Capillary Electrophoresis

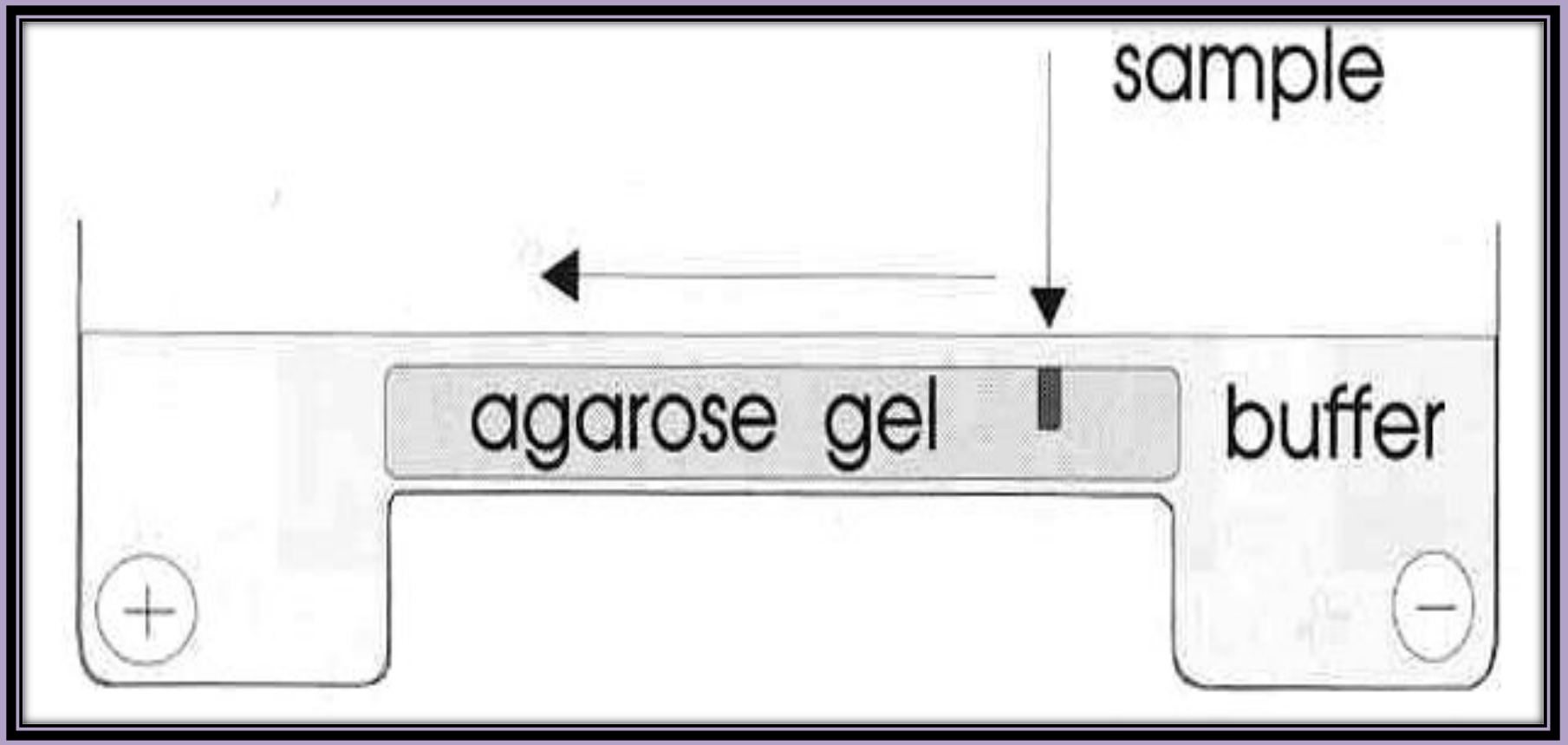
Electrophoresis

- *a separation technique that based on the mobility of ions in an electric filed towards its opposite charge electrode*

