Lecture 17:
Capillary Electrophoresis

CU- Boulder

CHEM 5181

Mass Spectrometry & Chromatography

Prof. Jose-Luis Jimenez

Fall 2002

R. Weinberger: Practical Capillary Electrophoresis, Academic Press, 1993

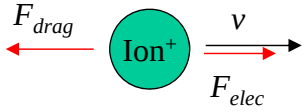
Concept of Electrophoresis

- Used (mostly) to separate charged molecules
- Based on differences on:
  - molecular movement through a fluid ("carrier electrolyte" or "buffer")
  - under an electric field
- No partitioning between mobile and stationary phases
  - Not a chromatographic technique
  - Result called an "electropherogram"

Physical Basis of Electrophoresis

- Solutions can be electrically conductive
  - Due to the migration of individual ions
- Different ions migrate at different rates
  - Electrophoresis means “ion migration”
  - Analytical EP uses the differential ion migration rates as a means of separation

Definition of Ionic Mobility in Solution



v: relative velocity between the ion and the fluid

$$F_{drag} = 6\pi\eta r v$$

$$F_{elec} = qE$$

Ionic Mobility

$$\mu = \frac{v}{E} = \frac{q}{6\pi\eta r}$$

Units: $cm^2/V{\cdot}s$

Mobilities for Group 1A Metals

Table 2.1. Physical Properties of the Group 1A Metals

Metal	Metal Radius(Å)	Crystal Radius(Å)	Approx. Hydrated Radius(Å)	Hydration Number	Ionic Mobility[a]	Observed Current(μa)[b]
Li	1.52	0.86	3.40	25.3	33.5	111
Na	1.86	1.12	2.76	16.6	43.5	147
K	2.27	1.44	2.32	10.5	64.6	195
Rb	2.48	1.58	2.28	na	67.5	204
Cs	2.65	1.84	2.28	9.9	68.0	215

[a] At infinite dilution. Units are 10^5 cm^2/Vs.

[b] 100 mM buffer solution, 20 kV, 50 cm × 75 μm i.d. capillary at 20°C.

Data from *J. Liq. Chromatogr.* **13,** 2517 (1990).

Electrophoresis vs. Chromatography

Table 4.1 Comparison of Electrophoretic and Chromatographic Terms

Capillary electrophoresis	Chromatography
Electropherogram	Chromatogram
Applied potential	Flow rate
Carrier electrolyte or buffer	Eluent or mobile phase
Injection mode (hydrostatic or electromigration)	Injector
Migration time	Retention time
Electrophoretic mobility	Column capacity factor
Velocity	—
Electroosmotic flow	—
High-voltage power supply	Pump
Capillary	Column

Classical Slab-Gel Electrophoresis

- Multiple lanes
- One or more lanes used for standard mixtures (calibration)
- Often used to classify according to molecular size, due to the distribution of gel pores (like size exclusion C)
- Gel plate should also dissipate the generated heat

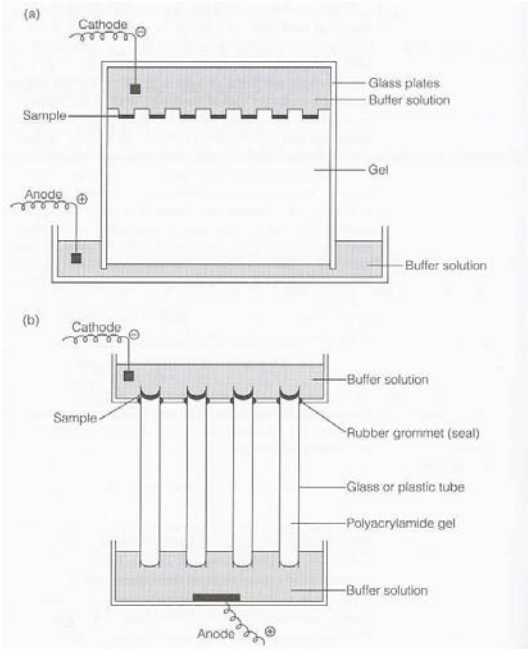


Fig. 1. Typical formats for classical gel electrophoresis. (a) Slab gel. (b) Tube gel. Reproduced from M. Melvin, Electrophoresis, 1987 with permission from Wiley-VCH and from M. Melvin.

Slab Gel Electrophoresis II

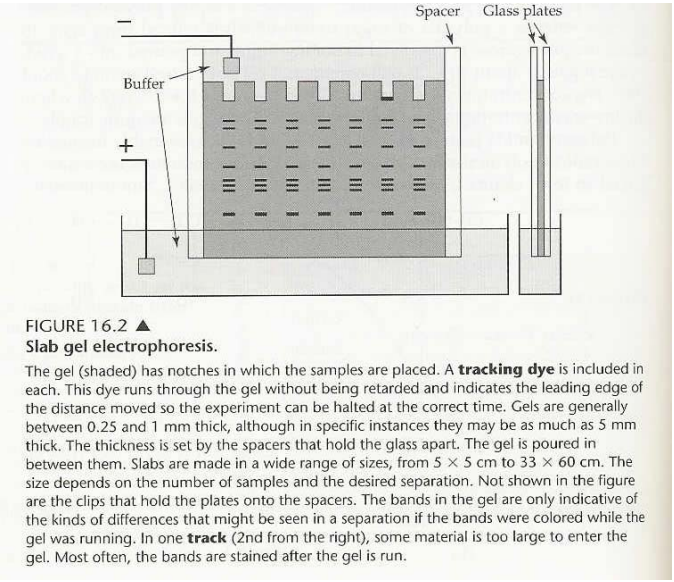


FIGURE 16.2 ▲
Slab gel electrophoresis.

The gel (shaded) has notches in which the samples are placed. A **tracking dye** is included in each. This dye runs through the gel without being retarded and indicates the leading edge of the distance moved so the experiment can be halted at the correct time. Gels are generally between 0.25 and 1 mm thick, although in specific instances they may be as much as 5 mm thick. The thickness is set by the spacers that hold the glass apart. The gel is poured in between them. Slabs are made in a wide range of sizes, from 5 × 5 cm to 33 × 60 cm. The size depends on the number of samples and the desired separation. Not shown in the figure are the clips that hold the plates onto the spacers. The bands in the gel are only indicative of the kinds of differences that might be seen in a separation if the bands were colored while the gel was running. In one **track** (2nd from the right), some material is too large to enter the gel. Most often, the bands are stained after the gel is run.

Example of Gel Electrophoresis

- Separation of PCR products
- Sizing standards on outer lanes
- Three replicate lanes of our sample

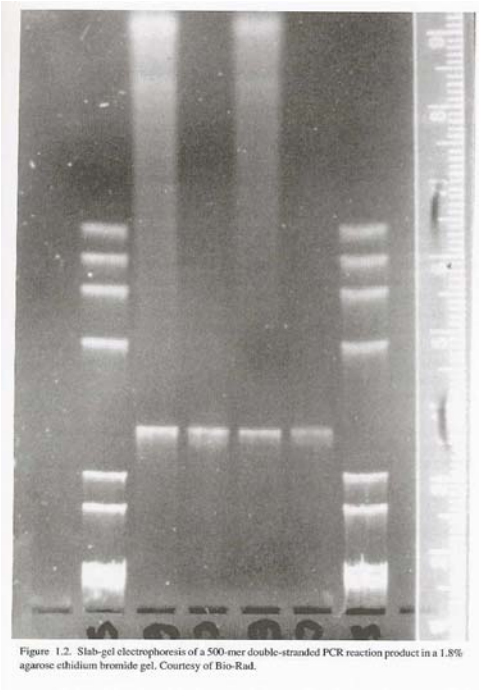


Figure 1.2. Slab-gel electrophoresis of a 500-mer double-stranded PCR reaction product in a 1.8% agarose ethidium bromide gel. Courtesy of Bio-Rad.

