Liquid Chromatography

1. Introduction and Column Packing Material

2. Retention Mechanisms in Liquid Chromatography

3. Method Development

4. Column Preparation

5. General Instrumental aspects

6. Detectors

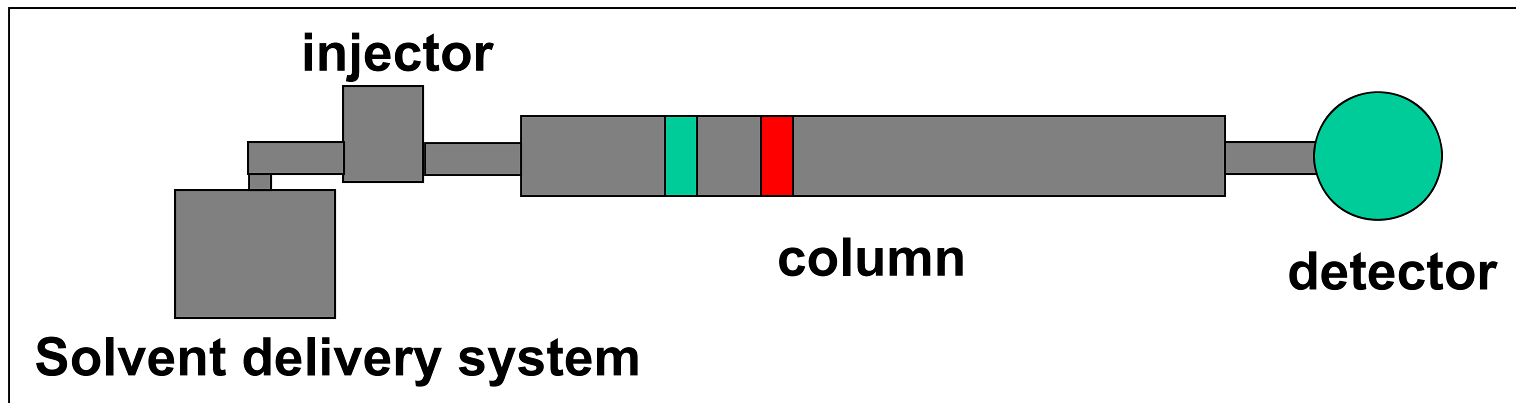
(Chapter 4 and 5 in The essence of chromatography)

Introduction

A. What is LC?

1. Liquid chromatography (LC) is a chromatographic technique in which the mobile phase is a liquid.

2. The technique of LC is much older than GC but was overshadowed by the rapid development GC in the 1950's and 1960's. However, LC is currently the dominate type of chromatography and is even replacing GC in some of GC's more traditional applications.



3. Advantages in using LC instead of GC.

(a) LC can be applied to the separation of any compound that is soluble in a liquid phase. This is a much less stringent than needed for GC, where solutes must be naturally volatile or convertible to a volatile to a volatile form. This makes LC more useful in the separation of many biological compounds, synthetic or natural polymers, and inorganic compounds.

(b) The use of a liquid mobile phase allows LC to be used at lower temperatures that typically required by GC, where the temperature must be high enough to convert solute into a volatile (gas soluble) form. This makes LC better suitable than GC for separating compounds which may be thermally labile.

(c) Retention of solute in LC depends on their interaction with both the mobile phase and stationary phase. In contrast, retention in GC is based mainly on solute volatility and interactions with the stationary phase. This makes more flexible than GC in optimizing separations, since retention of solutes can be varied by changing either of the column or mobile phase.

(d) Most LC detector are non-destructive while most GC detector are destructive. This makes LC better suited for preparative or process-scale separations.

