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# Gradient Elution Moving Boundary Electrophoresis for High-Throughput Multiplexed Microfluidic Devices

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**A novel method for performing electrophoretic separations is described—gradient elution moving boundary electrophoresis (GEMBE). The technique utilizes the electrophoretic migration of chemical species in combination with variable hydrodynamic bulk counterflow of the solution through a separation capillary or microfluidic channel. Continuous sample introduction is used, eliminating the need for a sample injection mechanism. Only analytes with an electrophoretic velocity greater than the counterflow velocity enter the separation channel. The counterflow velocity is varied over time so that each analyte is brought into the separation column at different times, allowing for high-resolution separations in very short channels. The new variable of bulk flow acceleration affords a new selectivity parameter to electrophoresis analogous to gradient elution compositions in chromatography. Because it does not require extra channels or access ports to form an injection zone and because separations can be performed in very short channels, GEMBE separations can be implemented in much smaller areas on a microfluidic chip as compared to conventional capillary electrophoresis. Demonstrations of GEMBE separations of small dye molecules, amino acids, DNA, and immunoassay products are presented. A low-cost, polymeric, eight-channel multiplexed microfluidic device was fabricated to demonstrate the reduced area requirements of GEMBE; the device was less than 1 in.$^2$ in area and required only $n + 1$ fluidic access ports per $n$ analyses (in this instance, nine ports for eight analyses). Parallel separations of fluorescein and carboxyfluorescein yielded less than 3% relative standard deviation (RSD) in interchannel migration times and less than 5% RSD in both peak and height measurements. The device was also used to generate a calibration curve for a homogeneous insulin immunoassay using each of the eight channels as a calibration point with less than 5% RSD at each point with replicate analyses.**

As the field of micro total analysis systems matures and moves in the direction of further integration, it is important to consider how many analysis elements can be accommodated into the small footprint of a microfluidic chip. Much of the work in the field has been to miniaturize conventional analysis techniques (e.g., capillary zone electrophoresis, CZE) onto a planar chip format with minimal modifications to the basic operation mode of the technique.[1] However, conventional techniques are often optimized using macroscale, benchtop instruments. If device footprint is considered as a key parameter, the optimal process is likely to be different for microfluidic platforms. In this work, we take the example of electrophoretic separations and consider footprint as the primary limiting factor.

Conventional electrophoretic separations begin with the injection of a discrete zone of analyte(s) and proceed with the electrokinetic migration and separation of that zone; microchip electrophoretic separations are performed similarly. However, if chip area is limited, two serious drawbacks to the conventional method exist: additional channels and fluid reservoirs are required to form the injection zone; and long channels are required for high-resolution or high peak capacity separations. Consider a common CZE device employing a cross[2] or double-T type injector,[3] requiring four ports for a single analysis (two ports for injection and two ports for separation). Simple duplication for replicate analyses would lead to $4n$ ports per $n$ analyses.[4,5] Use of shared background buffer inlets and waste ports can reduce requisite numbers to $2n + 2$ or even $2n + 1$ when paired sampling is used, as shown by Mathies' group.[6−9] Alternatively, the use of injections made with single ports (either electrokinetically or hydrodynamically) along with common reservoirs will result in $n + 2$ ports per analysis,[10,11] although less defined injection plugs

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will be formed, sample leakage may occur, and injection bias can be introduced.[2,12] The use of optically gated injections on a microchip by Xu et al.[13] reduced port numbers down to $2n$ and could be lowered to $n + 1$ if a common waste reservoir were to be used. However, optical gating requires the use of high-powered lasers and alignment of multiple optical paths and is limited to photobleachable fluorophores. In addition to the ports and extra equipment necessary for forming an injection, all current microchip electrophoresis methods suffer from the requirement of long separation channels. Although relatively long channels can be fit in a small area using a serpentine pattern, each turn of the channel introduces additional dispersion and band broadening.[12]

We have developed a new technique specifically avoiding these drawbacks to yield a much smaller footprint for electrophoretic separations: gradient elution moving boundary electrophoresis (GEMBE). Moving boundary electrophoresis (MBE) was chosen because it does not require injection structures and so can be implemented with the smallest possible number of access ports ($n + 1$); the sample solution is continuously introduced into the separation channel, and analytes are detected as steps when the sample boundary migrates past the detector. The stepwise output is differentiated yielding the familiar electropherogram. While MBE was the predominant mode of tubular electrophoretic analysis during the 1930s and 1940s,[14,15] it has only seen limited use in recent decades, either in a format similar to the original[16,17] or as applied as a continuous frontal analysis technique.[18]

