Electrophoresis Part 2

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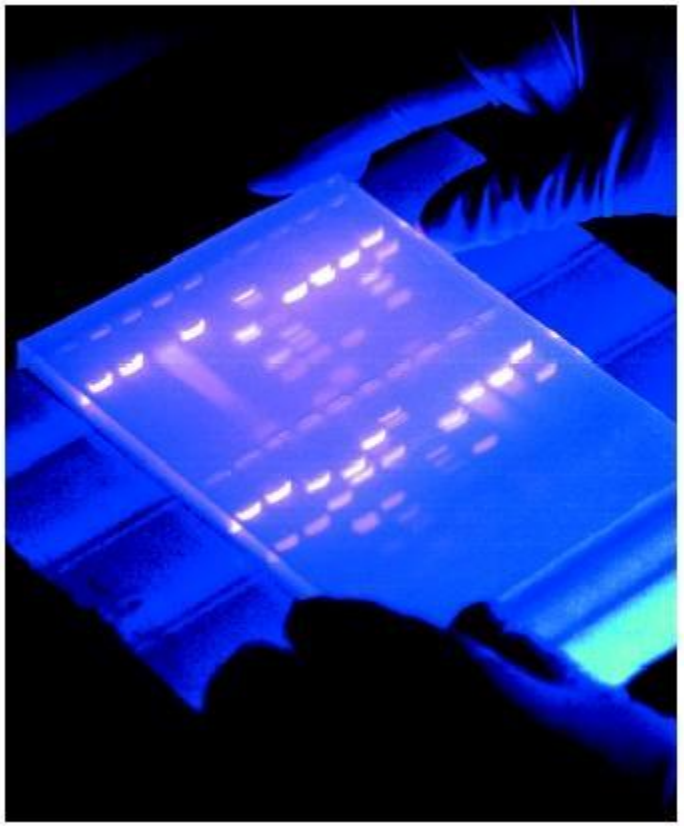
Zonal Electrophoresis

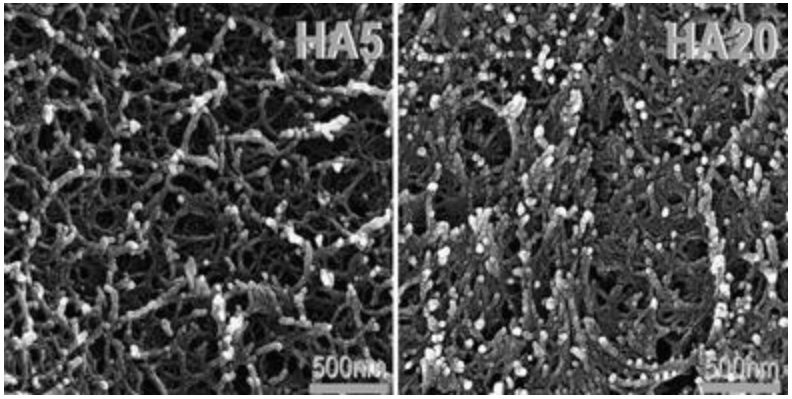
- Most common form of electrophoresis in biological studies
- Uses a support system, most commonly gel to separate proteins by their properties
- We will cover methods to separate by:
  - Size (Through Frictional Properties)
  - Charge
  - Both



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



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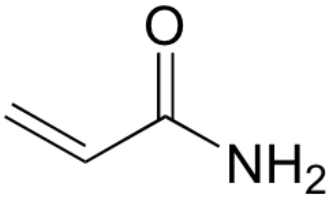
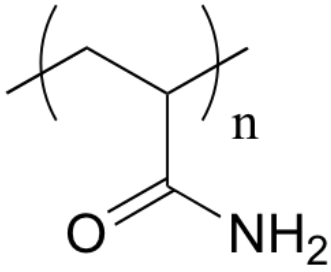