4. **One disadvantage of LC is that it has a greater band-broadening than GC.**

B. Types of LC

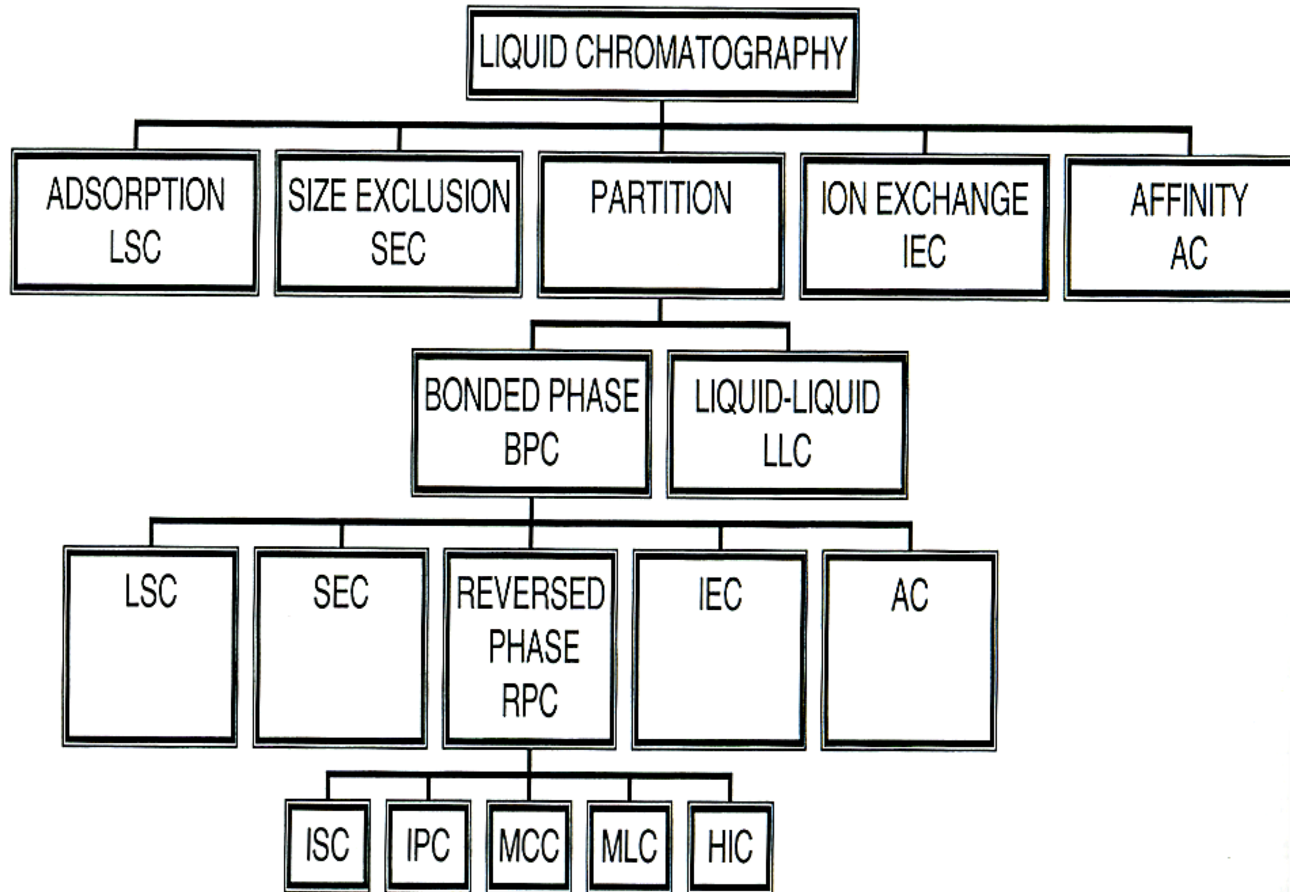


Figure 4.1. Family tree of liquid chromatographic separation modes. LSC = liquid-solid (or normal-phase) chromatography; SEC = size-exclusion chromatography; IEC = ion-exchange chromatography; AC = affinity chromatography; BPC = bonded-phase chromatography; LLC = liquid-liquid chromatography; RPC = reversed-phase chromatography; ISC = ion-suppression chromatography; IPC = ion-pair chromatography; MCC = metal-complexation chromatography; MLC = micellar-liquid chromatography; and HIC = hydrophobic-interaction chromatography.

C. Band Broadening

$$H_{tot} = Au^{1/3} + B/u + (C_S + C_M)u \quad \text{(For LC, Knox equation)}$$

$$\left(\frac{2\lambda\, d_p^{4/3}}{(D_m)^{1/3}}\right) u^{1/3} + \left(\frac{2\, D_m}{(1+\varepsilon_p/\varepsilon_e)}\right)\left(\frac{1}{u}\right) + q_s\left(\frac{k}{(1+k)^2}\right)\left(\frac{d_f^2}{D_s}\right)u + f(k)\left(\frac{d_p^2}{D_m}\right)u$$

$$\gamma = \frac{1}{(1+\varepsilon_p/\varepsilon_e)}$$

Typically, D_m in a gas phase is much larger than that in a liquid phase.

11. Calculate the plat height contributed by longitudinal diffusion in the mobile phase of a column for which γ = 0.60 and in which the mean flow velocity is 2.0 cm/s. First assume that the column is a GC column with a typical solute diffusivity of D_m = 0.10 cm^2/s; second, assume a LC column with D_m = 1.0 X 10^{-5} cm^2/s.