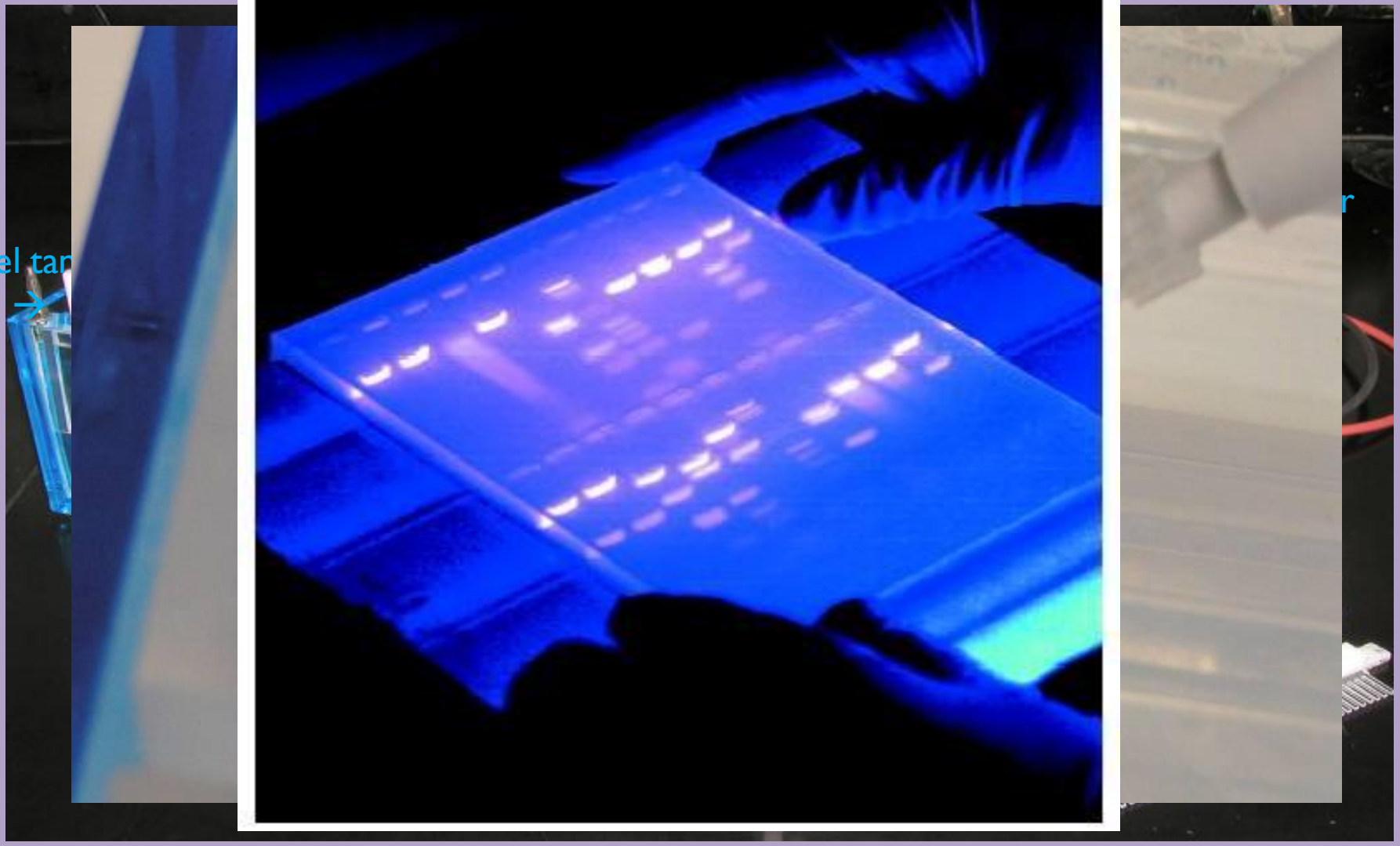
Arne Tiselius

in 1937
Invented "moving boundary electrophoresis" to separate proteins based on charge
1948. Awarded the Nobel Prize in Chemistry for his invention

GEL ELECTROPHORESIS



Electrophoresis Equipment



The term gel in this instance refers to the matrix used to contain, then separate the target molecules.

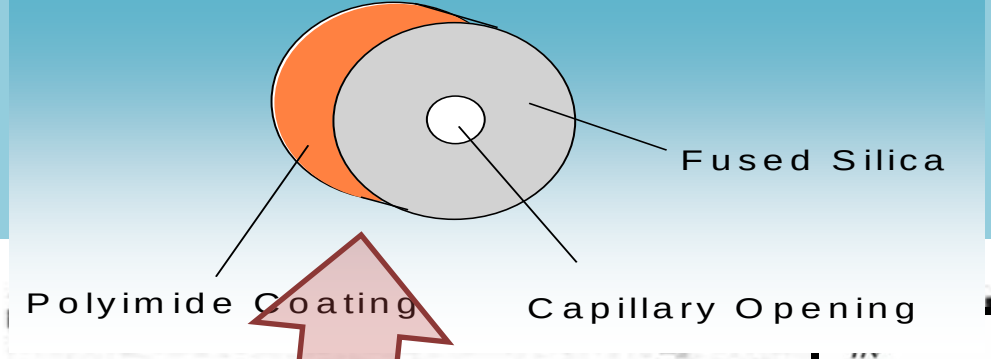
In most cases, the gel is a crosslinked polymer whose composition and porosity is chosen based on the specific weight and composition of the target to be analyzed.

