

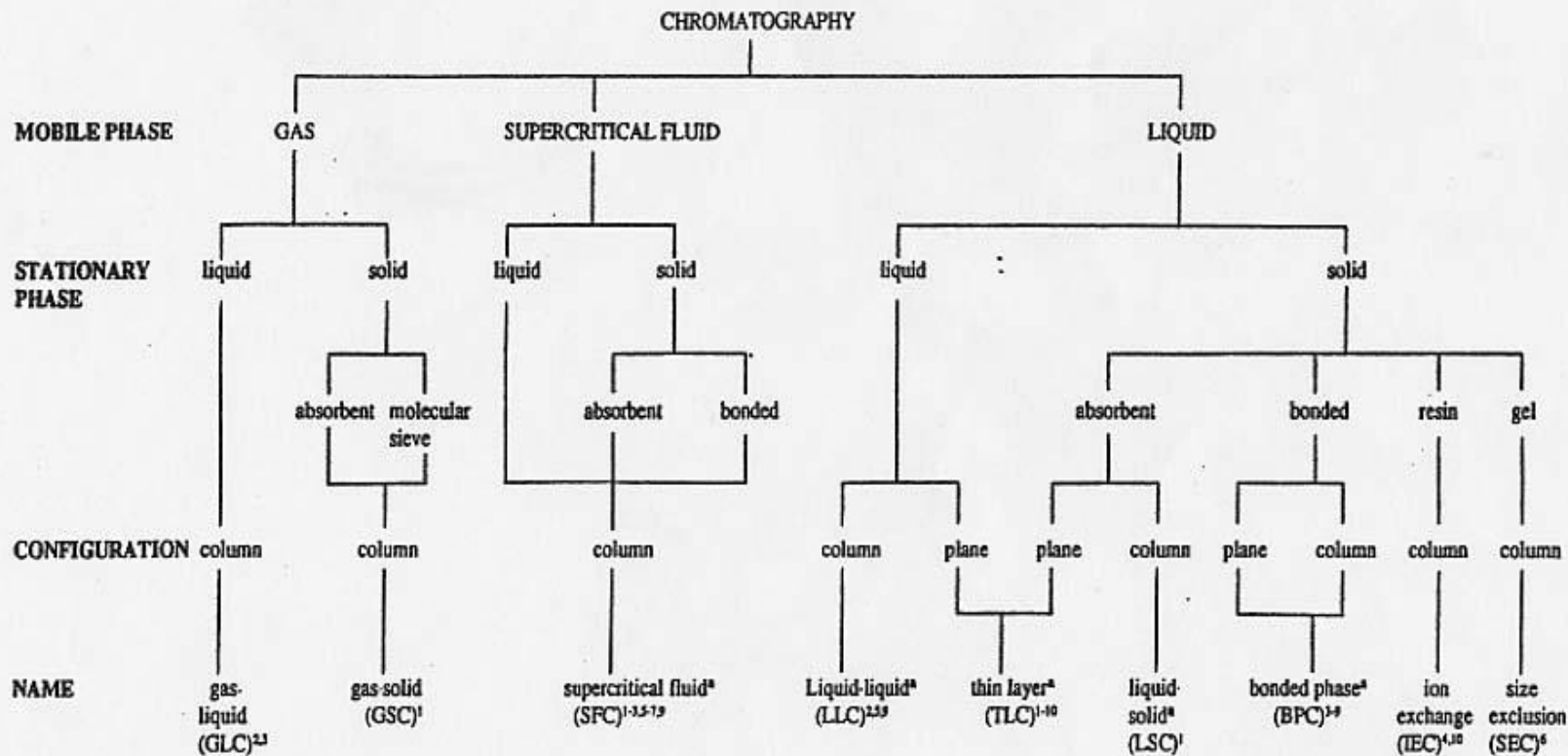


# CHEMISTRY 5570

## Advanced Analytical Chemistry Lecture 17



# Chromatography

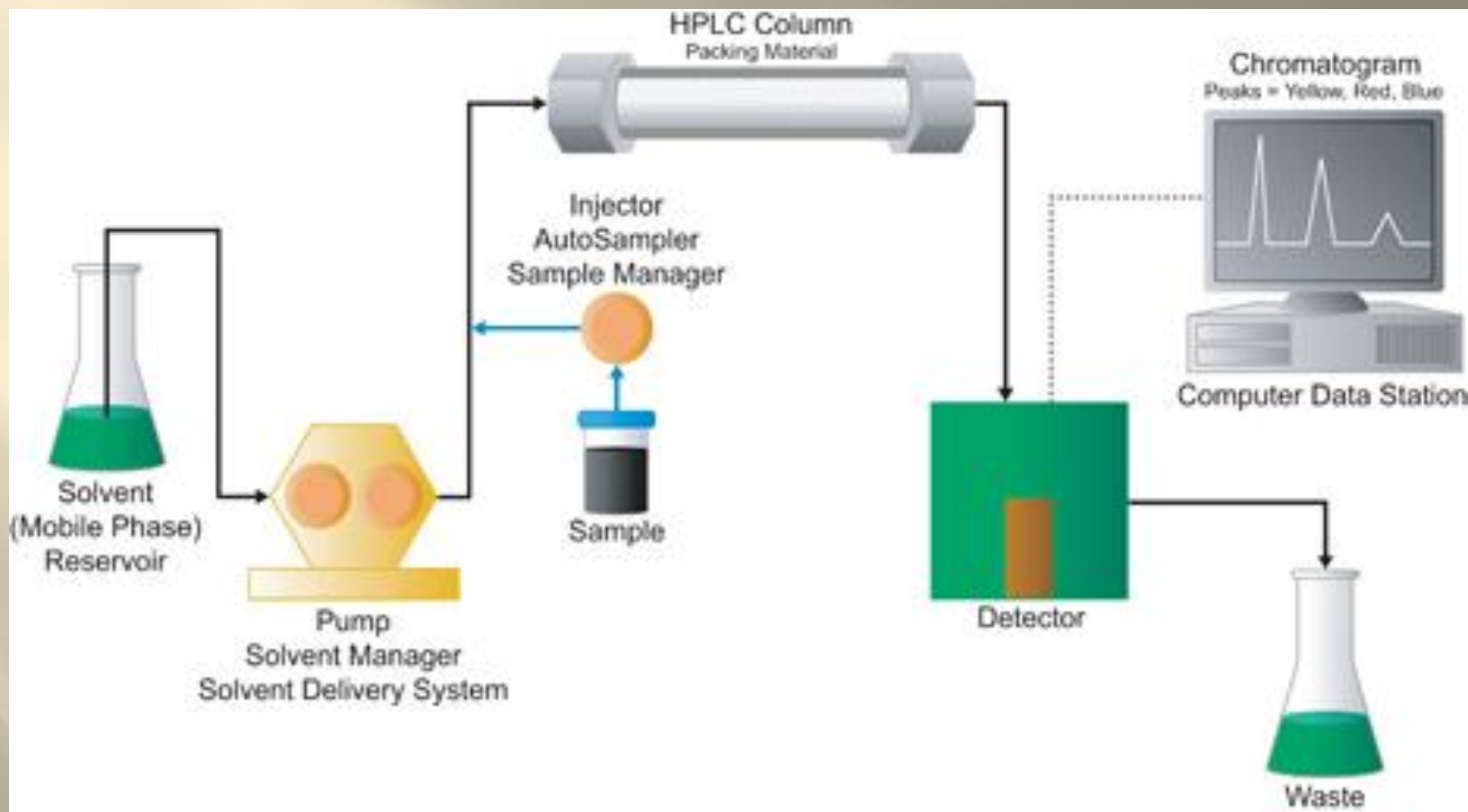


\*For these techniques the combination of mobile and stationary phase can be varied to generate either a normal phase or reversed phase system. Mechanisms which have been exploited in the various techniques are identified as: <sup>1</sup>adsorption, <sup>2</sup>partition, <sup>3</sup>bonded phase, <sup>4</sup>ion exchange, <sup>5</sup>ion interaction, <sup>6</sup>size exclusion, <sup>7</sup>affinity, <sup>8</sup>micellar, <sup>9</sup>chelation, <sup>10</sup>ion exclusion.

