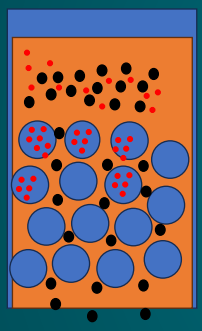
Gel Chromatography



Chromatography
Instrumental Analysis

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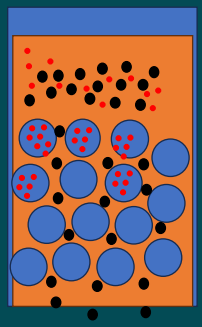
Gel Chromatography

Introduction & Principle

- Gel chromatography is a type of chromatography that separates molecules based on their size.
- The technique involves pumping dissolved molecules through columns containing a microporous gel.
- The smaller molecules pass through the pores,
- while larger molecules pass through the void volumes



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Gel Chromatography

Introduction

- Gel chromatography is also known as:
  - Gel-permeation chromatography
  - Gel-exclusion chromatography
  - Size-exclusion chromatography
  - Molecular-sieve chromatography
- Gel chromatography can be used to:
  - Fractionate molecules into size ranges
  - Remove molecules larger than a particular size
  - It is one of the effective methods used to isolate and analyze the Bio-macromolecular substances.

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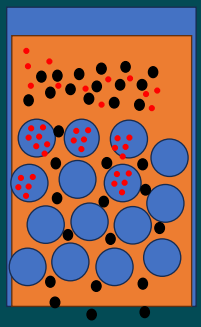
Gel Chromatography

Principle

- It's a technique that separates dissolved molecules on the basis of their size by pumping these molecules through specialized columns containing a microporous packing material (gel).
- Stationary phase is a porous polymer matrix whose pores are completely filled with the solvent to be used as the mobile phase.
- The flow of mobile phase will cause larger molecules to pass through the column unhindered, without penetrating the gel matrix.
- whereas smaller molecules will be retarded according to their penetration of the gel.



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Gel Chromatography

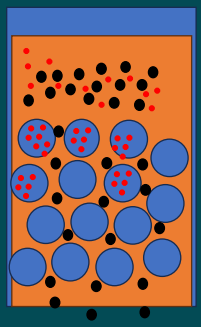
Instrumentation

Stationary Phase

- Nature of Gel
  - Chemically inert, Mechanically stable, Uniform particle size.
  - Gel should have carefully formed reproducible structure.

There are mainly two type of gel materials:

- 1. Xerogels: Which consists consist cross-linking polymers which swell when come in contact with the solvent to form a relatively soft porous medium.
  - If liquid removes the structure collapse and cannot be restored.
- 2. Aerogels: Rigid material which is not a gel at all.
  - Porous material, which does not collapse when solvent is removed. eg: porous glass, porous silica



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Gel Chromatography

Instrumentation

- **Stationary Phase**
- The gel which commonly used included cross-linked dextrants, agarose, polyacrylamide, polystyrene, polyacryloylmorphine
- **Dextrants**
  - A homopolysaccharide of glucose residues.
  - The trade name is Sephadex.
  - It's mainly used for separation of small peptides and globular proteins with small to average molecular mass.

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Gel Chromatography

Instrumentation

- **Stationary Phase**
- **Polyacrylamide**
  - These gels are prepared by with cross linking acrylamide N,N- methylene bis acrylamide.
  - The separation properties of polyacrylamide gels are mainly the same as those of dextrans.
  - They are sold as bio-gel P. They are available in wide range of pore sizes.

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Gel Chromatography

Instrumentation

- **Stationary Phase**
- **Agarose**
  - Linear polymers of D-galactose and 3,6 anhydro-1-galactose.
  - It forms a gel that's held together with H bonds. It's dissolved in boiling water and forms a gel when it's cold.
  - The concentration of the material in the gel determines the pore size.
  - The pores of agarose gel are much larger than those of Sephadex or bio-gel p.
  - It's useful for analysis or separation of large globular proteins or long linear molecules such as DNA.

