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Chromatography

Group of techniques used to separate complex mixtures on basis of different physical interactions between a stationary and mobile phase



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Chromatography (cont)

Basic components

- mobile phase (gas or liquid)



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Chromatography (cont)

- stationary phase (solid or liquid)



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Chromatography (cont)

- column holding stationary phase



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Chromatography (cont)

- separated components (eluate)



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Chromatography (cont)

- complex mixture (sample)



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Chromatography (cont)

Forms

- Planar



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Chromatography (cont)

- Paper



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Chromatography (cont)

Column - Gas (GC)- can be mobile or stationary (polymer silica)



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Chromatography (cont)

- Liquid (LC) – mobile phase – high pressure and connected to mass spec



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Chromatography (cont)

Modes of Separation

- Adsorption – liquid-Solid (hydrophobic and Hydrophilic bonds)



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Chromatography (cont)

- Partition – Liquid-Liquid



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Chromatography (cont)

- Steric exclusion – Variation of liquid-Solid



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Chromatography (cont)

- Ion-exchange chromatography – Magnitude and charge (separation of amino acids)



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Chromatography (cont)

- Affinity



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CHROMATOGRAPHIC PROCEDURES

Thin-layer chromatography (TLC)

- A variant of column chromatography



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CHROMATOGRAPHIC PROCEDURES (cont)

- Thin layer of sorbent is uniformly coated on glass/plastic plate.



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CHROMATOGRAPHIC PROCEDURES (cont)

- Samples are applied as spots near edge of plate.



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CHROMATOGRAPHIC PROCEDURES (cont)

- One edge of plate is placed in solvent.



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CHROMATOGRAPHIC PROCEDURES (cont)

- Solvent migrates up thin layer by capillary action.



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CHROMATOGRAPHIC PROCEDURES (cont)

- After solvent reaches predetermined height, plate is removed and dried.



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HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY (HPLC)

- Uses pressure for fast separations, controlled temperature, in-line detectors, and gradient elution techniques



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HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY (HPLC) (cont)

- Components: pumps, columns, sample injectors, detectors, recorders



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