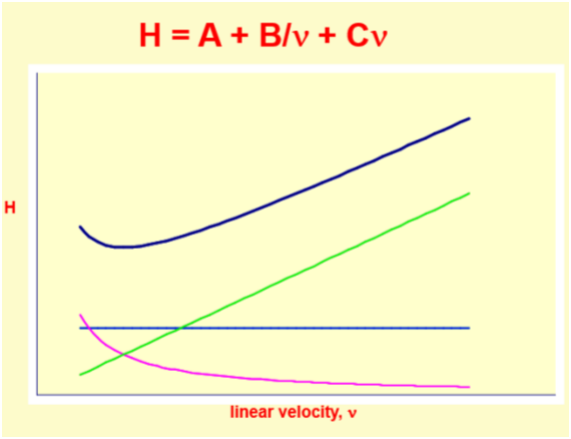
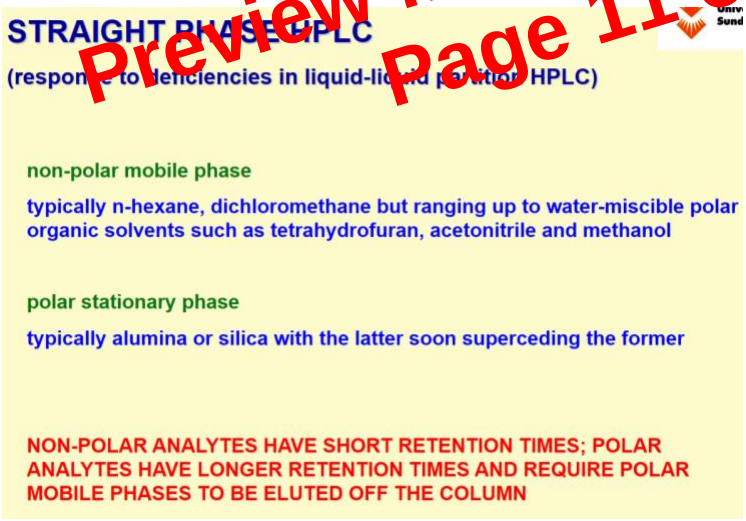


7. **HOW DO YOU REDUCE BAND BROADENING???**
   Operate at the flow rate corresponding to the minimum of the van Deemter plot.
   Use small spherical porous particles (5 microns) with a narrow particle size distribution.

8. What are the 3 key issues with band broadening?
   Laminar flow in inter-particle channels
   Knox equation to compare assess packing method
   Extra column band –broadening

9. What is straight phase HPLC?



10. What are the advantages of straight phase HPLC?

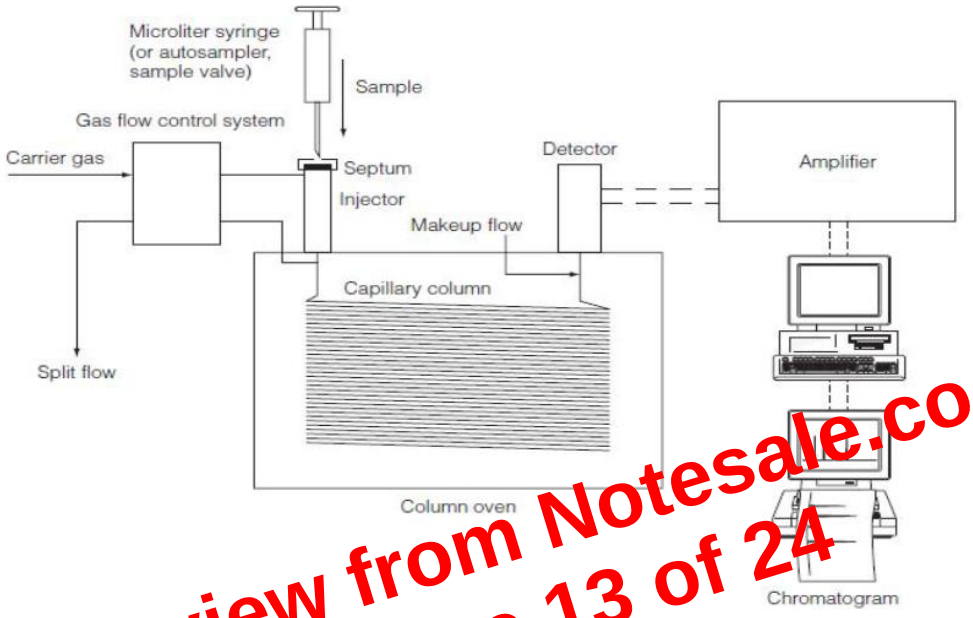
    Polar analytes are more strongly retained than non-polar analytes.
    Mobile phase solvents are volatile, making normal phase suitable for preparative work
    Stationary phases are cheap and highly selective.