Combined TLC- Gel Electrophoresis

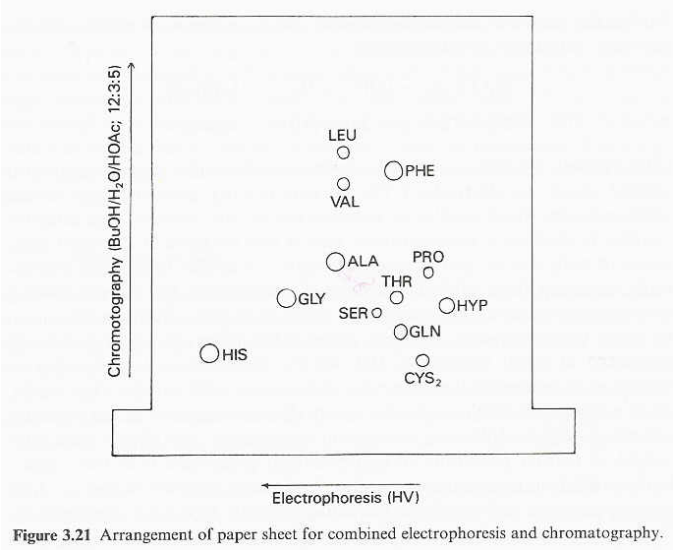


Figure 3.21 Arrangement of paper sheet for combined electrophoresis and chromatography.

Conceptual Schematic of HPCE

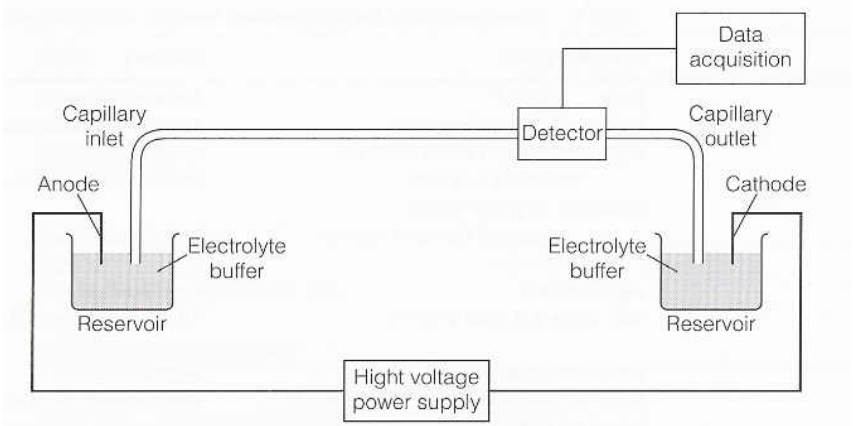


Fig. 2. Schematic diagram of a high performance capillary electrophoresis system.

Capillary for HPCE

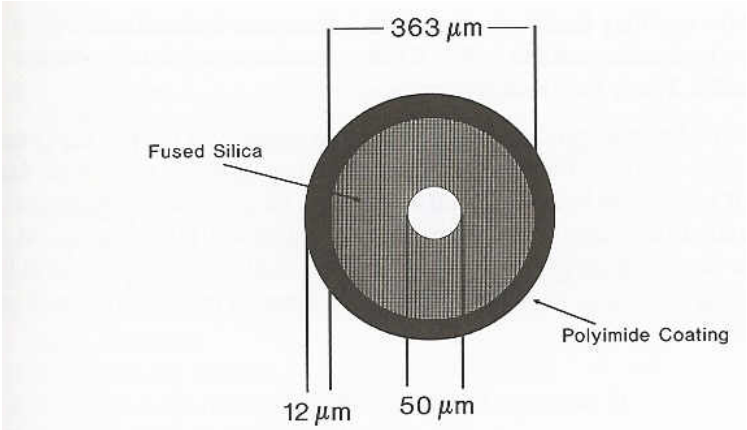


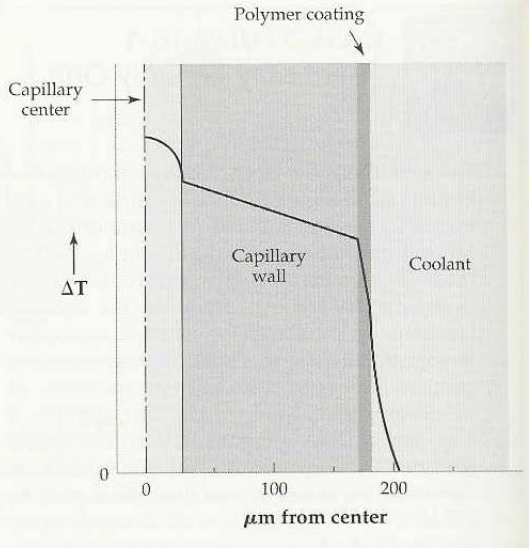
Figure 2.18. Cross-sectional view of a fused-silica capillary.

Why small capillaries in HPCE?

Temperature in HPCE

FIGURE 16.8 ▶
A plot of the approximate relative temperatures across the capillary during an electrophoretic separation.

The details depend on the capillary internal diameter, the voltage, the conductivity of the buffer, and the coolant (gas or liquid). The center of the capillary is in the range of 10° to 40°C above the temperature of the bulk of the coolant during a regular run. The temperature falls off faster in the polymer coating than in the wall because the polymer is a better conductor of heat. The rapid falloff at the outside surface occurs because the coolant is circulating. If the coolant were still, the temperature would drop more slowly in it, and the buffer temperature would be higher.



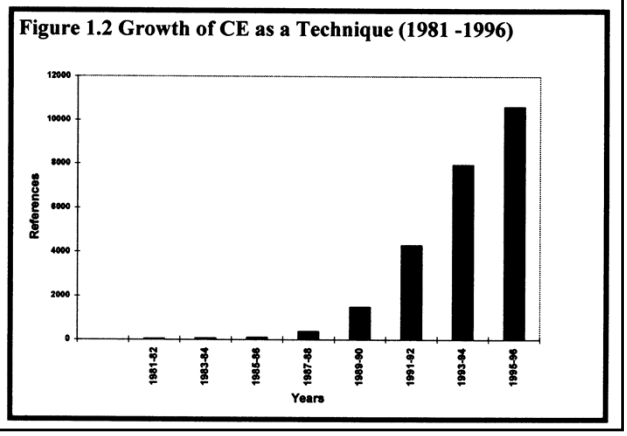
History of HPCE

Table 1.2. History of HPCE

Year	Technique	Detection	Other
1967	CZE in 3 mm i.d. rotating tubes (20)		
1971	CITP (35)		
1974	CZE in 200–500 µm i.d. glass capillaries (21) Electroosmotic chromatography (36)		commercial instrumentation for CITP
1979	CZE in 200 µm i.d. teflon cappillaries (22)		stacking (22)
1981	CZE in 75 µm i.d. capillaries (23)	fluorescence (23)	
1983	CGE (24)		
1984	MECC (26)	ultraviolet (27)	
1985	CIEF (25)	laser fluorescence (28)	chiral recognition (28) coated capillaries (32)
1986			repulsion of proteins from capillary walls (33)
1987		mass spectrometry (29) electrochemistry (30)	
1988		indirect fluorescence(31)	commercial instrumentation for HPCE
1990			field-effect electroosmosis (34)

Growth of HPCE

Number of mentions of CE in Chemical Abstracts
(Research Phase, use continues to increase after abstracts taper off)