Gradient elution is accomplished by applying a controlled hydrodynamic counterflow that is varied over time. While several examples exist of controlling bulk flow in capillary electrophoresis (CE), either hydrodynamically[19−23] or through electric field gradients,[24,25] these methods have mainly been applied in a static, constant-flow manner with discrete injection zones in order to improve resolution and separation reproducibility. McLaren and Chen have demonstrated continuous electrophoretic purification by countering electrophoresis with hydrodynamic flow;[26,27] however, a gradient elution method has not been previously shown. In a static counterflow method, the flow rate can be optimized for two species; however, additional components may be unresolved or even excluded from the separation column. In GEMBE, a continuous change in counterflow is utilized. At any given time, only analytes with electrophoretic mobilities greater than the counterflow can enter the separation channel. As the counterflow is varied from high to low flow rates, the different analyte boundaries begin their migration along the channel at different times, allowing for high-resolution separations in very short channels. In this work, we investigate the fundamental benefits of GEMBE over CZE as applied to microfluidic devices for a variety of analyte classes including small dye molecules, amino acids, DNA, and immunoassay products.

## EXPERIMENTAL SECTION[28]

**Chemicals and Reagents.** Fused-silica capillary (30-$\mu$m i.d.; 360-$\mu$m o.d.) was from Polymicro Technologies, LLC (Phoenix, AZ). Polycarbonate (PC) and poly(methyl methacrylate) (PMMA) were from McMaster-Carr (Atlanta, GA). Fluorescein, 6-carboxyfluorescein (FAM), and fluorescein isothiocyanate-labeled insulin (I*) were purchased from Invitrogen (Carlsbad, CA). Monoclonal antibody to insulin C-terminal (Ab; $k_d$ = 1 nmol/L) was from Biodesign (Saco, ME). Fluorescein-labeled single-stranded DNA (ssDNA) of 15 and 18 lengths of cytosine were from Sigma-Genosys (St. Louis, MO). Fluorescein-labeled ssDNA of 72 length of random bases was from Qiagen Operon (Huntsville, AL). All solutions were made from Milli-Q (Millipore, Bedford, ME) ≥18 MΩ·cm deionized water. All other chemicals were obtained from Sigma-Aldrich (St. Louis, MO) and were of the highest purity available.

Capillary experiments utilized a background electrolyte (BGE) consisting of 0.5 mol/L Tris and 0.5 mol/L boric acid for fluorescent dye and amino acid analyses. DNA analyses used BGE consisting of 89 mmol/L Tris, 89 mmol/L boric acid, 5 mmol/L EDTA, adjusted to pH 8.15, to which 1% (w/w) hydroxyethylcellulose (HEC) was slowly dissolved; the solution was then mechanically agitated overnight. Microfluidic experiments utilized BGE consisting of 0.5 mol/L Tris and 0.5 mol/L boric acid to which 0.1% (w/v) Tween 20 was added. Insulin immunoassay protocol involved mixing of I* diluted from stock 1 $\mu$mol/L in BGE and insulin diluted from stock 10 $\mu$mol/L in BGE, followed by addition of Ab from stock 500 nmol/L in BGE to a total final volume of 100 $\mu$L. Solutions were agitated after each reagent addition and allowed to stand for 5 min at room temperature prior to analysis. Final I* and Ab concentrations were 500 and 200 nmol/L, respectively; insulin concentrations varied from 0 to 1 $\mu$mol/L.

**Instrumentation.** Both capillaries and polymeric microdevices were utilized.

Capillary experiments employed an apparatus previously described for use in scanning temperature gradient focusing (Figure 1a).[29] Briefly, a 5-mm-wide optical window was burned into a 3-cm capillary prior to enclosure between two PC sheets; the sandwich assembly was then placed in a hydraulic press at 500 kg, heated to 200 °C, and then cooled to 120 °C prior to releasing pressure. This process ensured good mechanical stability and thermal conductivity of the exposed silica region. The device was placed in the holding apparatus consisting of two copper anchor blocks maintained at 25 °C. One capillary end was



