capillary. In combination with electrokinetic or hydrodynamic injection, the combination of optical trapping with tapered tips might offer more control and reproducibility in introducing small samples into the separation column. The tapered capillary should also find use in sampling nanoenvironments and in introducing ultrasmall samples to cells by electrokinetic release of the capillary content. Although numerous micropipet methods exist to introduce substances into cells, these methods are mostly limited to introducing one substance at a time. Tapered capillaries permit the introduction of many compounds after electrophoretic separation.

**EXPERIMENTAL SECTION**

**Optical Trapping and Fluorescence Imaging.** The optical trapping and fluorescence imaging system were built in-house (Figure 1a). The optical trap was formed by sending the output of a single-mode MOPA laser diode (Model SDL-5762-A6, SDL, Inc., San Jose, CA) through a spatial filter (Model 900, Newport Corp., Irvine, CA) so that the output was then reflected from a near-IR mirror. The reflected laser light was passed through a dichroic mirror, reflected from a polychroic mirror (Chroma Technology Corp., Brattleboro, VT) that is placed in a microscope (Nikon Diaphot, Technical Instrument Co., San Francisco, CA), and subsequently brought to a diffraction-limited focus with a high numerical aperture objective (100×, NA 1.4, Nikon Model 85025, Technical Instrument Co.). Fluorescence imaging was achieved by sending the output of an argon ion laser (Model 95, Lexel Corp., Fremont, CA) through a telescope, followed by a spinning disk. The presence of the spinning disk is to scatter the laser light so that uniform illumination is achieved for fluorescence imaging.

was grounded through a platinum wire in the waste reservoir. The outlet of the capillary High Voltage, Inc., Whitehouse Station, NJ) was applied to the reservoir, which was enclosed in Teflon. Positive high voltage (30-kV dc power supply from Glassman o.d. 200 µm-o.d. or less) were found to form better and smaller tapered tips than thick-walled capillaries. Pulling of large outer diameter capillaries also appears to be more difficult and often results in annealed tips. Laser-heated capillary pullers should permit quality tapered tips to be manufactured in large quantities. A tapered capillary was mounted on a micromanipulator (Model Narashige M HW-3, Adams & List Associates, Westbury, NY) for fine motion control while it was viewed through the microscope.

Suction Syringe. To enable controlled flow and suction in the column during injection, a precision syringe was coupled to the CE capillary. The syringe was made by replacing the plungers of a 1- or 3-mL syringe with a micrometer. By turning the micrometer, controlled and reproducible pressure can be applied to the capillary column. The ability to control small pressure changes in the capillary is essential to inject ultrasmall sample volumes in a reproducible manner.

Chemicals. All chemicals were purchased from Sigma Chemical Co. (St. Louis, MO) unless otherwise noted. Carboxy-rhodamine 6G, fluorescein, and YOYO dye molecules were received from Molecular Probes (Eugene, OR). DNA was from New England Biolabs (Beverly, MA), and glucose oxidase, catalase, and glucose from Fluka Chemical Corp. (Ronkonkoma, NY).

Preparation of Vesicles and DNA. The preparation of vesicles was described elsewhere. Briefly, 700 µL of chloroform, 200 µL of methanol, and 7 µL of phosphatidylcholine (0.1 M in chloroform) were sequentially placed in a 50-mL round-bottom flask. Buffer (10 mM HEPES at pH 7.4) was then carefully added alongside the flask walls. The content was rotovaporated (Buechi R-124) under reduced pressure (Cole-Parmer aspirator pump 7049-00) for 2 min at 40 °C (Buechi Waterbath B-481), after which the vesicles formed. Molecules (carboxyrhodamine 6G and fluorescein) to be entrapped in the vesicles were added to the buffer solution prior to evaporation of the organic phase. The buffer containing the vesicles was next run through size exclusion columns (Model 73202010, Bio Rad, Hercules, CA) to remove dyes or unwanted molecules external to the vesicles.

