What Every Analytical Chemist Should Know about CE-MS

Summer Teaching Series 2019
Part 1

Scott Mellors

You should leave here understanding these things:

- CE is really simple
- CE separates molecules based on *mobility*
  - **A function of molecular charge/size**
- CE column diameters have to be small because of Joule Heating
- CE has no fundamental speed limit or size limit!
- CE + MS is a great match, but coupling is a little tricky
- Surface chemistry is very important

Thanks JJ!

1983

Capillary Zone Electrophoresis

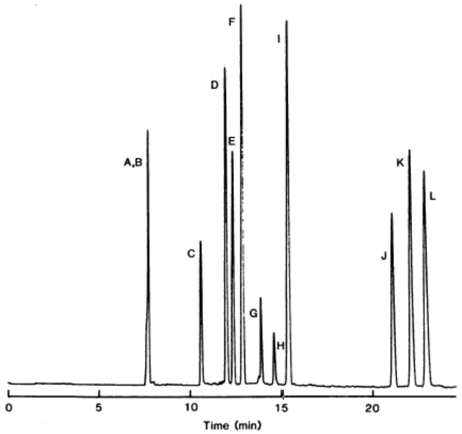
James W. Jorgenson and Krynn DeArman Lukacs

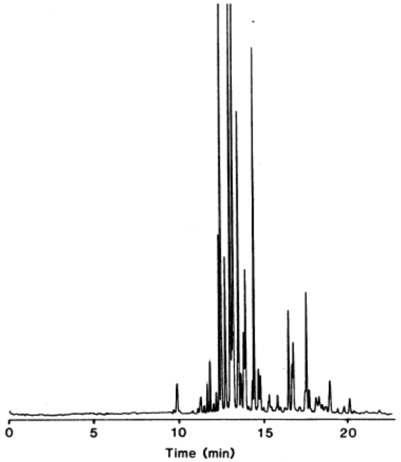
Electrophoresis has developed into a powerful technique for the separation and analysis of charged substances, especially biopolymers. In large part, the success of modern electrophoresis rests on the effective utilization of stabilizing media such as polymer gels. These gels stabilize the separation medium against convection and flow, which would otherwise disrupt separations. A large part of the science of modern electrophoresis is devoted to understanding and controlling the formation of these gels (*1*, *2*). Still, electrophoresis as commonly practiced would not be considered an instrumental method of analysis. True instrumental versions of electrophoresis analogous to modern column chromatography are rather rare. In part, this can be traced to the essential role of stabilizing gels in electrophoresis. Because of the presence of gels, the method has not been easily adapted to on-line sample application, detection, quantification, or automated operation. Instead, modern electrophoresis is a powerful and yet manual-intensive methodology.

Instrumental versions of electrophoresis have been developed. Among these the "rotating tube" system of Hjerten (*3*) and the "transient-state isoelectric focusing" technique of Catsimpoolas (*4*) are particularly notable. These techniques, although novel and quite powerful, have failed to come into routine usage due to their complexity. Capillary isotachophoresis is probably the only instrumental version of electrophoresis to see extensive application, although here, too, acceptance has been slow. The unconventional format of data output in isotachophoresis, coupled with the fact that it appears better suited for separations of relatively small molecules, is the probable reason for its slow acceptance (*5*).

In the course of considerations of causes for zone broadening in zone electrophoresis, it occurred to us that an "open" capillary tube—that is, one containing buffer without stabilizer—offered a unique and simple situation in which to study electrophoresis. In such a system electrophoresis could be studied with minimal interferences, and at the same time causes of zone broadening could be

J. W. Jorgenson is an assistant professor of chemistry and K. D. Lukacs is a graduate student in the Department of Chemistry, University of North Carolina, Chapel Hill 27514.





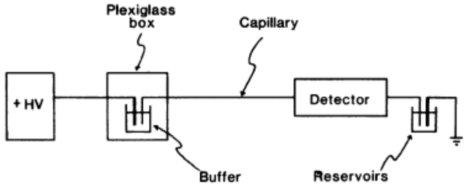


Fig. 1. Schematic of a capillary electrophoresis system.

phoresis in open tubular capillaries. A buffer-filled capillary is suspended between two reservoirs filled with buffer. Samples are introduced at one end and, under the influence of an applied electric field, migrate toward the other end of the capillary. Just before leaving the capillary, sample zones migrate through a detector, which senses their passage, yielding a recording of detector response versus time which is analogous to a chromatogram but is called an electropherogram. Because of the parallels between this system and a chromatographic system, it is natural to borrow such concepts as migration time (retention time), theoretical plates, and resolution from chromatography.

In capillary zone electrophoresis, the migration time for a solute is given by

$$t = \frac{L^2}{\mu V} \quad (1)$$

where t is a solute's migration time, L is the tube length, μ is the solute's electrophoretic mobility (electrophoretic velocity in a unit electric field), and V is the applied voltage. The separation efficiency, in terms of the total number of theoretical plates, N, is

$$N = \frac{\mu V}{2D} \quad (2)$$

where D is the solute's diffusion coefficient. These two equations are the basis of some interesting predictions. First, high separation efficiencies are best achieved through the use of high voltages. Electrophoretic mobilities and diffusion coefficients are independent of voltage; heat dissipation is decreasing. At some point significant thermal effects will ultimately appear, placing a practical limit on how short a tube can be used with a particular applied voltage. This conclusion assumes that the capillary diameter, voltage, and buffer composition have remained constant.

In the previous equations of migration time and separation efficiency an important phenomenon, electroosmosis, was neglected. Electroosmosis is the flow of liquid that occurs when an electrical potential is applied to a liquid-filled porous medium. In an unobstructed capillary the shape of the electroosmotic flow profile is piston-like. The flow velocity is constant over most of the tube cross section and drops to zero only near the tube walls (*11*). This is fortunate as the flat flow profile of electroosmosis will add the same velocity component to all solutes, regardless of their radial position, and will thus not cause any significant dispersion of the zone. The more familiar parabolic laminar flow profile, such as occurs in capillaries in ordinary hydraulic flow, would lead to serious zone spreading. Electroosmotic flow does, however, modify the equations for migration time and separation efficiency. The migration time becomes

$$t = \frac{L^2}{(\mu + \mu_{osm})V} \quad (3)$$

and the separation efficiency is now

$$N = \frac{(\mu + \mu_{osm})V}{2D} \quad (4)$$

way. The only effect of rapid electroosmotic flow is to sweep all solutes quickly through the capillary, leaving little time for zones to separate. The resulting zones will be sharper (increased theoretical plates) but more poorly resolved. It is resolution of zones that we ultimately wish to accomplish. Following the approach of Giddings (*12*), we have derived an equation to predict the resolution of two zones in capillary zone electrophoresis.

$$R_s = 0.177\,(\mu_1 - \mu_2)\left[\frac{V}{D(\bar{\mu} + \mu_{osm})}\right]^{1/2} \quad (5)$$

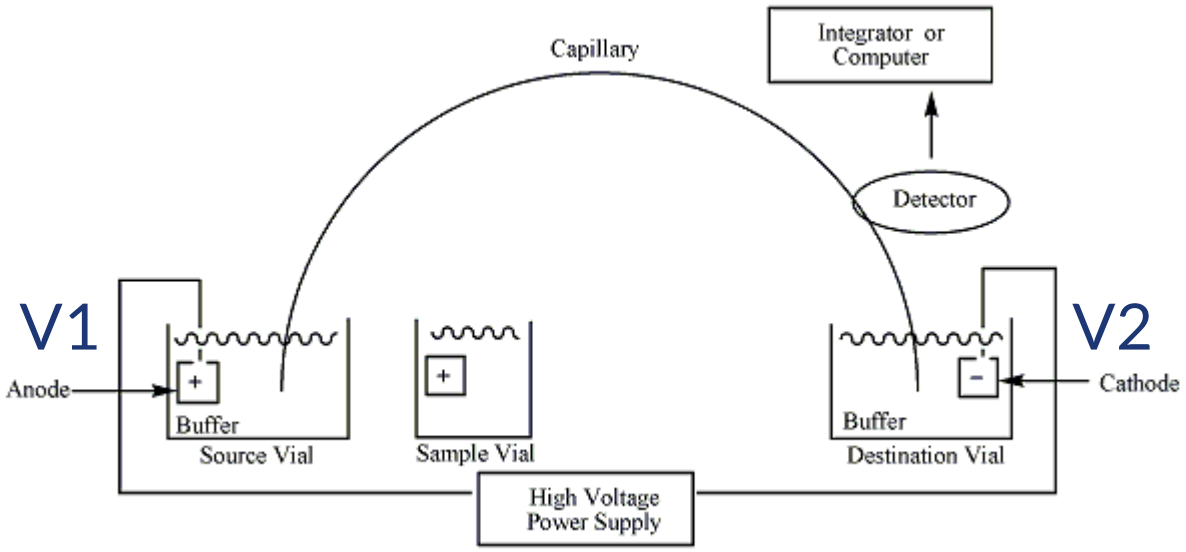
where R_s is the resolution, μ_1 and μ_2 are the electrophoretic mobilities of the two solutes, and $\bar{\mu}$ is their average mobility (*10*). From this equation it is clear that the resolution of two zones will be poorer if there is a large component of electroosmotic flow in the same direction as electrophoretic migration. In fact, good resolution of substances having very similar mobilities can be achieved by balancing electroosmotic flow against electrophoretic migration ($\mu_{osm} = -\bar{\mu}$). The cost of this approach to higher resolution is long analysis time. This is readily apparent by referring to Eq. 3 and assuming that the electroosmotic flow coefficient and the electrophoretic mobility are equal in magnitude but opposite in sign (direction).

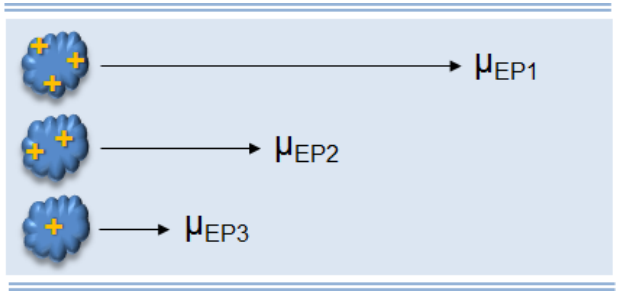
Description of System

A schematic diagram of the system we use to perform electrophoresis in capillaries is shown in Fig. 1. In almost all cases the strong electroosmotic flow carries solutes, regardless of charge, toward the negative (grounded) electrode. For this reason samples are usually introduced at the positive (high-voltage) end, and a detector capable of sensing zones within the capillary (on-column detector)