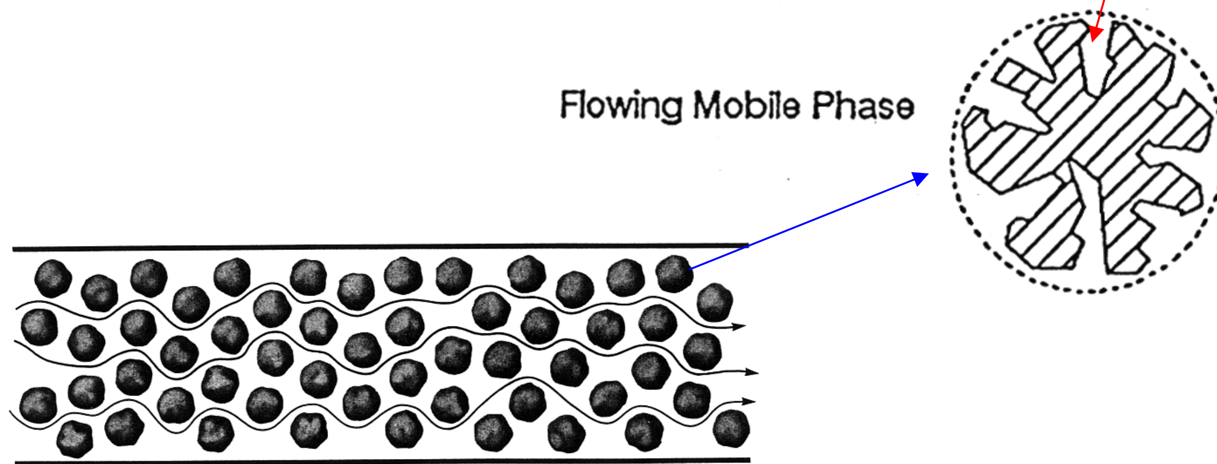
$$H = \frac{2\,\gamma\, D_m}{u}$$

H_g = 0.06 cm

H_l = 0.000006 cm → Negligible!

Resistance of Mass transfer in mobile phase

Stagnant mobile phase



(1) Resistance of mass transfer in stagnant mobile phase is a dominant term in mobile phase of LC. It is due to differences in the rates of diffusion of solute molecules as they go from the flowing mobile phase to the stagnant mobile phase in the column. Since a solute does not travel down the column that solute remaining in the flowing mobile phase. This leads to some solute molecules leaving the column before others and creates band broadening.

(2) The plate height contribution due to stagnant mobile phase:

$$H_{sm} = f(k)\left(\frac{d_p^{\,2}}{D_m}\right) u$$

$$f(k) = \frac{q\, \varepsilon_e\, k_z^2\, (1+\varepsilon_p/\varepsilon_e)}{\varepsilon_p\, (1+k_z)^2}$$

$k_z = (\varepsilon_p/\ \varepsilon_e)k + (\varepsilon_p/\ \varepsilon_e) + k$

q = shape factor (1/30 for a sphere)

d_p: average size of the filling particles

D_m: solute diffusion coefficient in mobile phase

u: linear velocity

In most chromatographic systems, H_{sm} makes a significant contribution to H_{tot}. (30 ~ 50%)