acrylamide



polyacrylamide

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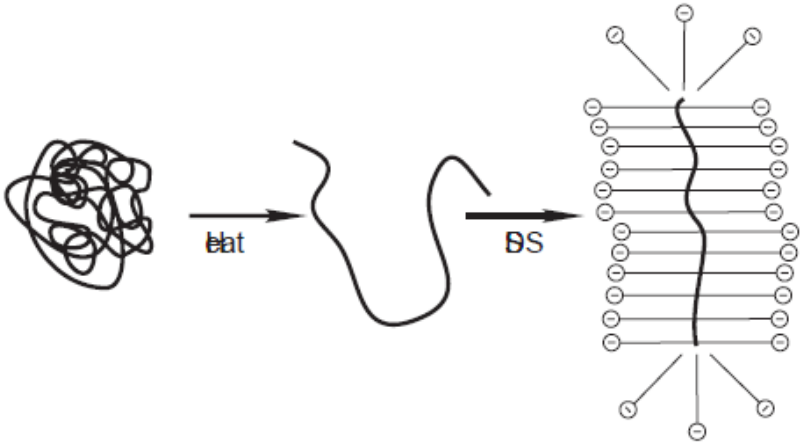
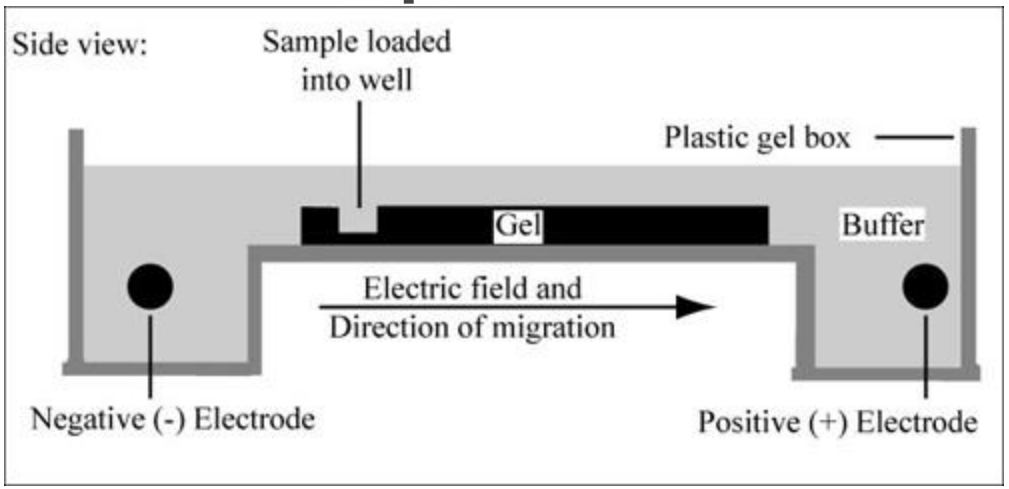
SDS Gel Electrophoresis