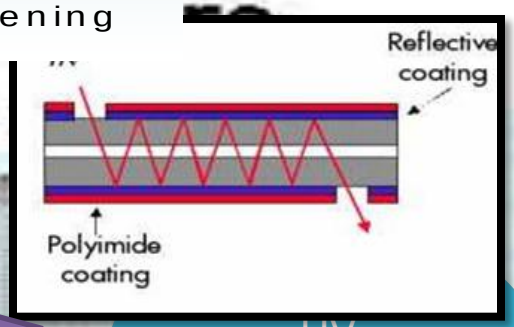
When separating proteins or small nucleic acids (DNA, RNA, or oligonucleotides) the gel is usually composed of different concentrations of acrylamide, producing different sized mesh networks of polyacrylamide.

Capillary Electrophoresis

known as capillary zone electrophoresis (CZE), can be used to separate ionic species by their charge.

In traditional electrophoresis, electrically charged analytes move in a conductive liquid medium under the influence of an electric field.





compartment, 20

Capillary

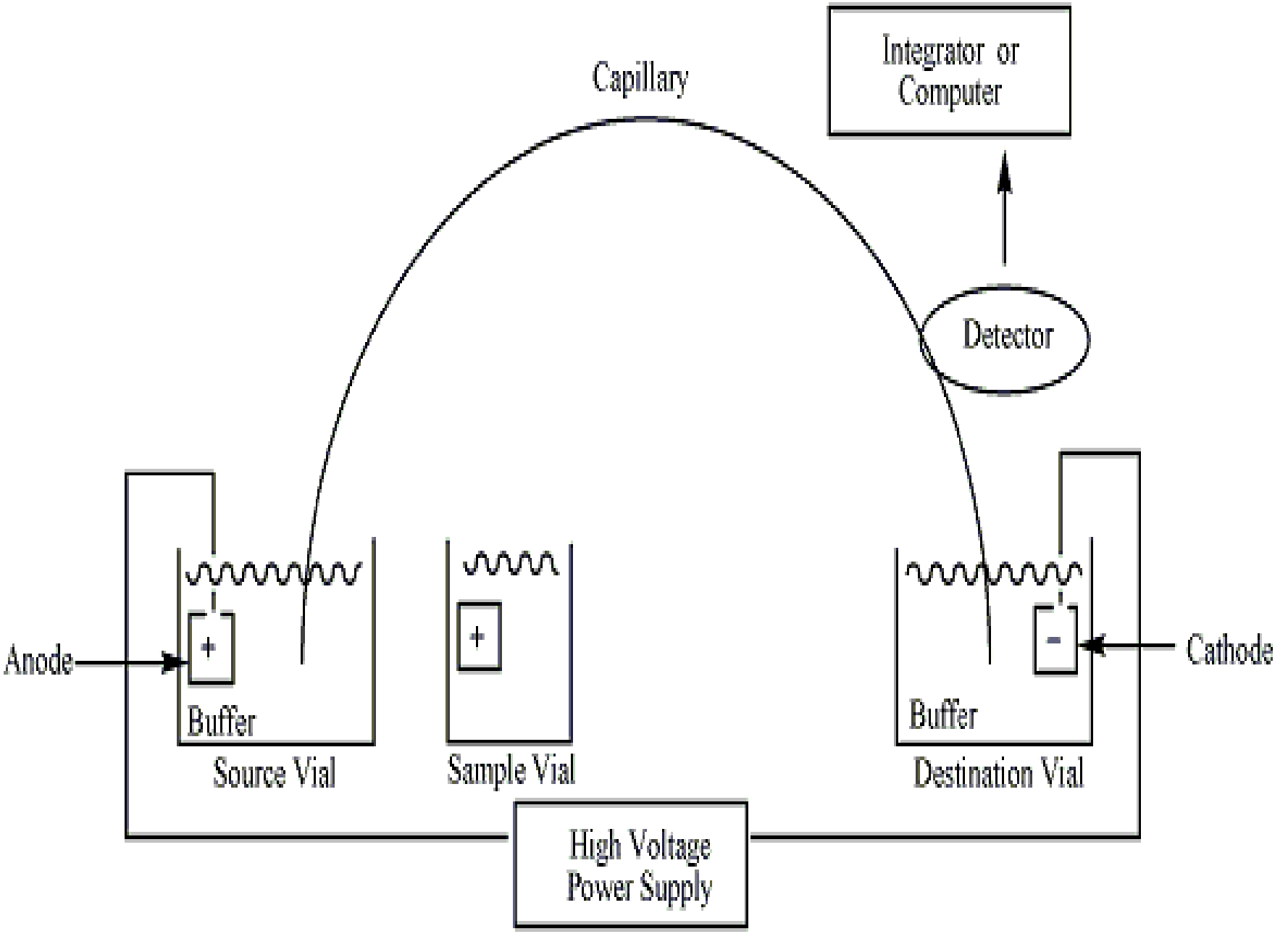
UV Detector

High voltage power supply

Buffer reservoirs

electrolyte

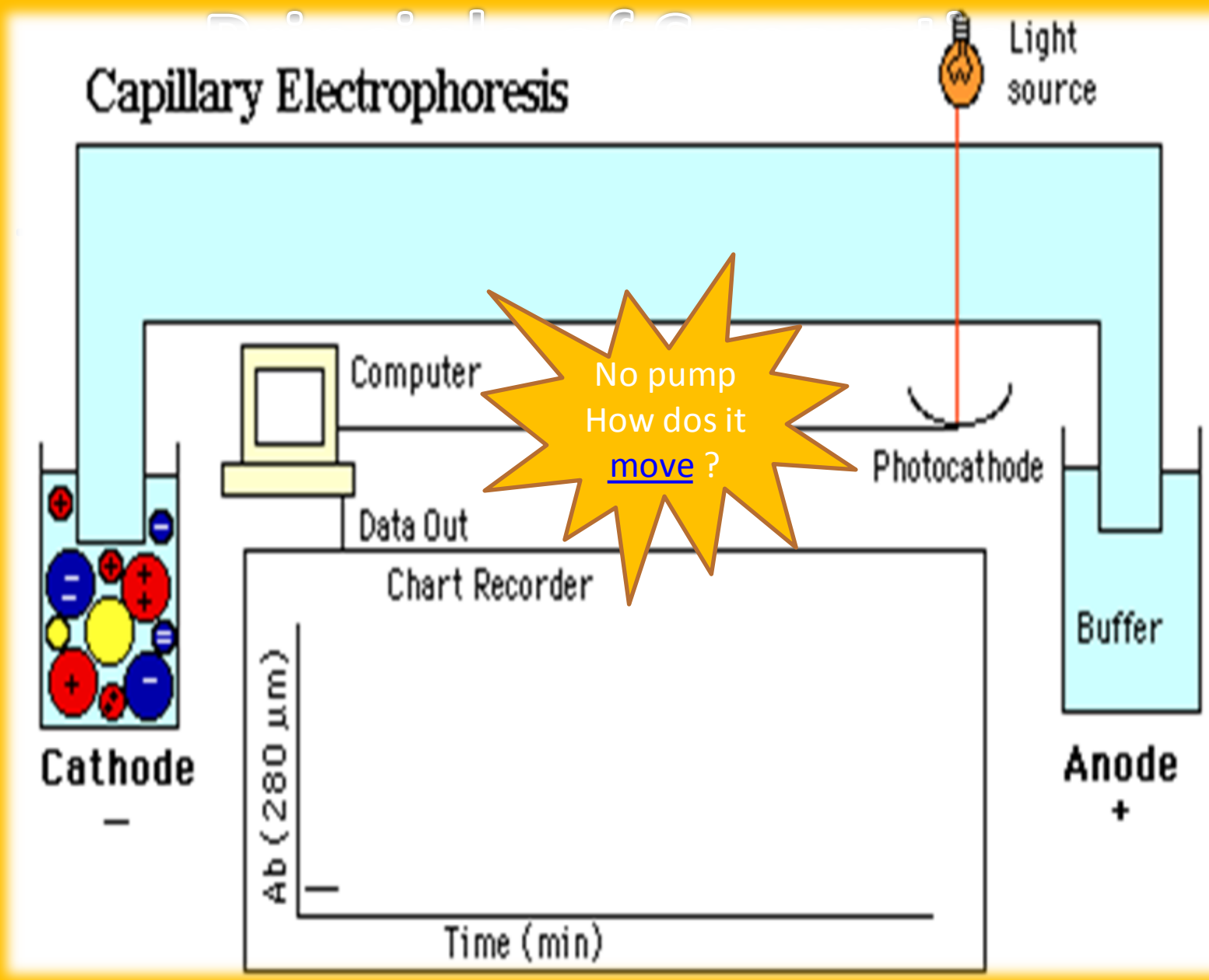