CE Basics

- Capillary Zone Electrophoresis (CZE)
  - **About the simplest method you could imagine!**





$$\mu_{EP} = \frac{q}{6\pi\eta a}$$

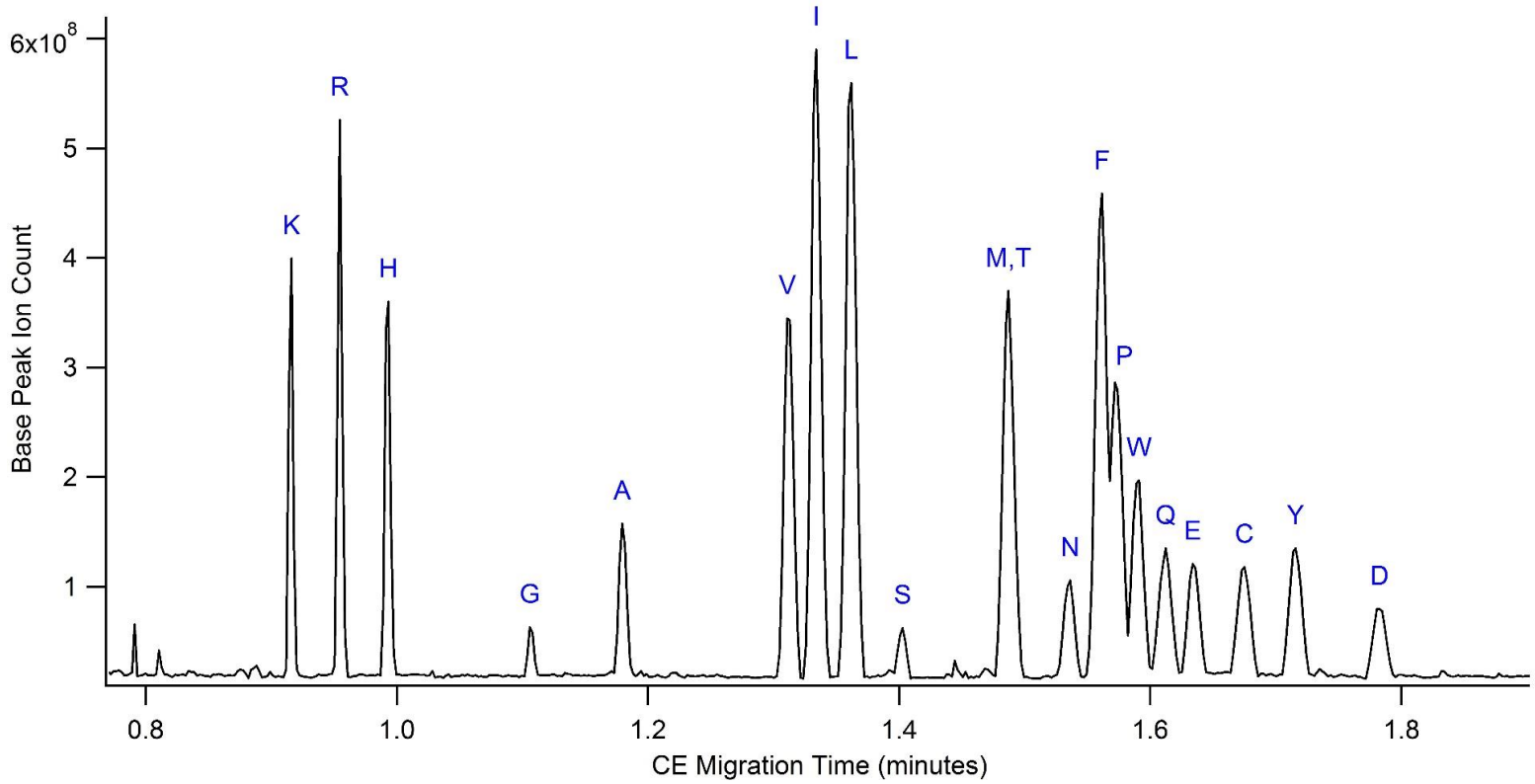
q - charge
η - viscosity
a - hydrodynamic radius

- More charge = faster migration
- Bigger hydrodynamic radius = slower migration

CE Basics - Definitions

- Electropherogram
- Migration time
- Background Electrolyte (BGE)
- Field strength
- Mobility

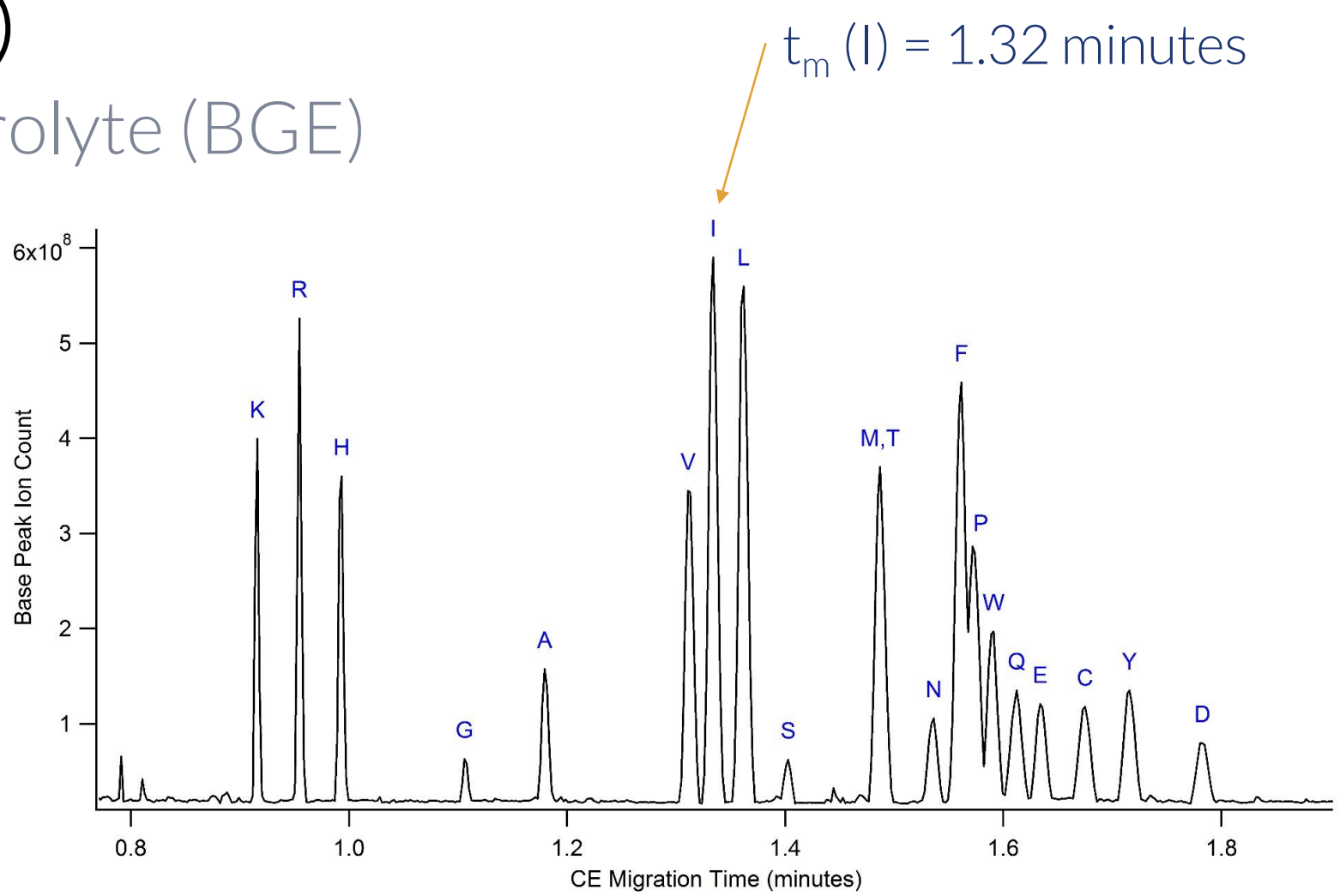
The CE equivalent of a chromatogram



CE Basics - Definitions

- Electropherogram
- Migration time (t_m)
- Background Electrolyte (BGE)
- Field strength
- Mobility

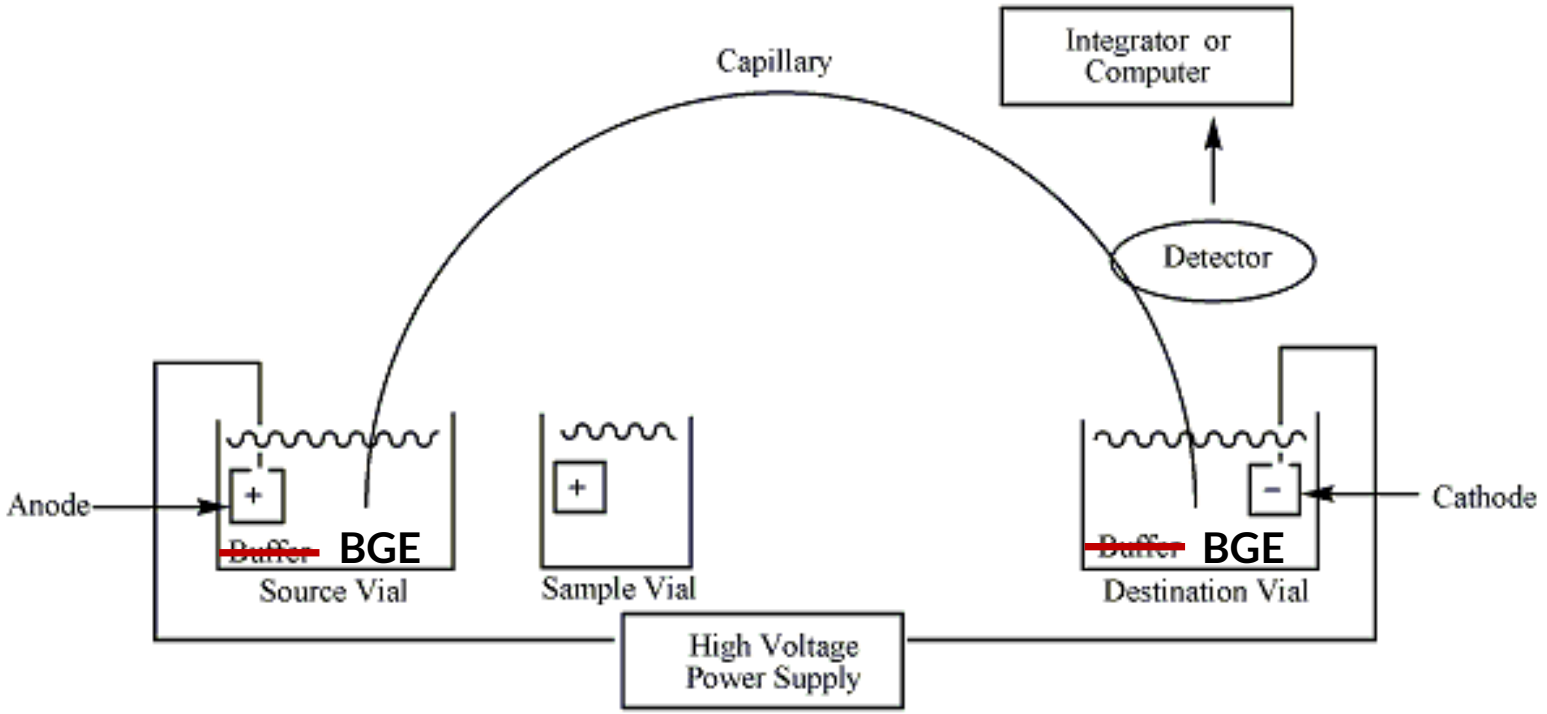
The CE equivalent of retention time



CE Basics - Definitions

- Electropherogram
- Migration time
- Background Electrolyte (BGE)
- Field strength
- Mobility

