

FPLC

(Fast protein liquid chromatography)

or

(Fast performance liquid chromatography)

A decorative horizontal bar at the bottom of the slide, consisting of a thick teal line on top, followed by a thin white line, and then two thin teal lines below that.

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Introduction

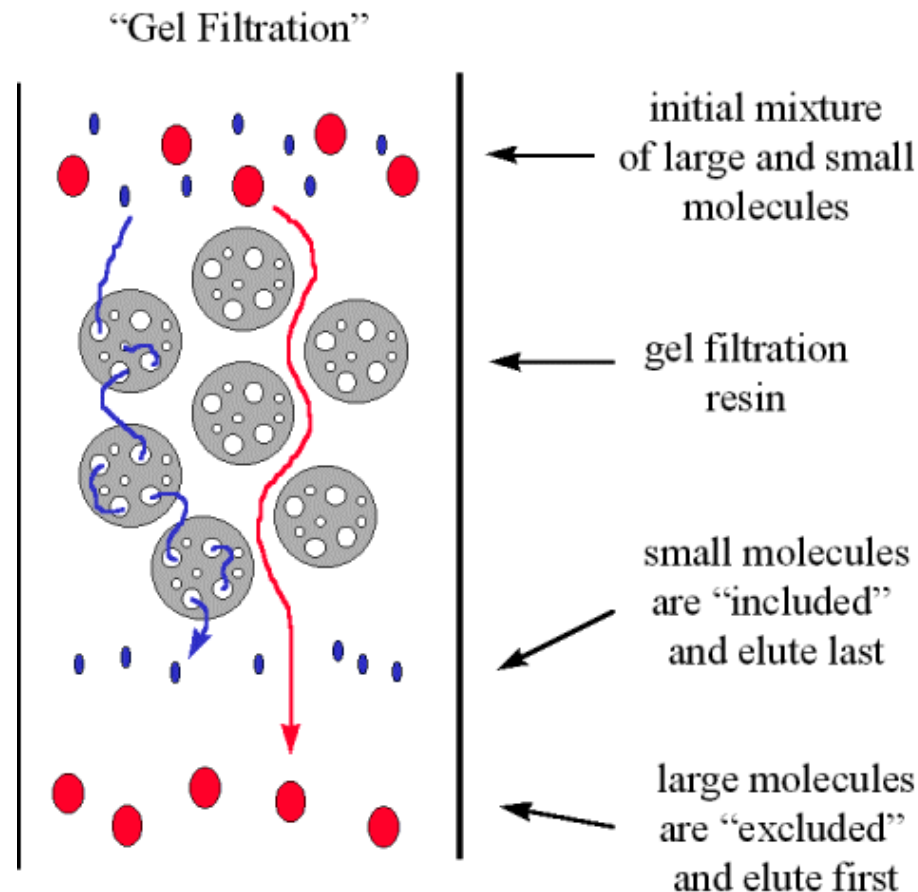
- FPLC is basically a “protein friendly” HPLC system.
- FPLC is an intermediate between classical column chromatography and HPLC.
- It is a complete system for chromatographic separations of proteins and other biomolecules such as nucleic acids.
- It is commonly used in biochemistry and enzymology .