Comparison of LC and Electrophoresis

Table 1.1. Comparison of Slab-Gel Electrophoresis, μ-LC, conventional LC, and HPCE

	Slab-Gel	μLC	HPLC	HPCE
Speed	slow	moderate	moderate	fast
Instrumentation Cost	low	high	moderate	moderate
Operating Cost	low	moderate	high	low
Sensitivity				
CLOD	poor	poor	excellent	poor
MLOD	poor	good	poor	excellent
Efficiency	high	derate	moderate	high
Automation	little	yes	yes	yes
Precision	poor	good	excellent	good
Quantitation	difficult	easy	easy	easy
Selectivity	moderate	moderate	moderate	high
Methods Development	slow	moderate	moderate	rapid
Reagent Consumption	low	low	high	minimal
Preparative Mode	good	good	excellent	fair
Ruggedness	good	good	excellent	fair
Separations				
DNA	excellent	fair	fair	excellent
Proteins	excellent	fair	fair	excellent
Small Molecules	poor	excellent	excellent	excellent

Types of HPCE

- Capillary Zone Electrophoresis (CZE)
- Capillary Isotachophoresis (CITP)
- Capillary Isoelectric Focusing (CIEF)
- Micellar Electrokinetic Capillary Chromatography (MEKC)
- Capillary Gel Electrophoresis (CGE)
- Capillary Electroosmotic Chromatography (CEC)

Classification

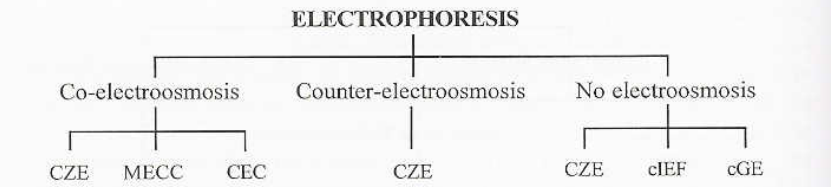


Figure 4.2 Classification of electrophoresis according to the contribution of the electroosmotic flow. CZE, Capillary zone electrophoresis; MECC, micellar electrokinetic capillary chromatography; CEC, capillary electrochromatography; cIEF capillary isoelectric focusing; cGE, capillary gel electrophoresis.

Selecting a HPCE Mode

Table 11.1. Selecting the Mode of Capillary Electrophoresis

Small Ions	Small Molecules	Peptides	Proteins	Oligonucleotides	DNA
CZE	MECC	CZE	CZE	CGE	CGE
CITP	CZE	MECC	IEF	MECC	
	CITP	IEF	CGE		
		CGE	CITP		
		CITP			

Double Layer in EOF

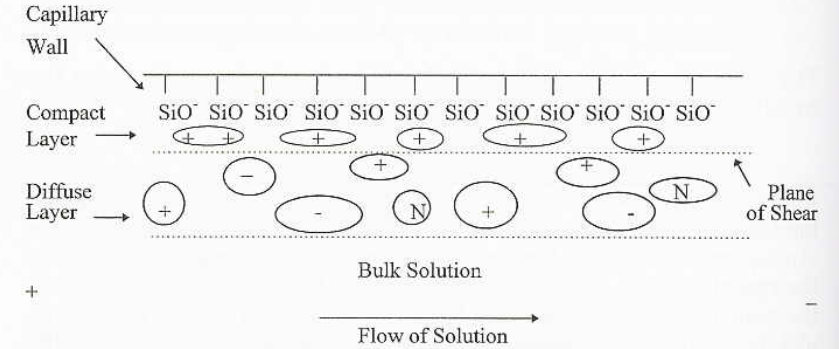


Figure 4.3 Electrical double layer at the capillary wall and creation of electroosmotic flow. (N = neutral analyte.)

Electro-Osmotic Flow (EOF)

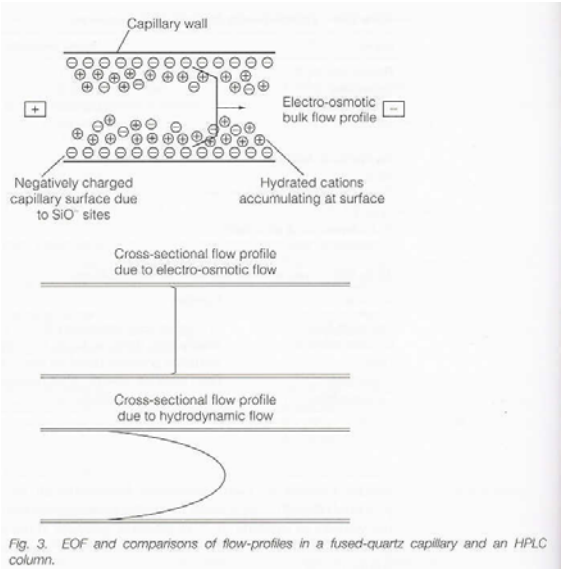


Fig. 3. EOF and comparisons of flow-profiles in a fused-quartz capillary and an HPLC column.

Flow Profiles

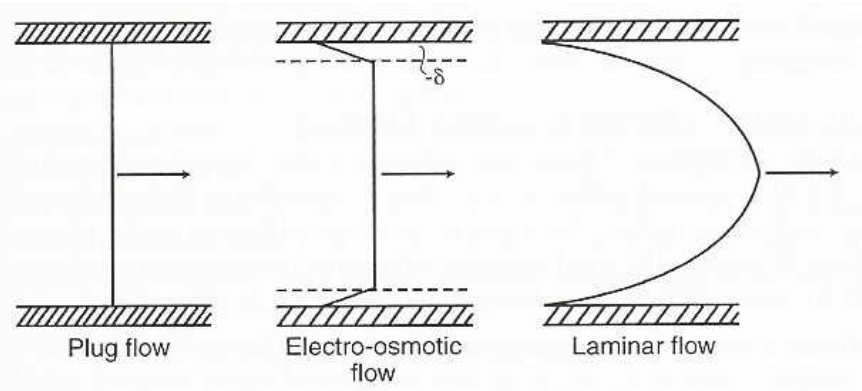
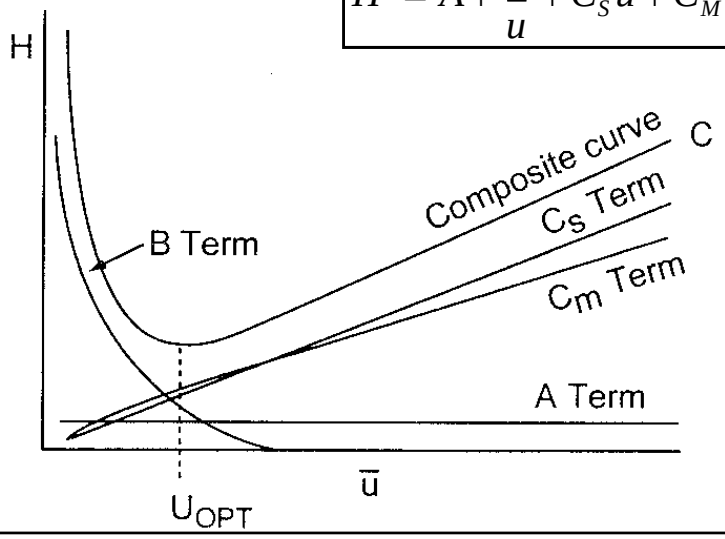


Figure 4.7 Flow profile under various types of flow.

