Zonal Electrophoresis

- Most common form of electrophoresis in biological studies
- Uses a support system, most commonly gel to separate proteins by their properties
- We will cover methods to separate by:
  - Size (Through Frictional Properties)
  - Charge
  - Both

http://www.biologyreference.com/images/biol_02_img0140.jpg
Gels

- Two main types of gels
  - **Agarose**
    - Seaweed based linear polysaccharide
    - Mechanical properties are determined by the percentage of Agarose
  - **Polyacrylamide (PAGE)**
    - Cross-linking acrylamide polymer
    - Firmness and pore size are determined by percentage of PAGE present and bisacrylamide
SDS Gel Electrophoresis

- Separates proteins by size
- Proteins are denatured and negatively-charged sodium dodecyl sulfate (SDS) is added
  - SDS binds to every two amino acids causing the protein to have a negative charge
  - SDS polypeptides move through a gel at a rate dependent on their mass
SDS Gel Electrophoresis

- Protein is placed into a gel in a conductive solution and then pulled towards a positive electrode.
- The distance travelled in the gel is related logarithmically to the size.
- A control sample with known sizes is used to determine the sizes of the unknown samples.

SDS Gel Electrophoresis

• Log of the molecular weight versus the distance traveled through the gel is plotted on a semi log plot
• Known sample is used to create a line
• Unknowns are determined using the equation of the line

http://www.thermoscientificbio.com/uploadedImages/Products/Protein_Electrophoresis/Protein_Ladders/26610-ladder-002.jpg
Isoelectric Focusing (IEF)

- Separates proteins by charge
- Separates amphoteric molecules in a pH gradient
  - Amphoteric molecule are molecules whose charge is dependent on pH
  - They all have a pI point where they have neutral charge
- When a pH gradient is created across a gel, the amphoteric molecule will be pulled by the electrode until it reaches its pI point
- Once it is neutral, it is no longer affected by the electrodes
Understanding IEF

- pKa of the carboxylic acid is around 2.2
- pKa of the amine group is 9.4
- When pH > pKa, the group is deprotonated
- When pH < pKa, the group is protonated
Two-Dimensional Gel Electrophoresis

- Separates by both size and charge
- IEF is first used on a gel strip
- The strip is then mounted on a gel slab and SDS PAGE is used
- Proteins can be later removed from gel to identify
- Great for large scale comparisons (proteomics)
Capillary Electrophoresis (CE)

- Alternative method to gel electrophoresis
- Proteins are dragged through a capillary tube rather than a gel
- Detector uses UV light absorbance readings to identity whether a protein separation has passed through
CE Injection

- First the capillary tube has to be loaded with buffer
- Three methods:
  - Apply pressure or vacuum to one side
  - Use a gravity siphon
  - Drive the buffer in using a potential difference
CE Formulas

- There are two equations for CE
- First we define the electrophoretic mobility:
  \[ \mu = \frac{L^2}{Vt} \]
- So to minimize \( \mu \), high voltage and short capillary are ideal
  - However we are constrained due to heat production from our power source
- Then we can look at the separation efficiency:
  \[ N = \frac{\mu V}{2D} \]
- This is in terms of the theoretic plates, which is an evaluation of the resolution of the separation
Electroosmotic Flow (EOF)

- Driving force in CE
- Drives both anions and cations towards the cathode
  - Walls of the capillary are negatively charged
  - This binds cations from the buffer
  - The cation layer that forms is attracted to the cathode and drives the flow towards the cathode
  - EOF can be reversed or completely removed by changing the charge on the capillary walls

http://micromachine.stanford.edu/~dlaser/images/eof_capillary.jpg
Ultrafast Capillary Electrophoresis

- It is possible to speed up separation
- Using an hourglass shape in the capillary increases the electric field at a specific point (reducing the risk of overheating)
- As cross-sectional area decreases electric field magnitude increases, this allows very large electric field at the mid point with relative low input voltage

![Graph showing electrophoretic resolution of 5HT and 5HTp photoproducts in 10 μs. A field, which was estimated to be 0.15 MV cm⁻¹ (35 kV), was used to fractionate components over a separation distance of 9 μm. (After Plenert and Shear, 2003.)](image)
Two-Dimensional CE

- Performed similarly to 2-D gel electrophoresis
- CE is performed in a one-dimensional capillary
- The capillary is then connected to a series of parallel capillaries
- Then a second separation is performed
Chirality

- Chirality is the “handedness” of a molecule
  - Chiral molecules are non-superimposable mirror images of each other
  - While identical in structure, chiral molecules can have vastly different properties

- Pharmaceutical applications
  - Thalidomide
    - One enantiomer helps with morning sickness
    - The other enantiomer causes birth defects
Separation of Chiral Molecules

• CE is performed with Cyclodextrin in the buffer
• Cyclodextrin is a non-ionic, cyclic, chiral molecule
• One enantiomer will react much more strongly with cyclodextrin
• This enantiomer will reach the detector later
  ▫ Interaction with the cyclodextrin slows down the migration of this enantiomer

Fig. D5.20 Chemical structure of $\beta$-cyclodextrin. (After Ward, 1994.)
Capillary Electrochromatography (CEC)

- Uses EOF to move through a column
- Sorbent in column will interact differently with different molecules
- Molecules with migrate at different rates based on their charge and based on their interactions with the sorbent in solution
- By adding an additional determining factor, it becomes easier to distinguish between molecules that would otherwise look similar (in CE)
Sources