**Fig. 1.3.** Classification of chromatographic systems.

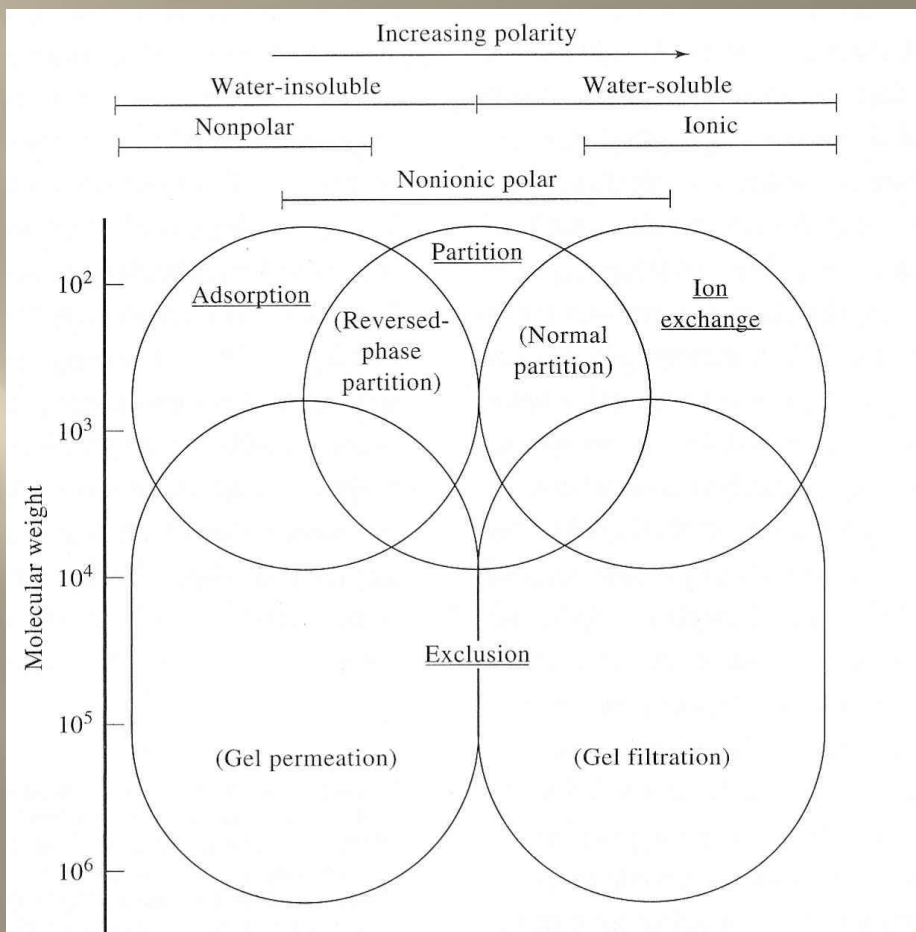
# Chromatography

## High Performance Liquid Chromatography



# Chromatography

## Stationary/Mobile Phase



**Figure 28-1** Applications of liquid chromatography. (From D. L. Saunders, in *Chromatography*, 3rd ed., E. Heftmann, Ed., p. 81. New York: Van Nostrand Reinhold, 1975. With permission.)

# Chromatography

## Normal Phase Chromatography

**Normal Phase - a polar stationary phase with a less polar mobile phase.**

**Neutral solutes are separated on the basis of their polarity. More polar solute, longer retention time.**



# Chromatography

## Normal Phase Chromatography

### Mechanism of Retention

Dominant retention mechanism is adsorption. Also called adsorption chromatography.

Selectivity depends on relative strength of the polar interaction.

The spatial configuration - how the solute is positioned on the stationary phase is important (sensitive to spatial or steric differences).

# Chromatography

## Normal Phase Chromatography

### Stationary Phase for Normal-Phase

Wide range of stationary phase materials have been used:

sucrose

starch

fluorisil

magnesium oxide

cellulose

silica gel

charcoal

alumina

# Chromatography

## Normal Phase Chromatography

### Stationary Phase for Normal-Phase

**Most common - silica or alumina that have polar hydroxyl groups on the surface.**

**Silica is preferred - available, low cost, allows higher sample loadings, less likely to catalyze decomposition of sample components.**

**Bonded phases with polar ligands (amino ( $-\text{NH}_2$ ), cyano ( $\text{C}\equiv\text{N}$ ), and diol (2-OH groups) or functional groups attached by alkyl chains to silica**



# Chromatography

## **Normal Phase Chromatography**

### **Stationary Phase for Normal-Phase**

**Most common - silica or alumina that have polar hydroxyl groups on the surface.**

**Alumina is used for basic compounds (i.e. amines) which are strongly retained on silica.**

**In general, both retain more polar compounds strongly.**

# Chromatography

## Normal Phase Chromatography

### Stationary Phase for Normal-Phase

Order of elution: saturated hydrocarbons < alkenes (olefins) < aromatic hydrocarbons  $\approx$  organic halides < sulfides < ethers (ROR) < nitro compounds < esters ( $\text{RCO}_2\text{R}$ )  $\approx$  aldehydes  $\approx$  ketones < alcohols  $\approx$  amines < sulfones < sulfoxides < amides < carboxylic acids ( $\text{RCO}_2\text{H}$ ).

# Chromatography

## Normal Phase Chromatography

### Stationary Phase for Normal-Phase

Silica adsorption sites (-Si-OH, -OH groups) can be controlled by pretreatment of column.

Usually silica for HPLC treated between 200-300 °C

Drawback of normal phase - lack of separation selectivity between packing materials despite difference in particle shape, size, or surface area.

(Compounds elute in same order regardless of column selected).

So selectivity must be achieved through changing the mobile phase.

# Chromatography

## Normal Phase Chromatography

### Mobile phase - solvent strength

Variation in retention is generally achieved by altering the mobile phase.

The interaction of a solvent molecule with an analyte depends on several forces: dispersion, dipole, hydrogen bonding and dielectric interactions (electrical insulator).

The extent of a molecule interacting in these four ways is referred to as the polarity of the compound.

The chromatographic strength of a solvent is directly related to its polarity.

# Chromatography

## Normal Phase Chromatography

Mobile phase - solvent strength

There are 4 main factors involved in the choice of solvents for normal-phase chromatography:

- Solvent strength,
- Localization - measure of the interaction of the solvent with the stationary phase,
- Basicity,
- UV cutoff.

The strengths of various solvents are determined empirically and are listed in a eluotropic series ( $\epsilon^\circ$ ).



# Chromatography

## Normal Phase Chromatography

### Mobile phase - solvent strength

Index of solvent strength is given by the experimental adsorption solvent strength parameter,  $\epsilon^\circ$ .

$\epsilon^\circ$  - A measure of adsorption energy per unit area of solvent.  
A listing of these values is called an eluotropic series.

Range from  $-0.25$  to  $+1.2$ .

An increase in  $\epsilon^\circ$  means a stronger solvent giving lower retention of a solute. Solvents with high  $\epsilon^\circ$  values are strong solvents, and will more easily elute the more polar analytes.

# Chromatography

## Normal Phase Chromatography

### Mobile phase - solvent strength

Solvent	Strength $\epsilon^0$	Localization	Basic?	UV cutoff
Hexane	0.00	no	Not relevant	201
Chloroform	0.26	no	Not relevant	247
Methylene chloride	0.30	no	Not relevant	234
Ethyl ether	0.38	yes	yes	219
Methyl t-butyl ether	0.48	yes	yes	225
Ethyl acetate	0.48	yes	no	256
Acetonitrile	0.52	yes	no	192
Tetrahydrofuran	0.53	yes	yes	230
1- or 2-Propanol	0.60	yes	Proton donor	214
Methanol	0.70	yes	Proton donor	210

# Chromatography

## Normal Phase Chromatography

### Mobile phase - solvent strength

Solvent	Solvent strength, $\epsilon^\circ$	
	<u>Silica</u>	<u>Alumina</u>
n – Heptane	0.01	0.01
Chloroform	0.26	0.40
Methanol	0.7	0.95

Hexane, Heptane - nonpolar - add polar solvent to adjust solvent strength. Choose solvent with low  $\epsilon^\circ$  and add solvent with greater  $\epsilon^\circ$  until desired separation occurs.

# Chromatography

## Normal Phase Chromatography

### Mobile phase - solvent strength

Solvent molecules with polar functional groups will prefer a specific position relative to nearby silanol groups (or other polar group on the stationary phase).

Therefore the stationary phase is covered with a well defined layer of solvent molecules.

The competition between analytes and these solvents for adsorptive sites is an important factor in normal-phase selectivity.

Solvents that are not polar or weakly polar interact with the stationary phase very weakly and the coverage of the surface is random.