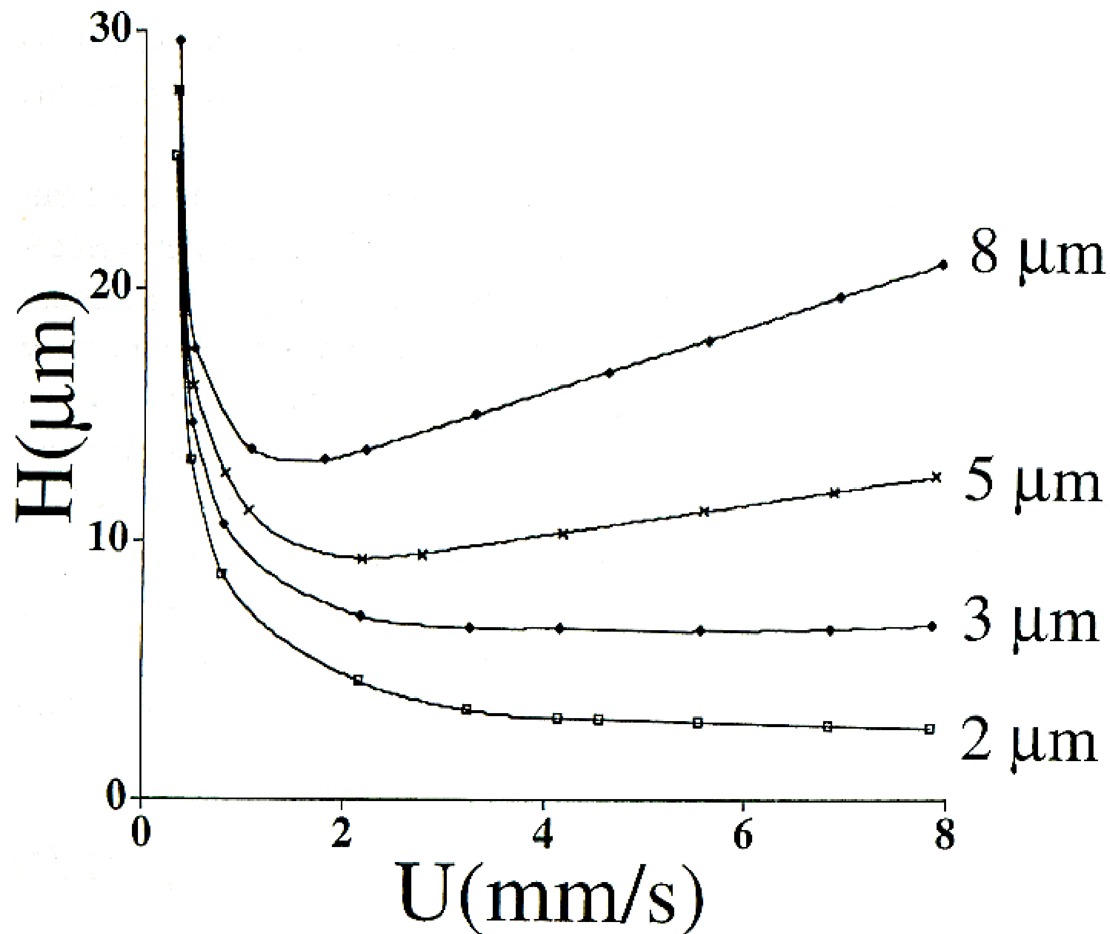
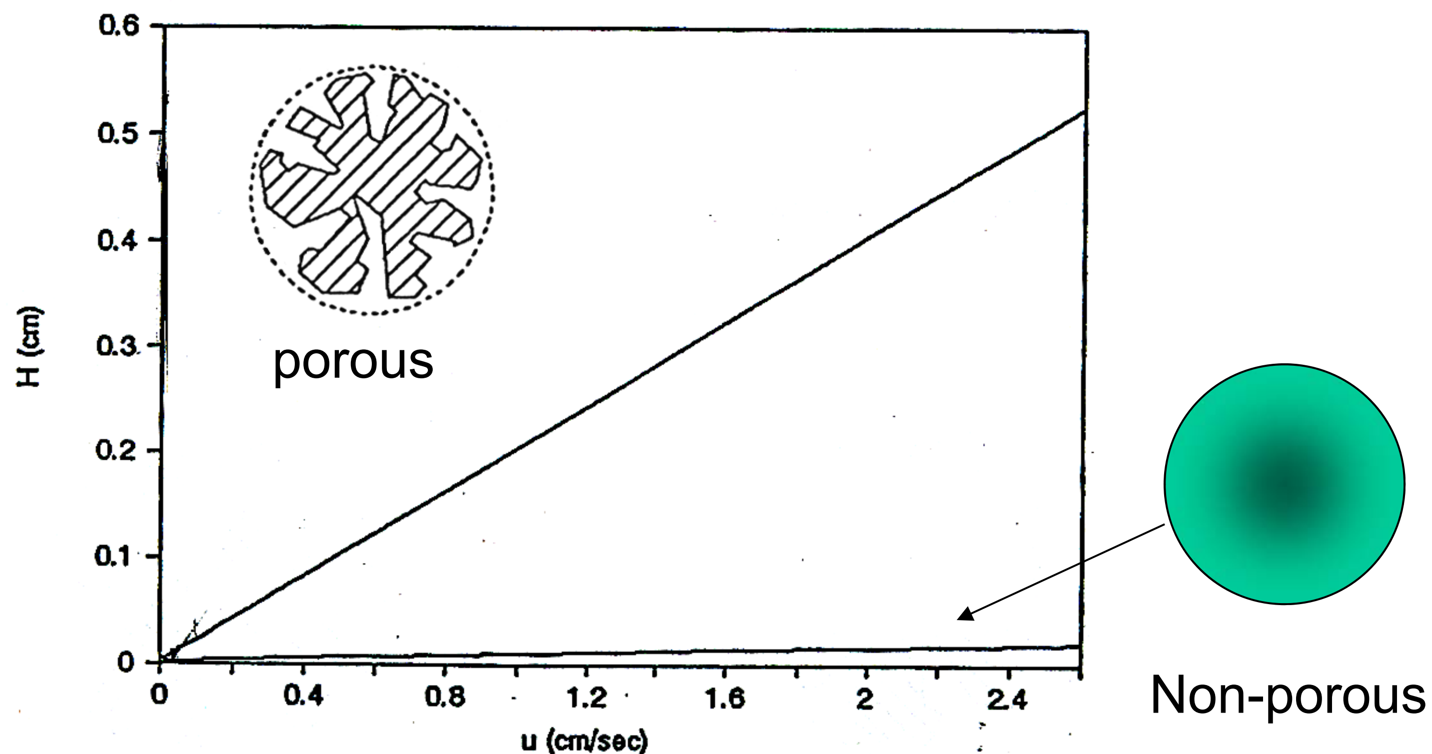


Figure 1.9. Plot of the plate height H (μm) against the mobile phase velocity u (mm/s) for columns of different particle diameters (and different column lengths) in liquid chromatography.