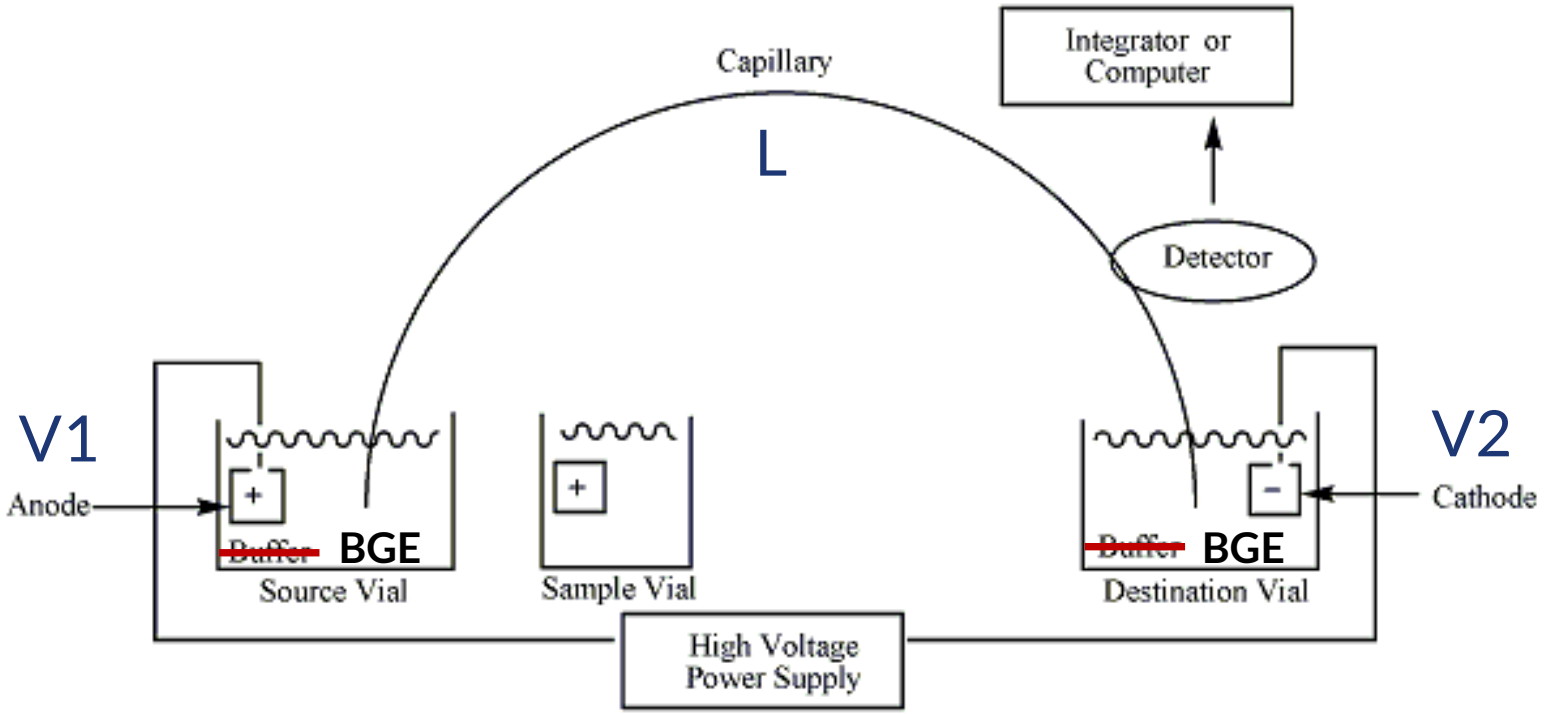
BGE is just the liquid that fills the CE column. For CE-MS this liquid is often not technically buffered, so calling it a buffer is not accurate. It's the CE equivalent of a mobile phase.



CE Basics - Definitions

- Electropherogram
- Migration time
- Background Electrolyte (BGE)
- Field strength (*E*)
- Mobility

$$E = (V1-V2)/L \quad \text{(Volts/cm)}$$



CE Basics - Definitions

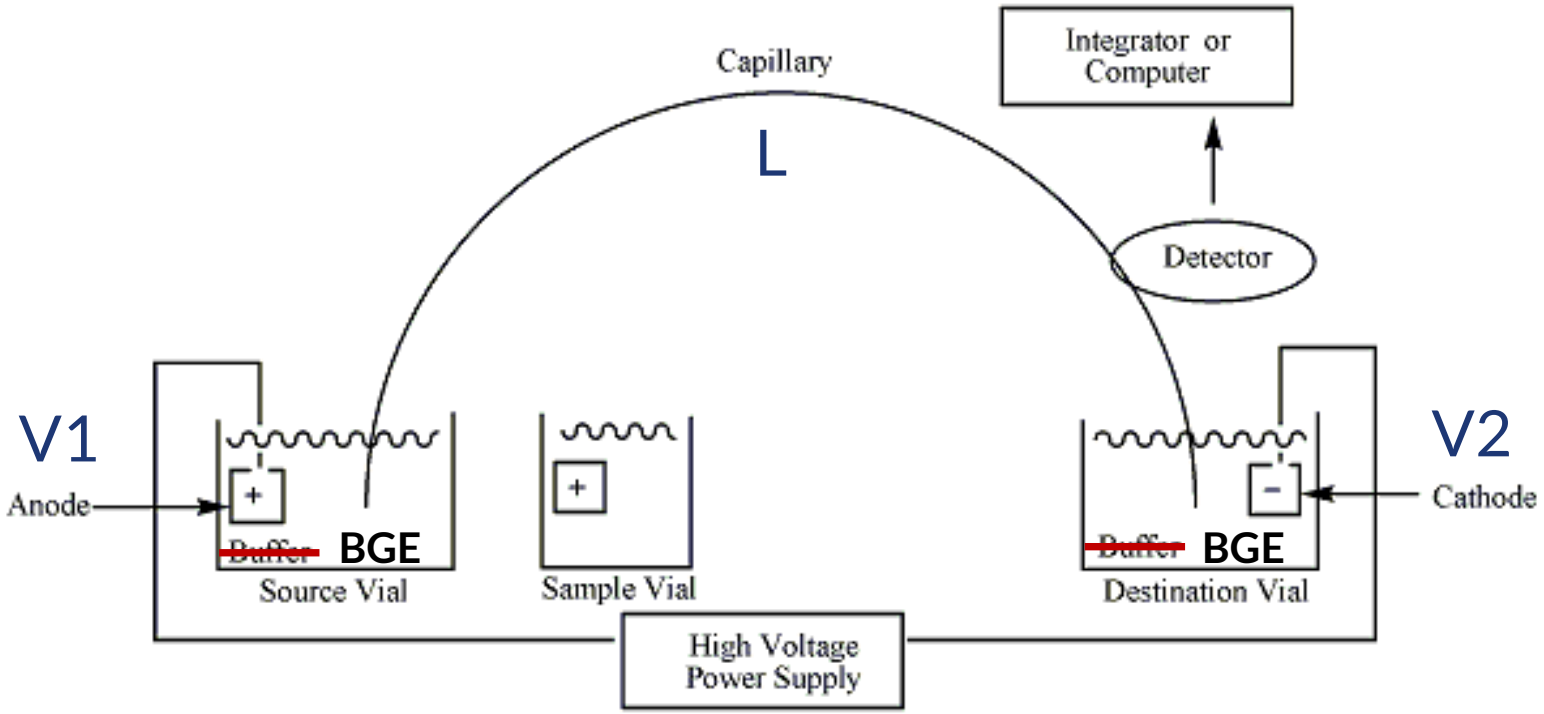
- Electropherogram
- Migration time
- Background Electrolyte (BGE)
- Field strength (*E*)
- Mobility (μ)

Mobility defines the velocity of a molecule as a function of field strength

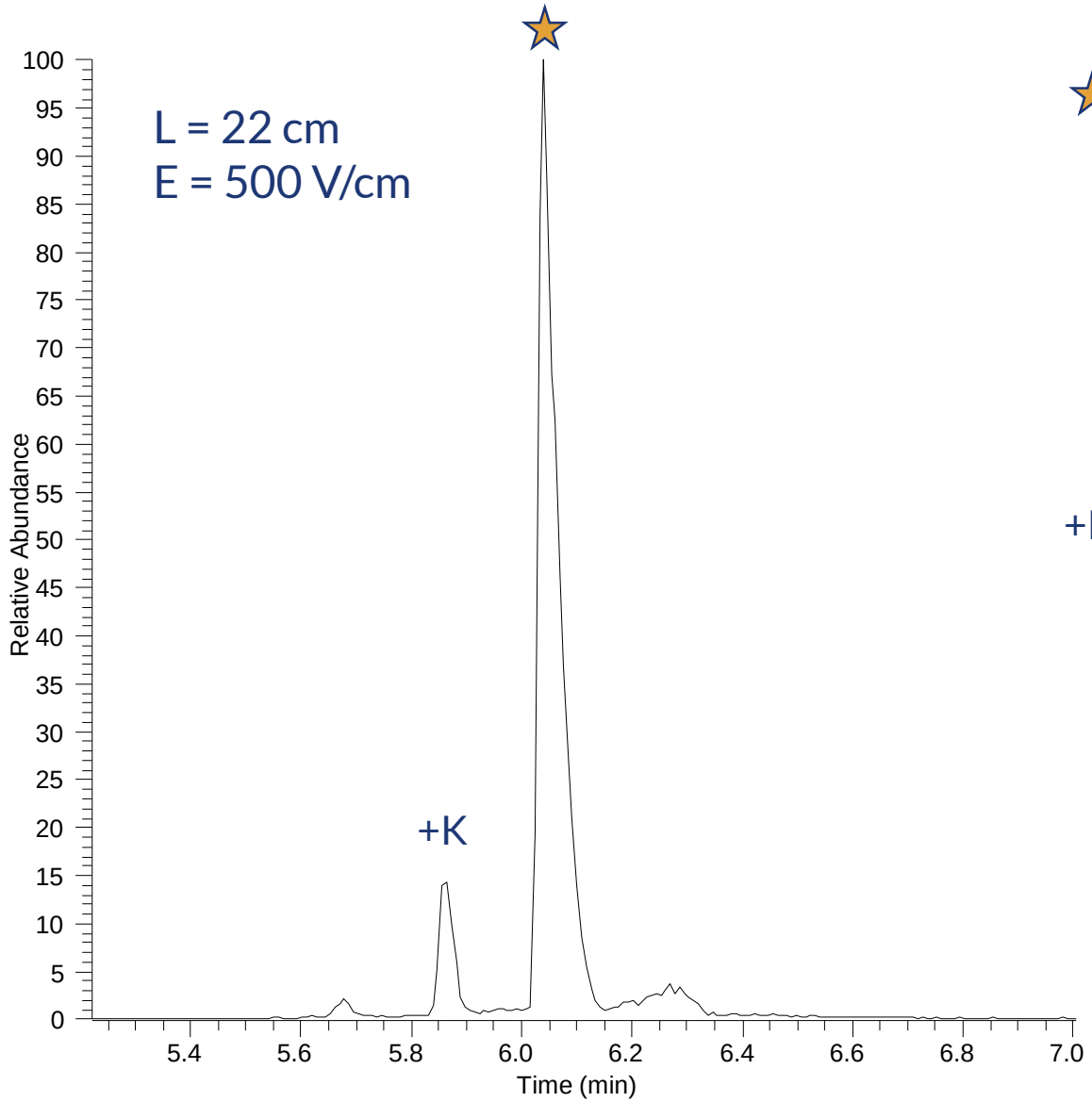
$E = (V1-V2)/L$ (Volts/cm)

Velocity (v) = L/t_m (cm/s)

$v = \mu E$



A Quick Example



★
- t_m = 6.05 m = 363 s
- $v = L/t_m$ = 22 cm ÷ 363 s = 0.061 cm/s
- $v = \mu E$, so
- $\mu_1 = v/E$ = 0.061 cm/s ÷ 500 V/cm = 1.21 E-4 cm^2/Vs

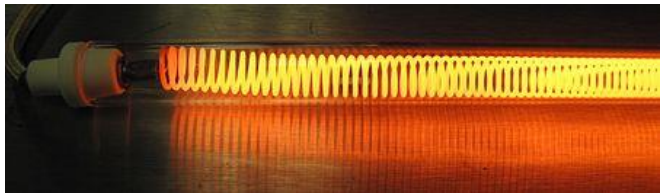
+K
- t_m = 351.6 s
- v = 0.0626 cm/s
- μ_2 = 1.25 E-4 cm^2/Vs

This is a CE-MS separation of a monoclonal antibody. The +K peak is identical to the main peak except is has an additional lysine. That gives it an extra positive charge, which increases its mobility

