25/5/22 # Capillary Electrophoresis.

Slab/plate electrophoresis:

1) It is slow, labour intensive, difficult to automate and does not ~~need~~ yield definitive Quantitative results.

★ **Capillary electrophoresis** : is an instrumental version and a substitute for slab electrophoresis.

② It displays high speed performance, high resolution separation, requires small sample size ie; 0.1 to 10 nanometres.

③ Quantitative detectors can be used instead of staining

Instrumentation.

Requirement:

① Buffer filled fused silica capillary 10 to 100 micrometres in internal diameter and 40–60 cm long.

② Two platinum electrodes.

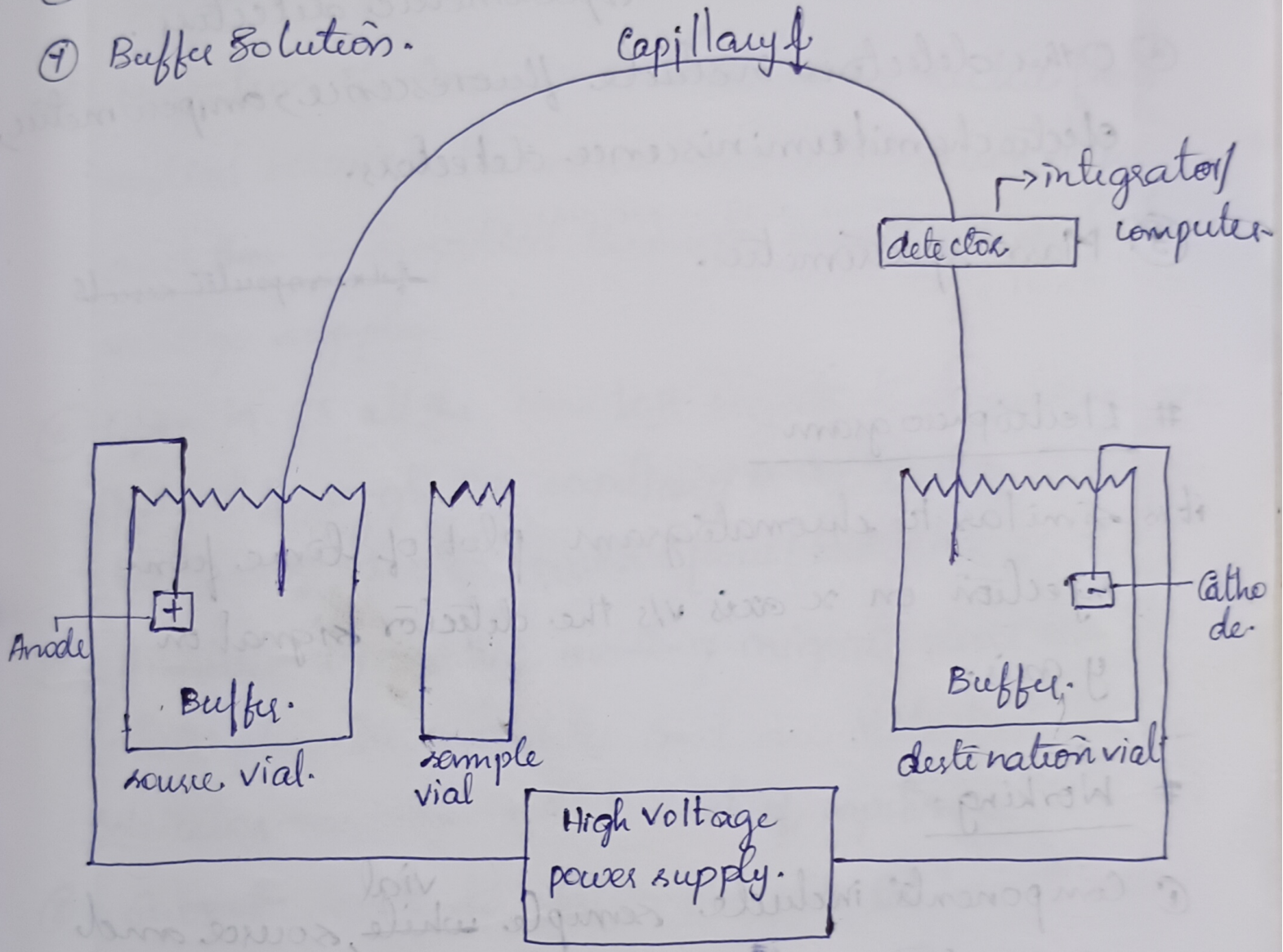
③ High Voltage supply.

④ Sample injector

⑤ Sample inlet and outlet vial

⑥ Detector.

⑦ Buffer Solution.



* sample injection.

① hydrodynamic injection

a) by applying pressure.

b) by applying vaccum.

c) by gravitation.

② Electro kinetic injection: ie; by using electric supply.

Detectors:

① UV- Vis absorbtion spectrometric detectors.

② Other detectors include fluorescence, amperometric, electrochemiluminiscence detectors.

③ Mass spectrometric.

Electrophoregram

It is similar to chromatogram plot of time from injection on x axis v/s the detector signal on y axis.

Working:

① Components include sample vial, source and destination vial, capillary, electrodes, data output and handling devices.

② source vial, destination vial and capillary all filled with an electrolyte (buffer solution).

③ To introduce the sample, capillary inlet is placed into the sample vial.

④ Sample is introduced in capillary by a

Capillary action, pressure, or Electrokinetically and then capillary is returned to source vial.

(5) Migration of Analites is initiated by electric field applied between the source and destination vials and ~~the~~ is supplied through electrodes by high voltage supply.

(6) Often in CE all the ions be it negative or positive are pulled through the capillary in the same direction because of electro osmotic flow (EOF).

(7) Analites separated as they migrate due to electrophoretic mobility and are detected using detector near the outlet end of capillary.

(8) Output sent to data output ~~&~~ handling device such as integrator or computer.

(9) Data displayed as electropherogram.

(10) separated compounds appear as peaks with different retention time in electropherogram.

(11) Retention time is the time taken for a solute to pass through the Column

Applications.

1) Illicit drug analysis
2) Analysis of GSR and explosive constituents.
3) To investigate in composition.
4) Analysis of small molecule pharmaceuticals and in their preparations.
5) Presence of pharmaceutical products in standard solutions and biological fluids.