Sample Reservoir


Integrator or Computer
Capillary
Detector
Anode
Cathode
Buffer
Source Vial
Sample Vial
Buffer
Destination Vial
High Voltage Power Supply

Principle of separation by capillary electrophoresis

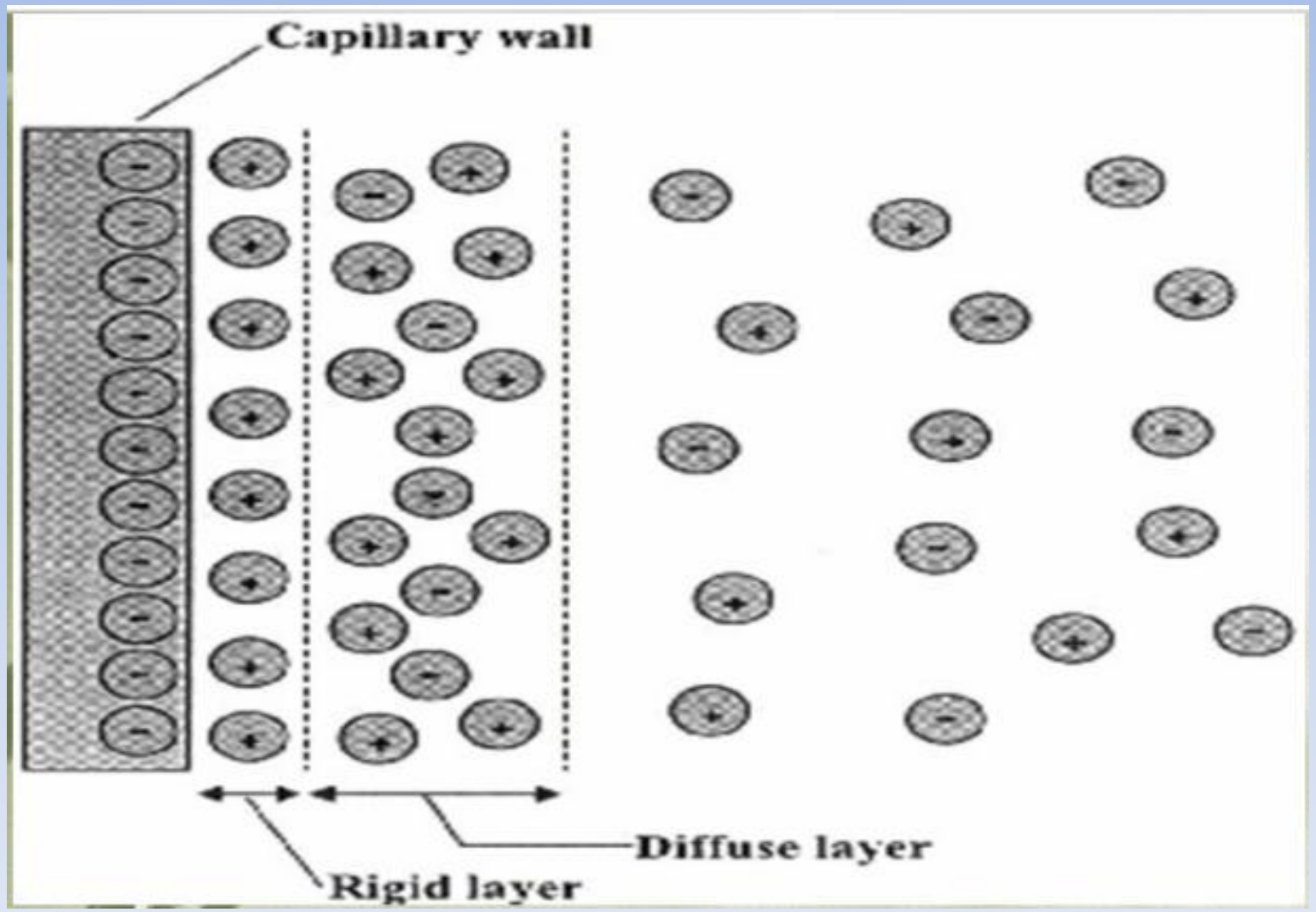
In practical term,a positive (anode) and negative (cathode) electrode are placed in a solution containing ions.

