

# Electrophoretic Separations in Small Spaces: Gradient Elution Moving-Boundary Electrophoresis (GEMBE)

Jonathan G. Shackman and David Ross

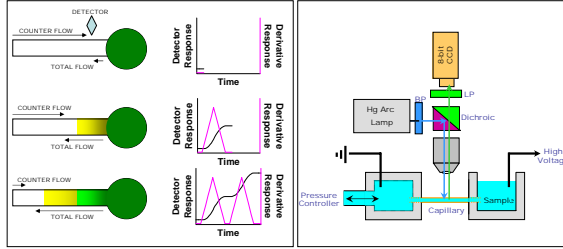
NATIONAL INSTITUTE OF STANDARDS AND TECHNOLOGY; GAITHERSBURG, MD 20899; USA

## I. Introduction

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 onto a planar chip format with minimal modifications to the basic operation mode of the technique. However, conventional techniques are often optimized using macroscale, bench-top 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 sample 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. We have developed a new technique in order to alleviate the limitations of injection based separations that can be implemented in very short separation lengths.

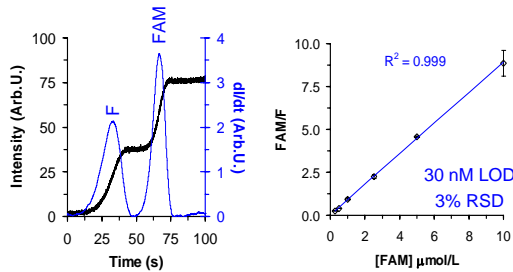
## II. What is GEMBE?

In Gradient Elution Moving-Boundary Electrophoresis (GEMBE) 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. Gradient elution is accomplished by applying a controlled counter flow that is varied over time. Only analytes with electrophoretic mobilities greater than the counter flow can enter the separation channel. As the counter flow 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.



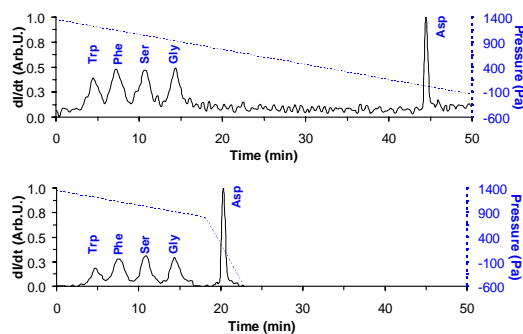
Concept of GEMBE and capillary apparatus for performing separations. (Left) As the counter flow is decreased, analytes are sequentially introduced onto the separation column, resulting in a step-wise increase in detector response that can be differentiated to yield a conventional electropherogram. As the separation point occurs at the sample inlet, very short separation lengths can be used. (Right) High voltage is applied across a capillary spanning a sample reservoir and a pressure controlled waste reservoir. Fluorescence excitation is performed using arc lamp illumination passed through a bandpass filter (BP), a dichroic split, and a microscope objective. Emission is collected back through the same objective and dichroic, filtered by a longpass filter (LP) and measured by an imaging 8-bit CCD camera.

## III. Quantitative One Centimeter Separations



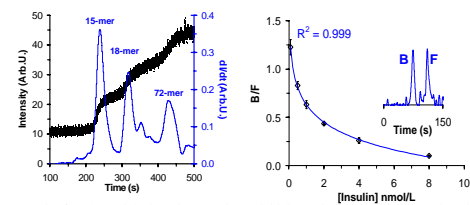
Quantitative single channel GEMBE separation of fluorescein (F) and FAM. (Left) Example GEMBE output. Black trace is raw fluorescence intensity versus time for 1 μmol/L each of F and FAM. Blue trace is Savitzky-Golay transformation of raw data. (Right) Calibration curve by peak area of FAM using 1 μmol/L F as an internal reference. Error bars are 1 standard deviation (n=3). Separation conditions: 3 cm capillary (30 μm i.d.); 1 cm separation length; 1000 V/cm; 0 Pa initial pressure; -40 Pa/s acceleration.

## IV. Non-linear Gradients for Faster Analyses



GEMBE separation of five dmsp-labeled amino acids. (Top) Separation using a linear gradient of -0.5 Pa/s. (Bottom) 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 the two gradient parameters. Solid traces are Savitzky-Golay transformations of raw data; dashed lines are applied pressure. All amino acids were 1 mmol/L. Separation conditions: 3 cm capillary (30 μm i.d.); 1 cm separation length; 1000 V/cm; 1350 Pa initial pressure.

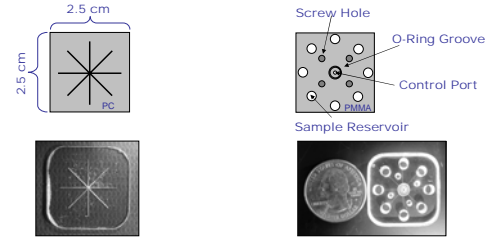
## V. GEMBE for Biomolecules



GEMBE separation of ssDNA 15 and 18 poly(cytosine)-mers and a random sequence of 72 bases using 1% hydroxyethyl cellulose as the sieving matrix. Black trace is raw intensity data. Blue trace is Savitzky-Golay transformed derivative. 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 μm i.d.); 1 cm separation length; -900 V/cm; 2500 Pa initial pressure; -10 Pa/s acceleration.