$H_{tot} = Au^{1/3} + B/u + (C_S + C_M)u$ (For LC, Knox equation)

$u_{Avg} = \Delta P\, d_p^{2*}\, \varepsilon_e^{2}/[180\, \eta\, (1- \varepsilon_e)^{2} L]$ (Darcy equation)

Band-Broadening in LC



$H_{tot} = Au^{1/3} + B/u + (C_S + C_M)u$ (For LC, Knox equation)

Optimizing Parameters for A Better Separation

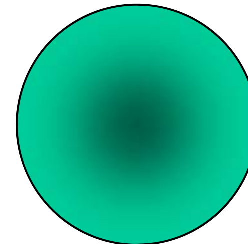
(R_s and time)

$$R_s = [N^{1/2}/4][(\alpha - 1)/(\alpha)]/[k_2/(1+ k_2)], \quad \alpha = k_2/k_1$$

$$N = L/H$$

$$R_s = [(L/H)^{1/2}/4][(\alpha - 1)/(\alpha)]/[k_2/(1+ k_2)]$$

$$k = K \frac{V_s}{V_m}$$



Small molecules (K)
Biomolecules, and polymers (K)

D. Column Packing Materials

1. Mesoporous inorganic oxides

Sol-gel methods

(a) silica, SiO_2

Sol: sodium silicate or a tetraalkoxylsilane to aqueous acidic solution.

↓

Hydrolysis

↓ **aging**

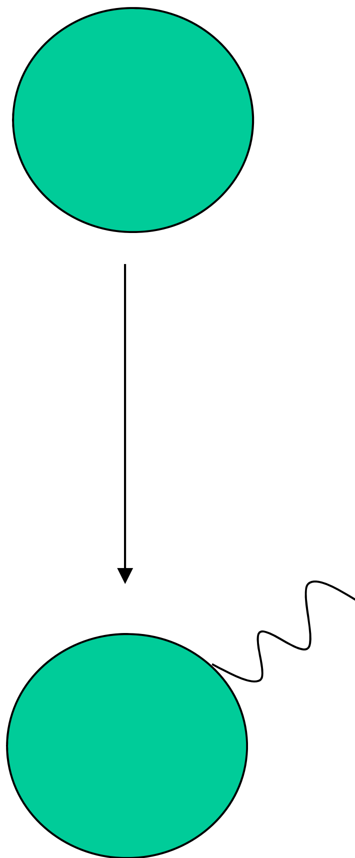
particles

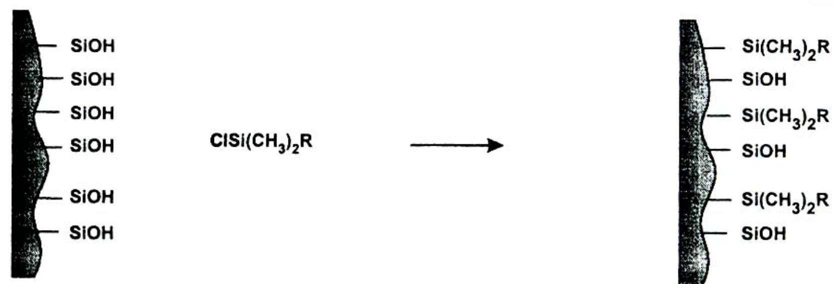
(b) Alumina, titania and ziconia (stable to extreme pH conditions)

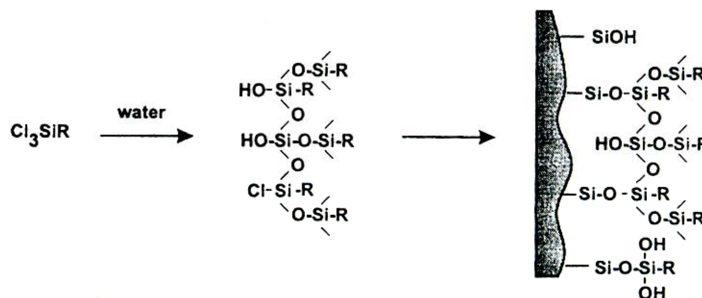
(Al_2O_3, TiO_2, ZrO_2)

pH: 2-14 (for Al_2O_3); 1-14 (for TiO_2, ZrO_2)

2. Chemically bonded inorganic oxides







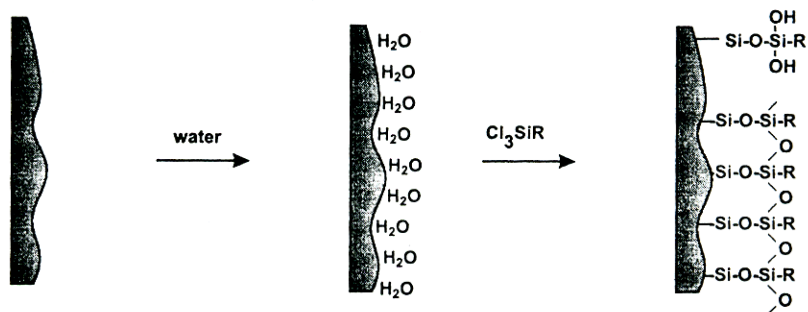
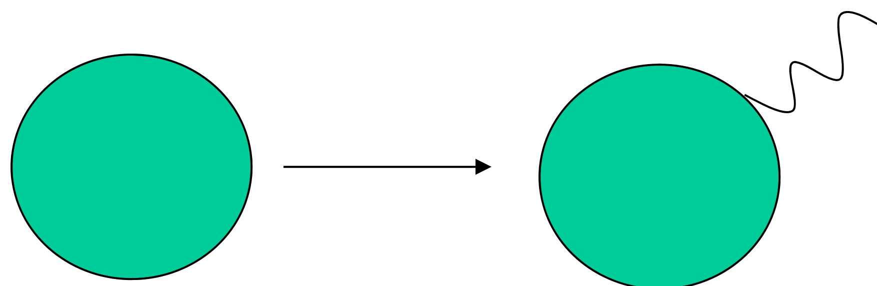