- 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

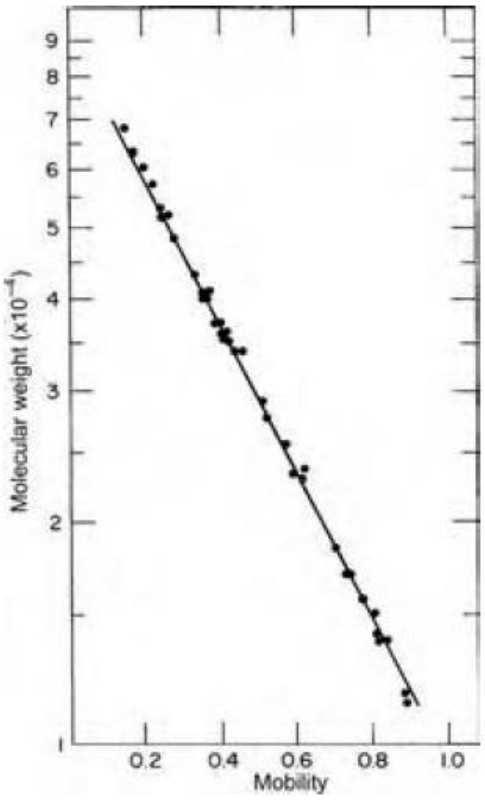


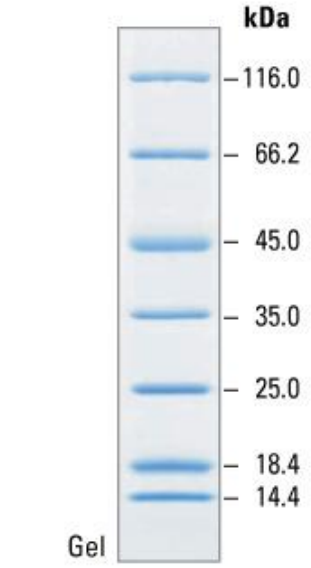
http://ocw.mit.edu/courses/biological-engineering/20-109-laboratory-fundamentals-in-biological-engineering-fall-2007/labs/mod1_2_photo.jpg

- 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

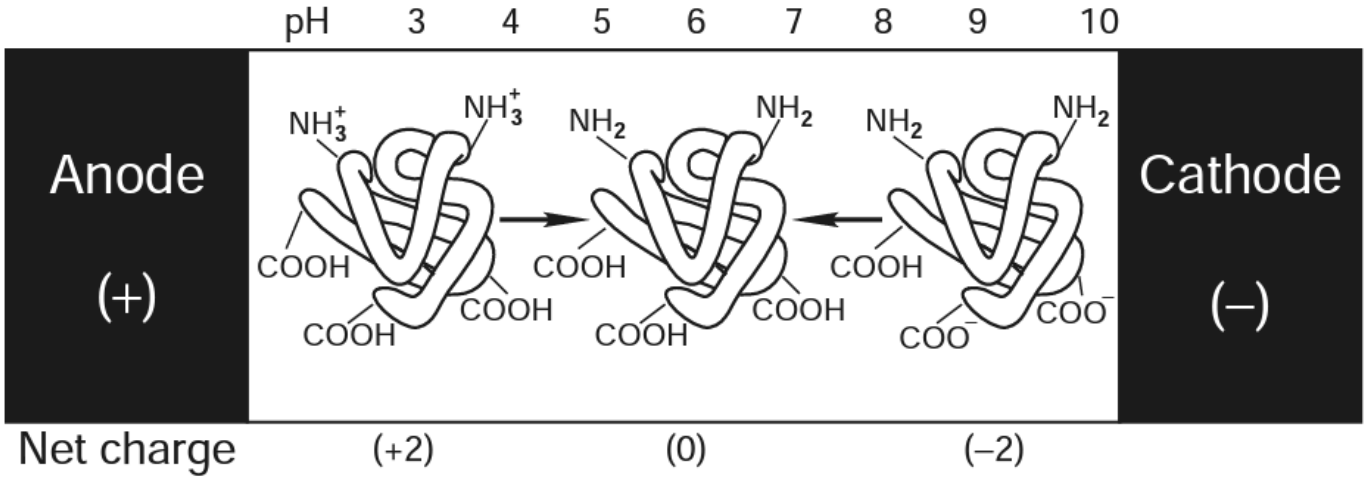
- Log of the molecular weight versus the distance traveled through the gel is plotted on a semi log plot
- Known sample is used to create a line
- Unknowns are determined using the equation of the line





http://www.thermoscientificbio.com/uploadedImages/Products/Protein_Electrophoresis/Protein_Ladders/26610-ladder-002.jpg

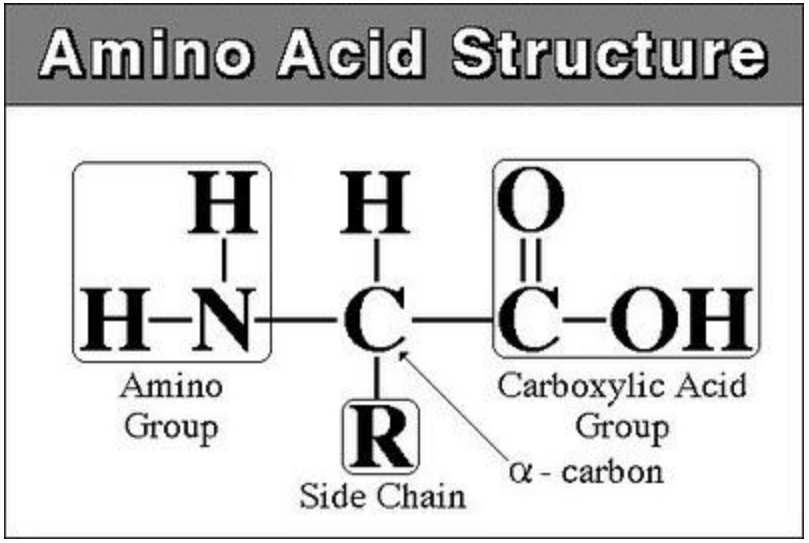
Isoelectric Focusing (IEF)