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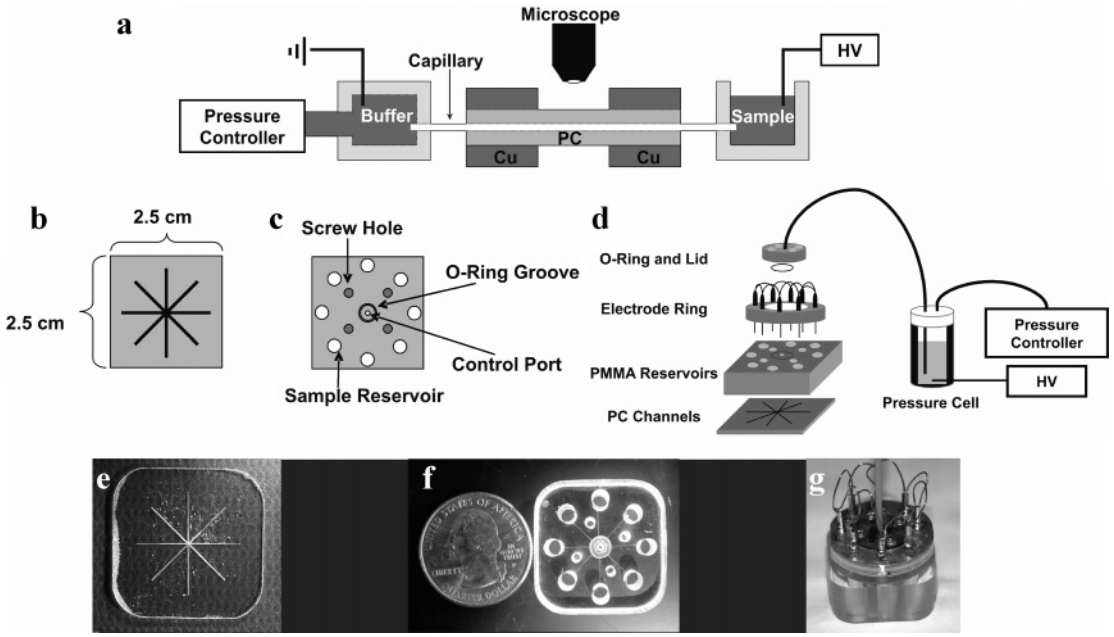


**Figure 1.** Capillary and microfluidic instruments for performing GEMBE. (a) Capillary assembly held in temperature-regulated copper blocks. Sample is maintained at atmospheric pressure; pressure controller regulates counterflow at the waste buffer reservoir. (b) Microdevice schematic diagram of the channel pattern in the PC layer. (c) Microdevice schematic diagram of the fluid reservoirs and connections in the PMMA layer. (d) Schematic of the entire microfluidic assembly. (e) Photograph of milled channels in PC. (f) Photograph of a bonded device; a U.S. quarter is included for size reference. (g) Photograph of complete microdevice assembly including the grounding electrode ring and pressure connections.



inserted into a 360-$\mu$m-diameter hole drilled into a polypropylene analyte reservoir maintained at high voltage while the other end was inserted through a Teflon-backed silicone septum into a grounded polypropylene block containing ~1 mL of buffer connected to a ±69 kPa (10 psi) precision pressure controller (Series 600, Mensor, San Marcos, TX).

Microdevices were fabricated by computer numerical control (CNC) milling into 380-$\mu$m-thick PC sheets. Channel design patterns were drafted in TurboCAD v4.1LE (IMSI, Inc., Novato, CA) and translated into G-codes by VisualMill 5.0 (MecSoft Corp., Irvine, CA). Channels were cut using a Taig model 2019 CNC milling machine (Carter Tools, Philomath, OR) with Mach 3 (ArtSoft Inc., Halifax NS, Canada) control software and a 100-$\mu$m-diameter end mill (LPKF, Garbsen, Germany). Milled channels were 40 $\mu$m deep, 150 $\mu$m wide, and 1 cm long. A PMMA piece was milled to contain a common waste access port and 75-$\mu$L-volume sample reservoirs as well as an O-ring groove and screw pilot holes, which were tapped after bonding. Prior to bonding, channels were deburred and cleaned by repeated application and removal of common adhesive tape, followed by sonication in methanol for 15 min. The PC and PMMA pieces were placed in a milled aluminum jig to aid in alignment of the channels to the reservoirs. Two glass microscope slides were placed on top of the PMMA, and the entire assembly was clamped with binder clips; thermal bonding was accomplished en vacuo at 145 °C for 1 h. A pressure cell containing background buffer and regulated by a pressure controller was connected via 1.0-mm (0.04-in.)-i.d.,1.6-mm ($^1/_{16}$-in.)-o.d. PEEK tubing to an O-ring sealed lid at the common waste access port. High voltage was applied to the pressure cell, and sample reservoirs were grounded by a machined electrode ring, simplifying electrical connections by having single high voltage and grounding points spaced far apart, thus preventing arcing. The complete microdevice assembly is shown in Figures 1b–g.

