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Introduction

Electrophoresis - an electric field is applied across a tube containing a conductive solution and solutes, the solutes migrate though the solution.

The rates and directions of the solute migration depend on the signs and magnitude of their charges.











Introduction

Capillary electrophoresis is similar to chromatography but some of the terms are different:

Chromatography

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Chromatogram Flow rate Mobile phase Injector Retention time Column capacity factor Pump Column

Capillary Electrophoresis

Electropherogram Applied potential Carrier Electrolyte(buffer) Injection mode Migration time Electrophoretic mobility High- voltage power supply Capillary

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Introduction

Technique

CE

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Advantages

Disadvantages

- Minimal sample consumption and waste- Limited capacity for production. separating large molecules.
 - High separation efficiency due to microscale dimensions and uniform flow front.
 - Rapid analysis and reduced run times. Sensitive to changes in
 - Excellent for charged molecules and fast separations.

- May require specialized
- equipment for certain applications.
- buffer composition.
- LC - Versatile separation of a broad range of - Longer analysis times molecules. compared to CE.
 - Enhanced control over separation conditions.
 - Suitable for complex samples and biomolecules.
- Consumes large sample volumes.
- Risk of column deterioration and band broadening.

- Many mobile and stationary phases are - More complex available, as well as columns of different instrumentation. lengths and packings.

Introduction

over LC

when

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- **Choose CE** Analytes are charged or ionic in nature.
 - Rapid analysis is crucial.
 - High separation efficiency is required for small molecules.
 - Minimal sample consumption is required.
- Choose LC over CE when
 - Analytes vary in size, charge and hydrophobicity. - Superior separation control and flexibility are needed.
 - Complex samples with a diverse range of compounds are being analyzed.
 - Longer separations times are acceptable.







Electrophoretic mobility

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The movement (migration) of a charge species under the influence of an applied field, $\mu e(\ cm^2/sec\ V)$

Mobility depends on the charge density of the solute (valence & size), the dielectric constant and viscosity of the electrolyte, and the temperature.

Under electroosmotic flow

 $\mu = \mu_e + \mu_{eo}$

 μ_{e} - electrophoretic mobility of the analyte

 μ_{eo} - electroosmotic flow mobility









electroosmosis).



Fig. 2.2. Drawing of an electropherogram showing the order of migration due to electroosmotic flow and electrophoretic mobility. Small, highly charged cations are the first to elute from the capillary. Neutral solutes are not separated from each other. In this example, the outlet of the capillary is at the negatively charged cathode.



Electroosmotic Flow (EOF)

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For fused silica capillaries - acidic silanol groups on the surface dissociate when in contact with an electrolyte solution:

SiOH(s) \rightarrow SiO-(s) + H+(aq)

Hydrated cations in the electrolyte are attracted to the negatively charged SiO- groups.

When an electric field is applied - diffuse layer breaks away and moves toward the cathode - dragging with it the bulk solution of electrolyte (viscous drag).

This flow of the bulk solution is called electroosmosis.



Chem., 1989, 61, 294A. With permission.)



Fig. 2.3. Representation of electroosmotic flow in a capillary. Electroosmotic flow is caused by the negatively charged $Si-O^-$ groups on the inner wall of the capillary attracting the positively charged cations, represented by the circled +'s, forming the fixed layer. The mobile layer of cations is pulled toward the cathode, dragging the bulk buffer solution with it. The anions and the solvation of the cations are not shown.



Between the 2 layers is the shear plane where there is a potential difference known as the zeta potential.

Electroosmotic flow is proportional to the thickness of the double layer.

The thickness of the double layer is inversely proportional to the concentration of the buffer, 10 mM concentration gives a 1nm thick layer.



Electroosmotic Flow (EOF) - Measurement of Electroosmosis Flow

To calculate electrophoretic mobility of an analyte - need to know contribution from the electroosmotic flow to the mobility.

Common way - record the migration time of an injected uncharged marker solute - which is carried to detector only under EOF.

For a neutral marker: $\mu_{eo} = 1/Et$

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t - time for the marker to migrate to the detector E - electric field



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Electroosmotic Flow (EOF) - Measurement of Electroosmosis Flow

Example of neutral markers: methanol, acetone, benzene, dimethyl sulfoxide

Disadvantage: may have adsorption of marker on wall.