Principle

1. Size(size exclusion chromatography)
2. Charge distribution(ion exchange)
3. Affinity chromatography

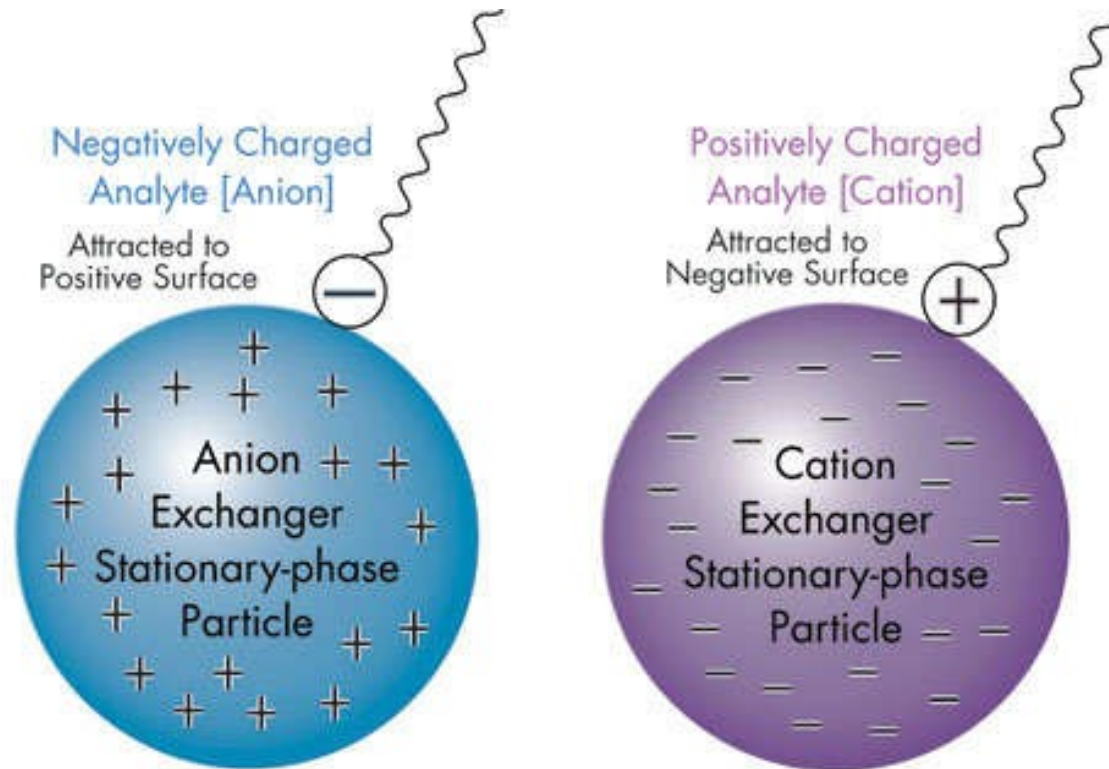
1. Size (size exclusion chromatography)

separate proteins according to their size. Also termed as “Gel Filtration Chromatography”



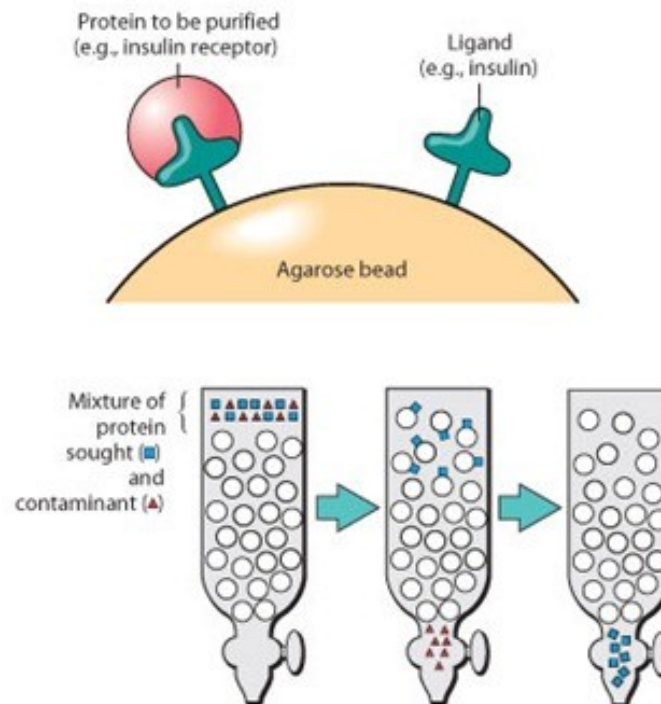
2. Charge distribution(ion exchange)

separate proteins based on surface-charges.



3. Affinity chromatography

separate protein based on a highly specific interaction such as that between antigen and antibody, enzyme and substrate, or receptor and ligand.



Instrumentation

- **Stationary Phase** : It is typically a resin composed of cross-linked agarose beads with varying surface ligands.
- **Mobile Phase** : Mostly buffers, organic solvents.
- **Pump** : Constant controlled flow by peristaltic pumps. The flow rate is varied based on scale of preparation ie; analytical or preparative chromatography.
- **Mixer**: Powered and controlled by the pump. Especially important when forming gradients between buffer sources. The mixer ensures that the buffers used are in the correct proportion relative to each other during the course of the FPLC run.

Instrumentation

➤ **Injection valve:**

- Pumps are connected to valves which send the buffers in the desired direction.
- The Inject position is for injecting contents of the sample loop onto a column.