Capillary experiments were performed on a fluorescence microscope (DMLB, Leica Microsystems, Bannockburn, IL) equipped with a long-working distance 10× objective (numerical aperture, NA = 0.3), Hg arc lamp, color CCD camera, (DXC-390, Sony, New York, NY), and appropriate fluorescence filter sets. Microfluidic experiments were performed on an inverted Axioscope 300 (Carl Zeiss, Thornwood, NY) with a 5× objective (0.12 NA), Hg arc lamp, and CCD detection. All instrument control and data acquisition was performed using Java 5.0 software (Sun Microsystems, Santa Clara, CA) written in-house. Data acquisition was performed at 60 Hz. Raw data were transformed by Savitzky–Golay smoothing[30] implemented into LabVIEW by SciWare (http:// www.sciware.com.au) using the Gorry algorithm[31] to obtain a derivative function and evaluated with Cutter software.[32] Immunoassay data were fit to a four-parameter logistic model[33,34] using Microsoft Excel Solver for weighted least-squares curve fitting.[35]

## RESULTS AND DISCUSSION

GEMBE relies upon continuous, sequential sample introduction through the variation of the bulk flow under electrophoresis conditions. With the bulk flow and electrophoretic migration in opposite directions, the method is useful for providing improved separation resolution, particularly in very short channels. For the

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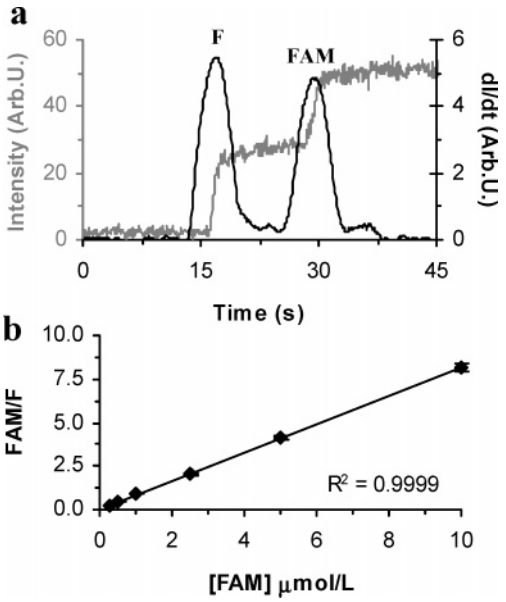


**Figure 2.** Quantitative single-channel GEMBE separation of fluorescein (F) and FAM. (a) Example GEMBE output. Gray trace is raw fluorescence intensity versus time for 1 $\mu$mol/L each of F and FAM. Black trace is Savitzky−Golay transformation of raw data (order = 1; window = 30). (b) Calibration curve by peak area of FAM using 1 $\mu$mol/L F as an internal reference. Error bars are 1 standard deviation ($n$ = 3). Separation conditions: 3-cm capillary (30-$\mu$m i.d.); 1-cm separation length; 1000 V/cm; 0 Pa initial pressure; −40 Pa/s acceleration.

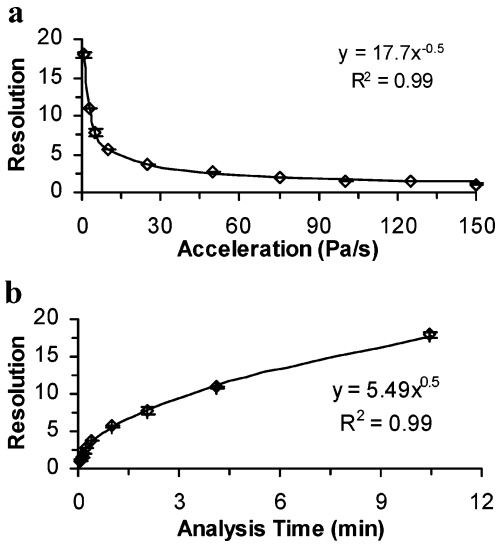


**Figure 3.** Relationships between (a) resolution and counterflow acceleration and (b) resolution and analysis time using 10 $\mu$mol/L each of fluorescein and FAM as the analytes. A square root relationship was found to fit well to both resolution versus acceleration and analysis time. Error bars are 1 standard deviation ($n$ = 3). Separation conditions: 3-cm capillary (30-$\mu$m i.d.); 1-cm separation length; 1000 V/cm; 500 Pa initial pressure; Savitzky−Golay parameters were order = 1 and window = 1% of total data point number.

separations described here, the bulk counterflow is primarily driven by the native electroosmotic flow (EOF) of the channel or capillary used, and the variation of the counterflow is accomplished by varying the pressure applied to one end of the channel. At any given time during the separation, only analytes with electrophoretic velocities greater than the bulk counterflow velocity can enter the separation column and be detected as boundary zones. As the counterflow is swept from high to low, analytes of lower mobilities are successively introduced, allowing for separation of compounds based upon electrophoretic mobility variations. A slower sweeping rate (acceleration) results in higher resolution. The variation of the bulk flow velocity over time ensures that the optimal bulk flow velocity is used for each portion of the separation.