Electroosmotic Flow (EOF) - Measurement of Electroosmosis Flow

Alternative method:

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-Fill capillary & receiving vial with buffer at concentration ,C -Injection vial filled with buffer at 0.95C is pulled into capillary by applied voltage.

-since injection buffer is more dilute, the current will fall as buffer fills capillary.

-time for current to stabilize, Δt , represents the complete filling of the capillary.

L - length of capillary v_{eo} - electroosmotic flow rate.



Speed of EOF is related to the magnitude of the "zeta potential"

 ξ -- potential at the sheer plane

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Zeta potential depends on the nature of the solid surface and the ionic state of the liquid.

Polar solvents-i.e. - water-give a high ξ value (~100 mV) when in contact with polar surfaces.

Non-polar, non-conducting solventsi.e. heptane – do not exhibit zeta potentials



Effect of pH

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Acidic silanol groups at surface of capillary wall dissociate when in contact with solution.

At high pH – silanol groups are fully ionized, generating a dense compact layer and increasing zeta potential.







Effect of Buffer cation & Buffer anion

EOF is proportional to potential drop across the diffuse layer of counterions.

Highest mobility obtained with smallest cations.

Buffer anions affect mobility but do not seem to show any trend.



Applied Voltage

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Mobility of EOF increases with increasing applied voltage.

But this affect is due mainly to increasing temperature (joule heating).



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Electroosmotic Flow (EOF) - Manipulation of Electroosmotic flow

Ways to suppress or reduce electroosmotic flow include:

 -coating (deactivating) the capillary walls with polymers (difficult to get uniform coatings)
 -adding chemical reagents to the solution to reduce solutecapillary action.







namic pressure.

адvancedchromotography Theory

Separation Efficiency

Migration time of a solute to migrate from injection to detection is:

$$t = I/\upsilon = I/\mu E = LI/\mu V$$

- I total length of capillary
- L length of capillary with charge applied (I \approx L)
- μ mobility

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- E electric field (V/L)
- V applied voltage



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N = 16(t/w)<sup>2</sup>
w - peak width at base
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 $N = \mu V/2D$

D - solute diffusion coefficient (zone broadening)


Injection length (volume-injection)

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Volume of sample plug introduced into capillary may affect efficiency

At small injection lengths - zone length is determined by diffusion

At large injection length - diffusion is not important - zone length determined by injection length



Diffusion

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Eddy (A), longitudinal (B), resistance to mass transfer (C)

For CE - no A - no packed capillaries - no C - no transfer between a stationary and liquid or liquid and gas phase B - only source of zone broadening



Joule Heating

If high voltages are applied to minimize diffusion excessive band broadening may occur because of joule heating of the liquid inside the capillary.

Joule heating - heat generated by collisions between solute and electrolyte ions as a result of the conduction of electric currents.



Joule Heating

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Since capillary is cooler at the outside than inside, the viscosity of the electrolyte can be lower in the center.

Viscosity decreases ~2.7% per degree Celsius, and the migration velocity of the analytes will be higher.

Smaller diameter capillary give more efficient separations.



A deviation from linearity shows that the temperature removal capacity of the system has been exceeded.



Fig. 2.6. Ohm's law plot. The voltage indicated by the dashed line is the maximum voltage that should be used.



Solute - Wall interactions

Interaction between solute and the capillary walls leads to band broadening, peak tailing, and irreproducibility.

Primary causes of adsorption:

-hydrophobic interactions

-ionic interactions between cations solutes (i.e. proteins) and SiO- groups.



Solute - Wall interactions

Reduce interactions by:

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coating capillary wall (drawback: uneven coating) increase ionic strength (competition adsorption sites) (drawback: increase ionic strength need decreasing voltage to avoid joule heating) use extreme pH's (pH 1.5 - silanol groups are not ionized).



These are influenced by: applied voltage, pH, ionic strength, capillary wall.

pH major factor to manipulate resolution

Resolution, Rs

$$Rs = 2(t_2 - t_1)/w_1 + w_2$$

t - migration time w - baseline width



Typically operating voltages for CE are between 10 – 30 kV (because of joule heating - not a large range)



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Instrumentation

Main components:

- high-voltage supply
- source and destination vials
- capillary

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detector



Fig. 4.1. Drawing of the main components of a capillary electrophoresis system. The capillary inlet is in the source vial and the outlet in the destination vial. For an injection, the capillary inlet and anode are placed in the sample vial.

