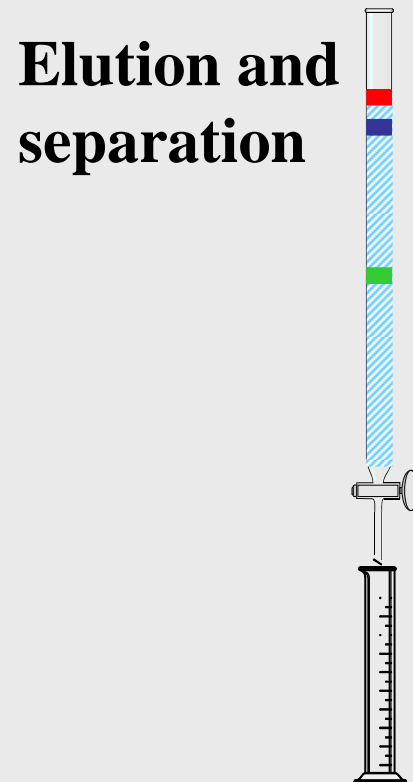
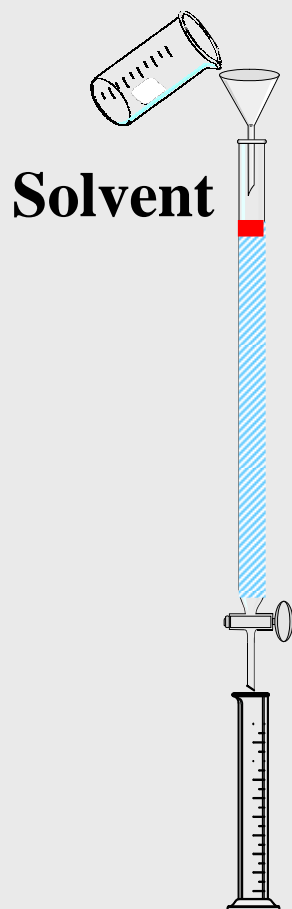
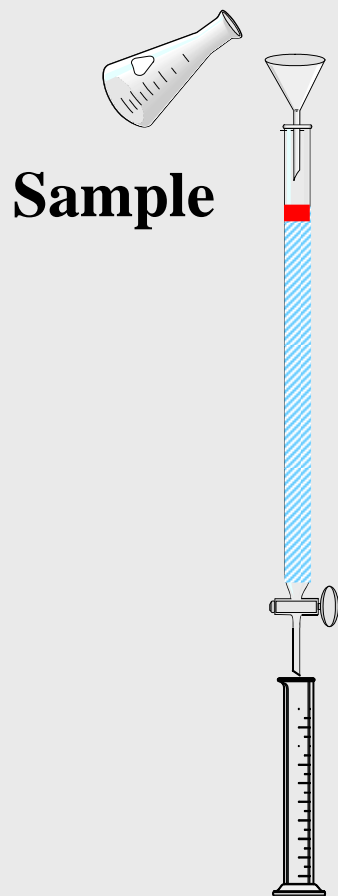
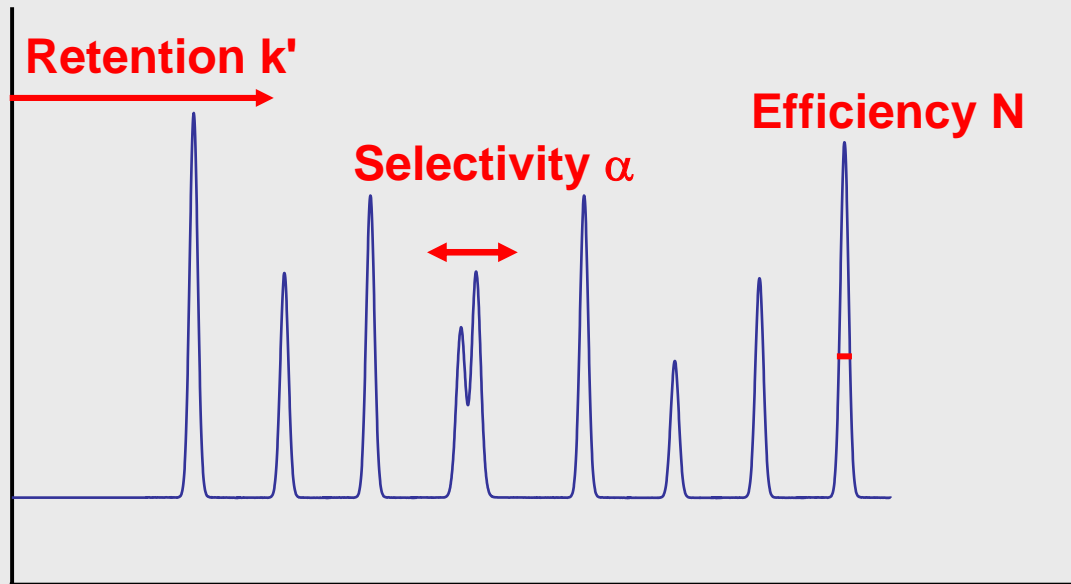