# Chromatography

## **Normal Phase Chromatography**

### **Mobile phase - solvent strength**

**Biggest problem with normal phase is the effect that water has on the activity of polar adsorbents.**

**Water adsorbs onto the strongest adsorption sites - leaving weaker sites open to retain the sample – decreasing solute retention.**



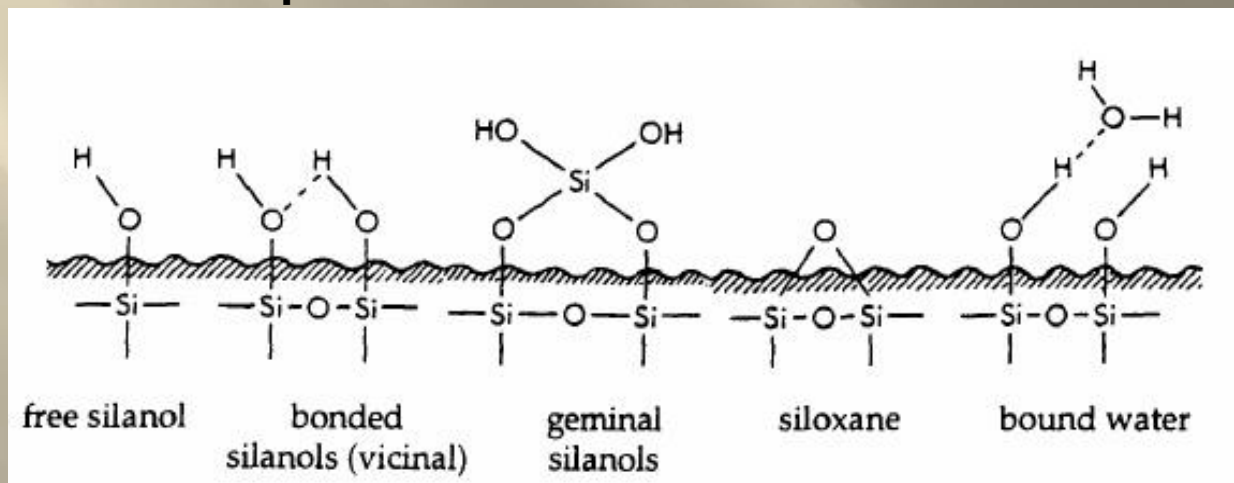
# Chromatography

## Normal Phase Chromatography

### Mobile phase - solvent strength

Difficult to control amount of water present in mobile phase - easy to pick up water from atmosphere.

Generally add small amount of water to mobile phase to give less variation in sample retention from run to run.



# Chromatography

## Normal Phase Chromatography

Mobile phase - solvent strength

Separation of Molecules with Different Functional Groups

The hydrophobic portion of an analyte molecule has little effect on separation, for example, butanol, hexanol, and octanol cannot be well separated using normal phase chromatography (but they can be easily separated using reversed-phase).

If a molecule has several functional groups, then retention is based on the most polar one.

# Chromatography

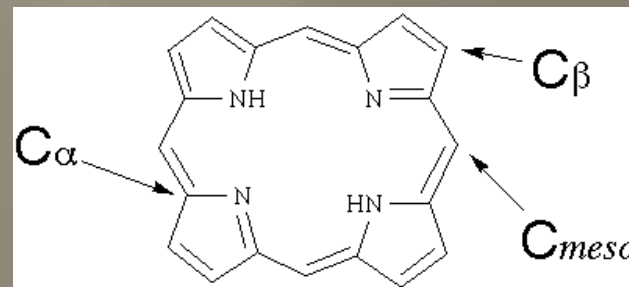
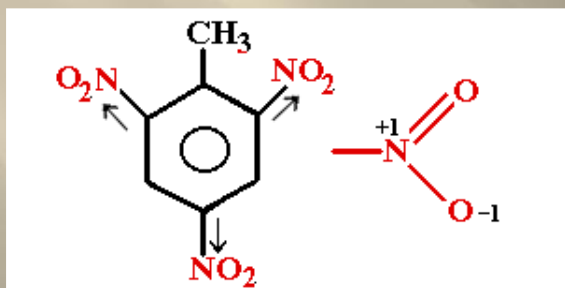
## Normal Phase Chromatography

### Applications

Normal phase used less than other HPLC techniques.

Used for analysis of samples that are soluble in non-polar solvents and for isomer separation.

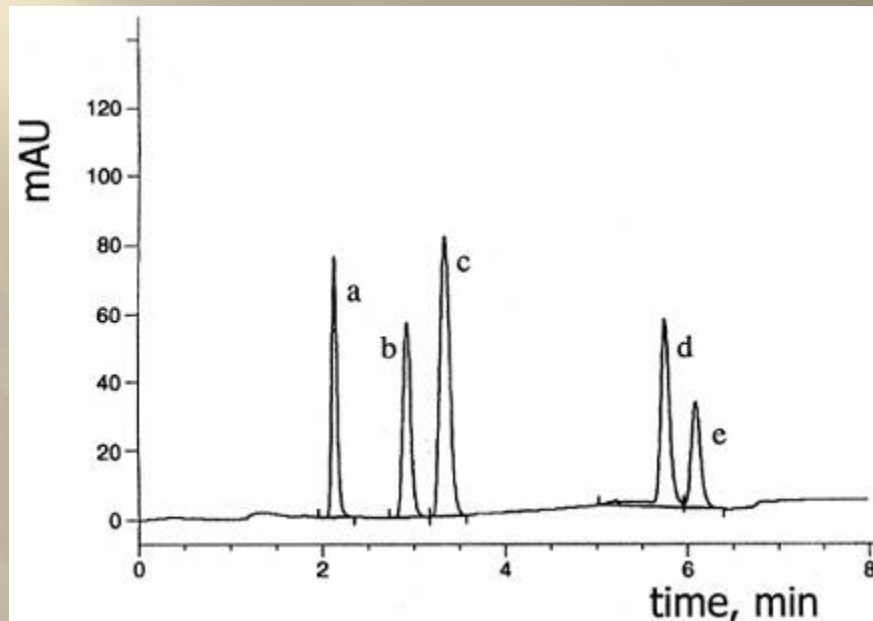
Examples:      fat & water – soluble vitamins  
Less-polar plant pigments –porphyrins  
Pesticides- still better with GC  
Separation of isomers (6 isomers of trinitrotoluene)



# Chromatography

## Normal Phase Chromatography

### Applications



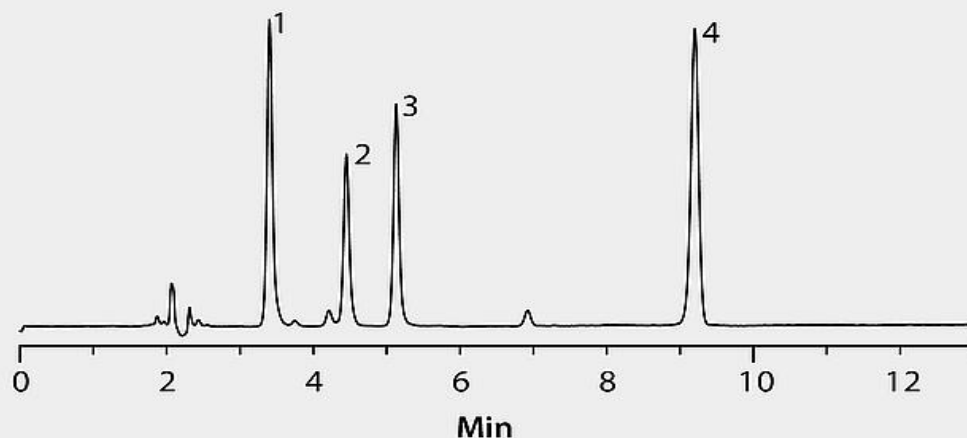
Separation of isomers of chloraniline in gradient elution on NP LC Conditions;  
Mobile phase: n-heptane/2-propanol, gradient elution from (99/1) to (19/81) in 4 min, Flow rate: 1.5 ml/min, Temperature: 43° C, Detection: UV, 245 nm, Solutes: (a) 2,3-Dichloroaniline, (b) 2-chloroaniline, (c) 2,4-dichloroaniline, (d) 3-chloroaniline, (e) 3,4-dichloroaniline.