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Gel Chromatography

Instrumentation

- **Mobile Phase**

Materials	Solvent
Synthetic elastomers (polybutadiene , polyisoprene)	Toluene
PS, PVC, Styrene-Butadiene Rubber , Epoxy resins	Tetrahydrofuran (THF)
Polyolefins	Tri- chloro -benzene
Polyurethane	Di- methylformamide (DMF)
Proteins, polysaccharides	Water/Buffer

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Gel Chromatography

Instrumentation

- **Column**

Commercially Available Columns:

  - analytical column- 7.5—8mm diameters.
  - Preparative columns-22—25mm.
- Usual column lengths-25, 30, 50, and 60 cm.
- Recently, narrow bore columns- 2—3mm diameter have been introduced, which save time and solve.

- **Pump**
- A) Syringe pump
- B) Reciprocating Pump

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Gel Chromatography

Instrumentation

- **Detectors**
- Different detectors used in identification of components
- 1. Concentration sensitive detectors
  - Bulk Property Detectors- Refractive Index (RI) Detector
  - Solute Property Detectors- Ultraviolet (UV) Absorption Detector
  - Evaporative Detectors- Evaporative Light Scattering Detector (ELSD)
- 2. Molar mass sensitive detectors
  - Light Scattering Detectors
  - Low Angle Light Scattering (LALS) Detectors
  - Multi-angle Light Scattering (MALS) detectors
- 3. Viscosity Detectors- Differential Viscometers

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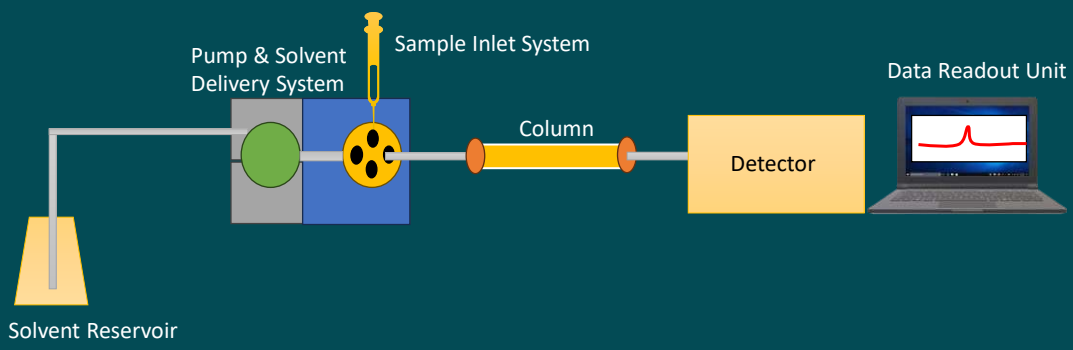
Gel Chromatography

Instrumentation



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Gel Chromatography

Applications

- Separating proteins and peptides
- Separating nucleic acids and nucleotides
- Removing endotoxins
- Removing inhibitors and cofactors from enzymes
- Determining molecular weight

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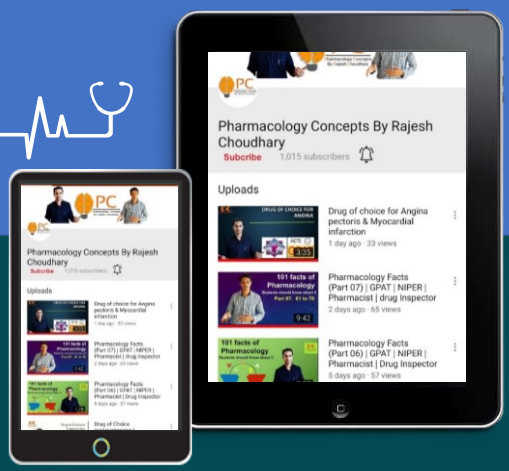

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