Figure 4.5. General outline for the synthesis of siloxane-bonded phases on a porous silica substrate. (From ref [73]. ©American Chemical Society).

Table 4.2
Common substituents for siloxane-bonded phases

Type	Substituent	Application
Alkyl	$-CH_3$	Reversed phase
	$-C_4H_9$	
	$-C_8H_{17}$	
	$-C_{18}H_{37}$	
	$-C_{30}H_{61}$	
Fluoroalkyl	$-(CH_2)_2(CF_2)_5CF_3$	Reversed phase
	$-(CH_2)_2C(CF_3)_2C_3F_7$	
Phenyl	$-C_6H_5$	Reversed phase
	$-C_6F_5$	
Cyano	$-(CH_2)_3CN$	Normal and reversed phase
Amino	$-(CH_2)_3NH_2$	Normal and reversed phase. Weak anion exchanger
Diol	$-(CH_2)_3OCH_2CH(OH)CH_2(OH)$	Normal phase and size exclusion
Amide	$-(CH_2)_3NHCOC_{13}H_{27}$	Reversed phase
Carbamate	$-(CH_2)_3OCONHC_8H_{17}$	Reversed phase
Sulfonic acid	$-(CH_2)_3SO_3H$	Strong cation exchanger
	$-C_6H_4SO_3H$	
	$-(CH_2)_3C_6H_4SO_3H$	
Carboxylic acid	$-(CH_2)_3OCH_2COOH$	Weak cation exchanger
	$-(CH_2)_3COOH$	
	$-(CH_2)_3C_6H_4CH_2COOH$	
Dimethylamine	$-(CH_2)_3N(CH_3)_2$	Weak anion exchanger
Quaternary amine	$-(CH_2)_3N^+(CH_3)_3$	Strong anion exchanger



3. Polymer coated inorganic oxides
4. Porous Polymers
5. Porous graphitic Carbon
6. Nonporous particles
7. Monoliths

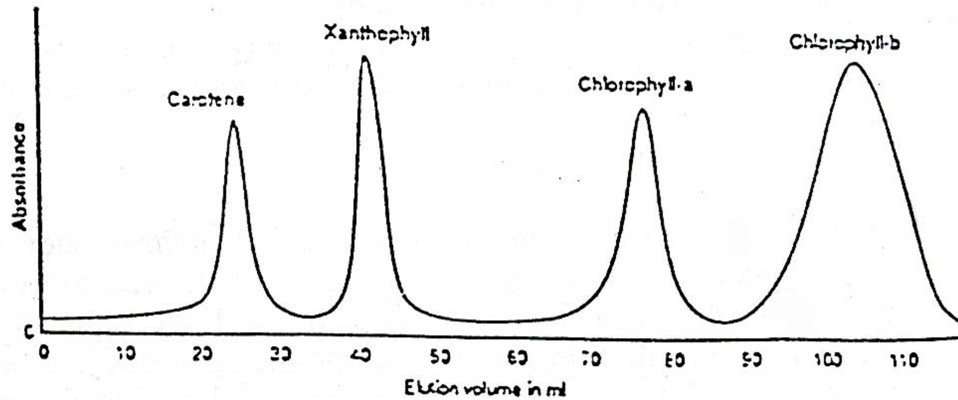
E. Low- and High-performance liquid chromatography

1. Many types of liquid chromatography are available based on different stationary phase and mobile phase combinations. Each type of liquid chromatography may be further characterized on its overall efficiency, or performance.

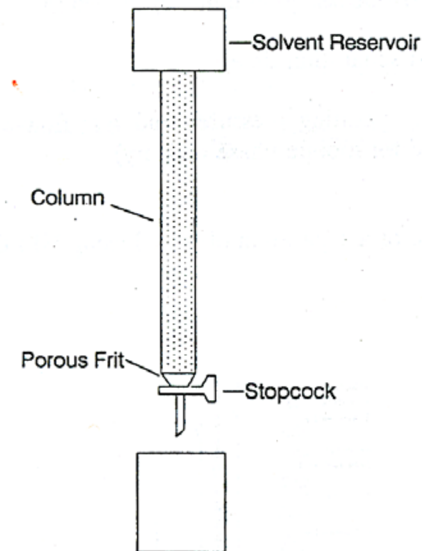
2. Low-performance liquid chromatography is a term used to describe LC methods which used large, non-rigid materials (i.e., particles > 40 µm in diameter). The use of such a support give rise to poor system efficiencies and large plate heights. This produces the following characteristics:

(a) Broad peaks, poor limits of detection
(b) Long separation times
(c) Low operating pressures and flow-rates

3. A typical separation obtained using low-performance liquid chromatography is shown below.



4. An example of the equipment used in performing low-performance liquid chromatography is a packed bed column is shown. In this format, low-performance liquid chromatography is also called column chromatography.