# Chromatography

## Normal Phase Chromatography

### Applications

1. alpha-Tocopherol
2. Menadione
3. gamma-Tocopherol
4. Cholecalciferol



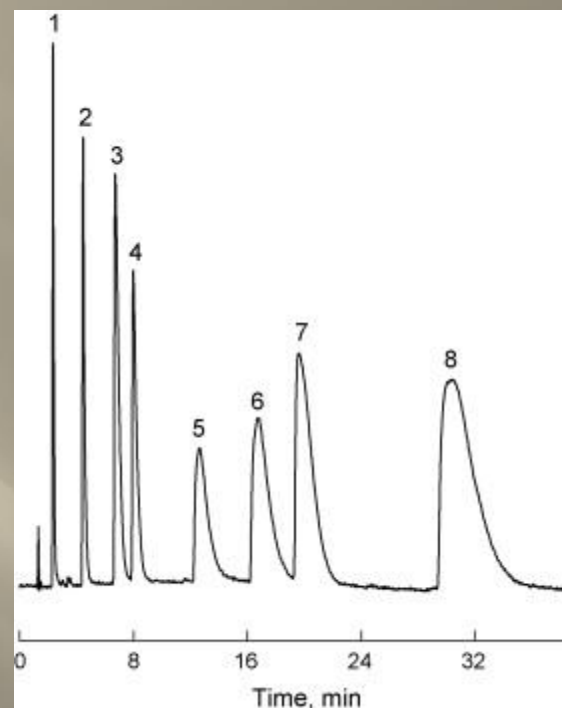
Analysis of fat-soluble vitamins using a normal phase, vitamin K 3 (menadione), vitamin E (alpha and gamma tocopherol), and vitamin D3 (cholecalciferol).

Column: Ascentis Si, 15 cm x 4.6 mm I.D., 5  $\mu$ m particles, mobile phase: [A] hexane; [B] ethyl acetate, gradient: 10 to 30% B in 10 min; held at 30% B for 2 min, flow rate: 1.0 mL/min, detector: UV, 290 nm, injection: 10  $\mu$ L.



# Chromatography

## Normal Phase Chromatography Applications



Separation of alkylbenzenes on MSND column. Solutes: (1) 1,3,5-triisopropylbenzene, (2) 1,3-diisopropylbenzene, (3) tert-butylbenzene, (4) isopropylbenzene, (5) benzene, (6) toluene, (7) n-amylnbenzene, (8) n-nonylnbenzene. Eluent n-pentane.

# Chromatography

## Reverse-Phase Chromatography

**Most widely used of LC modes (over 75%)**

**Uses a non-polar stationary phase with a polar mobile phase.**

**More polar the solute, the lower the retention time.**

**Mechanism of separation**

**More complex than for normal-phase.**

**Interaction between solute molecule and non-polar stationary phase is too weak to explain the degree of solute retention observed.**

**Mechanism - combination of partition and adsorption.**

# Chromatography

## Reverse-Phase Chromatography

### Mechanism of separation

### Solvophobic theory

#### Assumes:

- using aqueous mobile phases with low organic modifier
- stationary phase is uniform layer of a non- polar ligand
- solute binds to the stationary phase and reduces surface area of solute exposed to mobile phase. (Solute binds to stationary phase because it is solvophobic)

# Chromatography

## Reverse-Phase Chromatography

### Mechanism of separation

#### Solvophobic theory

Retention occurs because of interaction between mobile phase and solute - mobile phase composition influences separation selectivity more than stationary phase (behaves as a solid).

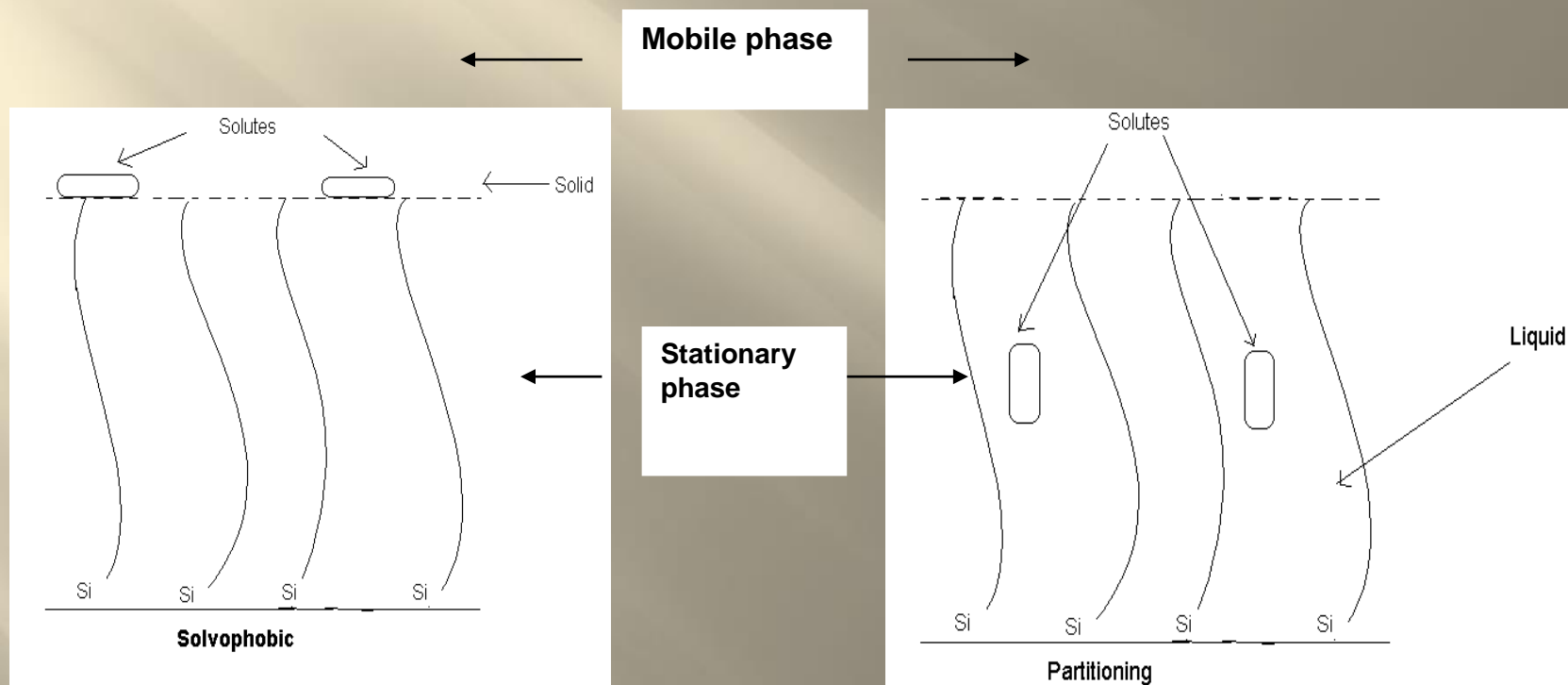
#### Partitioning theory

Solute is thought to fully interact with stationary phase and partitions between mobile phase and stationary phase (behaves as a liquid).

# Chromatography

## Reverse-Phase Chromatography

### Mechanism of separation





# Chromatography

## **Reverse-Phase Chromatography**

**Mechanism of separation**

**Partitioning theory**

**As the chain length of the bonded material increases the retention mechanism is more like partitioning.**

**Short chain lengths - more like solvophobic (adsorption).**

# Chromatography

## Reverse-Phase Chromatography

### Stationary Phases

Majority - bonded silica - also polymeric

# Chromatography

## Reverse-Phase Chromatography

**Stationary Phases - Bonded phase silica columns**

**Functional group is chemically attached to a silica support (like used for normal phase).**

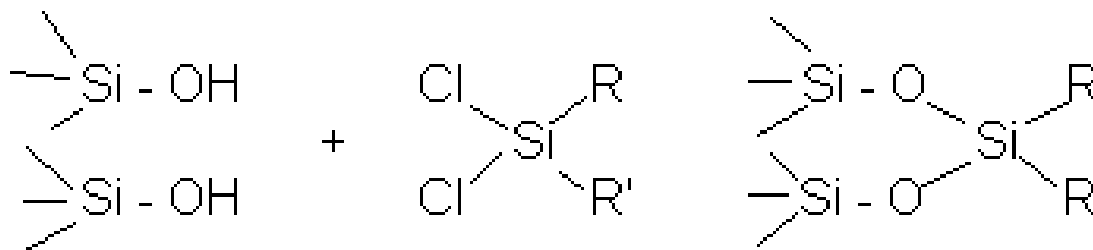
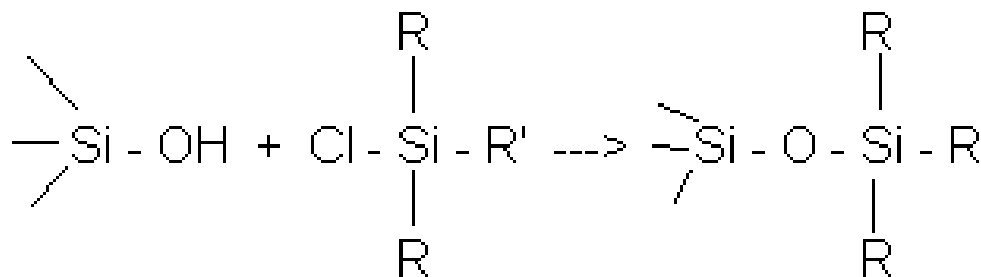
**Attaching groups to the silica depends on reaction with the surface silanol groups.**