By means of the gradient elution, GEMBE facilitates greater flexibility in selectivity when compared to conventional CZE, as the acceleration of the counterflow (analogous to gradient slope in chromatography) becomes a separation parameter. Furthermore, when analyzing high-mobility analytes, the use of counterflow can be utilized for the rejection of low-mobility species, such as large proteins, which could adhere to surfaces and degrade separation efficiency and reproducibility.

Figure 2 shows an example GEMBE separation of two fluorescent dyes, fluorescein and FAM. The detector response, which was the average fluorescence intensity within a selected region of interest at a fixed point along the separation channel, produced a "staircase" plateau pattern in the intensity profile as analytes were sequentially eluted, as shown in Figure 2a. The raw data could then be transformed to a smoothed derivative (dIntensity/dtime; d$I$/d$t$) to yield a more conventional electropherogram for determination of chromatographic figures of merit, such as peak areas and resolution. Using a constant concentration of 1 $\mu$mol/L fluorescein as an internal standard and varying FAM from 0.5 to 10 $\mu$mol/L, a calibration curve was generated using conventional peak analysis (i.e., peak area) as shown in Figure 2b. Percent relative standard deviation (RSD) at all FAM concentrations was less than 3% for peak area normalized to fluorescein ($n$ = 3). Limit of detection calculated at three times the standard deviation of the blank FAM run was 30 nmol/L, a reasonable value considering arc lamp illumination, a low NA objective, and 8-bit CCD detection.

As with the gradient slope in chromatography, the acceleration parameter in GEMBE can be reduced to improve the resolution. This is shown in Figure 3a for separations of fluorescein and FAM. A power law fit to the data indicates that the relationship between resolution, $R_s$, and acceleration, $a$, is proportional ($R_s \sim a^{-1/2}$). This square-root dependence leads to the conclusion that the resolution in GEMBE when employing a linear gradient slope is diffusion limited, similar to the limitation found in conventional CE.[36] A plot of resolution versus total analysis time (as defined as the difference in peak migration times) more explicitly demonstrates this relationship (Figure 3b). This result implies that any peak broadening due to Taylor dispersion[37] is negligible on the time and length scales involved, likely due to EOF being the dominant flow component.

Nonlinear or multistage gradients could also be implemented, as demonstrated with a separation of five dansyl-labeled amino acids (AAs): tryptophan (Trp); phenylalanine (Phe); serine (Ser); glycine (Gly); and aspartic acid (Asp). While four of the AAs were singly charged, Asp was a doubly charged species in the BGE, yielding a large window of unused separation space under linear gradient conditions (Figure 4a). A 50% reduction in analysis time

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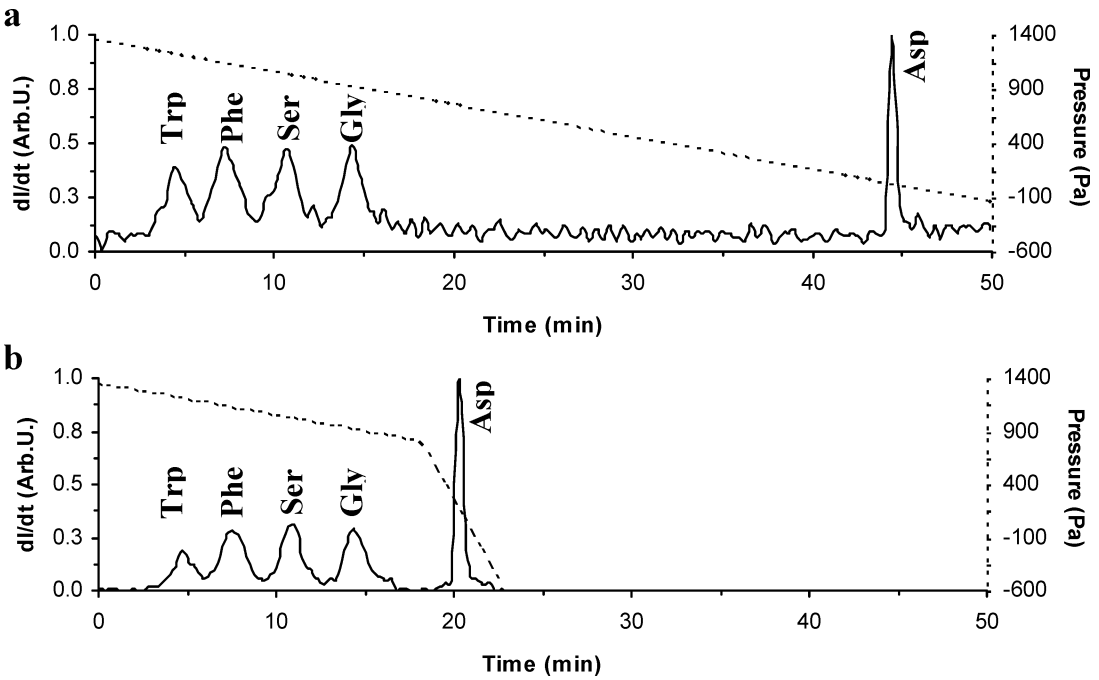


