Adsorption chromatography

By-

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Principle

- This is the classic form of chromatography, which is based upon the principle that certain solid materials, collectively known as adsorbents, have the ability to hold molecules at their surface.
- This adsorption process, which involves weak, non-ionic attractive forces of the van der Waals' and hydrogen-bonding type, occur at specific adsorption sites.
- These sites have the ability to discriminate between types of molecules and are occupied by molecules of either the eluent or of the analytes in proportions determined by their relative strength of interaction.
- As eluent is constantly passed down the column, differences in these binding strengths eventually lead to the separation of the analytes.

...Principle

- Silica is a typical adsorbent. It has silanol (Si-OH) groups on its surface, which are slightly acidic, and can interact with polar functional groups of the analyte or eluent.
- Other commonly used adsorbents are alumina and carbon.
- In general, an eluent with a polarity comparable to that of the most polar analyte in the mixture is chosen.
- Thus, alcohols would be selected if the analytes contained hydroxyl groups, acetone or esters would be selected for analytes containing carbonyl groups.
- Hydrocarbons such as hexane, heptane and toluene for analytes that are predominantly non-polar.
- Mixtures of solvents are commonly used in the context of gradient elution.

Hydroxylapatite chromatography

- Crystalline hydroxylapatite ($Ca_{10}(PO_4)_6(OH)_2$) is an adsorbent used to separate mixtures of proteins or nucleic acids.
- One of the most important applications of hydroxylapatite chromatography is the separation of single-stranded DNA from double-stranded DNA.
- Both forms of DNA bind at low phosphate buffer concentrations but as the buffer concentration is increased single-stranded DNA is selectively desorbed.
- As the buffer concentration is increased further, double-stranded DNA is released.
- The affinity of double stranded DNA for hydroxylapatite is so high that it can be selectively removed from RNA and proteins in cell extracts by use of this type of chromatography.

Hydrophobic interaction chromatography

- Hydrophobic interaction chromatography (HIC) was developed to purify proteins by exploiting their surface hydrophobicity which is a measure of their dislike of binding molecules of water.
- Groups of hydrophilic amino acid residues are scattered over the surface of proteins in a way that gives characteristic properties to each protein.
- In aqueous solution, these hydrophilic regions on the protein are covered with an ordered layer of water molecules that effectively mask the hydrophobic groups of the proteins the majority of which are in the interior of the folded molecule.
- These hydrophobic groups can, however, be exposed by the addition of salt ions, which preferentially take up the ordered water molecules.
- The exposed hydrophobic regions can then interact with each other by weak van der Waals' forces causing protein–protein aggregation.

… **Hydrophobic interaction chromatography**

- In HIC, the presence of hydrophobic groups such as butyl, octyl and phenyl attached to a matrix facilitates protein–matrix interaction rather than facilitating protein–protein interaction.
- Commercial materials include Phenyl Sepharose and Phenyl SPW, both for low-pressure HIC, and Poly PROPYL Aspartamide, Bio-Gel TSK Phenyl and Spherogel TSK Phenyl for HPLC HIC.
- To maximise the process, it is advantageous to adjust the pH of the protein sample to that of its isoelectric point.

… Hydrophobic interaction chromatography

- Once the proteins have been adsorbed to the stationary phase, selective elution can be achieved in a number of ways, including the use of an eluent of gradually decreasing ionic strength or of increasing pH (this increases the hydrophilicity of the protein) or by selective displacement by a displacer that has a stronger affinity for the stationary phase than has the protein.
- Examples include non-ionic detergents such as Tween 20 and Triton X-100, aliphatic alcohols such as 1-butanol and ethylene glycol, and aliphatic amines such as 1-aminobutane.