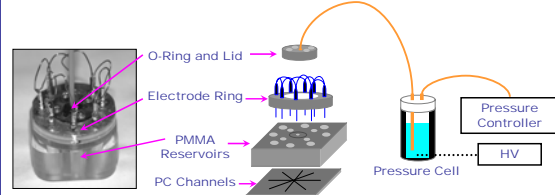
Serial GEMBE insulin immunoassay. Inset shows the zones arising from free FITC-labeled insulin (F) and FITC-labeled insulin bound to anti-insulin antibody (B). Calibration was generated by introduction of varying amounts of labeled insulin in the presence of 4 μmol/L FITC-labeled insulin and 2 μmol/L antibody. Error bars are 1 standard deviation (n=3). Separation conditions: 0.5 mol/L Tris-hydroxyl acid buffer; 3 cm capillary (30 μm i.d.); 1 cm separation length; -900 V/cm; 1850 Pa initial pressure; -20 Pa/s acceleration.

## VI. Multiplexed Microfluidic Separations

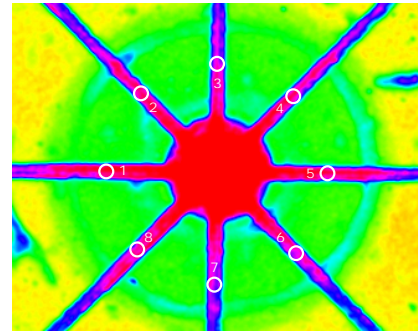


Microdevices were fabricated by computer numerical control (CNC) milling into 380 μm thick polycarbonate (PC) sheets. Milled channels were 40 μm deep, 150 μm wide, and 1 cm long. (Top) Microdevice schematic diagram of the channel pattern in the PC layer. (Bottom) Photograph of milled channels in PC.

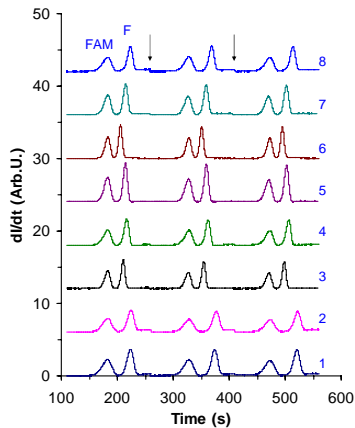
A poly(methylmethacrylate) (PMMA) piece was milled to contain a common waste access port and 75 μL volume sample reservoirs as well as an O-ring groove and screw pilot holes which were tapped after thermal bonding to the PC layer. (Top) Microdevice schematic diagram of the fluid reservoirs and connections in the PMMA layer. (Bottom) Photograph of a bonded device; a U.S. quarter is included for size reference.



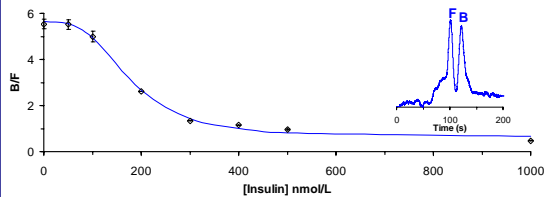
Complete microdevice for performing multiplexed GEMBE separations. (Left) Photograph of assembled device with lid and grounding electrode ring. (Right) Schematic of microdevice assembly. The single control port is connected by PEEK tubing to a pressure cell containing background electrolyte. The cell is controlled by both a pressure controller and a high voltage supply.



False color fluorescence image of detection region of chip (channels filled with fluorescein and FAM). The common waste port and the eight radially arranged channels are seen as red. The eight detection points are shown as the small white circles.



Derivative plots for parallel separation of fluorescein (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 (delimited by arrows). Separation conditions: 7.1 mm long channels; 1056 V/cm; 0 Pa initial pressure; -0.5 Pa/s acceleration.



Simultaneous microfluidic GEMBE generation of insulin immunoassay calibration curve. Each channel contained a mixture of 500 nmol/L FITC-labeled insulin, 200 nmol/L Ab and varying amounts of insulin (from 0 to 1 μmol/L). Inset shows Savitzky-Golay transformed derivative of the 500 nmol/L insulin sample showing the separation of free (F) and bound (B)-labeled insulin (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.

## VII. Conclusions

GEMBE is well suited to microfluidics when considering scalability as shown in parallel devices. The method provides high resolution separations in short microchannels without the additional access ports or equipment needed for injection formation. GEMBE allows for 'on-the-fly' resolution adjustment and gradient separations can more efficiently utilize the separation space. Additionally, sample zones can be interrogated for longer times and the counter flow allows for exclusion of matrix interferences.

## VIII. Acknowledgements

The authors gratefully acknowledge the financial support of the NIST/NRC Postdoctoral Research Program.