**Figure 4.** GEMBE separation of five amino acids. (a) Separation using a linear gradient of −0.5 Pa/s. (b) Separation using a two-stage gradient: −0.5 Pa/s from 0 to 18.3 min followed by −5.0 Pa/s. Note the reduced total analysis time between (b) and (a). Solid traces are Savitzky−Golay transformations of raw data (order = 1; window = 300); dashed lines are applied pressure. All amino acids were 1 mmol/L. Separation conditions: 3-cm capillary (30-$\mu$m i.d.); 1-cm separation length; 1000 V/cm; 1350 Pa initial pressure.



was achieved through the use of a two-stage gradient composed of a shallow gradient for the four similar AAs and a steep gradient to elute the final AA (Figure 4b). (Note that the low signal-to-noise ratio was due to the poor efficiency of dansyl-labeled AA detection on the instrument utilized.)

The elution mechanism allows for high resolution even on very short columns, which can be very beneficial in separations requiring surface modification or capillary filling (e.g., capillary gel electrophoresis). Shorter length modifications allow for faster flush and replenish cycles, as well as lower reagent usage. As an example, a separation of fluorescein-labeled ssDNA using a sieving buffer (1% HEC) was performed, with baseline resolution of 15- and 18-mers of poly(cytosine), as well as resolution of a 72-mer from the shorter lengths (Figure 5). The separation length used was just 1 cm. The use of mononucleotide ssDNA confirmed separation based solely upon sieving, rather than folding or minor charge differences between the lengths.

The lack of an injection scheme in GEMBE allows for high-density analysis components in microfluidic platforms. Through utilization of a single common control port for analysis elements in a radial pattern, $n$ analyses require only $n + 1$ fluidic connections or reservoirs on the device. The microdevice used herein contained eight analysis elements, all within an area less than 6.45 cm$^2$ (1 in.$^2$).

A demonstration of multiplexed analysis is shown in Figure 6. Figure 6a is a false color fluorescence image of the central region of the eight-channel GEMBE chip. The common waste port is shown in the center, with the eight separation channels extending radially outward from the port. For GEMBE separations, eight sample solutions were pipetted into the eight peripheral sample reservoirs (not shown in Figure 6a, see Figure 1f), and a single high-voltage power supply was used to apply a potential of 750 V between each of the sample reservoirs and the central port. As

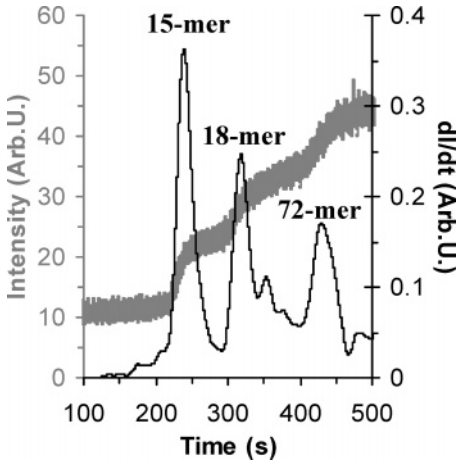


**Figure 5.** GEMBE separation of ssDNA 15 and 18 poly(cytosine)-mers and a random sequence of 72 bases using 1% HEC as the sieving matrix. Gray trace is raw intensity data. Black trace is Savitzk−-Golay transformed derivative (order = 1; window = 400). All concentrations were 200 nmol/L. The extraneous peaks were likely impurities arising from the DNA synthesis process. Peaks were identified by individual runs and spiking. Separation conditions: 3-cm capillary (30-$\mu$m i.d.); 1-cm separation length; −900 V/cm; 2500 Pa initial pressure; −10 Pa/s acceleration.

the pressure applied to the central port was reduced, each analyte in the samples eluted into its respective separation channel and then migrated toward the center of the chip where it was detected as a moving boundary at one of the eight detection points shown in Figure 6a. Each separation channel was just 7.1 mm long, and the detection spots were ~0.4 mm from the common waste port, so the separation distance was only 6.7 mm.