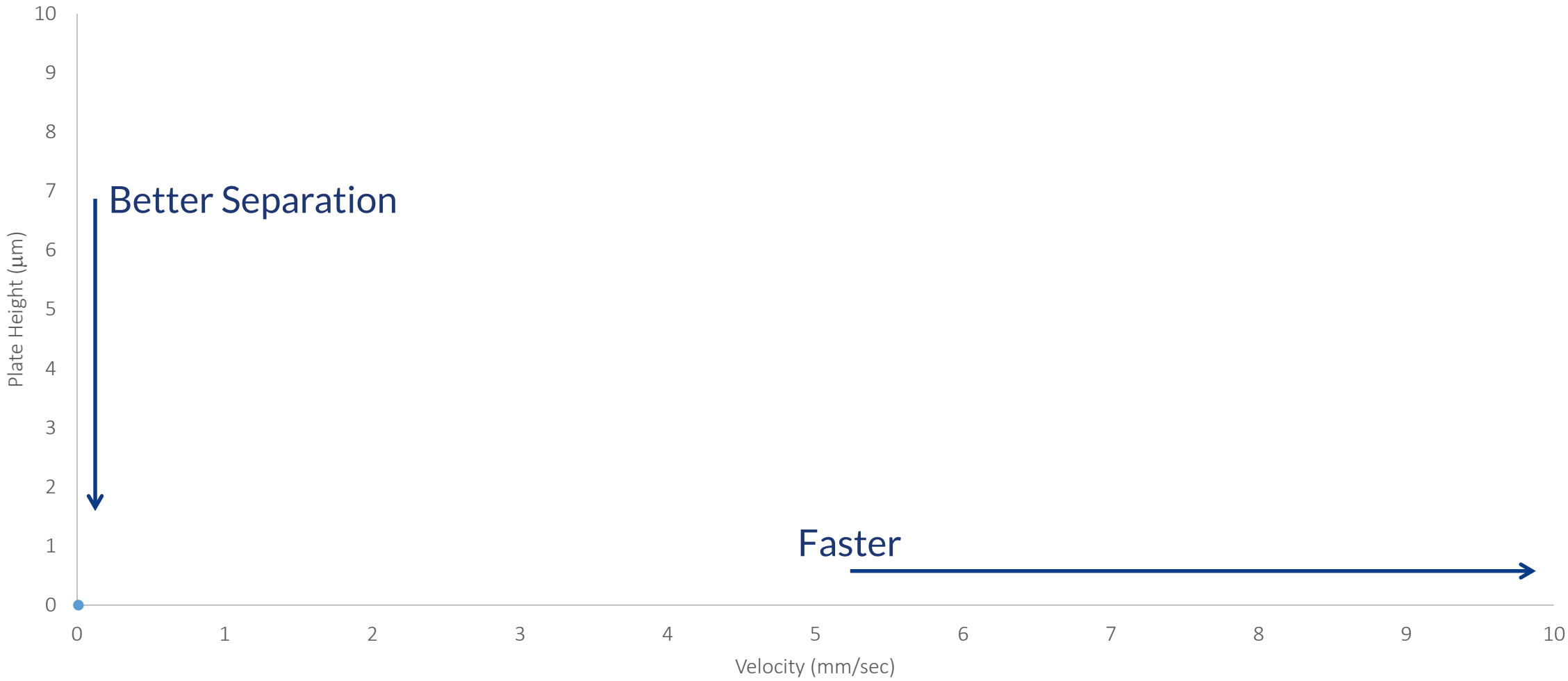
Why does it matter if CE is done in a narrow capillary?

- The CE column is a resistor in an electrical circuit
- All the electronics you need to know:
  - ***V = IR*** ***voltage = current x resistance***
  - ***P = IV*** ***power = current x voltage***
- From that you can combine to see that:
  - $P = V^2/R$
- In a CE separation:
  - **Too much heat in the column is bad**
  - **Resistance scales inversely with the square of the column diameter, *so***
  - **Power (and therefore heat generation) scales with d_c^2**
  - **Heat dissipation scales with $1/d_c$**
- The take home message: *Smaller diameter capillaries generate way less heat and are more efficient at dissipating that heat*
- Most CE is done with column diameters in the range of 30 μm to 75 μm

Joule Heating

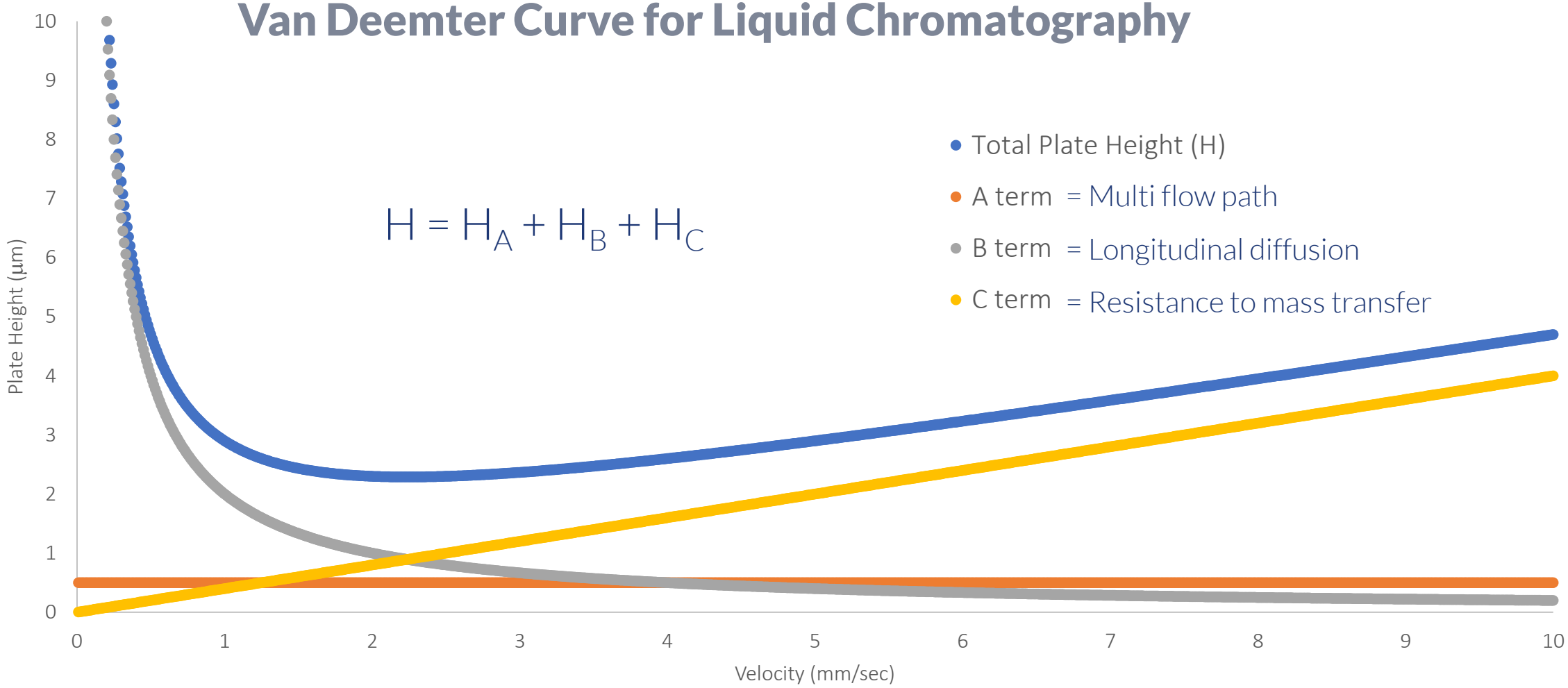


CE Basics – Separation Efficiency



CE Basics – Separation Efficiency

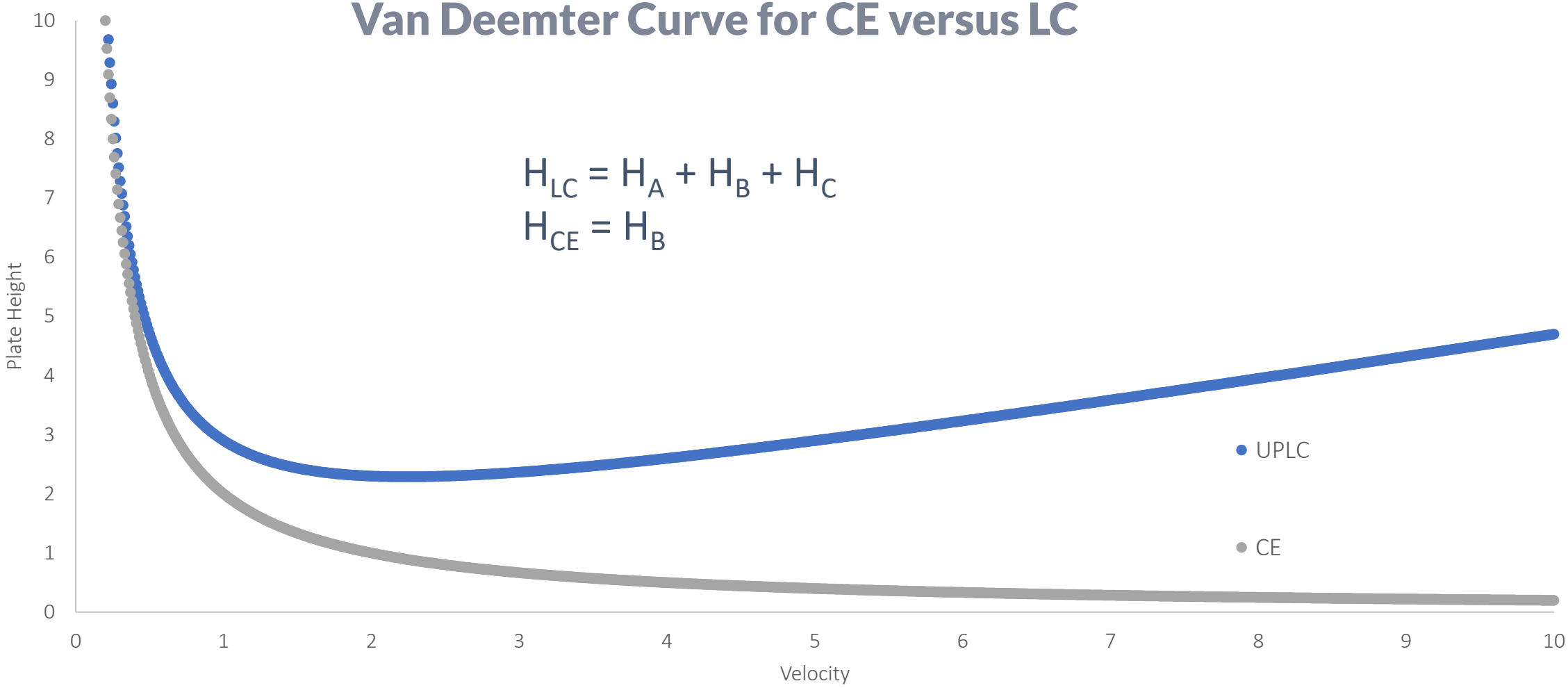
Van Deemter Curve for Liquid Chromatography