Efficiency: Chromatography

$$H = A + \frac{B}{u} + C_S \bar{u} + C_M \bar{u}$$



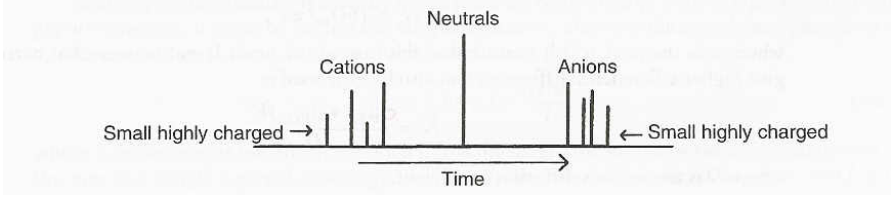
Efficiency: CE

$$N = \frac{t_R}{\sigma} \text{ and } H = \frac{L}{N}$$

Order of Elution with EOF

- What would be the order of elution between?
  - Small cations
  - Small anions
  - Large cations
  - Large anions
  - Neutral molecules

Figure 10.4 Drawing of an electropherogram indicating the order of elution due to EOF. Neutral molecules are not separated from each other. *(Reprinted courtesy of Hewlett-Packard Company.)*



Capillary Zone Electrophoresis

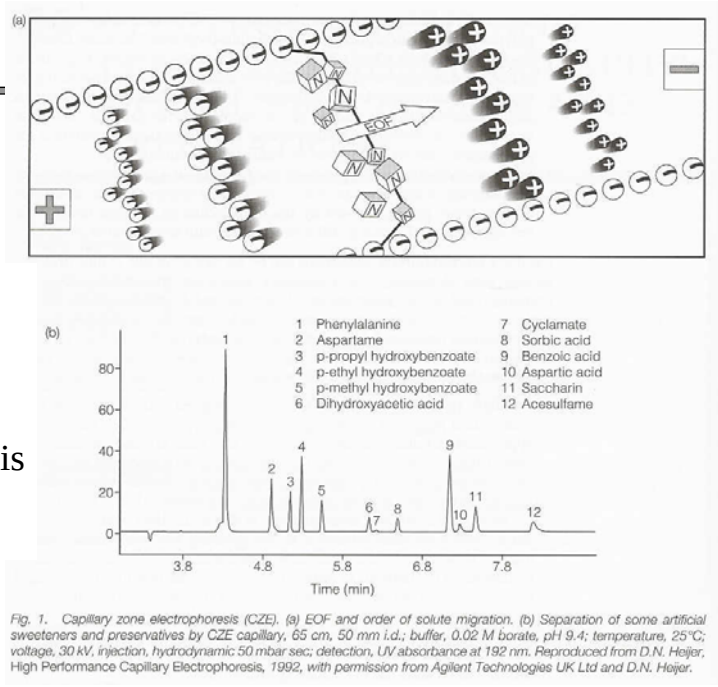


Fig. 1. Capillary zone electrophoresis (CZE). (a) EOF and order of solute migration. (b) Separation of some artificial sweeteners and preservatives by CZE capillary, 65 cm, 50 mm i.d.; buffer, 0.02 M borate, pH 9.4; temperature, 25°C; voltage, 30 kV, injection, hydrodynamic 50 mbar sec; detection, UV absorbance at 192 nm. Reproduced from D.N. Heijer, High Performance Capillary Electrophoresis, 1992, with permission from Agilent Technologies UK Ltd and D.N. Heijer.

Ions and Neutrals in EOF

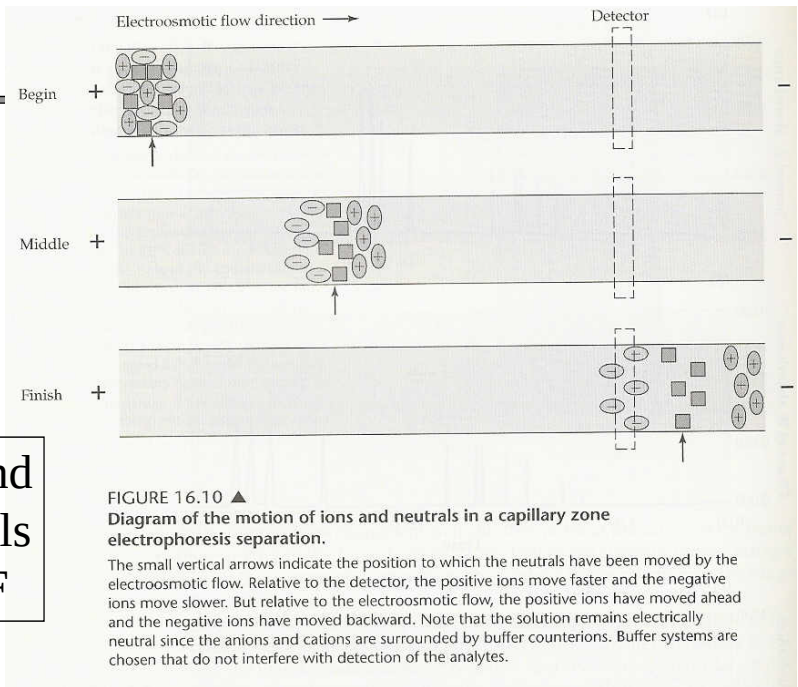


FIGURE 16.10 ▲
Diagram of the motion of ions and neutrals in a capillary zone electrophoresis separation.

The small vertical arrows indicate the position to which the neutrals have been moved by the electroosmotic flow. Relative to the detector, the positive ions move faster and the negative ions move slower. But relative to the electroosmotic flow, the positive ions have moved ahead and the negative ions have moved backward. Note that the solution remains electrically neutral since the anions and cations are surrounded by buffer counterions. Buffer systems are chosen that do not interfere with detection of the analytes.

Mobility vs. MW

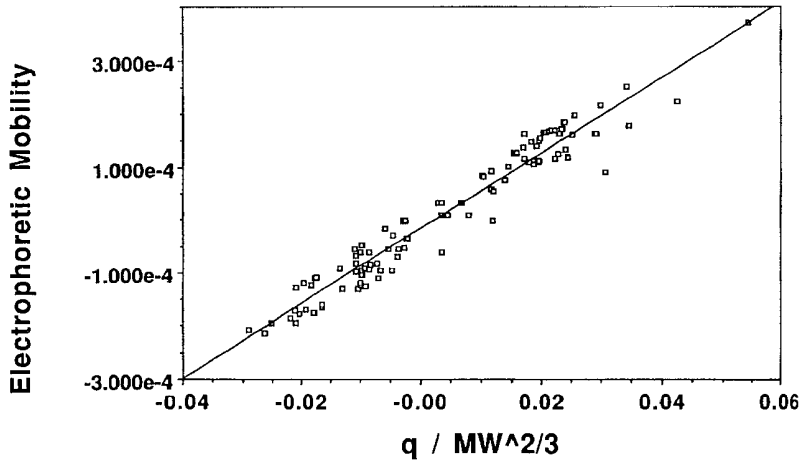


Figure 3.4. Correlation of mobility and $q/M^{2/3}$ for a human growth hormone digest (separated at pH 2.35, 8.0, and 8.15), insulin-like growth factor II digest (separated at pH 2.35 and 8.15). Reprinted with permission from *Anal. Biochem.* **197,** 197, copyright ©1991 Academic Press.