Figure 6b shows the resulting derivative plots for eight simultaneous separations of fluorescein and FAM. The applied pressure was scanned from 0 Pa at a rate of −0.5 Pa/s with an

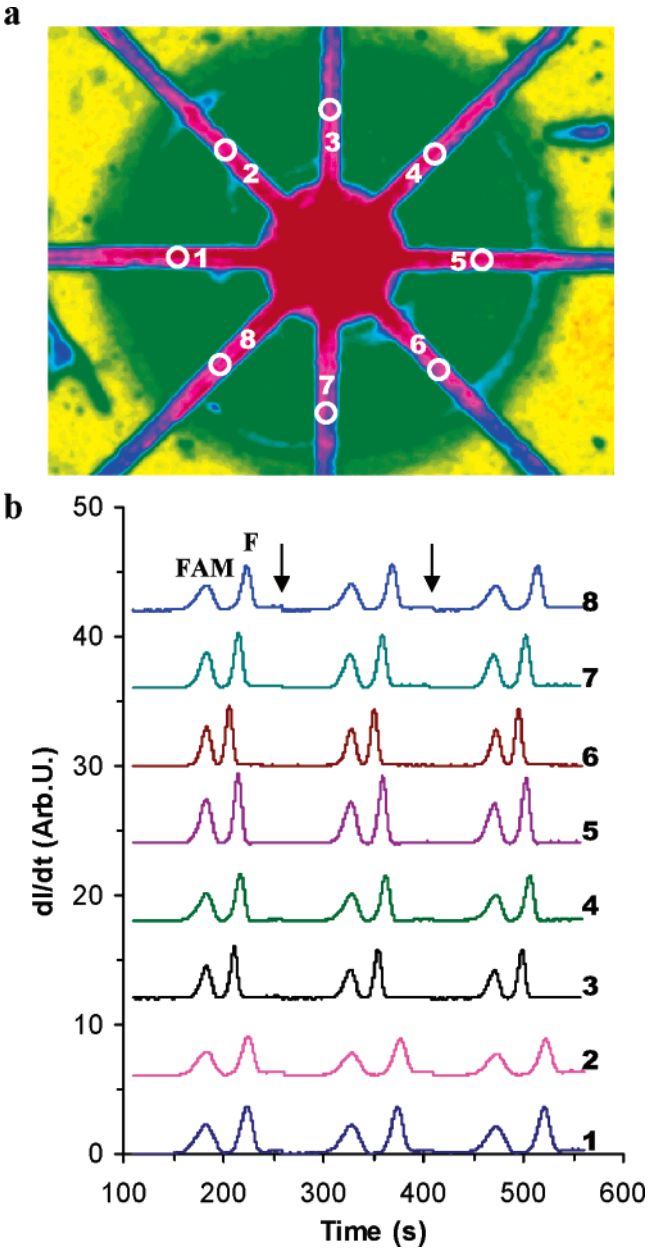


**Figure 6.** Multiplexed microfluidic GEMBE separation. (a) False color fluorescence image of detection region of chip (channels filled with F and FAM). The common waste port and the eight radially arranged channels are seen as red (blue at the outer edges of the image). The eight detection points are shown as the small white circles. The large green ring is signal due to the O-ring above the common port. (b) Derivative plots for parallel separation of F and FAM (500 nmol/L of each). Sample was loaded into each of the eight sample reservoirs; traces relate to each channel with three sequential runs (delineated by arrows). Separation conditions: 7.1-mm-long channels; 1056 V/cm; 0 Pa initial pressure; −0.5 Pa/s acceleration; Savitzky−Golay parameters were order = 1 and window = 200.