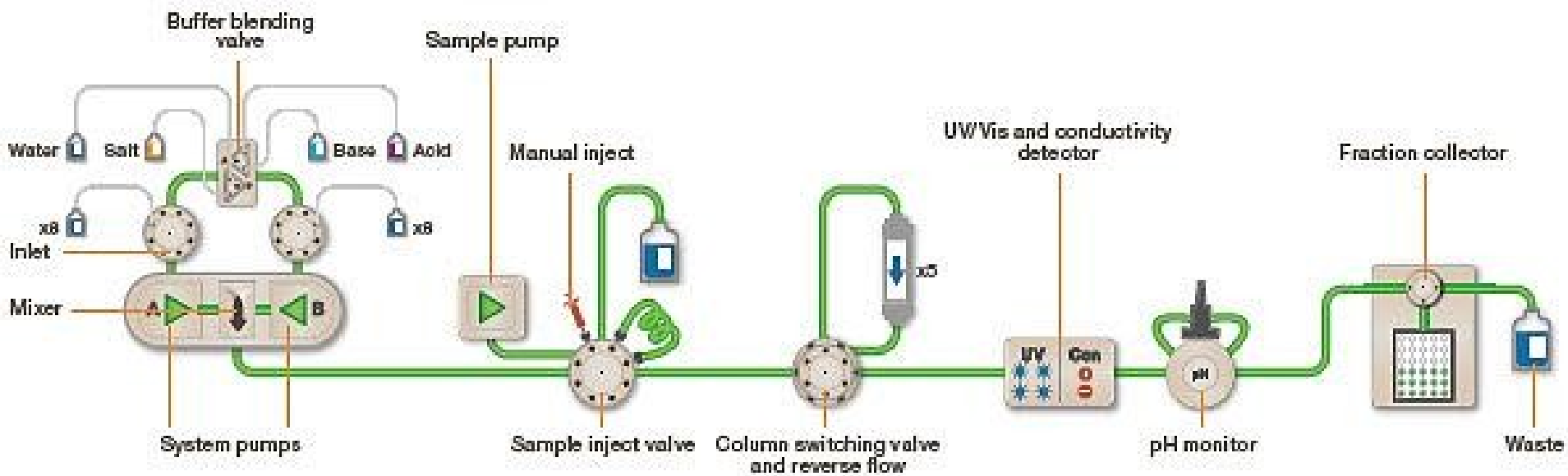
➤ **Column:**

- large [internal diameter, mm] tubes.
- contains small [13–15 μ] particles or gel beads.
- Typical column materials are inert plastic or inert fluid surfaces like Teflon, titanium, and glass.
- It is designed to operate at not more than 580 psi.
- Pre-packed columns are also available.
- Columns should be stored in 24% ethanol/H₂O when not in use.