CE Basics – Separation Efficiency

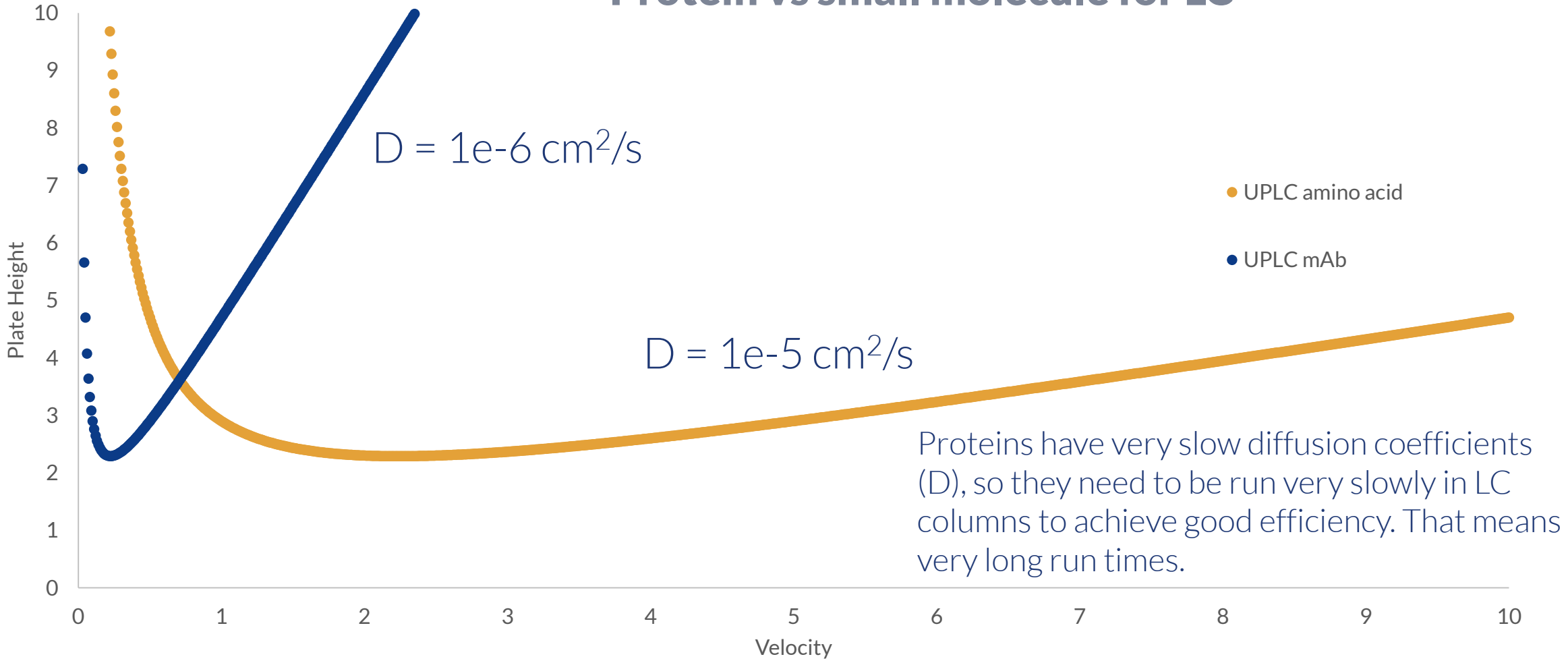
Van Deemter Curve for CE versus LC

$$H_{LC} = H_A + H_B + H_C$$
$$H_{CE} = H_B$$



CE Basics – Separation Efficiency

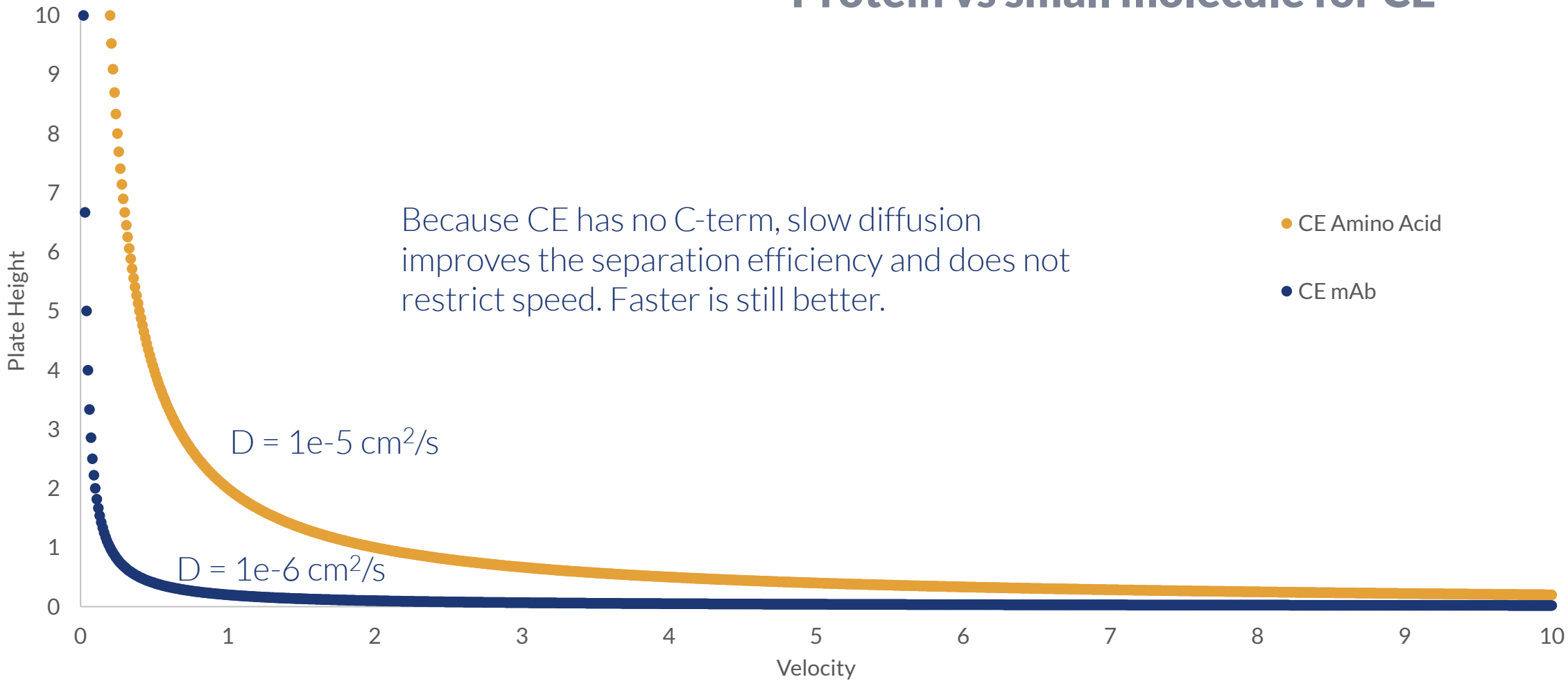
Protein vs small molecule for LC



Proteins have very slow diffusion coefficients (D), so they need to be run very slowly in LC columns to achieve good efficiency. That means very long run times.

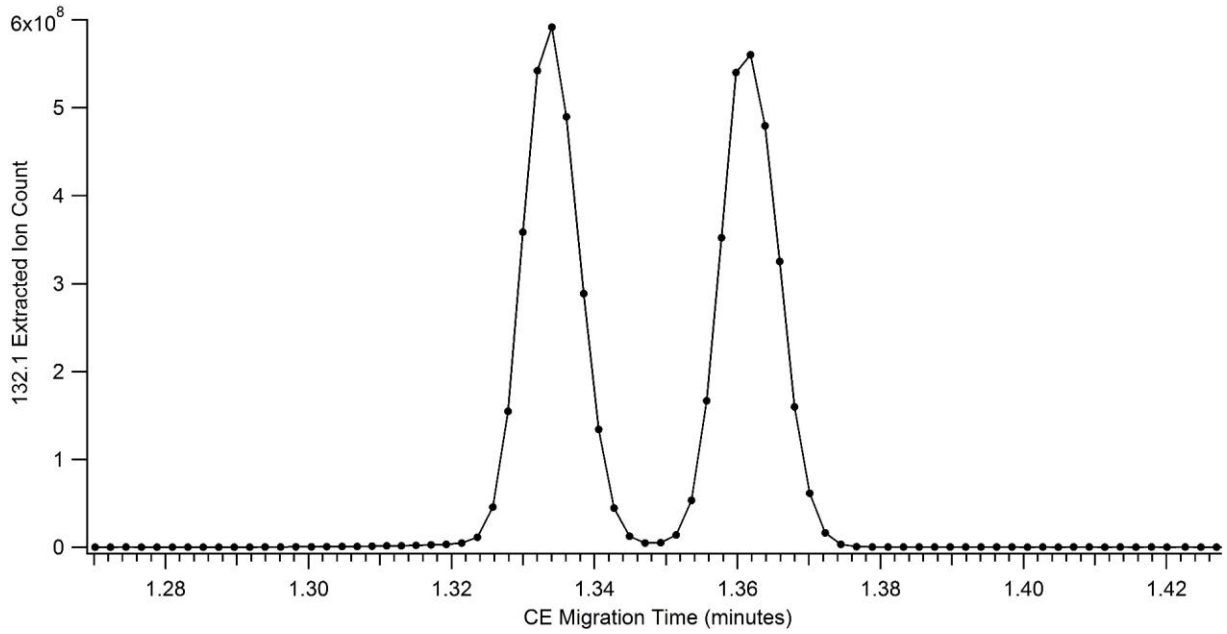
CE Basics – Separation Efficiency

Protein vs small molecule for CE



CE Basics – Separation Efficiency – Key Points

- Band-broadening is simpler for CE than for LC
- Good CE separations are “diffusion limited”
- Faster is better for CE!
- Bigger is better for CE!



Can we predict the efficiency of CE separations?

Theoretical Plates (N) = L^2/σ^2

Diffusion limited separation means that $\sigma^2 = 2Dt_m$

$v = L/t_m$

$v = \mu E = \mu \Delta V/L$

$N = L^2/2Dt_m$

$N = vL/2D$

$N = \mu EL/2D$

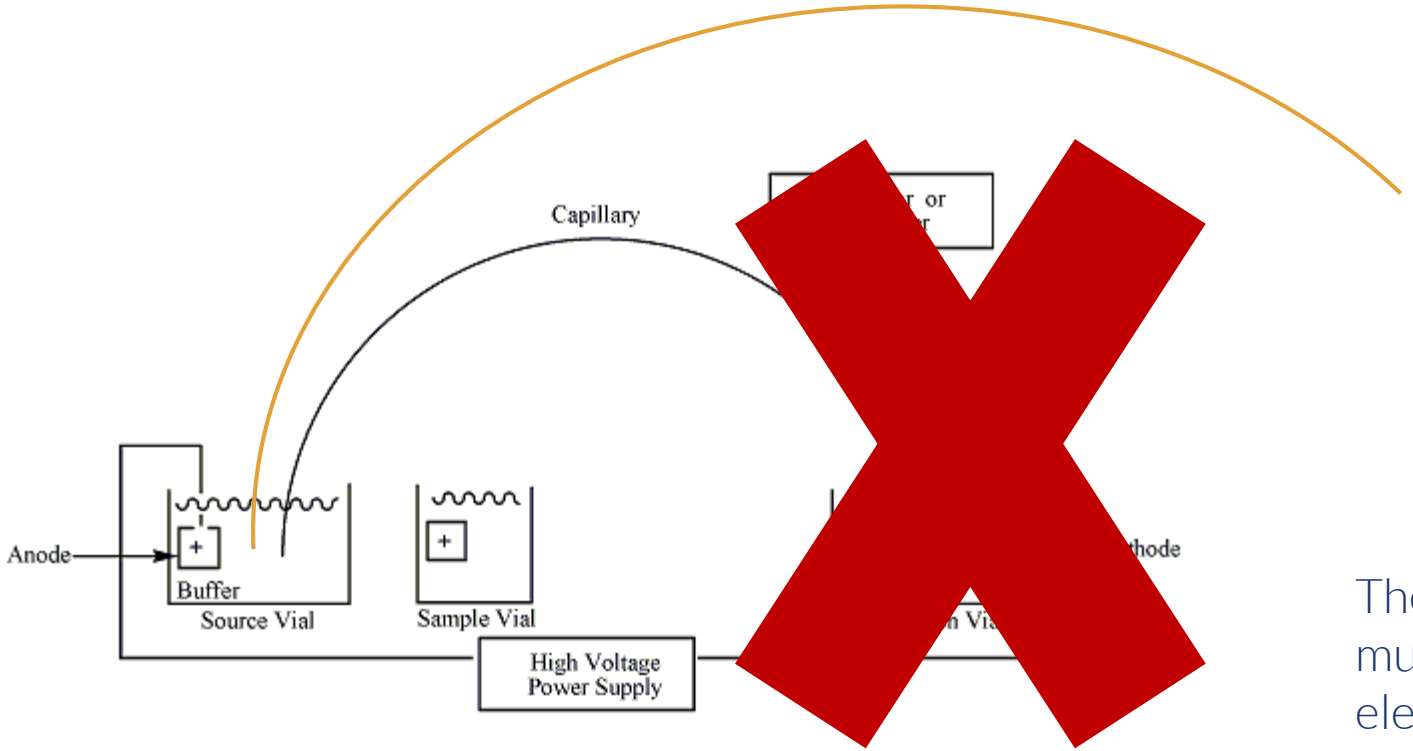
$N = \mu \Delta V/2D$ →

- Column length doesn't matter!
- To separate better, just turn up the voltage!

Efficiency is only a function of mobility, diffusion coefficient and voltage applied

Why is it hard to couple CE with MS?

Why can't we just do this?