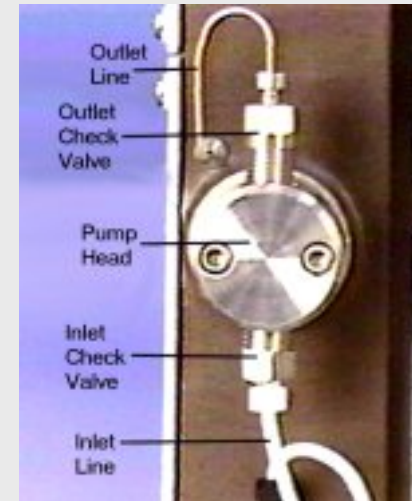
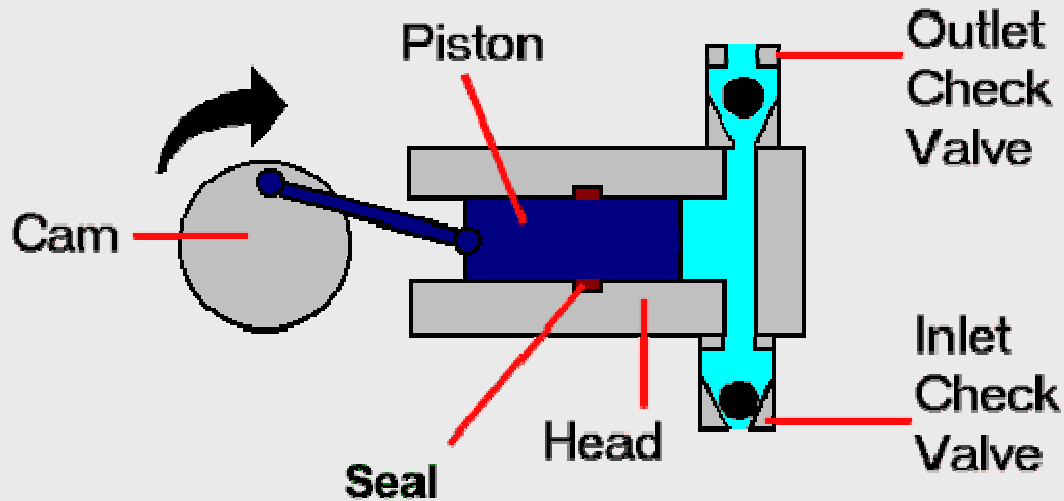
Chromatography - Twsett



