Sample Exam Question

1. ____ The ___ type of GC detector is selectively sensitive to halogen containing compounds

    A. FID

    B. TCD

    C. ECD

    D. SCD

HPLC Theory

Scope of HPLC – most widely used of all analytical separation techniques

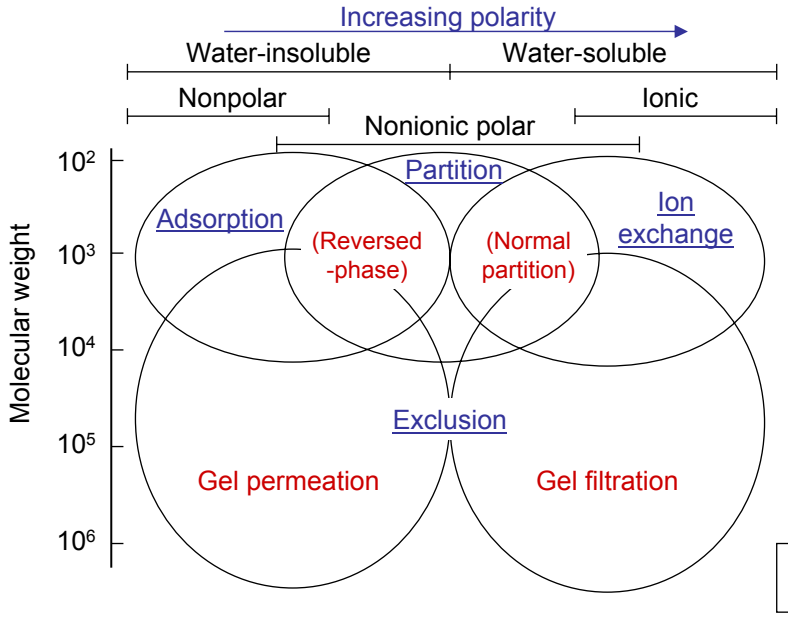
Good sensitivity, adaptable to quantitation, nonvolatile to fragile analytes

Amino acids, proteins, nucleic acids, hydrocarbons, carbohydrates, drugs, terpenoids, pesticides, antibiotics, steroids, metal-organic species, various inorganics

Covers wide range of molecular weights & solubilities

General band broadening principles for chromatography (Ch. 26) apply

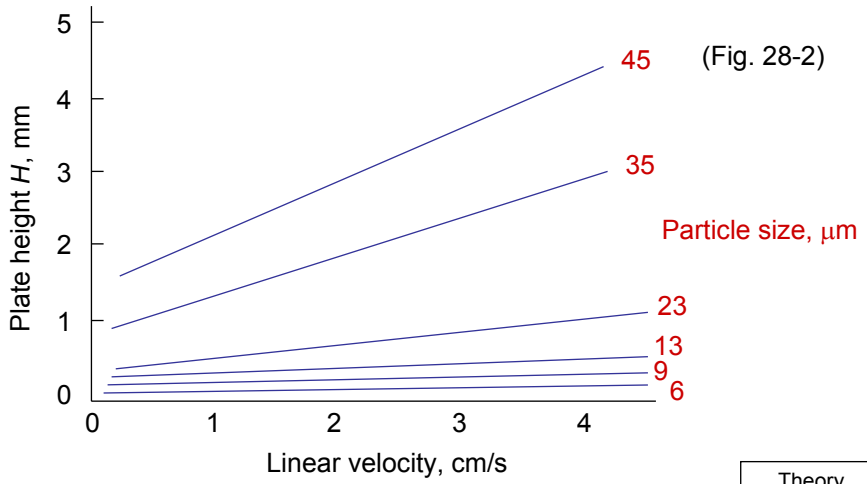
HPLC Separation Types



Theory
28A

Effect of Packing Particle Size

Column efficiency increases (C_M term in van Deemter eqn., Table 26-3, decreases) dramatically as diameter of packing particle size decreases



(Fig. 28-2)

Theory
28B

Extra-Column Band Broadening

Occurs as solutes are carried through open tubes outside column – connections to injector, detector, other components – serious for small bore columns, 0.1 inch max. diameter

Contribution to total plate height, $H_{ex} = \pi r^2 u / 24 D_M$

Effect of Sample Size

Larger samples cause increase in plate height (Fig. 28-3)