**Most common method - reaction of silica with organochlorosilanes to produce siloxane ( $\text{Si-O-Si-R}_3$ ) packings.**

# Chromatography

## Reverse-Phase Chromatography

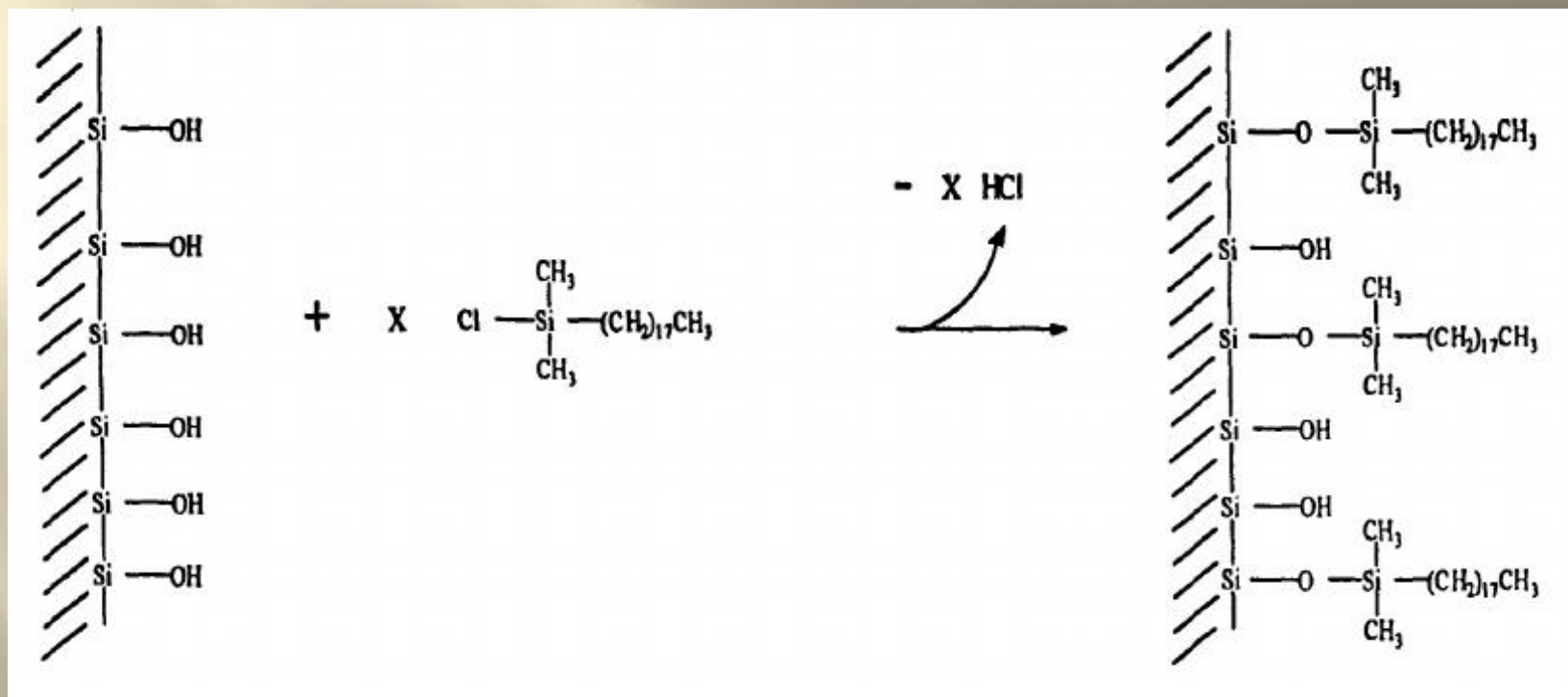
### Stationary Phases - Bonded phase silica columns



# Chromatography

## Reverse-Phase Chromatography

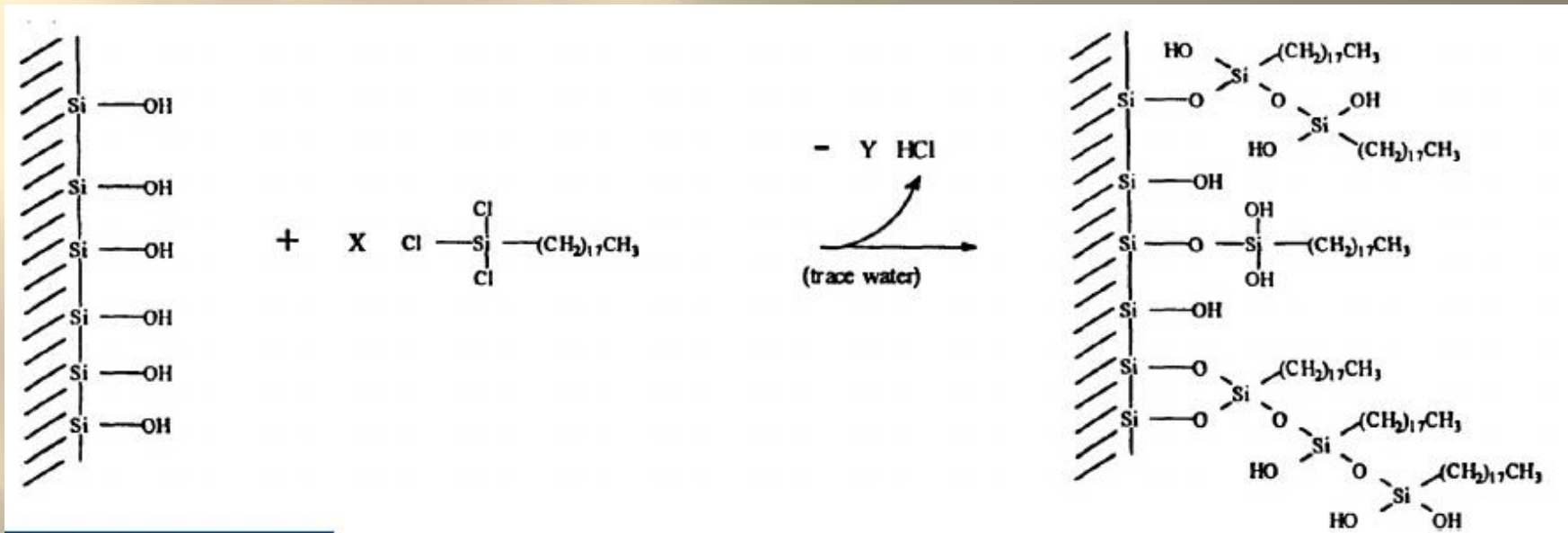
Stationary Phases - Bonded phase silica columns monomeric type



# Chromatography

## Reverse-Phase Chromatography

Stationary Phases - Bonded phase silica columns polymeric type





# Chromatography

## Reverse-Phase Chromatography

### Stationary Phases - Bonded phase silica columns

Amount of material bonded to the silica is described by the term carbon load - amount of carbon as a weight % of bulk silica packing.

Example: Monofunctional C<sub>18</sub> - carbon load = 7-15 % (w/w)

Monofunctional preferred - easier to control - less batch to batch variation.

Higher the carbon load, more hydrophobic the column.

The maximum sample loading for a C<sub>18</sub> functionalized column is about 2 mg sample/ g of stationary phase.