Response vs. Time and Length

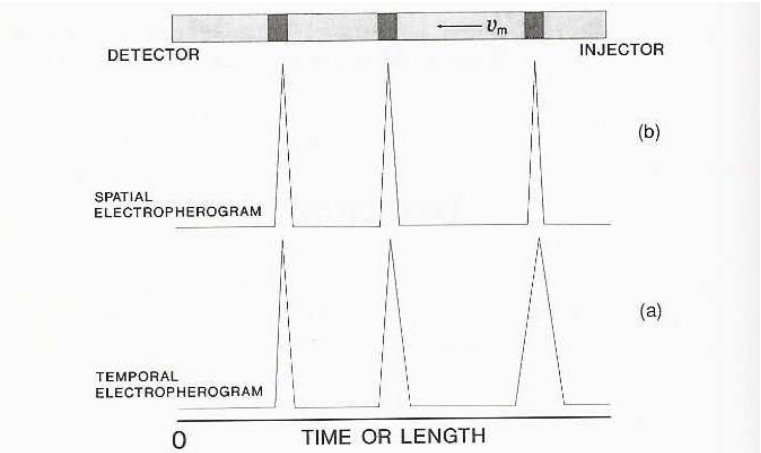


Figure 10.1. Plots of the detector response (a) as function of time and (b) as a function of zone length within the capillary. Redrawn with permission from *J. Chromatogr.* **480,** 95, copyright ©1989 Elsevier Science Publishers.

Species Ionic Mobility vs pH

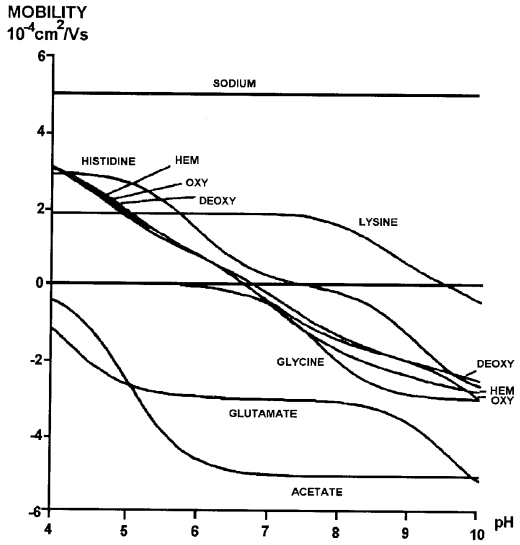


Figure 3.2. Effect of buffer pH on electrophoretic mobility. Reprinted with permission from *J. Chromatogr.* **480,** 35, copyright ©1989 Elsevier Science Publishers.

Effect of pH on CZE Separation

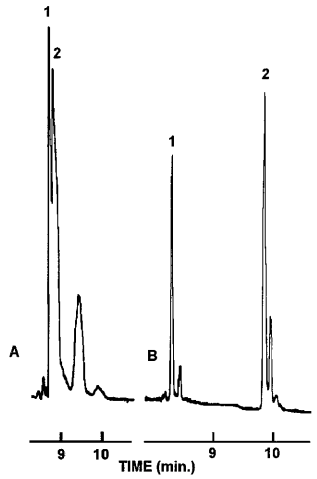
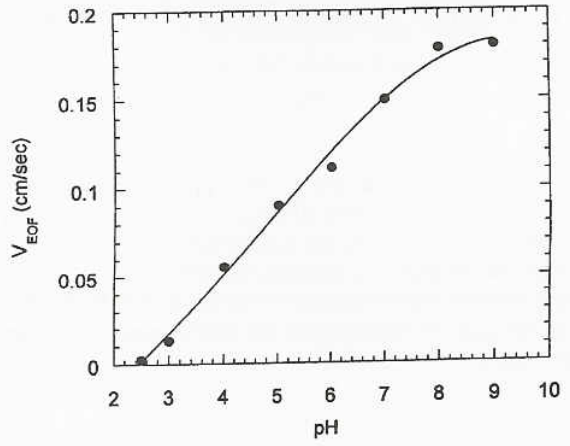


Figure 3.5. Effect of buffer pH on the selectivity of peptide separations by CZE. Capillary length: 45 cm to detector (65 cm total) × 50 μm i.d.; buffer: citric acid, 20 mM, (a) pH 2.5, (b) pH 4.0; field strength: 277 V/cm; current: (a) 24 μA, (b) 12 μA; temperature: 30°C; detection: UV, 200 nm; peptides: (1) AFKAING, (2) AFKADNG. Reprinted with permission from *Anal. Chem.* **61,** 1186, copyright ©1989 Am. Chem. Soc.

Electroosmotic Flow vs pH (Silica Column)

Figure 12.3 Magnitude of Electroosmotic Flow as A Function of pH



Printed through the courtesy of Bio-Rad Laboratories.

Typical Buffers and Additives for CE

Table 2(a). Typical running buffers for electrophoresis

Buffer	Useful pH range
Phosphate	1.1–3.1
Ethanoate	3.8–5.8
Phosphate	6.2–8.2
Borate	8.1–10.1
Zwitterionic buffers	
MES (2-(4-morpholino)ethanesulfonic acid)	5.2–7.2
TRIS ((2,3-dibromopropyl) phosphate)	7.3–9.3

Table 2(b). Typical buffer additives for electrophoresis

Additive	Function
Inorganic salts	Change protein conformations
Organic solvents	Modify EOF, increase solute solubilities
Urea	Solubilize proteins, denature oligonucleotides
Surfactants	Form micelles, cationic ones reverse charge on capillary wall
Cyclodextrins	Provide chiral selectivity

Ion Mobility Spectrometry

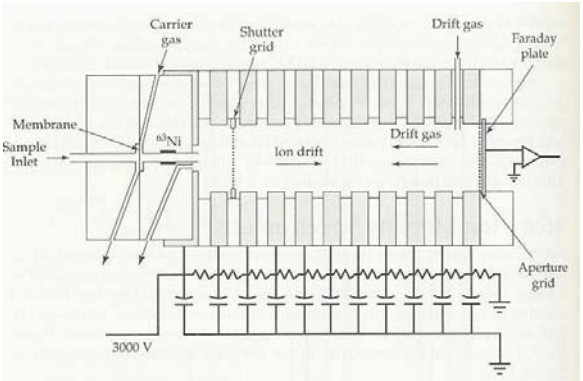


FIGURE 16.17 ▲
Illustration of the construction of an ion mobility spectrometer with a membrane input.

An example of a membrane is a 20 μm thick dimethylsilicone sheet. The organic analytes are soluble in the membrane and can pass through, but the membrane rejects water and ammonia to a high degree. The **shutter** or **gate** grid is an array of fine wires spaced about 1 mm apart that stops ions from traveling farther down the drift tube. It is pulsed open for times in the range 0.05–1 ms. Typical values are 0.25 ms pulse time and 25 ms drift time. The pulse time sets the minimum widths of the zones. The usual random diffusion then broadens the zones further. [Reprinted with permission from *Analytical Chemistry*. Copyright 1991 American Chemical Society.]

Isoelectric Points for Proteins

Table 16.1 Isoelectric Points of Some Proteins

	Isoelectric pH		Isoelectric pH
Pepsin	1.1	γ_1-Globulin	6.6
Casein	4.6	Hemoglobin	6.8
Egg albumin	4.7	Myoglobin	7.0
Serum albumin	4.9	Ribonuclease	9.5
Urease	5.0	Chymotrypsin	9.5
β-Lactoglobulin	5.2	Cytochrome *c*	10.65
Insulin	5.3	Lysozyme	11.0

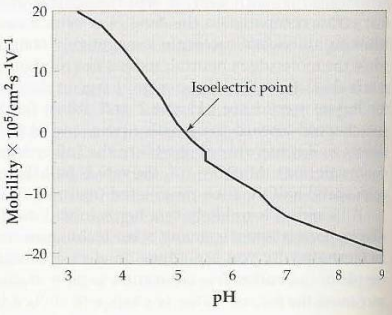


FIGURE 16.1 ▶
A graph of mobility *versus* pH for β-lactoglobulin, a protein from cow's milk.

The mobility is proportional to the charge on the protein and follows its changes with pH. Experiments suggest that the overall charge of the protein results from 57–60 carboxyl groups, 5–7 guanidinium groups, 33–35 amino groups, and 6 imidazole groups. The isoelectric point is 5.19 in the presence of acetate buffers. [Data from R. K. Cannan, A. H. Palmer, and A. C. Kibrick. 1942. *Journal of Biological Chemistry* 142:803–822.]

