Lecture 4. Electrokinetics and Electrohydrodynamics

- Electrophoresis
- Electroosmosis
- Capillary Electrophoresis (CE)
- Dielectrophoresis (DEP)
- AC Electroosmosis

DEP preconcentrator
**Electrophoresis**

- An ion with charge \( q \) in an electric field \( E \) moves toward opposite electrode due to Coulombic force. A steady-state speed is reached when the accelerating force equals the frictional force generated by the medium.

![Diagram of electrophoresis](image)

\[
F_E = qE \quad F_{Friction} = f \cdot u_E = 6\pi \eta r \cdot u_E
\]

\[
u_E = \frac{q}{6\pi \eta r} \cdot E \quad \mu_E = \frac{u_E}{E} = \frac{q}{6\pi \eta r}
\]

Electrophoretic mobility is a function of viscosity and charge to radius ratio.
Applications of Electrophoresis

• Many important biological molecules such as amino acids, peptides, proteins, nucleotides, and nucleic acids, possess ionisable groups (COOH, NH₂, phosphates) and, therefore, at any given pH, exist in solution as electrically charged species either as cations (+) or anions (-). DNA is negatively charged because the phosphates that form the sugar-phosphate backbone of a DNA molecule have a negative charge.

• Depending on the nature of the net charge, the charged particles will migrate either to the cathode or to the anode at different rates. Electrophoresis has been applied to a variety of analytical separation problems.
  – Amino acids
  – Peptides, proteins (enzymes, hormones, antibodies)
  – Nucleic acids (DNA, RNA), nucleotides
  – Drugs, vitamins, carbohydrates
  – Inorganic cations and anions
Gel Electrophoresis

• Gel electrophoresis is a separation technique widely used for the separation of nucleic acids and proteins. The separation depends upon electrophoresis and filtering effect by gel (molecular sieve). Under electric field, charged macromolecules are forced to move through the gel with pores.

• Their rates of migration depend on the field strength, size and shape of the molecules, hydrophobicity of the samples, and on the ionic strength, and pH, temperature of the buffer in which the molecules are moving. After staining, the separated molecules can be seen in a series of bands spread from one end of the gel to the other.
Electric Double Layer

• In general, a surface carries a net charge which comes about either through dissociation of the chemical groups on the surface or by adsorption of ions or molecules from the solution onto the surface.

• Glass surface is covered with silanol groups (Si-OH) that carry a negative charge (Si-O⁻) at pH>2. When the surface is immersed in an electrolyte, positive ions will be attracted toward the surface forming **diffuse layer.**
Electric Double Layer

- In a thin region between the surface and the diffuse layer, there is a layer of bound positive ions, referred to as **Stern layer**. In this region, it is assumed that the potential falls linearly. The potential decays exponentially in the diffuse layer.
- **Zeta potential**, $\zeta$ represents the value of electrostatic potential at the interface between stern and diffuse layers. The thickness $\delta$ of the diffuse layer is defined as the distance from the stern layer to a point at which the electrostatic potential has dropped to 37% of zeta potential.
- The zeta potential of an aqueous solution in contact with glass can have a magnitude as high as 100 mV. pH and amount of counter ions affect the zeta potential.
Electro-Osmotic Flow (EOF)

- In a tangential electric field, excess cations in the diffuse layer are attracted to the cathode, and this imparts a pumping action onto the whole fluid column which moves toward the cathode.
- pH, dielectric constant, and temperature of the electrolyte are important parameters for the electroosmotic mobility.

\[
\mu_{EOF} = \frac{\varepsilon \zeta}{4\pi \eta}
\]

\[
u_{EOF} = \mu_{EOF} E
\]
EOF vs. Pressure-Driven Flow

- Uniform flow over more than 99.9% of the cross section of a capillary column. The speed of the flow falls off immediately adjacent to the capillary wall.

Electroosmosis driven flow

\[ u_{EOF} = \mu_{EOF} E \]

\[ \mu_{EOF} = \frac{\varepsilon \zeta}{4\pi \eta} \]

\[ Q = u_{EOF} \cdot A \]

Pressure-driven flow

\[ u(y) = \frac{(P_o - P_i)}{2\mu L} \cdot (y^2 - h^2) \]

\[ \bar{u} = \frac{h^2}{3\mu} \cdot \frac{(P_o - P_i)}{L} = \frac{3}{2} \cdot u_{\text{max}} \]

\[ Q = \frac{2h^3}{3\mu} \left( -\frac{dP}{dx} \right) = \frac{2h^3}{3\mu} \cdot \frac{(P_o - P_i)}{L} \]
Capillary Electrophoresis (CE)

- Electrophoresis performed in glass capillaries
- Electroosmotic mobility > electrophoretic mobility
Working Principle of CE

\[ u_a = \frac{L_d}{t} \]

\[ \mu_a = \frac{u_a}{E} = \frac{L_d}{t} \cdot \frac{L_t}{V} \]

- \( L_d \): distance from injection to detector
- \( L_t \): Capillary total length
- \( t \): migration time from injection to detector

\[ \mu_a = \mu_E + \mu_{EOF} = \frac{q}{6\pi \eta r} + \frac{\varepsilon \zeta}{4\pi \eta} \]

\( \zeta \) can be obtained from \( \mu_a \)
Some Issues about CE

• Unlike chromatography, analytes pass through the detector at different rates. This results in peak areas that are somewhat dependent on retention time.