# Chromatography

## Reverse-Phase Chromatography

**Stationary Phases - Bonded phase silica columns**

**Identify the column by the nature of the bonded R' group.**

**C<sub>18</sub> - most common.**

**Others – C<sub>8</sub>, phenyl, C<sub>6</sub>, C<sub>4</sub>, C<sub>2</sub>, CN, NH<sub>2</sub>, and NO<sub>2</sub>.**

**Retention increases exponentially as alkyl chain length increases.**

# Chromatography

## Reverse-Phase Chromatography

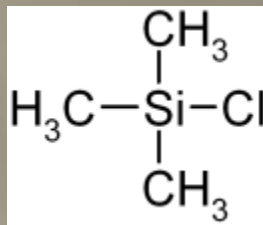
**Stationary Phases - Bonded phase silica columns**

**Not all silanol groups are functionalized.**

**These residual acidic silanol groups can cause tailing of basic compounds.**

**So usually after functional groups are added - an end-capping procedure is done.**

**This procedure uses chlorotrimethylsilane to cap open groups.**



# Chromatography

## Reverse-Phase Chromatography

### Stationary Phases - Bonded phase silica columns

#### Characteristic of bonded silica columns

Stability of bonded phases are largely limited by the silica support  
- mobile phase pH must be kept between 2-8.

Below pH 2 - hydrolysis of the bonded functional groups  
- decreasing retention.

Above pH 8, silica dissociates and the support starts to dissolve  
- creating voids in the packing material decreasing column efficiency.

# Chromatography

## Reverse-Phase Chromatography

### Mobile Phases - Solvent classification

Classification scheme developed by Snyder uses a triangle - the apexes represent solvent properties such as proton donor, proton acceptor, dipole interaction. There are eight groups of solvents.

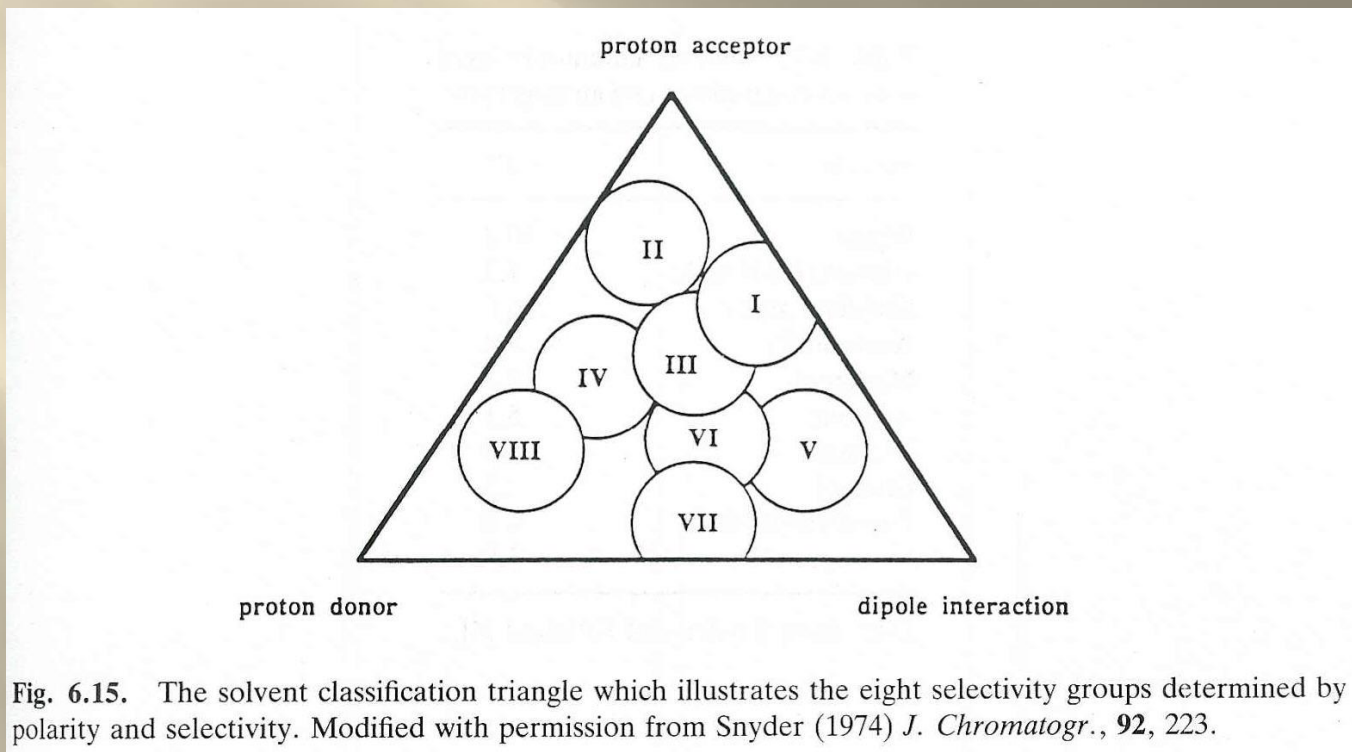


Fig. 6.15. The solvent classification triangle which illustrates the eight selectivity groups determined by polarity and selectivity. Modified with permission from Snyder (1974) *J. Chromatogr.*, **92**, 223.



# Chromatography

## Reverse-Phase Chromatography

### Mobile Phases - Solvent classification

**Table 6.6** Classification of solvents according to their groups.

Group	Solvents
I	Aliphatic ethers, hexamethylphosphoric acid amide, trialkyl amines
II	Aliphatic alcohols, methanol
III	Pyrene derivatives, amides, glycol ethers, sulfoxides, tetrahydrofuran
IV	Glycols, benzyl alcohol, acetic acid, formamide
V	Methylene chloride, ethylene chloride
VI	Tricresyl phosphate, aliphatic ketones and esters, polyethers, dioxane, sulfones, nitriles, polypropylene carbonate, acetonitrile
VII	Aromatic hydrocarbons, nitro compounds, aromatic ethers
VIII	Fluoroalkanols, <i>m</i> -cresol, chloroform, water

Data from Snyder [17].



# Chromatography

## Reverse-Phase Chromatography

### Mobile Phases - Solvent classification

Example:

Group VIII - good proton donors and interact preferentially with basic solutes (i.e. amines or sulfoxides).

Group I - good proton acceptor and interact preferentially with hydroxylated solutes (i.e. acids or phenols).

Group V - dipole interactions - interact preferentially with solutes that have a large dipole moment i.e. nitriles and amines.

# Chromatography

## Reverse-Phase Chromatography

**Mobile Phases - Solvent strength**

**Expressed as a function of polarity.**

**Solvent polarity parameter –  $P'$**

**Lower the value of  $P'$  - stronger the solvent.**

# Chromatography

## Reverse-Phase Chromatography

### Mobile Phases - Solvent strength

The eluotropic series are common solvents placed in order of relative chromatographic polarity.

Water is generally used as the base solvent and mobile phase strength determined by mixing water with another solvent (organic modifier).



# Chromatography

## Reverse-Phase Chromatography

### Mobile Phases - Solvent strength

Example:

50:50 H<sub>2</sub>O/CH<sub>3</sub>OH weaker mobile phase than  
50:50 H<sub>2</sub>O/tetrahydrofuran.

Generally solute retention decreases by a factor of 2 for every 10% volume addition of solvent to water.

Example:

50:50 H <sub>2</sub> O/ CH <sub>3</sub> OH	nitrobenzene	$t_r = 2$ min
45:55 H <sub>2</sub> O/ CH <sub>3</sub> OH	nitrobenzene	$t_r = 1$ min
55:45 H <sub>2</sub> O/ CH <sub>3</sub> OH	nitrobenzene	$t_r = 4$ min

# Chromatography

## Reverse-Phase Chromatography

### Mobile Phases - Solvent strength

Methanol is most widely used organic modifier since it has low u.v. cut-off (205 nm), reasonable eluting strength and is inexpensive.

Acetonitrile has a lower u.v. cut-off (190 nm), better mass transfer properties, more expensive.

Tetrahydrofuran next most used.

To find optimal mobile phase strength start with solvent-rich mobile phase (more organic modifier) and decrease organic modifier content until desired retention is obtained.



# Chromatography

**Table 6.7** Solvents commonly used with reversed-phase chromatography.

Solvent	$P'$
Water	10.2
Dimethyl sulfoxide	7.2
Ethylene glycol	6.9
Acetonitrile	5.8
Methanol	5.1
Acetone	5.1
Dioxane	4.8
Ethanol	4.3
Tetrahydrofuran	4.0
Isopropanol	3.9

Data from Snyder and Kirkland [4].