- 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



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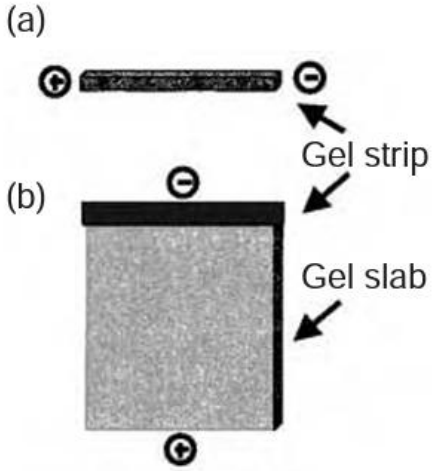
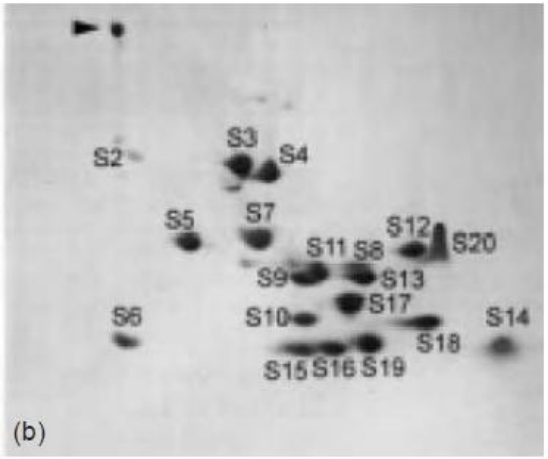
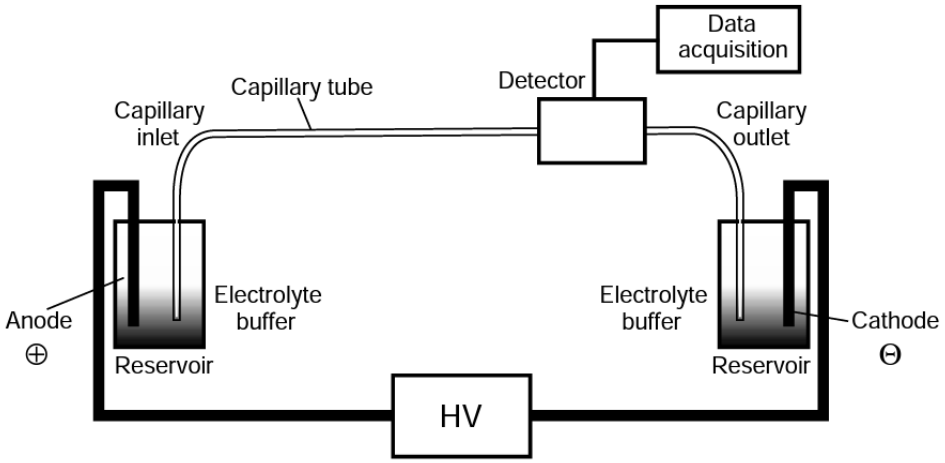
Two-Dimensional Gel Electrophoresis

- 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)