Isoelectric Focusing I

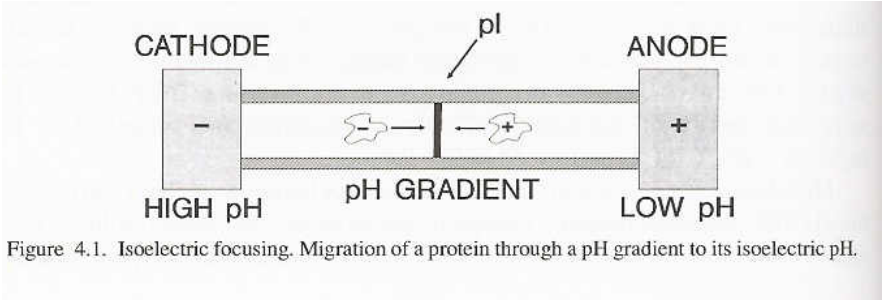


Figure 4.1. Isoelectric focusing. Migration of a protein through a pH gradient to its isoelectric pH.

Trick: modify the pH on-line => change the mobility on-line

Isoelectric Focusing II

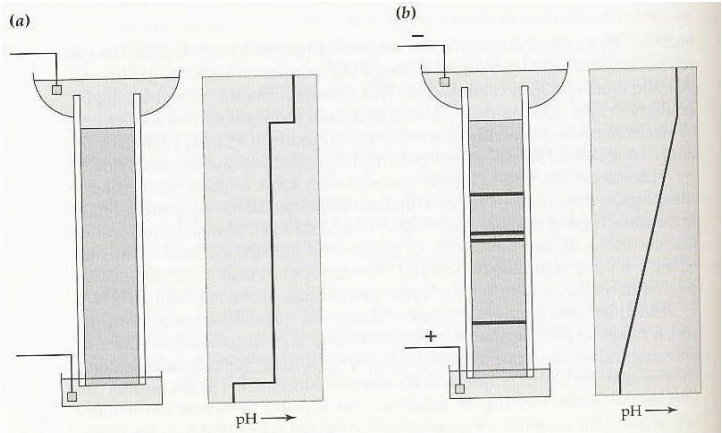


FIGURE 16.6 ▲
Diagram of an isoelectric focusing experiment.
(a) One type of experiment has the analytes dispersed throughout the gel with the ampholytes. The pH in the system is indicated in the graph to the right. **(b)** The voltage is applied and the ampholyte buffers migrate to establish a pH gradient along the gel, as shown in the pH plot. The proteins move until they become neutral at their respective isoelectric points in the pH gradient.

Isoelectic Focusing III

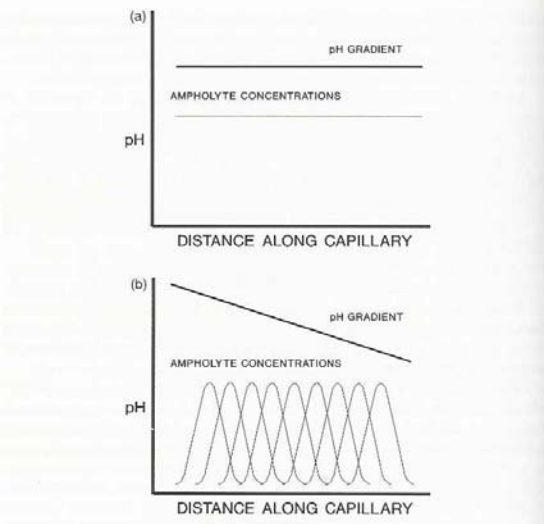


Figure 4.3. Representation of a pH gradient and individual ampholyte concentrations (a) before focusing and (b) after focusing.

Capillary Isotachophoresis

ITP means "electrophoresis at uniform speed"

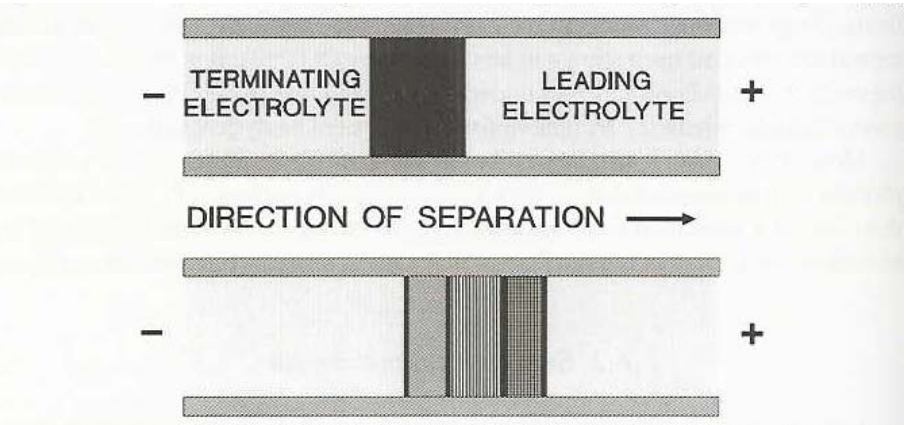


Figure 6.1. Schematic representation of a three-component separation by anionic CITP at the moment of injection (top) and after separation (bottom).

Electrofocusing

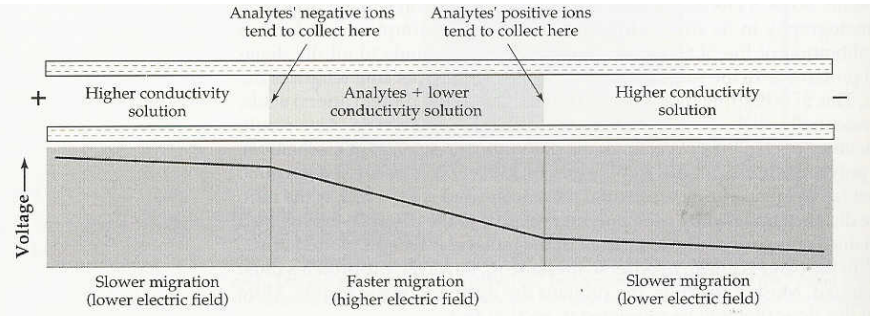
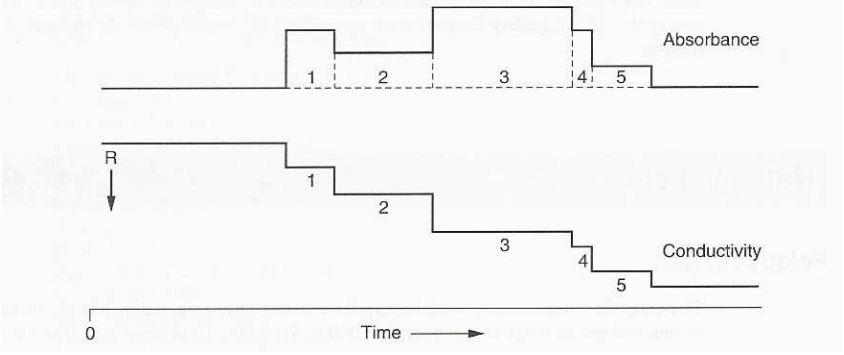


FIGURE 16.3 ▲
Electrofocusing.

Electrofocusing is done in both gel and capillary electrophoresis. The ions are focused because the high field in the sample region forces them to accumulate at the interface with a low-field region. The difference in voltage drop can be understood in analogy with a simple voltage divider, made with a number of resistors in series. The largest voltage drop occurs across the resistor with the highest resistance.