Discuss the use of Gas Chromatography (GC) for the analysis of drugs

GC is the method of separating mixtures – suited to volatile liquids

Very high efficiency through the use of capillary GC

What is needed for GC?



- Mobile phase – Gas
- Stationary phase – column packed with solid or high boiling point liquid

Originally used with 'packed' columns containing non-porous particles coated with a viscous, involatile, inert liquid; typically used nitrogen (or occasionally argon) carrier gas (as mobile phase) and hydrogen and air supplies for the flame of a flame ionisation detector.

Capillary-GC – Efficiency

- low resistance to flow; long columns
- no eddy diffusion
- thin film gives rapid mass transfer

However, sample 'loadability' is low compared to packed column GC and modified injection systems are required.

Capillary-GC – Injections

SPLIT INJECTION - for major components

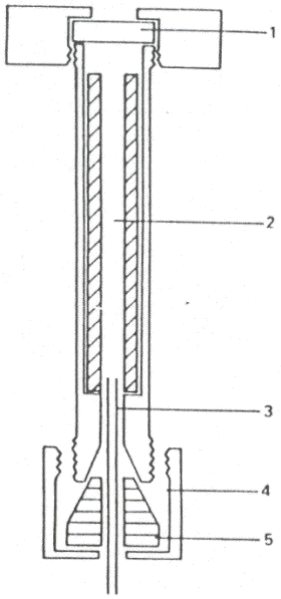
PURGE SPLITLESS INJECTION - for trace level analysis

DIRECT INJECTION - for wide bore open tubular columns

(For all of the above, injection is into a heated inert region provided by a glass liner which fits in the injection head)

ON-COLUMN (cool) INJECTION - for high boiling or thermally labile material

PACKED GC INJECTION PORT - adapted to accommodate wide bore capillary column



1. Septum
2. Glass liner
3. Wide bore fused silica capillary column
4. 1/4" nut
5. Reducing ferrule 1/4" x 1/16"

SPLIT / SPLITLESS INJECTION - For splitless injection, the valve is closed and a very volatile solvent is used; latent heat of evaporation of the solvent cools down the head of the column so that the trace analyte is 'focused' before warming up again and passing down the column.

Wide range of sensitive detectors available:

- FLAME-IONISATION DETECTOR
- NITROGEN-PHOSPHORUS DETECTOR
- ELECTRON-CAPTURE DETECTOR

The flame-ionisation detector: involves the combustion of organic compounds in a hydrogen/air flame. As a substance leaves the column, it burns in this flame producing ions which can be detected by measuring the electro conductivity of the flame. **(Most commonly used)**

Pros:

- has a wide linear range ($x10^7$)
- virtually universal for organic compounds, having a detector response that is relatively independent of structure
- gives low limits of detection (10-12 g)

What are the drawbacks of reverse phase of LC?

(a) very polar compounds are insufficiently retained (b) insufficient selectivity may be obtained even after mobile phase and stationary phase optimization (c) some analytes might be incompatible with the mobile phase and/or the stationary phase

What are the features of ion exchange?

silacious ion-exchangers now more common for LC, **-NHR, -CO_2H** used if weak exchange required, ionic strength may be moderate retention.

What are the applications of ion exchange?

Inorganic ions, strong organic bases, drugs in biological fluids, proteins

What are the features of ion pair?

Pairing ions used are e.g. long chain alkyl sulphonic acids, tetrabutyl ammonium hydroxide, selectivity may be controlled by pH, retention controlled by % organic, (ion-pair), carried out on alkyl-silicas

What are advantages and disadvantages of ion pair?

Advantages	**Disadvantages**
Large number of variable	Long equilibrium times
Very selective	Column not suitable for RPLC re-use
Retention varies with ion pair in a Predictable fashion	

What are applications of ion pair?

Applications: Hydrophilic ionic compounds, class separations, simultaneous determination

of acids, bases and neutrals (indirect UV visualisation)

What are the features of ligand exchange?

Feature: 7.5 < pH < 9.5, normally Cu^{2+}, large number of variables, v. selective, temperature may be used.

What are the applications of ligand exchange?

Applications: Amino acids, (amino alcohols), α-hydroxyl acids, chiral separations