5. In such a system, sample is usually applied to the column by direct application to the top of the column. Detection is usually performed by fraction collection, followed by later analysis of the fractions.

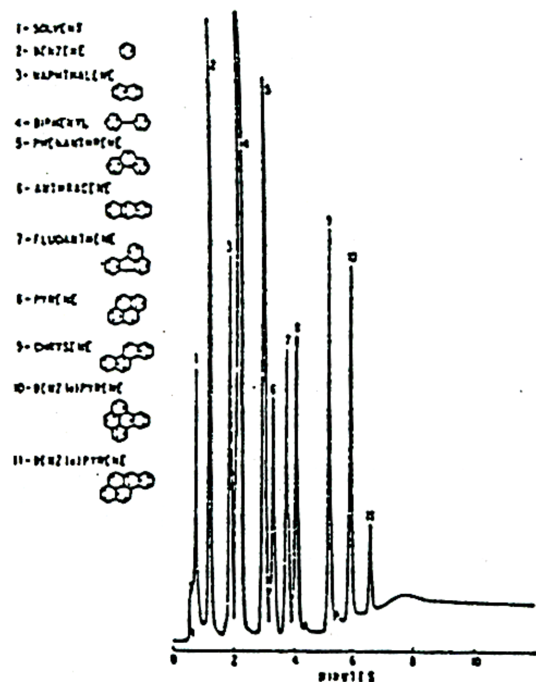
6. Due to its simple system requirements and low cost, low-performance liquid chromatography is popular in sample purification and in the removal of interferences from sample. It is also used in some analytical applications, but this is not as common due to its low efficiency, long analytical time, and poor limits of detection.

7. High-performance liquid chromatography (HPLC) is a term that describes LC techniques which use small, uniform, rigid supports (i.e., <40 μm in diameter in theory, but usually 3-10 μm in practice). The use of such a support gives rise to good system efficiencies and small plate heights. This results in the following:

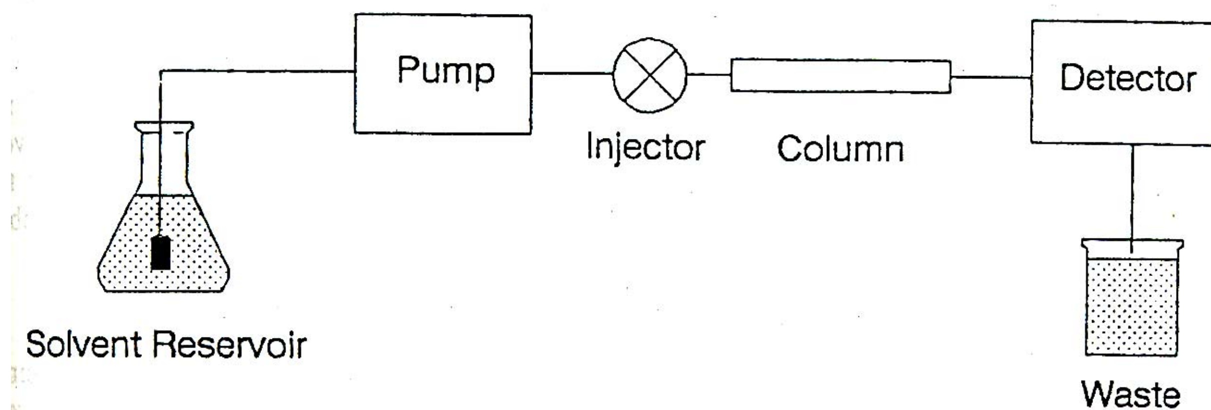
- **(a) narrow peaks, good limits of detection**
- **(b) short separation times**
- **(c) high operating pressures and fast flow-rates.**

$$u_{Avg} = \Delta P \, d_p^{2} * \varepsilon_e^{2}/[180\,\eta\,(1-\varepsilon_e)^2 L] \quad \text{(Darcy equation)}$$

8. An example of a separation obtained using HPLC is shown below



9. A typical system used for HPLC is as follows:



10. In such a system, the higher operating pressures needed for mobile phase delivery requires that special pumps and other system components be used. Sample is usually applied using a closed system (i.e., injection valve) and detection is typically performed using a flow-through detector

11. Due to its fast analysis times, good limits of detection, and ease of automation, HPLC is usually the LC technique of choice for analytical applications. These characteristics also make it popular in purification work.

12. Some disadvantages of HPLC in preparative work includes it greater expense and lower sample capacities the low-performance liquid chromatography.