• Band broadening occurs due to longitudinal diffusion, Joule heating, injection length, sample adsorption, etc.

• Electrokinetic injection leads to sampling bias, because a disproportionately large quantity of the species with higher electrophoretic mobility migrates into the tube and can cause problems for quantitative analysis.

• Electroosmotic flow rates tend to change over time because the zeta potential changes over time, thus leading to changes in mobility.
Microfabricated CE

- In electrophoresis, the separation efficiency (number of theoretical plates) and analysis speed depend on capillary length and diameter \((N \sim L/d, t \sim Lxd)\). This means, the only way of increasing the speed of an analysis and keeping the efficiency constant is to reduce the diameter and the length of a capillary by the same factor.

- High speed and efficiency, however, usually require small injection and detection volumes. These features can easily be arranged in microfabricated CE systems.

- Using planar micromachined channels instead of glass capillaries offers the potential for mass production, the choice of many materials (e.g., plastics), and integration of many processing step on one chip.
Microfabricated CE

Low-cost Glass or PMMA MicroCE

- PR spin-coating and soft baking
- UV Exposure
- Photoresist developing and hard baking
- BOE etching in ultrasonic bath
- PR stripping
- Cover glass drilling
- Cleaning and alignment
- Fusion bonding

CE + PCR + Optical sensor

Parylene MicroCE

- UV gel
- TBE Buffer
- Electrodes
- PCR chamber
Microfabricated CE

(A) Small channel $\rightarrow$ High E field $\rightarrow$ High speed separation in seconds or even milliseconds

(B) Geometrically defined injected volume

(C) Smallest possible sample plug + longer channel $\rightarrow$ High separation efficiency
AC Electrokinetics: Dielectrophoresis (DEP)

- A dielectric particle placed in an electric field becomes electrically polarized as a result of partial charge separation, which leads to an induced dipole moment. The dipole moment is a consequence of the generation of equal and opposite charges at the boundary of the particle.
- The induced surface charge is only about 0.1% of the net surface charge normally carried by biological cells and microorganisms and is generated within about a microsecond.
Dielectrophoresis (DEP)

- In a nonuniform electric field, the particle experiences a net dielectrophoretic force. The magnitude of the induced dipole depends on the polarizability of the particle with respect to that of the medium.
- If a suspended particle has polarizability higher than the medium, the DEP force will push the particle toward regions of higher electric field (positive DEP). If the medium has a higher polarizability than the suspended particle, the particle is driven toward regions of low field strength (negative DEP).

What if the bias voltage is reversed?
Dielectrophoresis (DEP)

Motion of a particle is determined by the magnitude and polarity of charges induced in the particle by an applied field.

AC field of a wide range of $\omega$.
Must be inhomogeneous

**Electrophoresis**
Motion of a particle is determined by a net intrinsic electrical charge carried by that particle.
DC field, usually homogeneous

**Dielectrophoresis**
Movement of a particle is determined by the net intrinsic electrical charge carried by that particle.
Effective Dipole Moment

- For a spherical particle of radius $r$ and complex permittivity $\varepsilon_p^*$, suspended in a fluid ($\varepsilon_m^*$), the effective dipole moment is derived as

$$\vec{p} = 4\pi\varepsilon_m \left( \frac{\varepsilon_p^* - \varepsilon_m^*}{\varepsilon_p^* + 2\varepsilon_m^*} \right) r^3 \vec{E}$$

- The effective dipole moment of the particle is frequency dependent. This dependence is described by the Clausius-Mossotti factor, which indicates the relative polarizability of the particle with respect to its suspending medium.

$$\varepsilon^* = \varepsilon - j\frac{\sigma}{\omega}$$

(Simplified)

$$K(\omega) = \frac{\varepsilon_p^* - \varepsilon_m^*}{\varepsilon_p^* + 2\varepsilon_m^*}$$

- The electrokinetic force on a particle subjected under an $E$ field is

$$\vec{F} = \vec{F}_{EP} + \vec{F}_{DEP} = q\vec{E} + (\vec{p} \cdot \nabla)\vec{E}$$