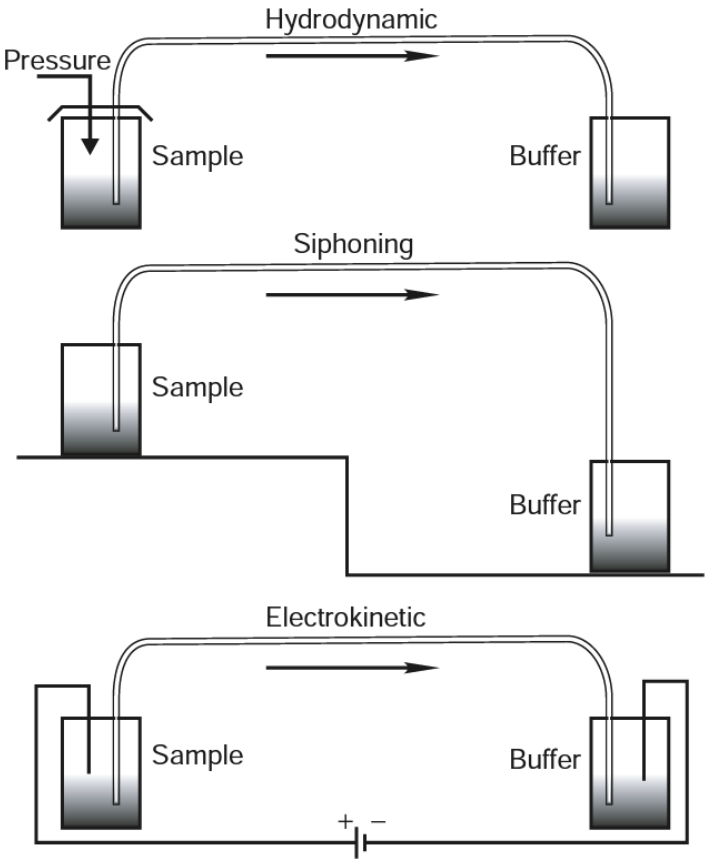
Capillary Electrophoresis (CE)



- 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:

$$\mu = {}^{L^2}/_{Vt}$$

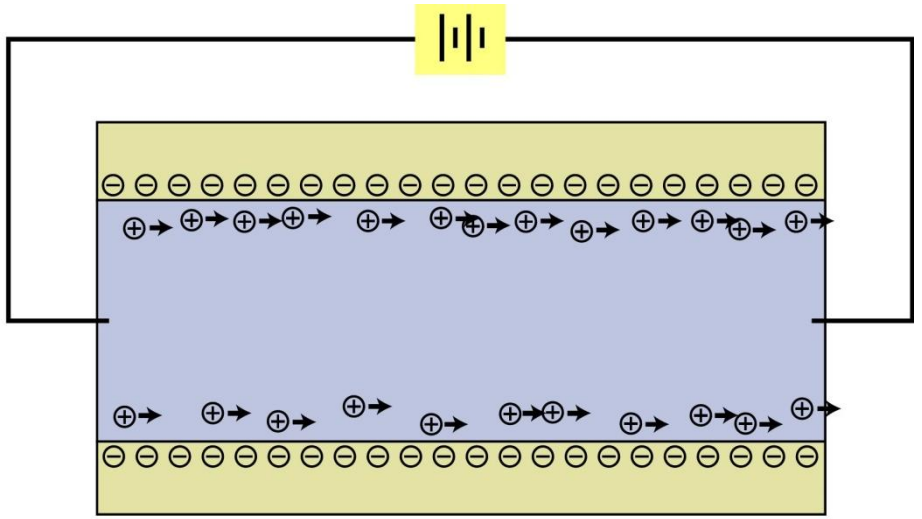
- So to minimize μ, high voltage and short capillary are ideal
  - However we are constrained due to heat production from our power source
- Then we can look at the separation efficiency:

$$N = {}^{\mu V}/_{2D}$$

- 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



[inside of capillary, cut along length]