F. Elution

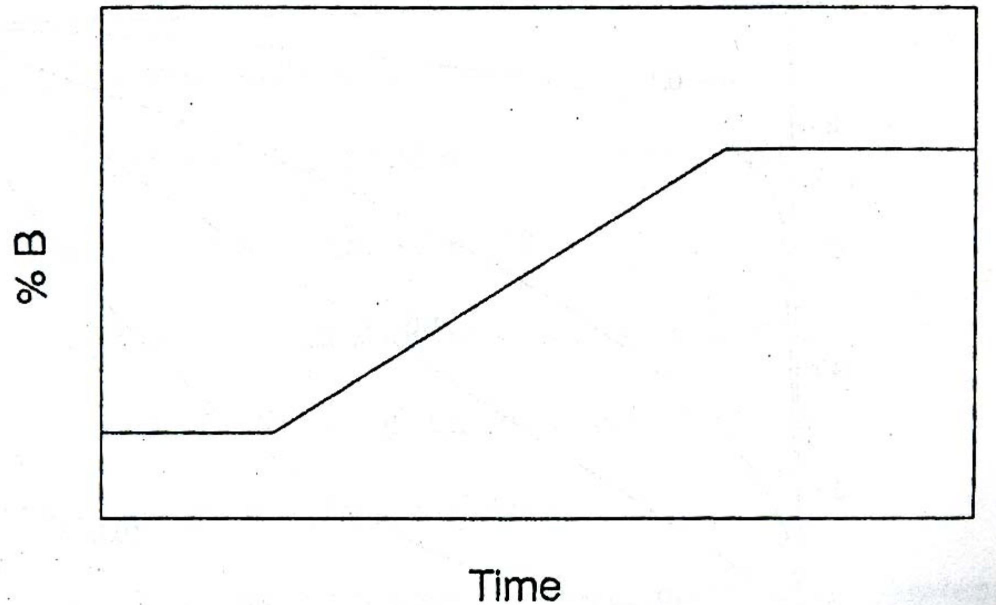
1. Retention and elution of solutes in LC depends on the interactions of solutes with both the mobile and stationary phases.

2. To describe how well solutes are retained on a column with different solvents, the terms *<u>weak mobile phase</u>* and *<u>strong mobile phase</u>* are use.

3. As in GC, solutes can be eluted from a column by using either constant column conditions or gradient elution.

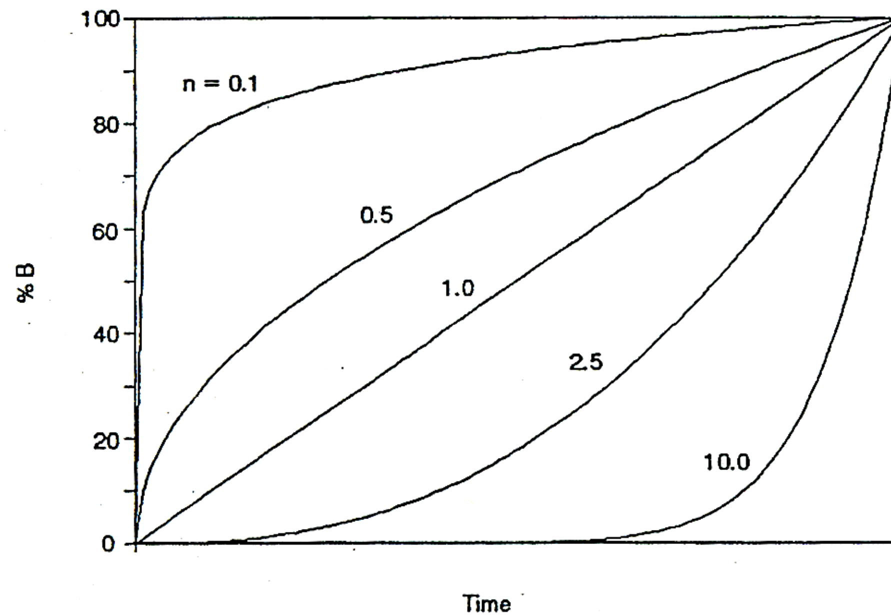
(a) The use of a constant mobile phase composition to elutes is known as isocratic elution.

(b) Gradient elution is LC is normally performed by changing the composition of the mobile phase with time. Known as solvent programming, this is done by going from a weak mobile phase to a strong one.



(c) Solvent programming can be done in one or more steps, each involving a stepwise, linear or nonlinear changes in mobile phase content.

(d) Examples of several types of solvents gradients are shown below



(e) The advantages of solvent programming vs. isocratic elution are:

i. Improved resolution of peaks.
ii. Faster analysis times
iii. Sharper peaks and better limits of detection
iv. Decrease peak tailing
v. Greater number of peaks can be separated
vi. Hi % D at end of run allows better column clean-up.

f. Disadvantages of solvent programing

i. Expensive
ii. Requires re-equilibration of column after each run.

g. Temperature-programming can be used in LC, but is much less common than GC

h. Flow-programming can be used in LC

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