- The time-averaged dielectrophoretic force is

$$< \vec{F}_{DEP} >= \frac{1}{2} \text{Re}[\nabla \cdot (\vec{p} \cdot \nabla \vec{E}^*)] = \pi\varepsilon_m r^3 \text{Re}[\frac{\varepsilon_p^* - \varepsilon_m^*}{\varepsilon_p^* + 2\varepsilon_m^*}] \nabla |\vec{E}|^2$$

Assume there is no spatially varying phase ($\vec{E}^* = -(\nabla \phi_R + i\nabla \phi_I) = -\nabla \phi_R = \vec{E}$)
Clausius-Mossotti Factor

- The real part of CM factor defines the frequency dependence and direction of the force.

\[ K(\omega) = \frac{\varepsilon_p^* - \varepsilon_m^*}{\varepsilon_p^* + 2\varepsilon_m^*} \]

\[ \varepsilon^* = \varepsilon - j\frac{\sigma}{\omega} \]

\[ \langle F_{DEP} \rangle = \frac{1}{2} \text{Re}[(\vec{p} \cdot \nabla)E^*] = \pi\varepsilon_m r^3 \text{Re}\left[\frac{\varepsilon_p^* - \varepsilon_m^*}{\varepsilon_p^* + 2\varepsilon_m^*}\right] V |\vec{E}|^2 \]

CM factor as a function of frequency

(A and B) \( \varepsilon_p = 2.4 \) \( \varepsilon_m = 81 \)
\( \sigma_p = 2e^{-4} \text{ S/m}, \sigma_m = 0 \text{ to } 0.05 \text{ S/m} \)

Theoretical \( K(\omega) \) values for bioparticles
(Huang Y. et al, Anal Chem 2001)
Dielectrophoretic Force

\[
<\vec{F}_{\text{DEP}} >= \pi \varepsilon_m r^3 \Re \left[ \frac{\varepsilon_p^* - \varepsilon_m^*}{\varepsilon_p^* + 2\varepsilon_m^*} \right] \nabla |\vec{E}|^2
\]

- The DEP force is zero if the electric field is uniform.
- The DEP force scales with the square of the voltage (or electric field). Reversing the bias does not reverse the force. Spatial dependence of the DEP force arise from electric field component.
- The DEP force scales inversely with the cube of the electrode gap. Decreasing the characteristic dimensions of the electrode by one order of magnitude can lead to a three orders of magnitude increase in the DEP force.
- The DEP force is proportional to the particle volume. Strong electric fields (\(10^4\)-\(10^5\) V/m) are required to manipulate micron-scale particles.
- Microfabricated electrodes can provide the required high E field only with several volts. Joule heating effect can also be minimized with microfluidic channels with high surface/volume ratio.
**Dielectrophoretic Mobility**

- If a particle moves under the influence of a DEP force, the equation of motion is

\[ m \frac{d\nu}{dt} = F_{\text{DEP}} - F_{\eta} \tag{Other forces ignored} \]

**Steady-state**

\[ F_{\text{DEP}} = F_{\eta} = 6\pi \eta r \nu \]

\[ \langle \bar{F}_{\text{DEP}} \rangle = \pi \varepsilon_m r^3 \text{Re}\left[ \frac{\varepsilon_p^* - \varepsilon_m^*}{\varepsilon_p^* + 2\varepsilon_m^*} \right] \nabla |\bar{E}|^2 \]

**DEP mobility**

\[ \mu_{\text{DEP}} = \frac{\nu}{\nabla |\bar{E}|^2} = \frac{\varepsilon_m r^2 \text{Re}\left[ \frac{\varepsilon_p^* - \varepsilon_m^*}{\varepsilon_p^* + 2\varepsilon_m^*} \right]}{6\eta} \]
DEP with a Spatially Dependent Phase

• For an AC field, such as that generated by the application of multiple potentials of different phase, the derivation of the dielectrophoretic force is more involved and the DEP force can be expressed as:

\[ \vec{E}^* = - (\nabla \phi_R + i \nabla \phi_I) \]

\[ \langle \vec{F}_{DEP} \rangle = \pi \varepsilon_m r^3 \text{Re}\left[ \frac{\varepsilon_p^* - \varepsilon_m^*}{\varepsilon_p^* + 2\varepsilon_m^*} \right] \nabla |\vec{E}|^2 + 2\pi \varepsilon_m r^3 \text{Im}\left[ \frac{\varepsilon_p^* - \varepsilon_m^*}{\varepsilon_p^* + 2\varepsilon_m^*} \right] (\nabla \times (\text{Re}[\vec{E}^*] \times \text{Im}[\vec{E}^*])) \]
Gravity and Brownian Motion

• Gravity and Buoyant forces

The effective mass of a spherical particle suspended in a medium

\[ \Delta m = \frac{4}{3} \pi r^3 (\rho_p - \rho_m) \]

Sedimentational force

\[ F_{grav} - F_{buoy} = \Delta m \cdot g \]

Sedimentation rate in a steady-state condition

\[ v = \frac{\Delta m \cdot g}{6 \pi \eta r} = \frac{2 r^2 (\rho_p - \rho_m) g}{9 \eta} \]

(What if there is a non-uniform heating in the system?)