Stock DNA (~48 kb) from New England Biolabs was heated to 60 °C for 5 min, followed by rapid cooling on ice for ~15 min. YOYO intercalator dye was then added at a concentration of ~1 YOYO/20 bases, and let sit for 2–3 h. The resulting YOYO-intercalated DNA was diluted in a solution that contains 10 mM Tris, 1 mM EDTA, 2 mM NaCl, and 1%/µL mercaptoethanol. The solution also contains 50 mg/mL glucose oxidase, 10 mg/mL catalase, and 0.1%/µL glucose for the enzymatic scavenging of oxygen. The pH of the solution was adjusted to 5.75 to induce the DNA to coil into a compact structure.

RESULTS AND DISCUSSION

Comparison between Etched and Tapered Capillaries.

The conventional way to create small-dimension capillary tips is to etch the injection end of the capillary with HF. Figure 2a shows an etched capillary with an inner diameter of 10 µm and an outer diameter of 375 µm. The inner diameter of the etched tip itself is larger than 10 µm because HF unavoidably also etched the inside of the tip even when the capillary is filled and flushed with water. In addition, the etched tip is seen to be uneven and jagged, which often causes puncture of biological membranes during injection. Although the smallest inner diameter capillary available is 2 µm, it is difficult to inject samples with an inner diameter of less than 10 or 15 µm. At this small inner diameter, the high resistance caused by the narrow capillary channel impedes suction. Figure 2b shows a tapered capillary made from a 100-µm-i.d. capillary. The tip itself is much smaller than that obtained by etching a 10-µm-i.d. capillary. The tip, in addition, is very smooth and rounded (see Figure 3). For the injection of ultrasmall

Figure 3. (A–D) A video sequence showing the injection of a cell-sized vesicle (~11 µm) containing 1 mM fluorescein in 10 mM HEPES at pH 7.4 into a tapered capillary (100-µm i.d./200-µm o.d.). The tip inner diameter is ~7 µm, and the tip appears smooth with fire-polished quality. In (E–H) the same vesicle is pushed from the capillary to reform a well-sealed vesicle, showing no puncture of the vesicle membrane.
samples, such as synaptic vesicles and mitochondria, the tip must be reduced to submicrometer dimensions so as to ensure that no more than one synaptic vesicle or mitochondrion is introduced into the column. This ultrasmall tip dimension cannot be achieved by etching. Because only the tip inner diameter is dramatically decreased, suction can be easily generated. In addition, the tip dimension and the column inner diameter are no longer correlated, which might prove advantageous in some situations. It is also much easier to pull a tapered capillary. Whereas etching a capillary took 5-6 h to complete, pulling took a few seconds.

Injection of Cell-Sized Vesicles with a Tapered Capillary.
Figure 3a–d show the injection of a cell-sized vesicle when suction is applied via a precision syringe. The same vesicle is pushed from the capillary in Figure 3e–h. The tip diameter is ~7 μm, and the vesicle is ~11 μm in size. It can be seen that the tapered capillary has a smooth, fire-polished quality and, consequently, does not puncture the membrane of the vesicle. The integrity of the vesicle is also seen when it is pushed from the capillary to reform a round, well-sealed vesicle as before injection (Figure 3e–h). This behavior is an important advantage in injecting large

![Image](https://example.com/image.png)
cells because membrane puncture will cause cell content to leak out, which prevents accurate quantitation of cellular content. A practical advantage is that the tapered capillaries are more robust under manipulation in the microscope than etched ones, which tend to break because of the thinness of the etched capillary walls. Because the vesicle is large, the Brownian motion that this cell-sized vesicle undergoes is very slow. Optical trapping of the vesicle is therefore not necessary.