Output from Capillary Isotachophoresis

Figure 10.6 Representation of the output from capillary isotachophoresis with absorbance and conductivity detectors. *R* represents increasing resistance. *(Reprinted courtesy of Hewlett-Packard Company.)*



2D Detection (=> 3D Data) in CITP

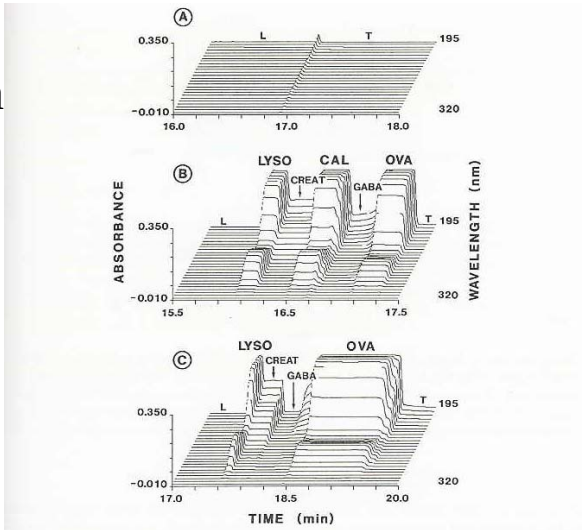
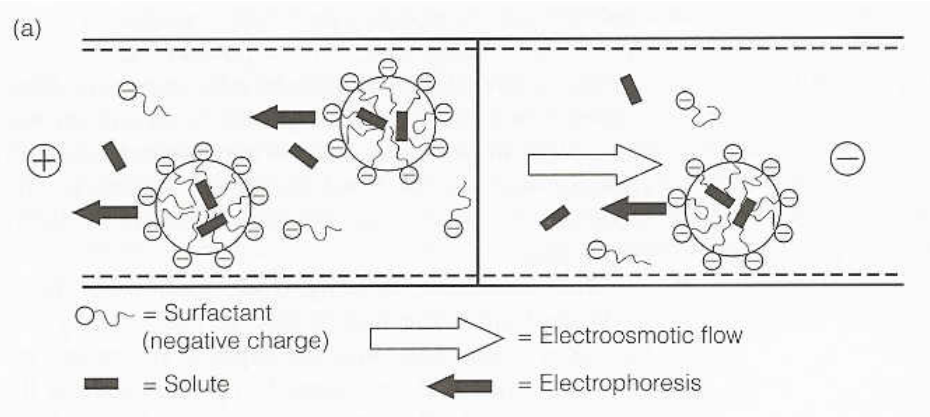


Figure 6.5. Three-dimensional cationic CITP of (A) blank, (B) lysozyme (LYSO), creatinine (CREAT), conalbumin (CAL), γ-amino-*n*-butyric acid (GABA), and ovalbumin (OVA), and (C) OVA spiked with CREAT and GABA. Capillary: 90 cm (length to detector, 70 cm) x 75 μm i.d.; leader: 10 mM potassium acetate and acetic acid with 0.3% HPMC, pH 4.75; terminator: 10 mM acetic acid; sample: proteins, 10–30 mg/mL dissolved in leader without HPMC; voltage: 20 kV; injection: gravity by raising end of capillary 34 cm; detection: multiwavelength UV; current: 12 μA declining to 2 μA. Reprinted with permission from *J. Chromatogr.* **558,** 423, copyright ©1991 Elsevier Science Publishers.

Micellar Electrokinetic Capillary Chromatography (MEKC)



MEKC: separation due to partitioning
between two mobile phases moving at different velocities

Partition in Micellar LC

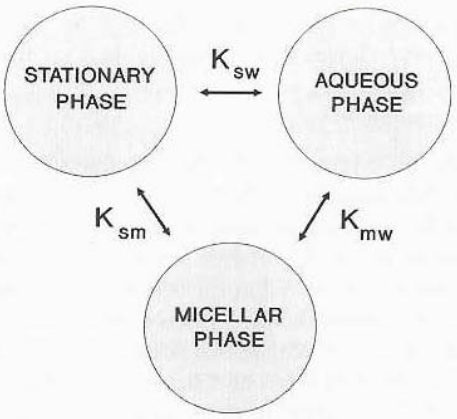
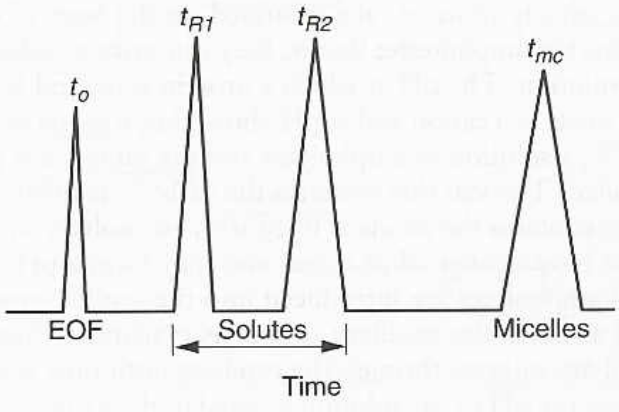


Figure 7.2. Partition coefficients for a solute in micellar liquid chromatography. K_{sw} = stationary phase–water, K_{mw} = micelle–water, and K_{sm} = stationary phase–micellar phase partition coefficients.

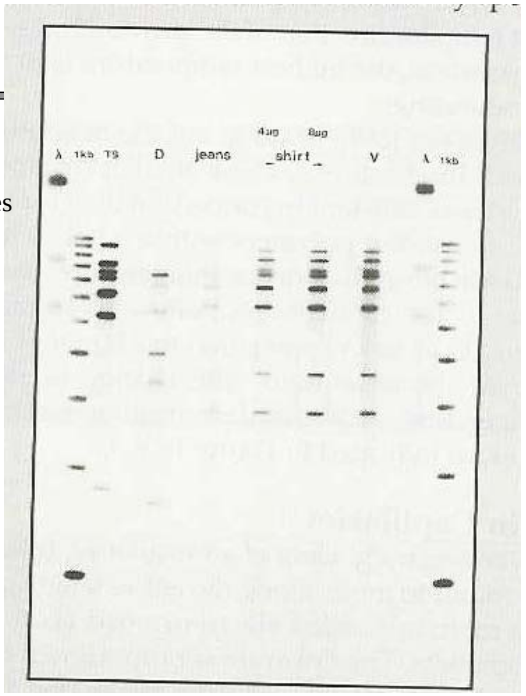
Micellar Electrokinetic CE

Figure 10.5 Representation of a micellar electrokinetic capillary chromatogram. *(Reprinted courtesy of Hewlett-Packard Company.)*



DNA Fingerprinting

- Some highly variable regions on genome: specific sequences are repeated a variable number of times (e.g CGA-CGA-CGA… 5 vs 66 times)
- Chop the DNA with a specific restriction enzyme
  - Millions of fragments
  - Sorted by size with GE
  - Chains are labeled with specific radiactive marks
  - Only e.g 1 in 20 people will show that pattern
- Using 6 to 8 well-chosen enzymes we can identify everyone in the world



Other Types of CE

- Capillary Gel Electrophoresis (CGE)
  - Like size exclusion chromatography
- Capillary Electroosmotic Chromatography (CEC)
  - Chromatography (partition)
  - Electroosmotic “pump”
  - Allows miniaturization and improved resolution

CE Detectors I

Table 3. Characteristics of CE and CEC detectors.