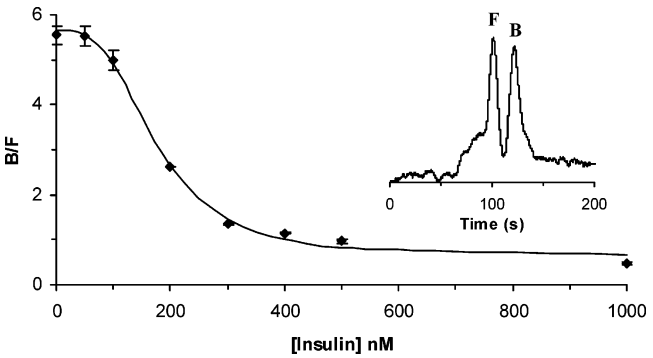


**Figure 7.** Multiplex microfluidic GEMBE generation of insulin immunoassay calibration curve. Each channel contained a mixture of 500 nmol/L I*, 200 nmol/L Ab, and varying amounts of insulin (from 0 to 1 $\mu$mol/L). Inset shows Savitzky−Golay transformed derivative (order = 1; window = 300) of the 500 nmol/L insulin sample showing the separation of free I* (F) and I* bound to Ab (B). Curve relates the ratio of F to B versus insulin present. Error bars are 1 standard deviation ($n$ = 3). Points were fit to a four-parameter logistic model. Separation conditions: 7.1-mm-long channels; 1056 V/cm; 0 Pa initial pressure; −1 Pa/s acceleration.

electric field of 1056 V/cm. A single run, consisting of the data from each of the eight channels, yielded less than 3% RSD variation in the spacing between the fluorescein and FAM peaks. The variation of the absolute migration times for the different channels was significantly greater, presumably due to variations in channel cross sections. Both FAM peak height and area variations were less than 5% RSD.

Three repeated analyses were also run to determine intrachannel variations. Each channel had less than 3% RSD in migration times of either fluorescein or FAM across the three runs. Furthermore, each channel had less than 5% RSD variation in peak height or area for both the fluorescein and FAM peaks.

The utility of the multiplexed device for high-throughput analyses was demonstrated by performing an insulin immunoassay product analysis. Immunoassays are widely used in a broad spectrum of applications, such as clinical, environmental, or pharmaceutical, due to the high specificity and sensitivity of the antibody−antigen interaction even in extremely complex matrixes.[38,39] Many immunoassay formats are both time and labor intensive; however, the development of microfluidic CE-based immunoassays has afforded much greater throughput, temporal resolution, and automation.[9,40,41] Insulin immunoassays are extremely important with regard to studying diabetes and other metabolic diseases.[42,43] Using the eight-channel device, a full calibration curve for a homogeneous competitive insulin immunoassay was generated in one step, with each channel producing one curve point (Figure 7). The eight-point calibration from 0 to 1 $\mu$mol/L insulin was run in triplicate with less than 5% RSD in each insulin concentration. Limit of detection was determined to be 50 nmol/L at the I* and Ab concentrations used.

## CONCLUSIONS

The novel electrophoresis technique presented here was demonstrated to be highly suitable for the production of high-throughput microfluidic devices while maintaining an extremely

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small device footprint. The lack of injection requirements and use of a common control port allowed for minimal sample and control access points on the device. These improvements minimize not only the requisite device features but also the accompanying external hardware, such as pumps, voltage sources, or relays. In addition, GEMBE offers the investigator a hitherto unexplored separation parameter to improve separations—the acceleration of the counterflow, which can be adjusted to provide high-resolution separations in very short microfluidic channels. As large area devices are both difficult to fabricate and require higher costs, the resulting reduction in chip area and complexity as well as the low-cost materials and methods (i.e., plastics and milling) should result in a significant reduction in the cost of device fabrication and operation.

It should be noted that GEMBE could be employed using other methods of controlling the bulk flow, such as by a secondary external field,[24] or should EOF be suppressed (e.g., through channel coatings[44]), a static hydrodynamic flow could be used while varying the applied separation voltage; these topics are currently being investigated. Also, use of spatial filtering (either directly incorporated into the device or in the excitation/emission optical path) could allow for even shorter channel lengths, which would further reduce device footprints and aid in reducing band broadening due to Taylor dispersion;[37] the lengths described were limited by the microscope field of view and the need to prevent the highly fluorescent sample reservoir signals from obscuring the analyte signal(s) in the channels.

The exclusion aspect of GEMBE should facilitate analysis of complicated mixtures that typically require a sample preparation step, as those species which would normally foul the separation column could be excluded while analytes of interest are eluted. The use of short separation lengths also should aid in electrophoretic separations utilizing channel modifications, such as coatings, packings, or gels. Further research is ongoing in several other areas including the following: development of a multichannel laser-induced fluorescence detector to improve the absolute fluorescence detection limits of the system and to increase the number of simultaneous separation channels (currently limited by microscope field of view); improvement of the detector sensitivity, data acquisition rate (currently 60 Hz), and dynamic range (currently 8-bit limited) used herein either by a photomultiplier tube or higher grade CCD, which will also reduce the effects of detector-related peak broadening; on-line reagent mixing for chemical monitoring by GEMBE, such as automated mixing of immunoreagents, for improved automation; and determination of the ability of GEMBE to perform analysis from complicated matrixes, such as immunoassays from blood serum.

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