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

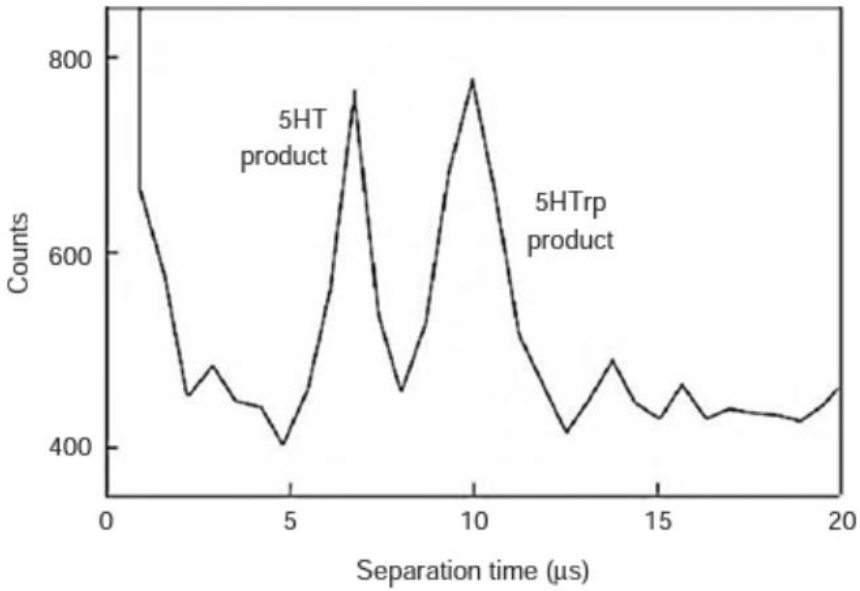
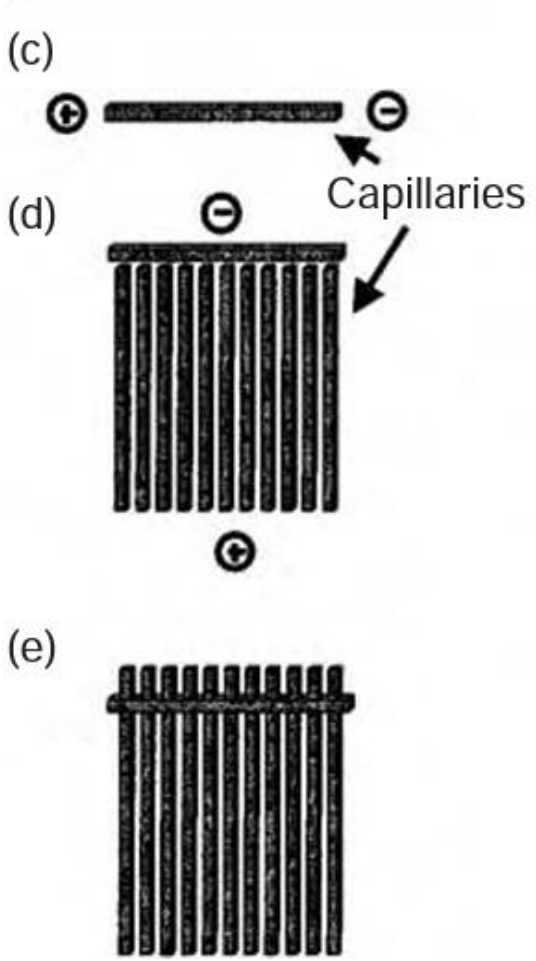


Fig. D5.17 Electrophoretic resolution of 5HT and 5HTrp photoproducts in 10 μs. A field, which was estimated to be 0.15 MV cm^{-1} (35 kV), was used to fractionate components over a separation distance of 9 μm. (After Plenert and Shear, 2003.)