# Chromatography

## Reverse-Phase Chromatography

### Mobile Phases - Solvent selectivity

Once a binary mixture of a desired strength is found - selectivity can be altered by changing the organic modifier - while still maintaining a constant eluotropic strength.

Example: 3 mobile phases: 30:70 CH<sub>3</sub>OH/H<sub>2</sub>O or 22:78 ACN/H<sub>2</sub>O or 16:84 THF/H<sub>2</sub>O -all give equal retention but different selectivity.

There are many different equations to calculate this but a simple rule to remember is:

$$\phi_{\text{ACN}} = 0.77 \phi_{\text{CH}_3\text{OH}}$$

$$\phi_{\text{THF}} = 0.66 \phi_{\text{CH}_3\text{OH}}$$

$\phi_{\text{M}}$  – represents the mobile phase volume fraction.

# Chromatography

## Reverse-Phase Chromatography

### Mobile Phases - Solvent selectivity

Once the best selectivity is found, the solvent strength is fine tuned by varying the percent of organic modifier.

**Table 6.8** Relative strengths of binary water:organic modifier mixtures.

Methanol (%)	Acetonitrile (%)	Tetrahydrofuran (%)
30	22	16
40	32	23
50	40	30
60	50	36
70	60	43
80	73	52
90	87	62

# Chromatography

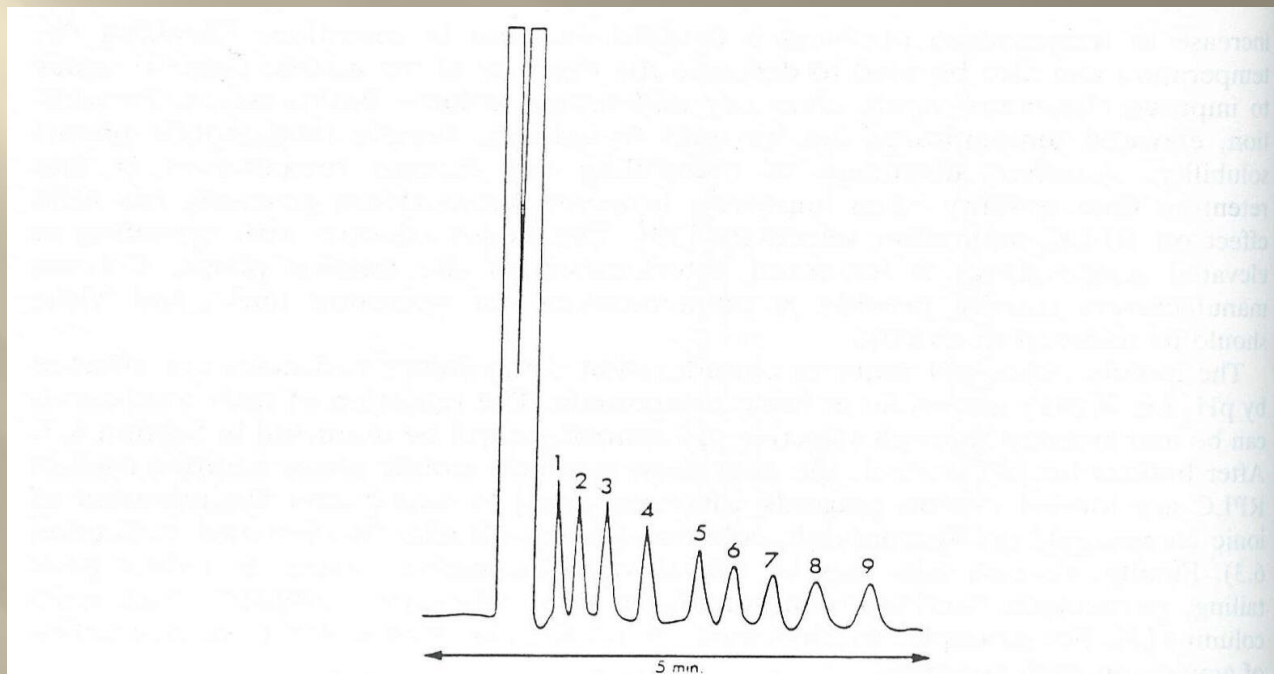
## Reverse-Phase Chromatography

### Applications

With RPLC best separations are of neutral solutes that are soluble in water or other relatively polar solvents with molecule weights less than 2000-3000.

Hydrocarbons was the first group separated with reverse phase.

Non-polar solutes or fatty acids resolved on the basis of their carbon chain length.



**Fig. 6.18.** Separation of fatty acids by reversed phase chromatography. A Waters Free Fatty Acid Analysis Column was used with a mobile phase of 45/20/35 ACN/THF/water at  $1.5 \text{ ml min}^{-1}$ . RI detection was used. Solute identities: 1, capric acid; 2, lauric acid; 3, myristic acid; 4, palmitic acid; 5, stearic acid; 6, nonadecanoic acid; 7, arachidic acid; 8, heneicosanoic acid; 9, behenic acid. Reprinted with permission from *Waters Sourcebook of Chromatography* (1992), Millipore Corporation, Milford.

# Chromatography

## Reverse-Phase Chromatography

### Applications

Largest HPLC reverse phase industry is pharmaceutical for drug analysis.

Clinic labs - analysis of plasma and urinary catecholamines with EC detection.

Also amino acids, proteins, peptides.

Analysis of food - beverage, agriculture, carbohydrates, food additives, aflatoxins, sweeteners.

Environmental samples - pesticides, pollutants

# Chromatography

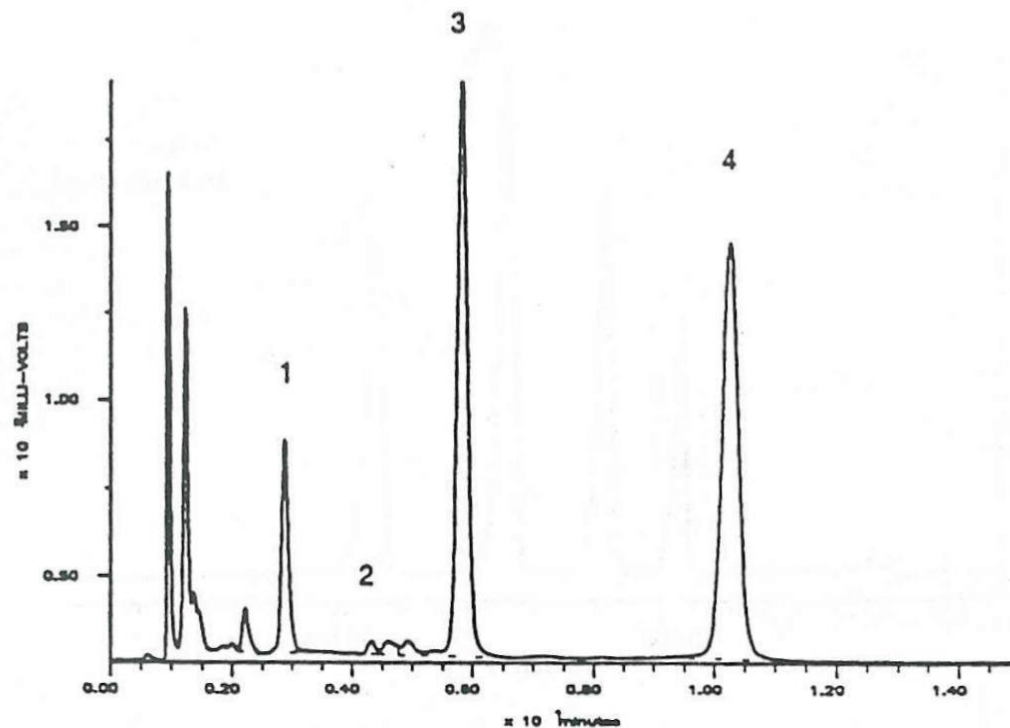


Fig. 6.19. Separation of catecholamines in urine by RPLC. A Waters Nova-Pak column was used with a mobile phase of 50 mM sodium acetate, 1 mM octane sulfonate, 0.1 mM EDTA in 96:4 water:MeOH. Amperometric detection was used. Solute identities: 1, noradrenaline; 2, adrenaline; 3, dihydroxybenzylamine; 4, dopamine. Chromatogram courtesy of Waters.



# Chromatography

## Basic Theory of HPLC

For HPLC, the separation is described by 4 major concepts:

Capacity, efficiency, selectivity and resolution

Capacity and selectivity of a column are controlled by the manufacturer.

Efficiency and resolution are controlled by the user.