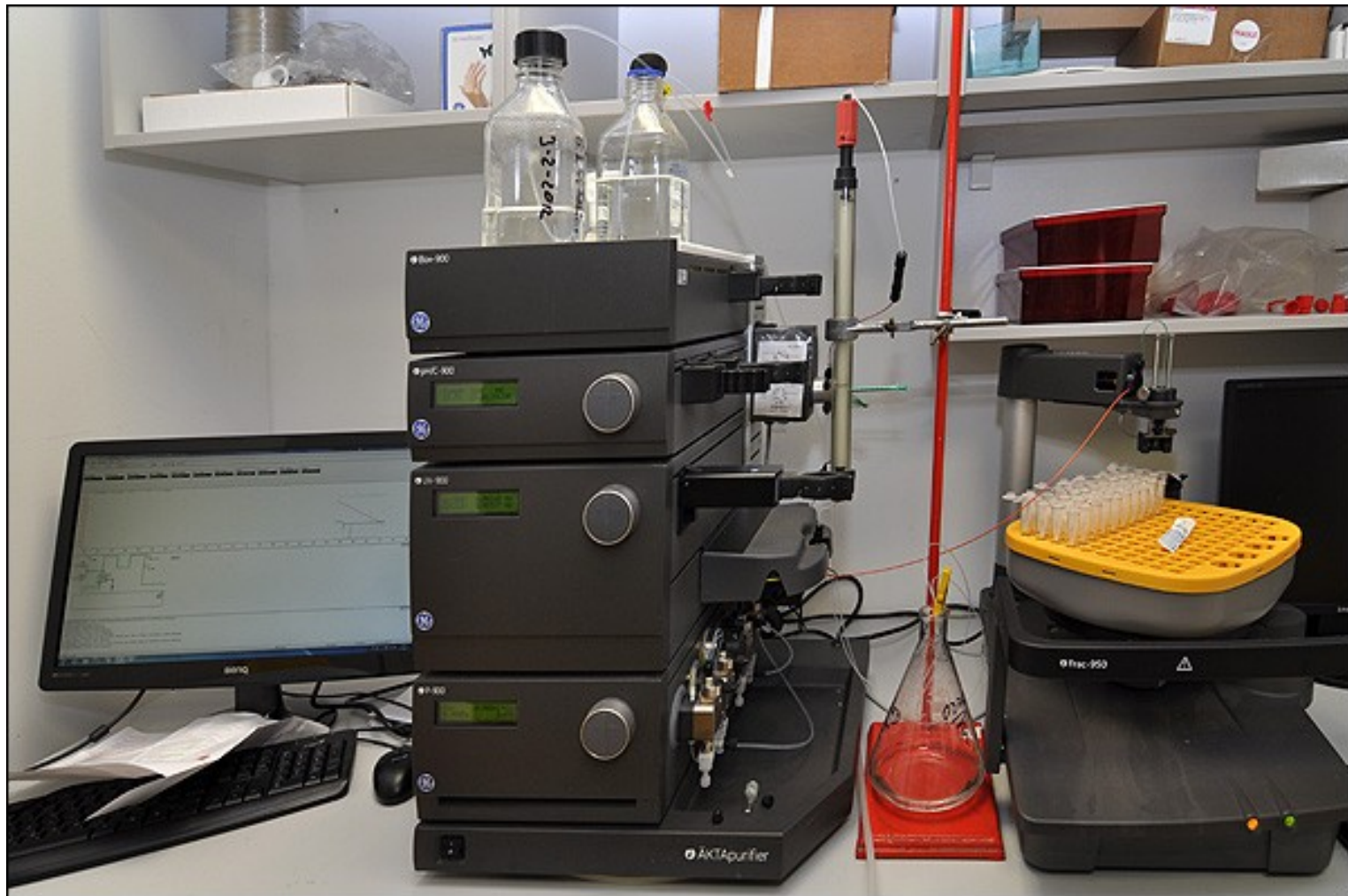
Instrumentation

- **Fraction collector:** Allows fixed volume fractionation.
- **Flow Restrictor:** Generates a steady back-pressure to prevent air bubbles being formed after the columns in the flow cells.
- **On-line Filter:** Rejects sample particulates that may clog the fluidic system by generating a maximum back-pressure of 0.5 MPa.
- **Detection system:** UV or UV/Vis spectrophotometer, Conductivity detector and Refractive Index (RI) Detector based on characteristics of the analyte of interest.

Instrumentation



Instrumentation



How does it differ from HPLC ??

- FPLC differs from HPLC in that the columns used for FPLC can only be used up to maximum pressure of 3-4 MPa (435-580 psi).
- Stainless steel components replaced with glass and plastic. Inert surfaces are necessary since many of the resolving buffers contain high concentrations of halide salts that attack and corrode stainless steel surfaces.
- FPLC pump delivers a solvent flow rate in the range of 1-499ml/hr HPLC pump gives a rate 0.010-10ml/min.
- FPLC system can use FPLC columns only but in HPLC system we can use both.

Advantages

- Reproducible with excellent resolution.
- Very simple system programming.
- Inert construction against the very high salt concentrations and corrosive liquids hence columns have longer lifetime.
- Since lower pressures are used in FPLC than in HPLC, a wider range of column supports are possible.
- The wide flow range makes it suitable both for analytical and preparative chromatography.

Drawbacks

- Needs glass columns.
- Can not handle high pressure.
- Instrument does not support HPLC columns.
- Purifying thermo labile (heat sensitive) proteins is a tough task.

Application

- Protein analysis.
- Lipoprotein separation by FPLC.
- Purification of animal venoms.
- Separation of Plasma Proteins in Urine and Cerebrospinal Fluid.
- Isolation of porphobilinogen deaminase from human erythrocytes
- Rapid purification of RNA.
- The Analysis of nitrogenous constituents of beer.
- Coupled to a double focusing inductively coupled plasma mass spectrometer for trace metal speciation in human serum and detectable levels of Cr, Al, Pb and Sn were present in uraemic sera.
- FPLC method could be used as a cost-effective method for routine β -thalassaemia diagnosis.

Conclusion

Thus FPLC SYSTEM is a very efficient system mainly for the separation of proteins and other biomolecules. Recent advances in this system makes it to conquer the field of protein analysis.

Thank You ...

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