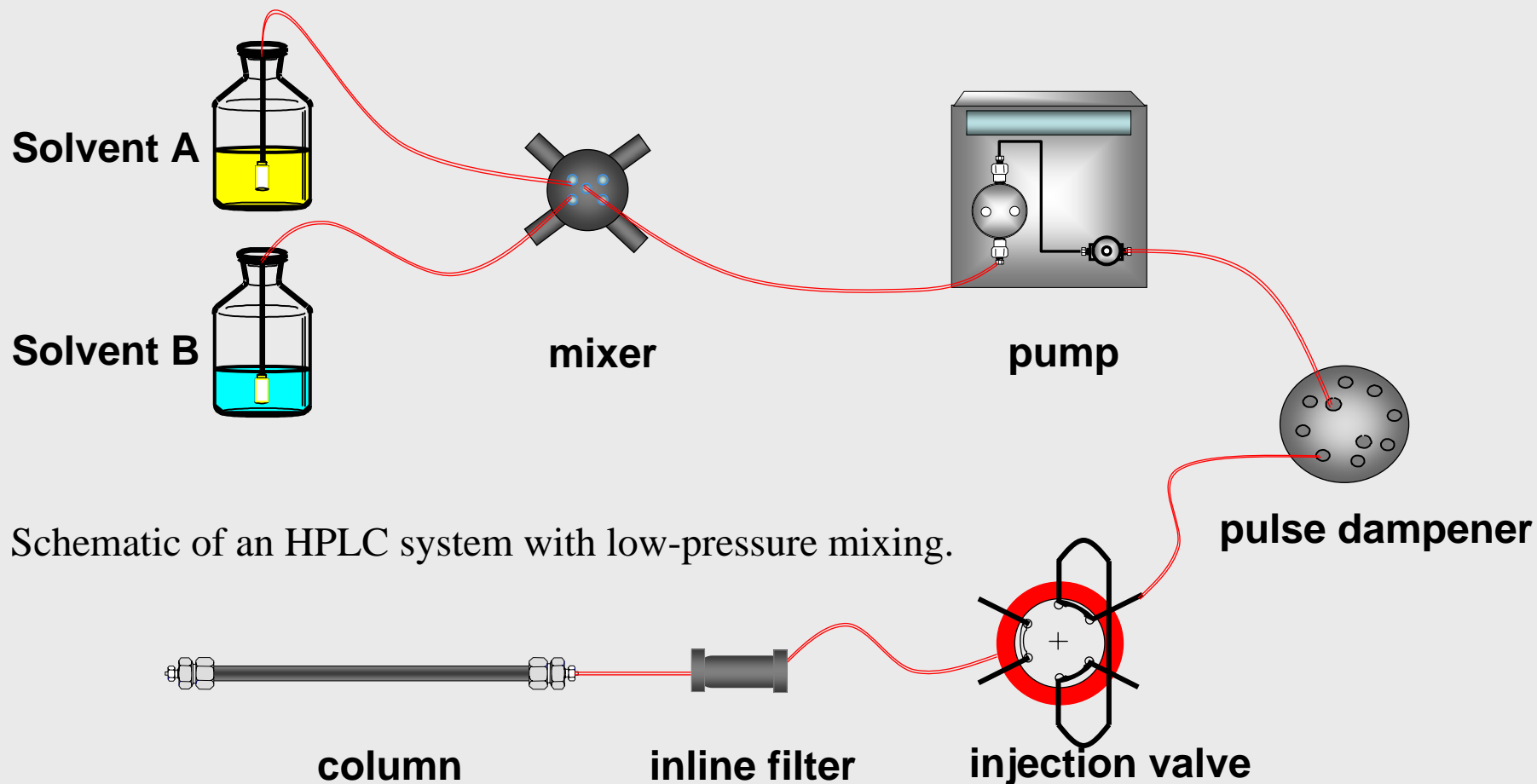
Resolution Factors



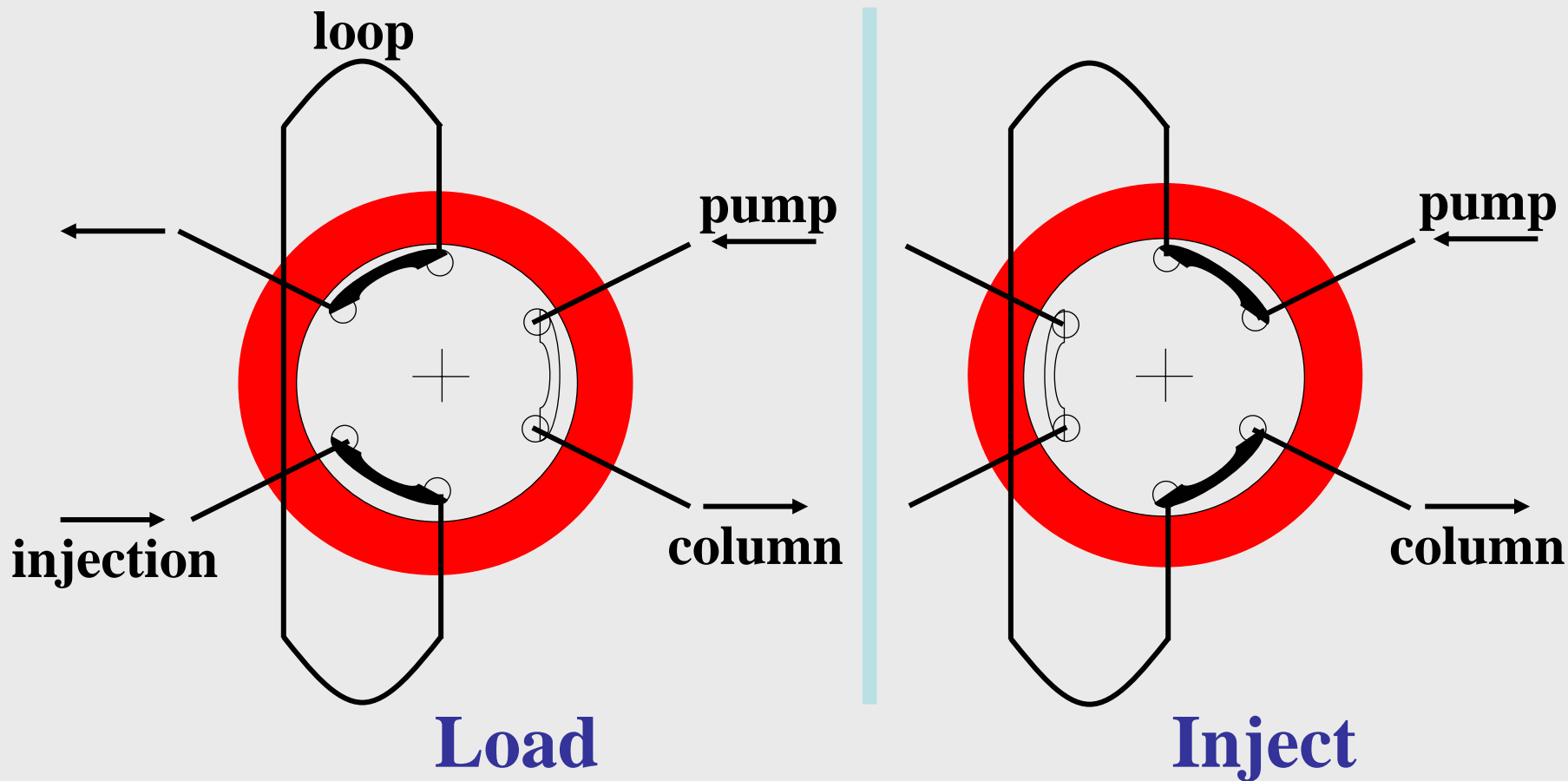
Single Piston Pump



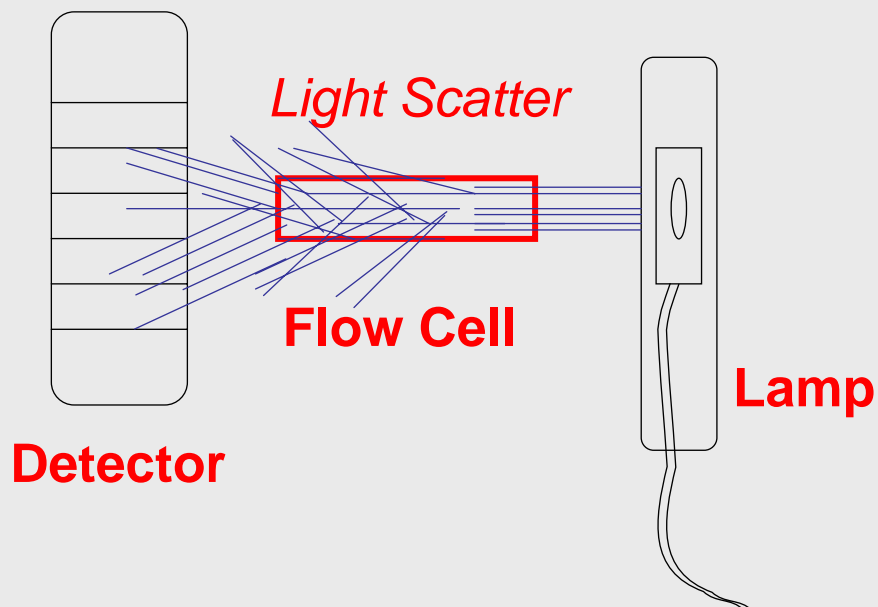
Delay Volume – quaternary pump



The Injector – Aim : to get the sample into the flow path while maintaining constant flow



HPLC Detection - UV



Lambert Beer's Law:

$$A = \epsilon \cdot l \cdot c$$

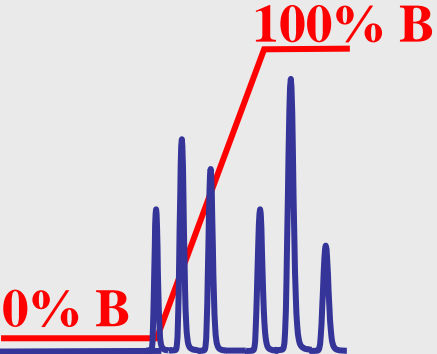
A = Absorbance

ϵ = molar absorptivity

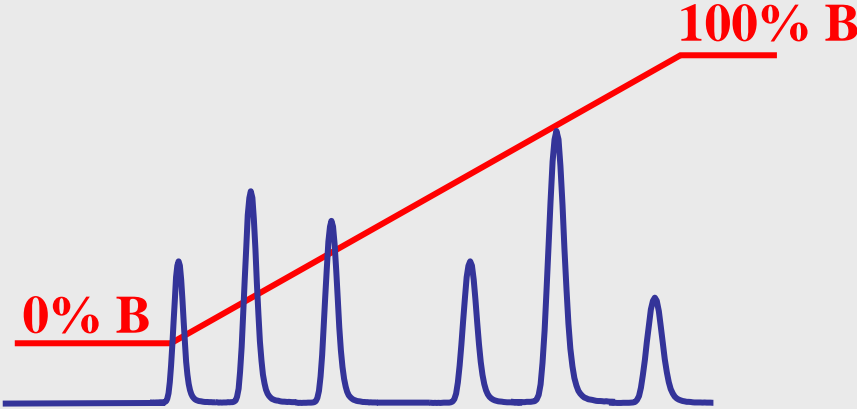
l = path length

c = concentration

Gradient Steepness



Steep



Shallow

How Buffers Work

- The ratio of A⁻ and HA, along with the pKa of HA, allows us to determine the solution pH
- If you add some acid to the solution, the ratio of A⁻ / HA will decrease
- Although the ratio changes, the log of the ratio does not change very much, as long as there is still A⁻ in solution.
 - This limits the pH change

$$\text{pH} = \text{pKa} + \text{Log} \left(\frac{[\text{A}^-]}{[\text{HA}]} \right) \quad \text{Ka} = \frac{[\text{A}^-] [\text{H}^+]}{[\text{HA}]}$$

Preventive Maintenance for Columns

- Filter and/or pre-treat dirty samples and solvents
- Use in-line filters, guard / preconcentration columns
- Change flow rate with care - do not pressure shock the column
- Keep mobile-phase pH close to 2.5 - 7
- Use a column oven where possible (30 - 60°C)
- Do not store column overnight in buffer solution
- Store columns in organic/water mixture (never 100% water – microbial growth) and cap