# Chromatography

## Basic Theory of HPLC

### Capacity Factor

For effective LC separations, a column must have the capacity to retain samples.

Capacity factor –  $k'$  - of a column is a direct measure of the strength of the interaction of the solute with the packing material.

$$k' = \frac{t_r - t_m}{t_m} = \frac{V_r - V_m}{V_m}$$

$t_r$  – ret. time of the analyte

$t_m$  - ret. time of unretained solute (marker compounds NaI)

# Chromatography

## Basic Theory of HPLC

### Capacity Factor

Capacity factor of a column is mostly a function of the packing material but can be manipulated to some degree by varying the solvent strength.

Higher  $k'$  - the greater the ability of the column to retain solutes, longer analysis time.

$k'$ , values range 1-10

Usually  $k'$  between 2-5 represents a good balance between analysis time and resolution.

$k'$  - too low - solutes not resolved

$k'$  - too high - analysis time too long

# Chromatography

## Basic Theory of HPLC

### Selectivity

Selectivity or separation factor,  $\alpha$ , describes the separation of two peaks relative to each other.

$$\alpha = \frac{k_2'}{k_1'} = \frac{t_{r_2} - t_m}{t_{r_1} - t_m}$$

Selectivity is a function of packing material.

Value of  $\alpha$  ranges from unity (1) when  $t_{r_2} = t_{r_1}$ ,  $\alpha \geq 1$ .

Easy way to increase  $\alpha$  is to change the composition (components) of the mobile phase.

# Chromatography

## Basic Theory of HPLC

### Resolution

Resolution,  $R$  or  $R_s$ - degree of separation between neighboring solute peaks.  $R_s$  is affected by selectivity ( $\alpha$ ), efficiency ( $N$ ), and capacity ( $k'$ ) of the column.

$$R = \frac{1}{4} \frac{\alpha - 1}{\alpha} (N^{1/2}) \frac{k'}{1 + k'}$$

$$R_s = \frac{2(t_{r_2} - t_{r_1})}{wb_1 + wb_2}$$

# Chromatography

## Basic Theory of HPLC

### Resolution

When the two peaks are just resolved at the baseline  $R = 1.5$ .

Increasing efficiency (double theoretical plates, longer columns) only gain 1.4 in resolution since  $R \propto N^{1/2}$ .

Better to change selectivity,  $\alpha$ , by varying the mobile phase or capacity factor,  $k'$  by varying mobile phase strength.

# Chromatography

## Basic Theory of HPLC

**Efficiency – can be defined as “mechanical separation power”**

**Determined by the column length and the particle size**

**Efficiency (N) describes peak broadening as a function of retention, and is described in terms of number of theoretical plates. More efficient the column - the less band spreading.**

$$N = 16 \left( \frac{t_r'}{W} \right)^2$$

$$N = 5.54 \left( \frac{t_r'}{W_{1/2}} \right)^2$$

**Typical ODS-C18 column ~ 12.5 cm long and 5 um packing  
N ≈ 6250    H = 0.02 mm**

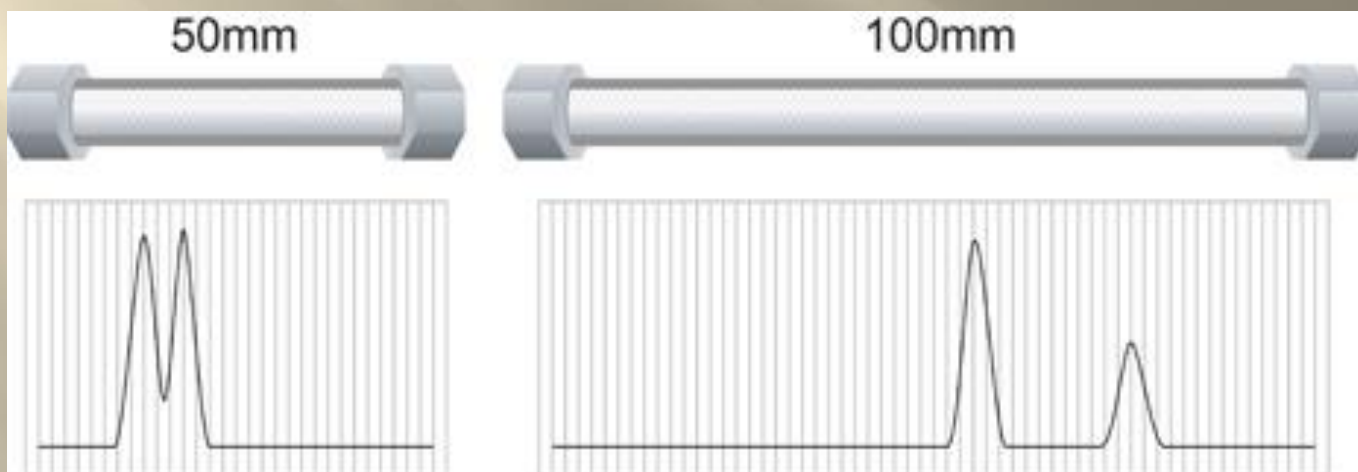


# Chromatography

## Basic Theory of HPLC

### Efficiency

For a given particle size, more mechanical separation power is gained by increasing column length. However, the trade-offs are longer chromatographic run times, greater solvent consumption, and higher backpressure. Shorter column lengths minimize all these variables but also reduce mechanical separation power.

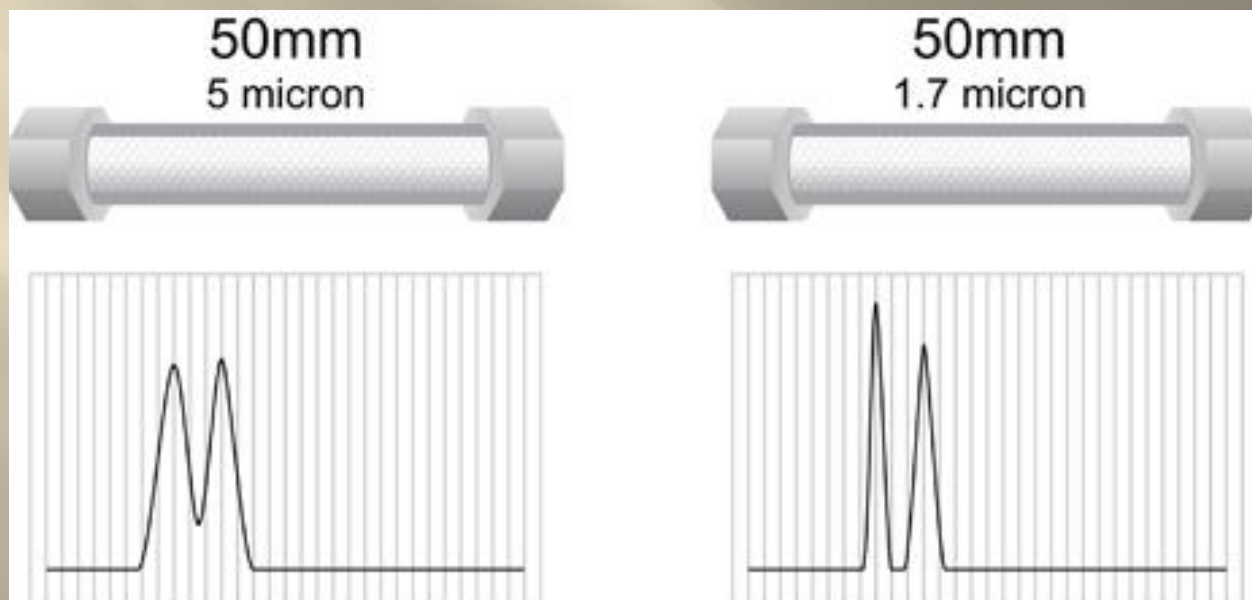


# Chromatography

## Basic Theory of HPLC

### Efficiency

A column of the same length and i.d., but with a smaller particle size, will deliver more mechanical separation power in the same time. However, its backpressure will be much higher.



# Chromatography

## Basic Theory of HPLC

### Fundamental Resolution Equation

$$R_s = \frac{\sqrt{N}}{4} \frac{(\alpha - 1)}{\alpha} \frac{k}{(k + 1)}$$

$\alpha$  = Selectivity (influenced by mobile and stationary phase)

$N$  = Column Efficiency (influenced by length and particle size)

$k$  = Capacity Factor (retention) (influenced by stationary and mobile phase)

# Assignment