• Brownian motion

Particles in solution experience a random force due to the thermal energy of the system, causing them to move in a random manner. The rms velocity of the particle is

\[ \langle |v|^2 \rangle^{1/2} = \sqrt{\frac{3 kT}{m}} \]
• Submicron particle manipulation requires very high field strength to overcome Brownian motion. The DEP potential must exceed the thermal energy.

\[ \pi \varepsilon_m r^3 \text{Re}\left[ \frac{\varepsilon^*_p - \varepsilon^*_m}{\varepsilon^*_p + 2\varepsilon^*_m} \right] |\vec{E}|^2 \geq kT \]

• Side effects: AC electroosmosis and possible cell damage or electroporation.
Particle-Particle Interaction

• The particles are not isolated entities and particle-particle interaction must be considered.

• Two or more particles with the same sign of charges suspended in an insulating medium will repel each other.

• However, in electrolyte the available free charges screens the particle’s charge and the field produced by the particle’s charge rapidly decays with distance. Therefore any long-range electrostatic interaction with other particles does not occur. But when the particles are very close, both electrostatic interactions and van der Waals may become visible, resulting in the formation of long chains.

![Diagram of attractive force between two fixed dipoles aligned by an applied uniform electric field.](image)
Microfabricated DEP

Separation, trapping, movement, levitation, rotation, identification, and other manipulations of cells and microorganisms
DEP: Electrode Design
DEP Applications

• The characterization of blood cell subpopulations on normal blood and the detection of perturbations of those subpopulations resulting from diseases.

• Sample collection (enrichment) for biological warfare agents or pathogens (bacteria, viruses) detection.

• Integrated isolation and molecular analysis of tumor cells.

• Bioparticle fractionation based on DEP-FFF or DEP chromatography.

• Cell patterning for in-vitro cell culture

• Particle focusing for flow cytometry

• Single cell manipulation

• Detection of molecular binding
Example: Single Cell Cage

Fig. 5. Image sequence of growing of yeast cells caged in a DFC. A single cell was initially trapped (a) and observed under permanent field exposition (ac drive at 1.4 \( V_{\text{rms}} \) and 7.7 MHz). Several cell divisions can be observed (b–e) until the cage region is almost filled with cells (f).
Example: Endothelial Cell Patterning

Fig. 1. Photographs of various stages of the dielectrophoretic patterning of cells. (A) Randomly distributed cells with electrodes turned off. (B) With a fluid flow rate of ~30 μl/min, energized traps attract single and multiple cells. (C) With fluid flow at the rate of ~150 μl/min, single cells remain trapped while additional cells are removed. (D) Cells spread on the electrode regions 1 h after DEP. In (B) and (C), flow is from left to right. Scale bars are 50 μm.
Example: 3D Particle Focusing for Microcytometry

Fig. 4. A schematic diagram of a microfluidic channel with a microelectrode array patterned on its circumference for dielectrophoretic particle focusing.

Fig. 8. A schematic diagram of the experimental setup for measuring the width of the particle stream.
Example: Molecular Separation Using DEP

**Fig. 1** Methods of biochemical assay based on B/F separation.

**Antigen-antibody reaction method**

- Label
- Fluorescence-labeled probe (Antibody)
- Mixture containing target molecule
- Antigen-antibody reaction
- B/F separation
- Detection: Target-probe complex
- Unbound molecules

**Hybridization reaction method**

- Label
- Fluorescence-labeled probe (short DNA)
- Mixture containing target molecule
- Hybridization reaction
- B/F separation
- Detection: Target-probe complex
- Unbound molecules

**Fig. 2** The Principle of DEP Chromatography

- a) Top view
- b) Cross-sectional view
- Electrode
- Fluid passage
- Negative DEP
- No DEP
- Positive DEP

**Fluorescence Intensity [arb.]**

- $\sigma_m = 0.45 \text{ mS/cm}$
- $\sigma_m = 0.25 \text{ mS/cm}$
- 6.6 kbp
- 48 kbp
- 6.6 kbp
- 48 kbp
AC Electroosmosis

- In the planar microelectrode arrays used for AC electrokinetics, divergent electric fields are generated, and as a result a component of the electric field lies tangential to the electrical double layer which is induced on the electrode surface. Therefore the ions in the diffuse double layer experience a force similar to DC electroosmosis.