Then when a voltage is applied across the electrodes, solute ions of different charge, i.e, anions (negative) and cations (positive), will move through the solution towards the electrode of opposite charge.


Capillary Electrophoresis
Light source
Computer
No pump
How dos it
move ?
Photocathode
Data Out
Chart Recorder
Buffer
Cathode
–
Anode
+
Ab (280 µm)
Time (min)

Electroosmotic Flow

This flow is a phenomena resulting when a solution is contained in a capillary with fixed charges along its wall. This is also known as the Electroendosmotic Flow.

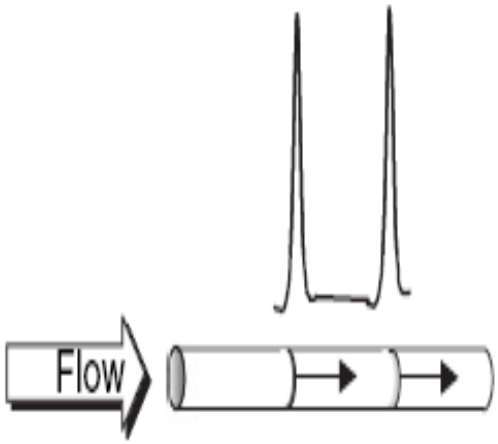

Capillary wall
Diffuse layer
Rigid layer

pH, Silanol Population, and the rate of EOF flow.

- *At very low pH, not many silanols are ionized and the EOF is slow.*
- *As pH increases the number of ionized sites also increases. The EOF speed rises steadily.*
- *At very high pH values, a maximum number of ionized sites is reached. The EOF speed also reaches a maximum*

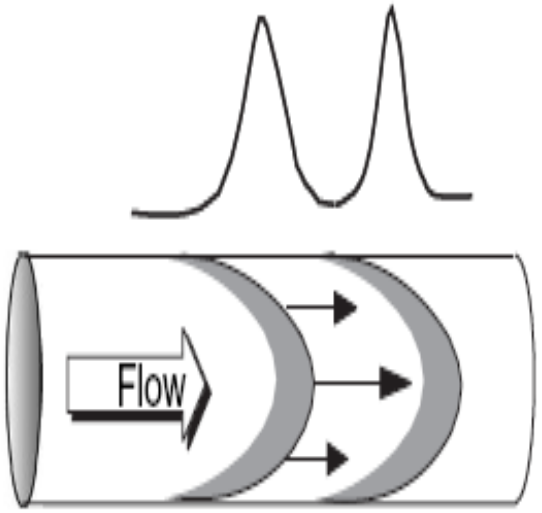
Electroosmotic Flow

Capillary electrophoresis or electrochromatography



Open tube, inner diameter: 50–150 μm

Liquid chromatography



Packed column, inner diameter: 2–4 mm

Optimizing electrophoresis

- ***Optimal electrophoretic separations*** *must balance speed and resolution :*

➢ ***Higher voltage*** *increases speed, but heat causes evaporation of the buffer and may denature molecules.*

➢ ***Higher ionic strength*** *(buffer) increases conductivity, but enhances endosmotic effects.*

Disadvantage of Capillary Electrophoresis :

- JOULE HEATING

Some buffers such as Tris are known to be pH-sensitive with temperature. For complex separations such as peptide maps, even small pH shifts can alter the selectivity.

- *Cannot identify neutral species.*

- *Cannot discern shape.*

To over come JOULE HEATING

- use a smaller-bore capillary (less current reduces the heat produced)
- a longer capillary (more surface area dissipates the heat

  generated).
- The use of low conductivity buffers is also helpful in this regard.
- effective cooling systems are required to ensure heat removal. Liquid cooling is the most effective means of heat removal and capillary temperature control

Advantage of CE

- *Requires minute amounts of sample.*
- *It is easily automated for precise quantitative analysis and ease of use.*
- *consumes limited quantities of reagents.*
- *It is applicable to a wider selection of analytes compared to other analytical separation techniques.*

- *Separation of large proteins differing in only one amino acid (ie. D-Lysine substituted for L-Lysine) and even an isotopic separation of ^{14}N and ^{15}N ammonium hydroxide have been reported.*