- CE current = microAmps
- ESI current = nanoAmps

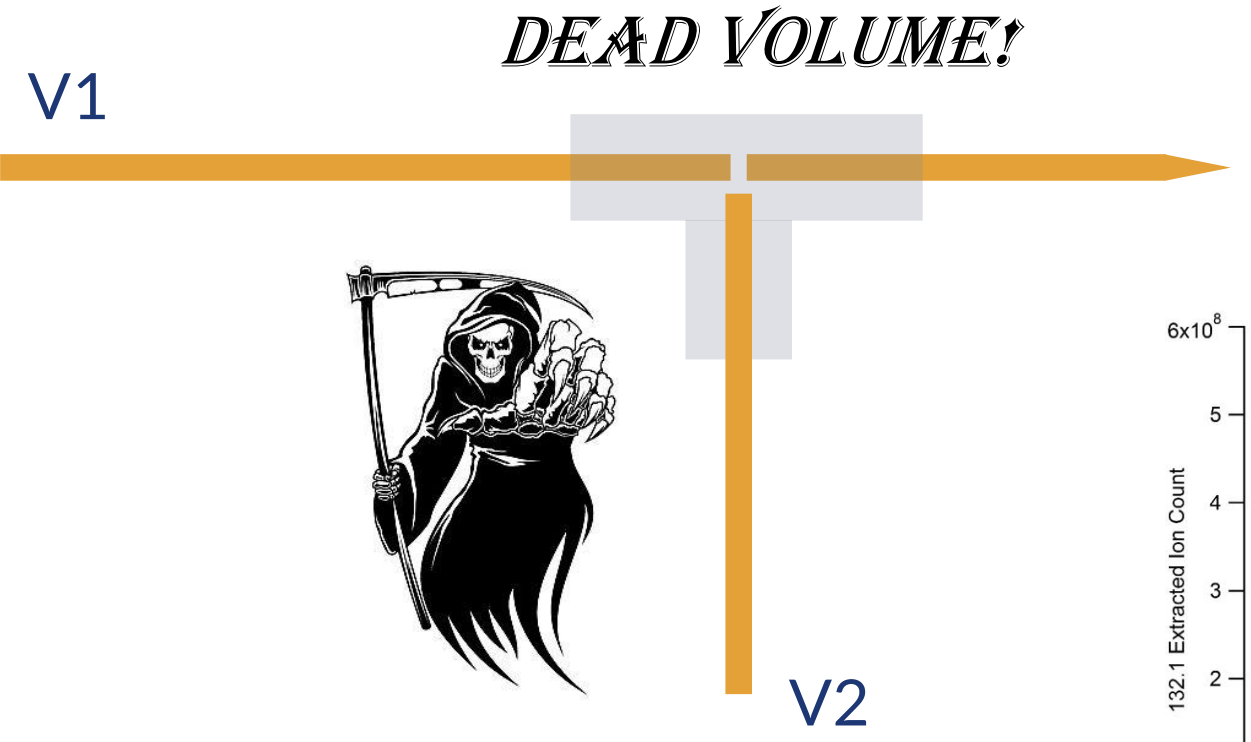
MS Inlet

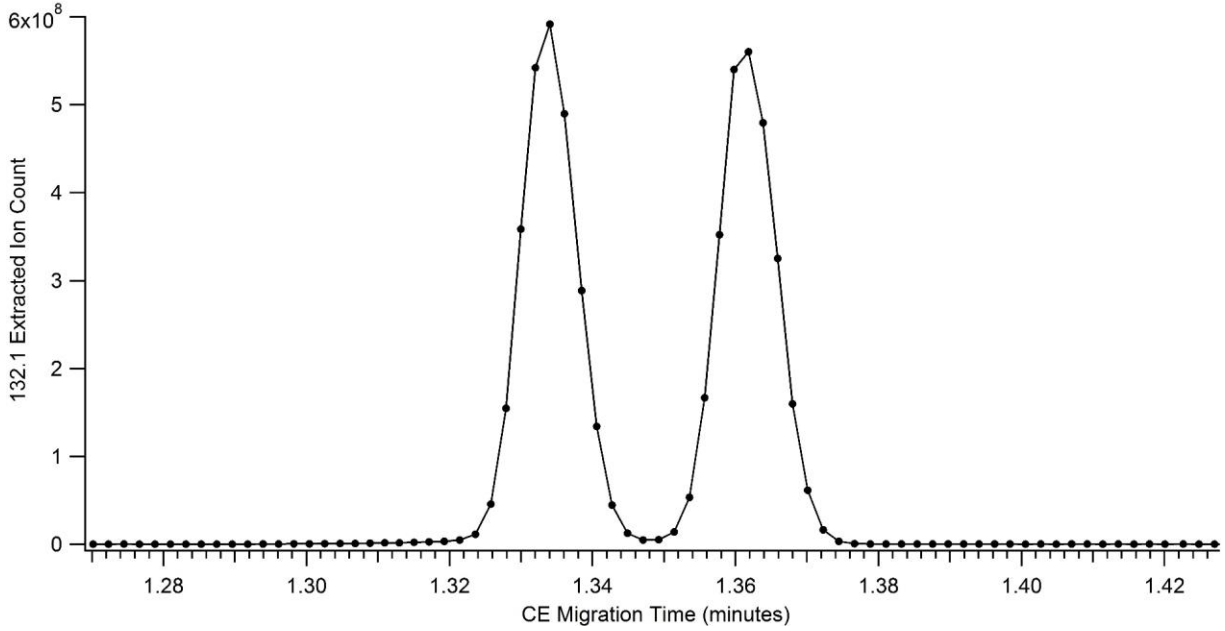
The electrical current of the CE separation is much greater than the current of the electrospray. So we need an electrode connected to the end of the separation column to complete the circuit.

Why is it hard to couple CE with MS?

Why can't we just do this?

- 10 cm x 30 μm i.d.
- Column volume = 70 nL
- *u* = 10 cm / 80 s = 0.125 cm/s
- Peak width = 1 s → 1.25 mm
- Volume of bands = 0.88 nL





What's a typical peak volume for LC?

- Nano-LC
  - **75 μm i.d. capillary column run at 300 nL/min (5 nL/s)**
  - **Peak width ~10 s**
  - **Band volume = 50 nL**
- UPLC
  - **1 mm i.d. column run at 100 μL/min (1.67 μL/s)**
  - **Peak width ~5 s**
  - **Band volume = 8.3 μL = 8300 nL**

Even the smallest LC columns generate peak volumes that are much bigger than good CE separations. So fittings that could be used to attach a spray tip to an LC column would ruin a CE separation.

The fundamental challenge for coupling CE with MS

- We need a junction to terminate the CE circuit, but…
- The column has to be very narrow to prevent joule heating, and…
- The separation is super efficient, so the peaks are very narrow, so…
- We can't afford to have any dead volume in the flow path, or else we'll ruin the great separation and defeat the whole purpose!!!

- So how do we create the junction without introducing too much dead volume?

Early attempts at CE-MS

436 Anal. Chem. 1988, 60, 436–441

Capillary Zone Electrophoresis–Mass Spectrometry Using an Electrospray Ionization Interface

Richard D. Smith,* José A. Olivares,[1] Nhung T. Nguyen, and Harold R. Udseth

Chemical Methods and Separations Group, Chemical Sciences Department, Pacific Northwest Laboratory, Richland, Washington 99352

Instrumentation developed for capillary zone electrophoresis–mass spectrometry (CZE–MS) is described. The interface is based upon direct electrospray ionization from the end of the CZE capillary. The electrospray ionization source functions at atmospheric pressure and provides excellent sensitivity for wide ranges of compounds, with detection limits generally in the femtomole range (although significant improvements appear feasible). The instrumentation allows the high separation efficiencies feasible with CZE to be exploited and offers potential advantages compared with LC–MS methods, particularly when only small samples are available or high-resolution separations are necessary. The performance of the electrospray interface and the techniques and operating conditions for CZE–MS separations are described. CZE–MS separations and mass spectra are shown for mixtures that include polypeptides and quaternary ammonium salts. Separation efficiencies and detection limits vary widely from compound to compound and are shown to be sensitive to buffer selection. Separation efficiencies exceeding half a million theoretical plates are demonstrated for some compounds. Wider application and improved performance are anticipated with minimization of CZE band spread (due to adsorption and possibly other processes) and optimization of CZE buffers (for both the separation and their compatibility with electrospray ionization).

detection methods (UV and fluorescence). Clearly, however, if detection limitations could be addressed CZE would provide a powerful analytical tool for a wide range of problems, particularly where only exremely small samples are available or where high separation efficiencies are required.

In a recent communication we described the first on-line combination of CZE with mass spectrometry (*5*), which also represented the first reported *direct* combination of any electrophoretic separation technique with mass spectrometry. This development was based upon the recognition that both ends of the CZE capillary did not have to be immersed in buffer reservoirs and provided a basis for new detection methods in which the electroosmotically induced flow could be analyzed at the column exit. The strong electroosmotic flow in CZE, which results from the substantial ζ potential of most suitable capillary surfaces, is suffficiently large under many conditions to result in elution of ions with both positive and negative electrophoretic mobilities. In our initial work it was shown that an electrospray could be produced at the capillary terminus, providing the basis for a viable CZE–MS interfacing method (*5*). Here we report details of the CZE–MS interface and describe new instrumentation and methods. The application of electrospray ionization (ESI) in CZE–MS is described, typical spectra and CZE–MS separations are presented, and current limitations related to both CZE and ESI are discussed. Future approaches to realizing the impressive potential of CZE–MS are briefly described.

EXPERIMENTAL SECTION

Figure 1. Schematic illustration of the CZE–MS instrumentation.

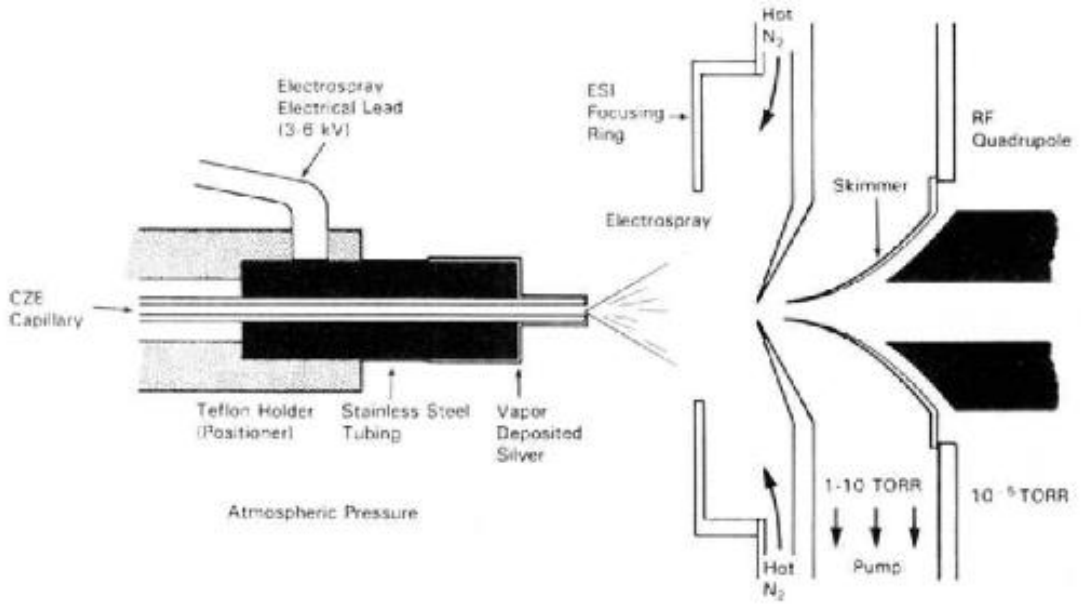


Figure 2. Detailed schematic of the electrospray ionization interface for CZE–MS (not to scale). The distance between the CZE capillary exit and the sampling nozzle is 1–2 cm.

Metal coatings applied to the surface of the capillary terminus solve the CE-MS junction problem, but they are not stable enough to be a practical solution.