High - Voltage Power Supply

Provides electric field to establish EOF of bulk solution and electromigration of the charged analytes.

Most power supplies provide ~-30 kV to +30 kV with current levels of ~200-300 $\mu A.$

Since detector end must be anodic or cathodic - needs to be possible to switch polarity.

For CE - stable voltage regulation is important to maintain migration time reproducibility.

Sample Injection

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Unlike GC or HPLC, the sample is loaded into the capillary while there is no flow of buffer through the capillary.

There are several injection methods:

- hydrodynamic or hydrostatic
- electrokinetic or electromigration



Sample Injection – Hydrodynamic Injection

Use either pressure or gravity.

Pressure injection by simply applying pressure to the vial.

Pressure injections can also be made by applying a vacuum to the destination valve.

Gravity injection done be simply raising the sample vial above the destination vial.



Fig. 4.3. Hydrodynamic injection by pressure. The sample vial is pressurized, forcing the sample solution into the capillary. The volume of sample injected depends on the magnitude and duration of pressure applied, sample solution viscosity, and capillary dimensions. After injection, the capillary is placed back into the source vial and an electric field applied.



Fig. 4.4. Hydrodynamic injection by vacuum. A vacuum is applied to the destination vial, pulling the sample solution into the capillary. The volume of sample injected depends on the magnitude and duration of vacuum applied, sample solution viscosity, and capillary dimensions. After injection, the capillary is placed back into the source vial and an electric field applied.



Fig. 4.5. Hydrodynamic injection by gravity (also called siphoning). The capillary is placed into the sample vial, and the vial and capillary are raised a distance, H, above the destination vial, causing the sample solution to siphon into the capillary. The volume of sample injected depends on H, the length of time the vial is raised, capillary dimensions, and the sample solution viscosity. After injection, the capillary is placed back into the source vial and an electric field applied.



Sample Injection – Electrokinetic Injection

The capillary and the anode are placed into a sample vial and a voltage is applied for a given period of time.

After the sample is introduced, the anode and capillary are placed back into the source vial, an electric field is applied and electrophoresis continues.



Fig. 4.6. Electrokinetic injection. The capillary and the anode (in this example) are placed in the sample vial. A voltage is applied which causes the sample ions to migrate into the capillary due to electroosmosis and electrophoretic mobility. The amount of sample injected depends on the electrophoretic mobility of the solutes, electroosmotic flow rate, applied voltage, capillary dimensions, and solute concentrations. After sample injection, the capillary and anode are placed back into the source vial and a voltage is applied.



Sample Injection – Electrokinetic Injection

There is a sampling bias with this type of injection.

Larger quantities of solutes with high mobilities are injected than solutes with lower mobilities.

i.e. doubly charged cations have higher mobilities than singly charged cations or neutrals or anions.





opposed to hydrodynamic injection. For this illustration, it is assumed that the solutes were initially present in equal concentrations and they all have the same detector response.

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Instrumentation

Capillary tubes

Capillary tube

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ID typically 25-75 µm.

Length varies based on application but is normally in the 20-50 cm range.

The small bore and thickness of the silica are important. When a current is applied, this leads to Joule heating.

Using a small ID and having a thick wall reduces this problem.



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Instrumentation

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Detection TABLE 30-1 Detection Modes Developed for Capillary Electrophoresis^a

Detection Principle	Representative Detection Limit ^b (moles detected)
Spectrometry	
Absorption ^c	$10^{-15} - 10^{-13}$
Fluorescence	
Precolumn derivatization	$10^{-17} - 10^{-20}$
On-column derivatization	$8 imes 10^{-16}$
Postcolumn derivatization	2×10^{-17}
Indirect fluorescence	$5 imes 10^{-17}$
Thermal lens ^c	$4 imes 10^{-17}$
Raman ^c	2×10^{-15}
Mass spectrometry	1×10^{-17}
Electrochemical	
Conductivity ^c	1×10^{-16}
Potentiometry	Not reported
Amperometry	$7 imes 10^{-19}$
Radiometry ^c	$1 imes 10^{-19}$

^aFrom A. G. Ewing, R. A. Wallingford, and T. M. Olefirowicz, Anal. Chem., 1989, 61, 298A. With permission. ^bDetection limits quoted have been determined with a wide variety of injection volumes that range from 18 pI to 10 nL. ^eMass detection limit converted from concentration detection limit using a 1-nL injection volume.