Detector	Sensitivity mass (moles)	Sensitivity concentration (molar)	Characteristics
UV-visible absorbance	10^{-13}–10^{-16}	10^{-5}–10^{-8}	Good sensitivity, most widely used. DADs are versatile and give spectral information.
Fluorescence	10^{-15}–10^{-17}	10^{-7}–10^{-9}	Sensitive, but many solutes need to be derivatized.
Laser-induced fluorescence	10^{-18}–10^{-20}	10^{-14}–10^{-16}	Extremely sensitive, but many solutes need to be derivatized. Expensive.
Electrochemical			Sensitive, require special electronics and capillary modification. Conductometric almost universal.
Amperometric	10^{-18}–10^{-19}	10^{-10}–10^{-11}	
Conductometric	10^{-15}–10^{-16}	10^{-7}–10^{-8}	

CE Detectors: Detection Limits

Table 10.1 Capillary electrophoresis detectors and their approximate detection limits.

Detector	Approximate Detection Limit, µg/mL
Absorbance, UV/Vis	10^{-1}
Indirect absorbance, UV/Vis	1
Fluorescence	10^{-3}
Indirect fluorescence	10^{-2}
Laser-induced fluorescence	10^{-6}
Mass spectrometer	10^{-4}
Amperometric	10^{-5}
Conductivity	10^{-3}

Reprinted courtesy of Hewlett-Packard Company.

Accuracy and Precision of HPCE

Table 11.3. Accuracy and Precision of HPCE

Application	Migration Time (%RSD)	Peak Area (%RDS)	Peak Height (%RSD)	%Recovery	Reference
CZE and MECC					
Analgesics	not reported	0.8–1.7[c]	1.4–2.6[c]	99–101	(18)
Domperidone and others	0.95–1.45	1.05–2.82	0.46–1.01	97–104[a]	(19)
Anti-inflammatory drugs	0.16–0.54	0.86–1.96	0.49–1.9	101–104[b]	(4)
Insulin	0.36–0.54	1.72–2.41	—	91–103[a]	(20)
Serum albumin	0.43	3.52	—	—	
Salicylamide	0.77	1.95	2.06	—	(21)
Dynorphins	0.57–0.63	0.95–1.39			(22)
CGE					
Proteins (polyacrylamide)	0.9–1.6	3.53	—	—	
Restriction fragments (polyacrylamide)	0.9[c]	—	—	—	(23)
Restriction fragments (HPMC)	0.16–0.22	5.32–9.16	4.79–6.00	—	(12)
CIEF					
Proteins	0.5–2.5[d]	—	—	—	(24)

[a]Compared to HPLC.
[b]% of labeled amount.
[c]Used internal standard.
[d]Corrected with a marker protein.

Tuning of HPCE

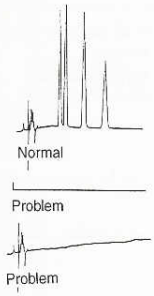
Table 11.2. Adjusting the Separation Parameters

Goal	Adjustment	Consequence	Compensation	Impact
Speed	Decrease capillary length	Joule heating	Decrease capillary diameter	Loss of sensitivity
	Increase field strength	Loss of resolution	Decrease buffer ionic strength[a]	Increased wall effects
	Increase temperature			Decreased loading capacity
Resolution	Increase capillary length	Longer run times	None[b]	n/a
	Reduce electroosmotic flow	Longer run times		
	Decrease injection size	Loss of sensitivity	None	n/a
Loading	Increase capillary diameter	Joule heating	Decrease field strength	Longer run times
	Increase buffer ionic strength	Loss of resolution	Decrease temperature	
	Increase injection size	Loss of resolution	Increase resolution	Longer run times
Sensitivity	Increase capillary diameter	Joule heating	Decrease field strength	Longer run times
	Increase buffer ionic strength	Loss of resolution	Decrease temperature	Longer run times
	Stacking buffers	Loss of resolution	Increase resolution	Longer run times
	"Z-cell"	Loss of resolution	Increase resolution	Longer run times
	Increase injection size	Loss of resolution	Increase resolution	Longer run times
	Laser fluorescence	Derivatization usually required	None	Validation and sample prep complicated
	On-line concentration (LC/CE)	Limited to CZE	None	n/a

[a]Decreasing the buffer ionic strength also increases both mobility and EOF.
[b]Assuming maximum voltage is already employed.
n/a: not applicable

CE Troubleshooting I

Table 6.2 CE Problems, Probable Causes, and Remedies

Problem No. 1: No peaks/very small peaks		
Problem	Probable cause	Remedy/Comments
	1. Capillary not aligned in detector	1. Align capillary.
	2. Plug/bubble in capillary	2. Purge with a syringe.
	3. No voltage	3. Confirm voltage setting. Ensure capillary ends are immersed in buffer.
	4. No sample injected	4. Confirm sample in vial. Be sure capillary extends into sample. Confirm injection time.
	5. Detector lamp off/ dead	5. Turn lamp on. Replace lamp.
	6. Incorrect detector wavelength	6. Confirm wavelength setting. Confirm wavelength accuracy.
	7. Wrong buffer	7. Confirm buffer composition.

CE Troubleshooting II

Table 6.2 *continued*

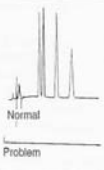
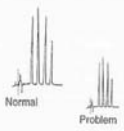
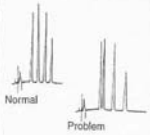
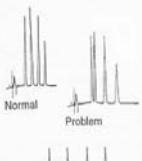
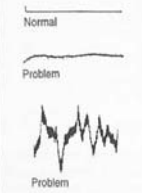
Problem No. 2: No Current		
Problem	Probable cause	Remedy/Comments
	1. Plug/bubble in capillary	1. Purge capillary with a syringe. Trim capillary ends. Replace capillary.
	2. Broken capillary	2. Replace capillary.
	3. Safety interlock not closed	3. Close interlock.
	4. Wrong buffer	4. Confirm buffer composition.
Problem No. 3: Poor sensitivity		
	1. Incorrect injection volume	1. Confirm injection time, voltage, pressure, etc.
	2. Aging detector lamp	2. Replace lamp.
	3. Incorrect detector wavelength	3. Confirm wavelength setting.
Problem No. 4: Current fluctuations		
	1. Bubbles in capillary	1. Flush capillary with a syringe.
	2. Temperature fluctuations	2. Monitor capillary/ room temperature.

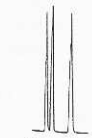
Table 6.2 *continued*

Problem No. 5: Variable migration times		
Problem	Probable cause	Remedy/Comments
	1. Temperature fluctuations	1. Measure capillary/ room temperature.
	2. Ion depletion of buffers	2. Replace buffers.
	3. Ionic strength differences between samples	3. Use reference compounds.
	4. Sample-wall interactions	4. Wash capillary thoroughly.
	5. Capillary not washed	5. Inject electroosmotic flow marker.
Problem No. 6: Baseline Noise		
	1. Aging detector lamp	1. Replace lamp.
	2. Wrong detector wavelength	2. Confirm wavelength setting.
	3. Mismatched buffers	3. Confirm buffer composition.
	4. Capillary not conditioned	4. Rinse with base, followed by water and buffer.
	5. Particles in buffer	5. Filter buffer.
	6. Bubbles in capillary, due to pinholes or broken capillary	6. Replace capillary.

(*continues*)

CE Troubleshooting III

Table 6.2 *continued*

Problem No. 7: Broad Peaks		
Problem	Probable cause	Remedy/Comments
 Normal  Problem	1. Sample-wall interaction	1. Wash capillary well.
	2. Too large an injection	2. Reduce injection time.
	3. Joule heating	3. Reduce voltage. Decrease buffer concentration.
	4. Buffer siphoning	4. Equalize buffer reservoir levels.
	5. Ionic strength of sample diluent too high	5. Dilute sample in water. Remove salt from sample. Increase concentration of buffer.