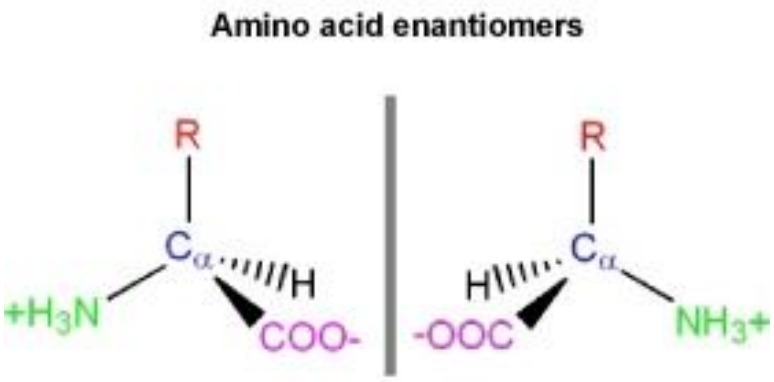
Two-Dimensional CE

- 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

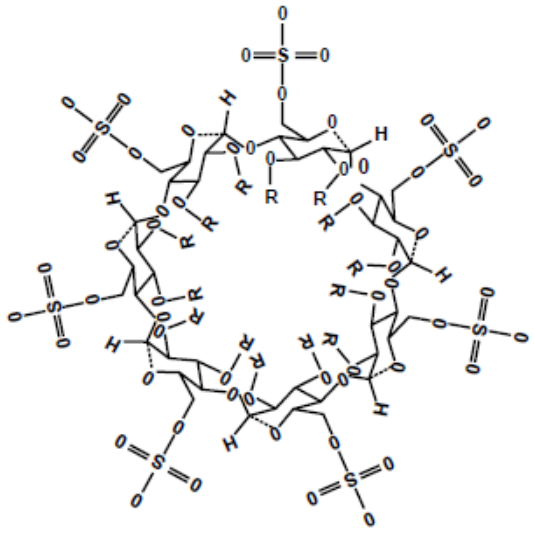
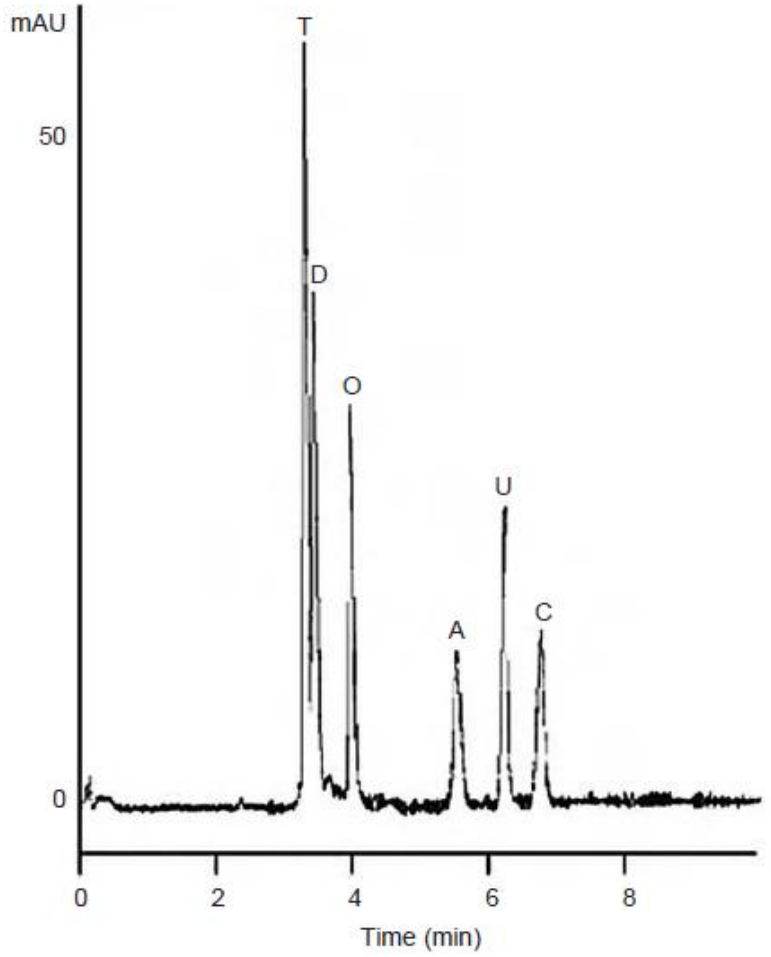


Fig. D5.20 Chemical structure of β-cyclodextrin. (After Ward, 1994.)

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

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3. "Capillary Electrophoresis." *Sam Houston State University*. N.p.. Web. 6 Oct 2013. <http://www.shsu.edu/chm_tgc/sounds/flashfiles/CE.swf>.
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