Reversed-phase separations most tolerant to large samples

Theory
28B

Partition Chromatography

Uses stationary phases chemically bonded to 3, 5, or 10 μm silica particles

Siloxanes derivatives w/ organochlorosilane → -Si-O-Si$(CH_3)_2$-R

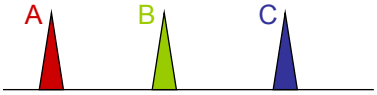
Normal phase – polar (water) stationary phase / nonpolar (hexane) mobile phase – least polar component (C) elutes first:

low polarity mobile phase

medium polarity mobile phase

Reversed phase – nonpolar (hydrocarbon) stationary phase / polar (water, methanol) mobile phase – most polar component (A) elutes first:

high polarity mobile phase



medium polarity mobile phase

Theory
28D

Method Development - Partition

Involves balancing interactions of three components – mobile phase, stationary phase, and solute; polarity of functional groups is key:

Hydrocarbons < ethers < esters < ketones < aldehydes < amides < amines < alcohols

Generally, polarity of solute is matched to stationary (not mobile) phase

Mobile phase is chosen to optimize N, k', and α – k' may vary from 0.5 to 20 (rather than 2 to 5) to accommodate entire sample mixture – relative polarity of mobile phase controlled by using mixtures of solvent with different polarity indices (Table 28-2, 0.1 for hexane, 10.2 for water) – selectivity may be altered by mixing up to 4 solvents (Fig. 28-16)

Separations can also be improved by using gradient elution (Fig. 28-18)

Theory
28D

Adsorption Chromatography

Liquid-solid chromatography – stationary phase is silica (usually) or alumina

Retention time order for either stationary phase is same: olefins < aromatic hydrocarbons < halides, sulfides < ethers < nitro compounds < esters ≈ aldehydes ≈ ketones < alcohols ≈ amines < sulfones < sulfoxides < amides < carboxylic acids

Optimization involves only changing mobile phase; eluent strength (Table 28-2) is better indicator of solvent strength; use mixture of two solvents, one too weak, the other too strong, and try various combinations

Solvent can be tested using TLC

Theory
28E

Ion-Exchange Chromatography

Stationary phase has ion-exchanger groups attached to solid support:

For cation-exchange, sulfonic acid group ($-SO_3^-H^+$), a strong acid, or carboxylic acid group ($-CO_2^-H^+$), a weak acid is used

For anion-exchange, tertiary amine group ($-N(CH_3)_3^+OH^-$), a strong base, or primary amine group ($-NH_3^+OH^-$), a weak base is used

Partition coefficient / distribution constant $K = c_S/c_M$

Cations: $Tl^+ > Ag^+ > Cs^+ > Rb^+ > K^+ > NH_4^+ > Na^+ > H^+ > Li^+$

Divalent: $Ba^{2+} > Pb^{2+} > Sr^{2+} > Ca^{2+} > Ni^{2+} > Cd^{2+} > Cu^{2+} > Co^{2+} > Zn^{2+} > Mg^{2+} > UO_2^{2+}$

Anions: $SO_4^{2-} > C_2O_4^{2-} > I^- > NO_3^- > Br^- > Cl^- > HCO_2^- > CH_3CO_2^- > OH^- > F^-$

Once ions are introduced to column, more H^+ or OH^- is added, replacing ions on exchanger, causing the ions to migrate to end / be eluted from column

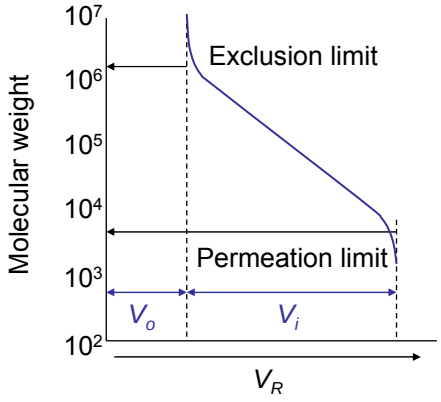
Theory
28F

Size-Exclusion Chromatography

Instead of chemical or physical interactions with stationary phase, solute molecules interact with pores (10^2 to 10^6 angstroms) in packing material (~10 μm silica or polystyrene-divinylbenzene particles, Fig. 28-6)

Larger solute molecules cannot fit into pores – elute first

Smaller solute molecules spend more time in pores – elute last



Total volume of column, V_t

$V_t = V_g + V_i + V_o$

V_g is volume of solid matrix

V_i is volume inside pores

V_o is free volume outside particles

$V_e = V_o + KV_i$

$K = (V_e - V_o)/V_i = c_S/c_M$

Theory
28G