Figure 30-7 An instrument for capillary electrophoresis/mass spectrometry. The voltage between the buffer solution on the left and the metalized silica capillary is 30 to 50 kV. The flow of nitrogen is 3 to 5 kV. The flow of nitrogen at \approx 70°C for desolvation is 3 to 6 L/min. *(From R. D. Smith, J. A. Olivares, N. T. Nguyen, and H. R. Udseth,* Anal. Chem., **1988**, 60, 437. *With permission.)*







Most common modes of capillary electrophoresis:

Micellar electrokinetic capillary chromatography

MEKC is a hybrid technique that merges electrophoresis and chromatography.

It involves adding surfactant micelles to the electrolyte.

Neutral analytes partition between the aqueous phase and the micelle core, while charged analytes migrate according to their charge-to-mass ratio.

MEKC's versatility allows for the separation of both neutral and charged compounds. However, micelle stability can affect reproducibility.



Most common modes of capillary electrophoresis: •Capillary isoelectric focusing (CIEF)

This technique exploits differences in the isoelectric points (pl) of analytes to achieve separation.

The capillary is filled with an ampholyte buffer, creating a pH gradient. Analytes migrate to their respective pl, where they become electrically neutral and halt.

This technique is excellent for proteins and peptides with distinct pls. But, CIEF is time-consuming due to the need for pl calibration, and complex samples can challenge resolution.





Most common modes of capillary electrophoresis: •Capillary isotachophoresis (CITP)

In CITP, the separation of analytes is based on their ionic mobilities in a stepwise manner.

A leading electrolyte with lower mobility and a trailing electrolyte with higher mobility sandwich the analytes, driving them toward the detector.

CITP excels in separating complex mixtures, ensuring that each analyte occupies a distinct migration zone. However, concentration effects can lead to distortion of peak shapes.





Most common modes of capillary electrophoresis:

• Capillary electrochromaotography (CEC) (recent technique)

CEC combines chromatography and electrophoresis principles. A stationary phase coats the inner capillary wall, interacting with analytes similarly to traditional chromatography. This interaction, combined with electrophoretic mobility, offers highresolution separations.

CEC is compatible with various detection methods and is capable of separating complex samples. However, the optimization of stationary phases and mobile phases can be intricate.

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Technique	Strengths	Limitations
CZE	Rapid, minimal preparation, suitable for ions/small molecules.	Limited resolution for complex mixtures.
CIEF	Separates based on pl, excellent for proteins/peptides.	Time-consuming, challenging with complex samples.
CITP	Efficient for complex mixtures.	Concentration effects.
MEKC	Versatile, separates neutral/charged compounds.	Micelle stability affects reproducibility.
CEC	High resolution, compatible with detectors.	Complex optimization.


Applications

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Food analysis:

CE contributes to food safety and quality control.

Detection of allergens, additives and contaminants, quantification of vitamins, amino acids and fatty acids, ensuring nutritional label accuracy.

CE also helps to detect food product adulterations.



CE electropherograms for the concurrent separations of inorganic cations, inorganic anions or artificial sweeteners, and organic anions. Channel 1, inorganic cations. Channel 2, option 1, inorganic anions; option 2, artificial sweeteners, aspartame (Asp), cyclamate (Cyc), saccharine (Sac), and acesulfame-K (Ace). Channel 3, organic anions (1) oxalate, (2) formate, (3) tartrate, (4) malate, (5) succinate, (6) citrate, (7) pyruvate, (8) acetate, (9) lactate, and (10) ascorbate.

Instrumentation

Applications

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Environmental monitoring:

CE used in environmental testing of pollutants, heavy metals and organic compounds in water and soil samples.

Detection of pesticide residues and pharmaceutical pollutants.

CE also assists in monitoring water quality, allowing the quantification of relevant anions like nitrates and sulfates



CE facilitates forensic DNA analysis, vital in criminal investigations and paternity testing.

The technique's sensitivity enables the analysis of minute samples, often crucial in solving cold cases.