Early attempts at CE-MS

Optimization of Capillary Zone Electrophoresis/Electrospray Ionization Parameters for the Mass Spectrometry and Tandem Mass Spectrometry Analysis of Peptides

M. A. Moseley* and J. W. Jorgenson
Department of Chemistry, University of North Carolina, Chapel Hill, North Carolina, USA

J. Shabanowitz and D. F. Hunt
Department of Chemistry, University of Virginia, Charlottesville, Virginia, USA

K. B. Tomer
Laboratory of Molecular Biophysics, National Institute of Environmental Health Sciences, Research Triangle Park, North Carolina, USA

The solution chemistry conditions necessary for optimum analysis of peptides by capillary zone electrophoresis (CZE)/electrospray ionization mass spectrometry and CZE electrospray ionization tandem mass spectrometry have been studied. To maximize the signal-to-noise ratio of the spectra it was found necessary to use acidic CZE buffers of low ionic strength. This not only increases the total ion current, but it also serves to fully protonate the peptides, minimizing the distribution of ion current across the ensemble of possible charge states.

The use of acidic buffers protonates the peptides, which is advantageous for mass spectrometry and tandem mass spectrometry analysis, but is problematic with CZE when bare fused silica CZE columns are used. These conditions produce positively charged peptides, and negatively charged silanol moieties on the column wall, inducing adsorption of the positively charged peptides, thus causing zone broadening and a loss in separation efficiency. This problem was circumvented by the preparation of chemically modified CZE columns, which, when used with acidic CZE buffers, will have a positively charged inner column wall. The electrostatic repulsion between the positively charged peptides and the positively charged CZE column wall minimizes adsorption problems and facilitates high efficiency separations. Full-scan mass spectra were acquired from injections of as little as 160 fmols of test peptides, with CZE separation efficiencies of up to 250,000 theoretical plates. *(J Am Soc Mass Spectrom 1992, 3, 289–300)*

Revolutionary developments occurred during the 1980s in the fields of separation science and mass spectrometry: capillary zone electrophoresis (CZE) and electrospray ionization (ESI). The development of CZE by Jorgenson and Lukacs [1, 2] yielded a separation system for ionic species based on their differential rates of migration in an electric field, offering a separation mechanism complementary to that of liquid chromatography (LC). The primary advantage of CZE over LC is a distinctly superior separation efficiency per unit time. CZE has been shown to be capable of generating in excess of 1,000,000 plates in less than 20 min [3], and in excess of 100,000 plates in less than 1 min [4]. While the high separation efficiencies inherent in electrophoresis have been recognized and utilized by biochemists for some time, it was the development of CZE that transformed electrophoresis from a slow, labor intensive, and highly variable separation technique into a rapid, fully automatable, and reproducible instrumental method of analysis [5, 6].

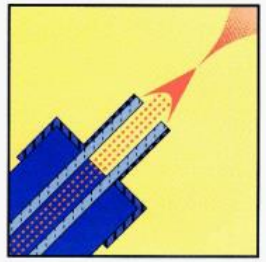
*Present address: Department of Drug Metabolism, Glaxo Research Institute, Research Triangle Park, North Carolina, USA.
Address reprint requests to M.A. Moseley, Department of Drug Metabolism, Glaxo Research Institute, 5 Moore Drive, Research Triangle Park, NC 27709.

1044–0305/92/$5.00

Received April 25, 1991
Revised October 29, 1991
Accepted October 29, 1991

Capillary Electrophoresis/ Mass Spectrometry

Richard D. Smith, Jon H. Wahl, David R. Goodlett[1], and Steven A. Hofstadler
Chemical Methods and Separations Group
Chemical Sciences Department
Pacific Northwest Laboratory
Richland, WA 99352



Many of the most difficult chemical, environmental, biochemical, and biomedical analytical problems require a combination of instrumental attributes, including speed, low detection limits, wide linear dynamic range, good sensitivity, and high selectivity. For such demanding applications, the on-line combination of separation methods with MS often provides the most practical or perhaps the only approach. The orthogonal nature of the selectivities provided by a chromatographic or electrophoretic separation in conjunction with MS has long been considered attractive. Indeed, GC/MS is firmly established as a definitive analytical technique for many environmental and clinical analyses.

The hallmarks of GC/MS are its speed, selectivity, and sensitivity. Unfortunately, however, both GC and the conventional ionization methods used in MS (primarily electron impact and chemical ionization) require sample volatilization. Thus, GC/MS is not amenable to many analytical problems without invoking often complex and problematic chemical degradation or derivatization procedures designed to modify sample components to "GC-able" forms. Interest in LC/MS has continued to grow, and the technique has begun to open new avenues for the characterization of biological and biomedical samples (*1*).

The 1980s saw the genesis and rapid development of a high-resolution separation method, capillary electrophoresis (CE), primarily because of the efforts of J. W. Jorgenson of the University of North Carolina (*2*). He and his co-workers have demonstrated that CE can generate both rapid and very high resolution separations, based on differences in the electrophoretic mobilities of charge-carrying species in an electric field, in small-diameter fused-silica capillaries.

The advantages of the capillary format for electrophoresis are multifold. First, small-diameter capillaries (generally 50–100-μm i.d.) generate less Joule heat and dissipate this heat more effectively, allowing higher electric fields than can be used with conventional electrophoresis and providing faster and higher resolution separations. Second, the capillary format allows for easy automation of sample handling and injection. The CE format allows ready implementation of a range of on-capillary detection methods; most effectively and broadly used are UV absorption and fluorescence emission detectors.

Since the first commercial CE instruments appeared in the late 1980s, CE technology and its applications have grown explosively. In fact, the rate of growth, use, and commercial implementation has considerably exceeded that seen earlier for LC methods. Improvements in injection methods, detector sensitivity, capillary surface deactivation, and coating technologies, as well as the introduction of new electrophoretic buffer systems, continue to drive further developments in CE for chemical, biological, and environmental applications.

The growth of CE as a viable analytical tool is primarily the result of advances in detection methods and an increasing recognition of its unique capabilities. CE would not be practical without the sensitivity improvements that have been demonstrated with on-capillary UV and fluorescence detection. Detectable amounts in the femtomole (10^{-15} mol) range can be obtained routinely, although optimized and specialized detection schemes have been reported for which detectable amounts extend to attomole (10^{-18} mol) and zeptomole (10^{-21} mol) levels. Thus, for CE with typical capillary diameters, in which effective injection volumes are generally in the range of 1–10 nL, routinely detectable concentrations are typically on the order of 10^{-6} M for the injected sample. Specialized detection systems allow these detectable concentrations to be extended to < 10^{-10} M, which is well into the regime of trace analysis. Improved detection limits can be obtained by using electrophoretic methods to concentrate sample components during injection.

The ability to manipulate and inject extremely small sample volumes, steps that are generally problematic with LC, provides a basis for using CE to confront extreme analytical challenges (e.g., the analysis of, or sampling from, single biological cells). In addition, CE has the flexibility provided by a range of formats (free-zone electrophoresis, electrokinetic micellar chromatography, isotachophoresis, gel electrophoresis, etc.) and a plethora of methods for manipulating injection conditions and separation specificity.

Moreover, methods have been developed or are being investigated for CE application to the analysis of practically any substance that can be dissolved or suspended in a liquid. Finally, from a pragmatic viewpoint, the small sample, buffer, and waste volumes required and generated by CE are much less than those used by

REPORT

[1] Current address: Immunobiology Research Institute, Route 22 East, P.O. Box 999, Annandale, NJ 08801

0003-2700/93/0365-574A/$04.00/0

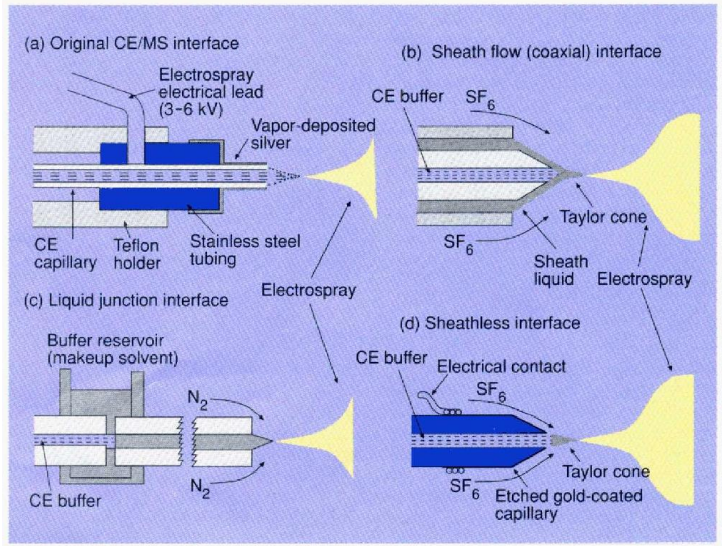


Figure 2. Schematic illustration of CE/electrospray interfaces.
(a) The original design utilizing a metallized capillary terminus, (b) sheath flow (coaxial) interface used for CE/MS, (c) ESI based on a liquid junction, and (d) a sheathless interface design.

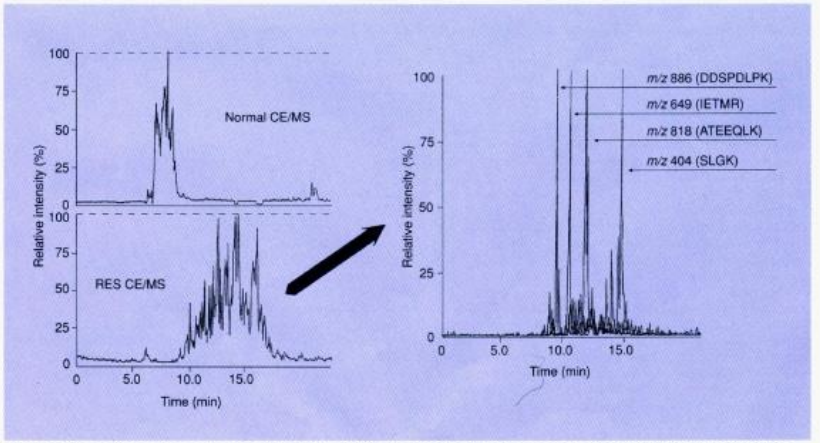


Figure 4. Comparison of normal constant field strength (top) and reduced elution speed (bottom) CE/ESIMS analysis of a tryptic digest of bovine serum albumin.
The reduced elution speed CE/ESIMS analysis was conducted at 300 V/cm until 1 min before elution of the first analyte, when the electric field strength was reduced to 60 V/cm. Single-ion electropherograms corresponding to several of the tryptic (polypeptide) fragments are shown on the right (designated by use of the single-letter code for amino acid residues). (Adapted with permission from Reference 17.)