Injection of Organelle-Sized Vesicles with Optical Trapping. For small vesicles that are comparable in size with subcellular organelles, Brownian motion tends to be very rapid. Under these conditions some form of trapping is essential in immobilizing the vesicle in solution for subsequent manipulation and injection. If the diameter of the tapered capillary tip is larger than that of the vesicle, the optically trapped vesicle can be directly moved and placed inside the capillary, as shown in Figure 4a.
This dexterity is crucial in analyzing and quantifying the content of single cellular organelles, such as synaptic vesicles, in which no more than one vesicle is introduced into the capillary column. The tip dimension can be further reduced so as to match even more closely the dimension of the vesicle. Panels b–e of Figure 4 are a video sequence that show the injection of a small vesicle into a 25-μm-i.d. capillary. The tip inner diameter is ~1.5 μm. A small suction results in the injection of the vesicle into the capillary. At this small tip diameter, the tip can be inserted into a single cell to analyze the cellular content or to inject cellular organelles into the column for subsequent separation and analysis. If CE is used to sample the environment surrounding a cell, the spatial resolution can be dramatically increased with a small inner diameter tip. Alternatively, CE can be used to introduce compounds to a cell to study its behavior and characteristics. Once again, spatial resolution will be greatly enhanced with a small inner diameter tapered tip.

Figure 4f is the resulting electropherogram of the vesicle injected in Figure 4b–e. Only one large peak is present, which indicates that the vesicle is not lysed. Because the vesicle contains 1 mM carboxyrhodamine 6G and 3 mM fluorescein, lysing would have led to the separation of the two dyes, which would give rise to two peaks. Although the high voltage applied is often adequate to lyse large cells, it is insufficient to lyse small vesicles or organelles because the membrane breakdown potential becomes greater with decreasing diameter of the liposome.\(^\text{(44)}\) The field, however, might have caused the vesicle content to leak out during separation, which would give rise to the broad envelope that extends from 6 to 7.5 min. The presence of only one peak also indicates that no more that one vesicle is injected into the capillary.

**Injection and Manipulation of a Single DNA Molecule with Ultrasmall Tips.** The dexterity of this injection technique is demonstrated with the loading of a single DNA molecule. Panels a–d of Figure 5 show a single λ DNA first being trapped by the laser beam, with an ultrasmall tip (~1 μm) subsequently being moved next to it. The application of a small controlled suction results in the loading of the DNA molecule. The ability to introduce a single DNA molecule with ease into the capillary opens many exciting possibilities. If coupled to an imaging system, the motion and behavior of this single DNA molecule in the capillary might be followed. It also suggests the ability to transfer single molecules between different environments. For example, by inserting the tip into the cell nucleus, single DNA molecules might be transferred outside the cell for subsequent manipulation and analysis. Other transfer or isolation procedures that involve attoliter-sized volumes or single molecules might also find optical trapping and tapered capillaries to be useful.

If the capillary tip is further reduced, it can be made into a mechanical manipulation device. Panels e–g of Figure 5 show the manipulation of a single λ DNA that is being sucked and attached to the submicrometer inner diameter tip. Once attached to the capillary, the DNA molecule can be moved at will in solution. The nature of this attachment is not clear, but it seems to be caused in part by the “stickiness” of the intercalated YOYO dye molecules. At this ultrasmall tip dimension, the electrophoresis capillary should also be able to manipulate cellular organelles, such as mitochondria and other cellular compartments.

**CONCLUSION**

In summary, we have developed and demonstrated the utility of tapered capillaries combined with optical trapping for sample injection. In etching, the tip dimension is determined by the column inner diameter, but the tip of tapered columns can be pulled into arbitrary dimensions. In addition, pulled tips have many desirable characteristics, such as their round, fire-polished quality and ease of fabrication. In combination with optical trapping, injection of attoliter-sized volumes or even single molecules can be accomplished with ease and control. Although we emphasized the use of tapered columns in the injection of ultrasmall samples, they should find many other important applications. These applications include the sampling of extracellular and intracellular environments and the delivery of chemicals or drugs to cells using electrokinetic release. Optical trapping and tapered capillaries might also be used to transfer, isolate, and manipulate ultrasmall samples. This described technique should make feasible manipulation and analysis at the single-organelle level.

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