Some newer examples of coupling strategies

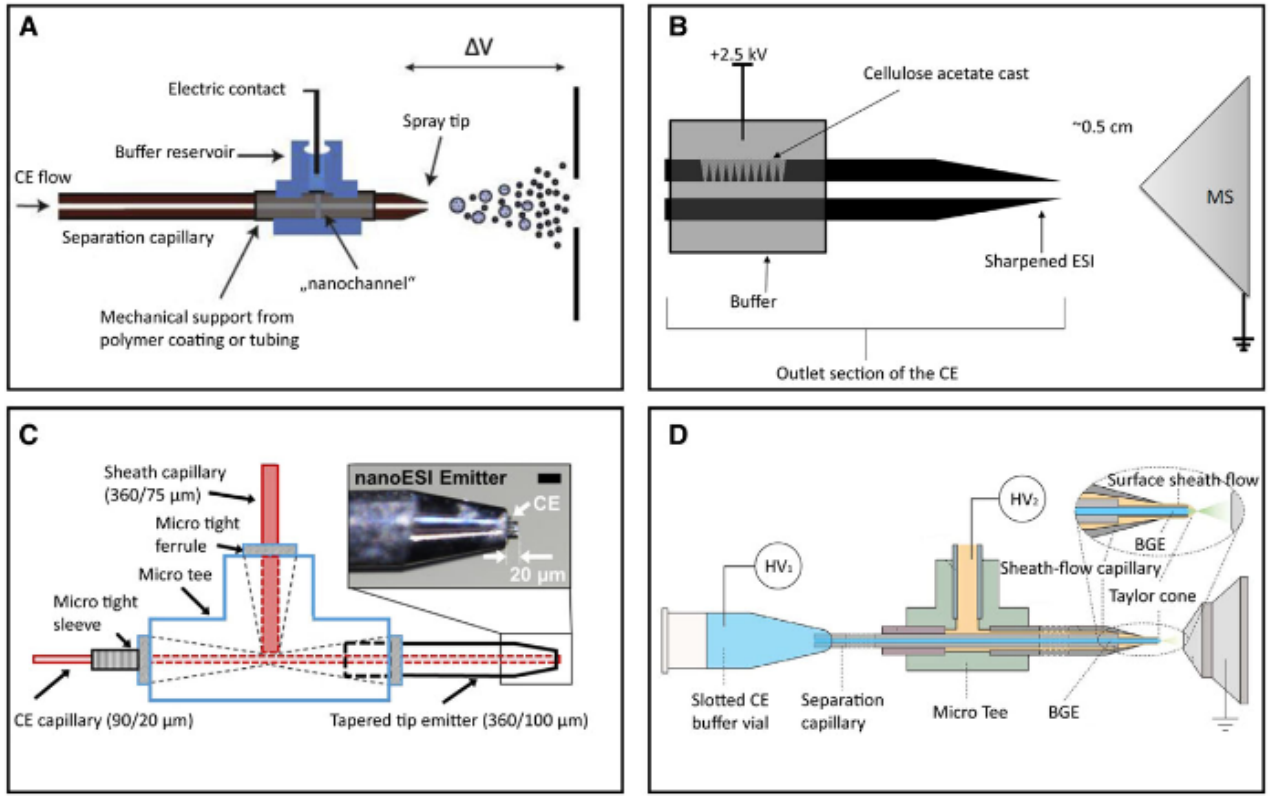
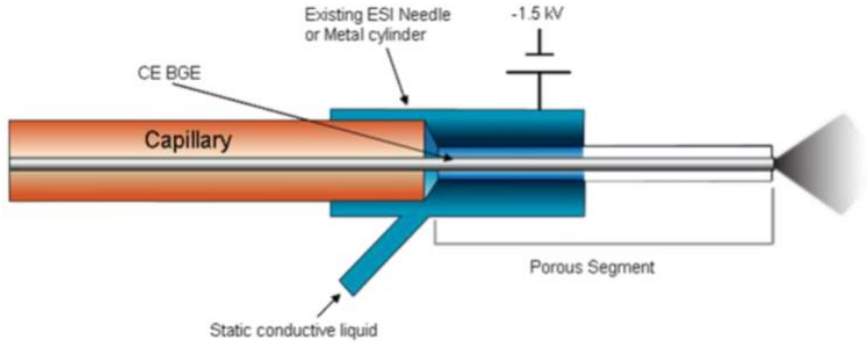
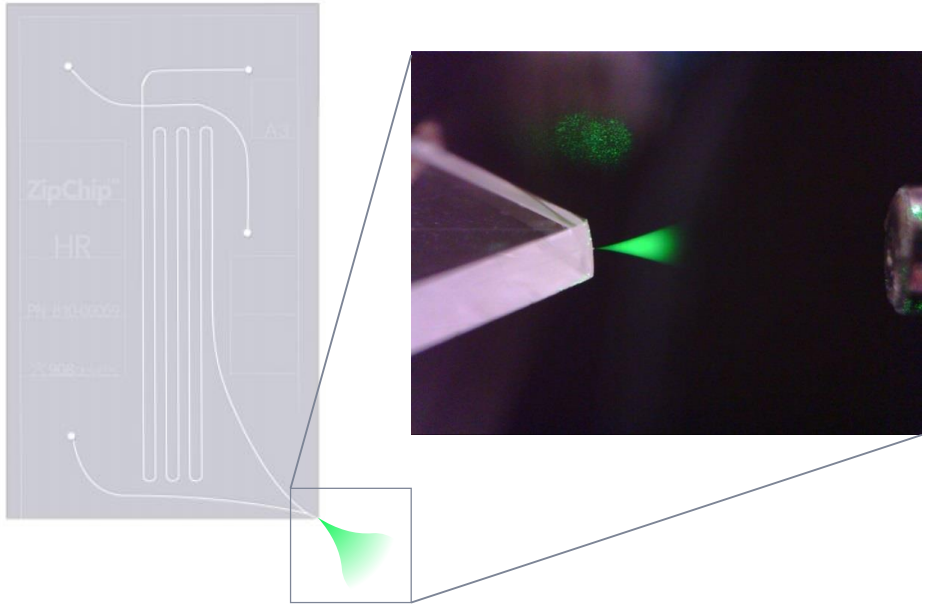
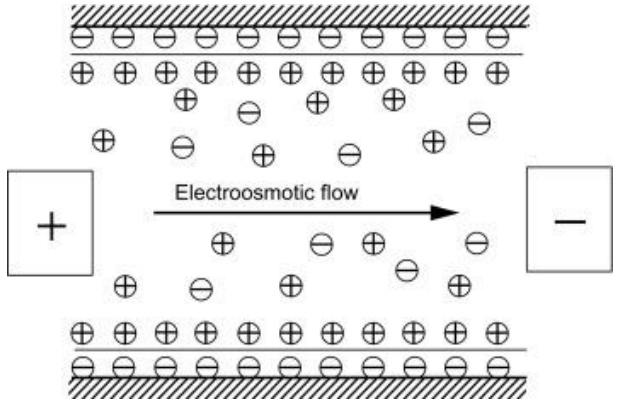


Figure 1. Schematics of (A) Sheathless interface with nanochannel, adapted from [8]. Copyright © 2016, with permission from Elsevier; (B) Sheathless interface with 24 holes using laser ablation with cellulose acetate, adapted by permission from Springer Nature [9], Copyright © 2017. (C) Tapered tip sheath liquid interface, adapted by permission from Springer Nature [21], Copyright © 2016; (D) Sheath flow interface with extendable separation capillary, adapted from [23]. Copyright © 2018, with permission from Elsevier.

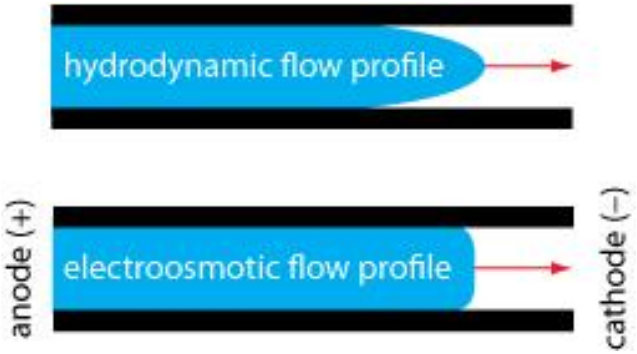




Electroosmotic Flow (EOF)



- Charge on the capillary surface causes a layer of complimentary ions to form near the wall (aka the electrical double layer).
- This layer moves in the electric field, dragging all of the BGE with it.



- EOF has a uniform (flat) flow profile.
- This means that it doesn't broaden the analyte bands as it moves them.

Electroosmotic Flow (EOF)

- The magnitude of EOF depends on:
  - **Surface chemistry**
  - **pH of BGE**
  - **Viscosity of BGE**
    - Organic content
    - Temperature
  - **Ionic strength of BGE**
- The velocity of EOF scales with field strength, just like electrophoresis, so it can be directly added to electrophoretic mobility
  - $\mu_{total} = \mu_{EOF} + \mu_{electrophoresis}$
  - **Or more simply:** $\mu = \mu_{EO} + \mu_{EP}$

Electroosmotic Flow (EOF)

- Unmodified fused silica or glass has a negative surface charge, due to the presence of silanol groups
  - **The mobile layer of complimentary ions is positively charged, so EOF moves from high positive voltage to lower voltage**
  - **We give this direction of EOF a positive charge**
- μ_{EO} for fused silica is relatively high compared to μ_{EP} for most analytes, therefore:
  - **The EOF of an uncoated capillary will push all analytes in the same direction, regardless of their charge**
- EOF can be controlled by altering the surface chemistry of the column

EOF and Surface Chemistry

- Connecting CE to ESI-MS limits the tricks available for controlling surface chemistry
  - **Need clean BGE for ESI**
    - Background/interfering ions
    - Ionization suppression etc..
  - **Need static surface coatings**
    - Could be covalent or ionic, but the coating needs be attached so that it doesn't interfere with ESI-MS
- Must prevent analyte sticking
- Must be uniform to prevent pressure gradients

EOF and Surface Chemistry – Some examples

- Uncoated silica/glass
  - **Strong negative surface charge**
- Aminopropyl-silane coating
  - **Strong positive surface charge**
- Neutral polymers
  - **Polyacrylamide**
  - **Polyethylene glycol**
- Charged polymers
  - **Polyethylenimine (++)**
  - **Dextran sulfate (--)**

Electroosmotic Flow (EOF) - Example

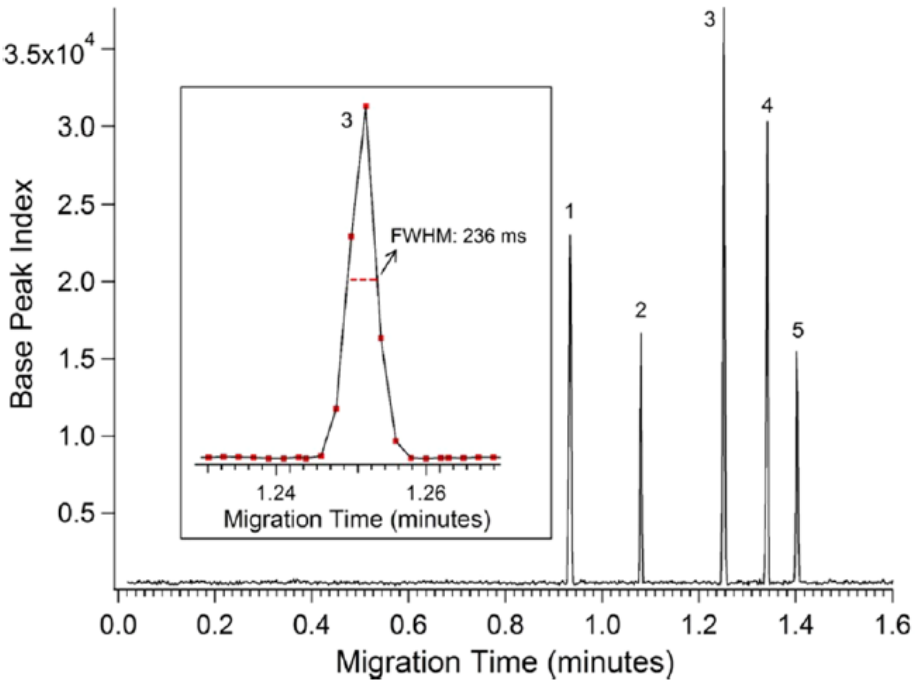


Figure 4. Base peak electropherogram showing: fluorescein (1), methionine enkephalin (2), angiotensin II (3), bradykinin (4), and thymopentin (5) separated using a CE-ESI microfluidic device coated with APDIPES using a field strength of 410 V/cm and 50% acetonitrile and 0.1% formic acid (pH 2.8) BGE. Mass spectra were acquired with a Synapt G2 mass spectrometer at a rate of 8 summed scans per second.

- We know that fluorescein is neutral at this pH, so its total mobility is equal to the electroosmotic mobility
- *Neutral Marker (fluorescein)*
  - t_m = 1.24 m = 74.4 s
  - $v = L/t_m$ = 23 cm ÷ 74.4 s = 0.309 cm/s
- $v = \mu E$, so
- $\mu = v/E$ = 0.309 cm/s ÷ -410 V/cm = -7.54 E-4 cm^2/Vs = μ_{EO}

- *Peak #5 (thymopentin)*
- t_m = 84 s
- v = 0.274 cm/s
- μ_5 = -6.68 E-4 cm^2/Vs
- $\mu = \mu_{EO} + \mu_{EP}$
- $\mu_{EP} = \mu - \mu_{EO}$ = +0.86 E-4 cm^2/Vs

N.G. Batz, J.S. Mellors, J.P. Alarie, and J.M. Ramsey, "Chemical Vapor Deposition of Aminopropyl Silanes in Microfluidic Channels for Highly Efficient Microchip Capillary Electrophoresis-Electrospray Ionization-Mass Spectrometry," Anal. Chem., 86, 3493 (2014).