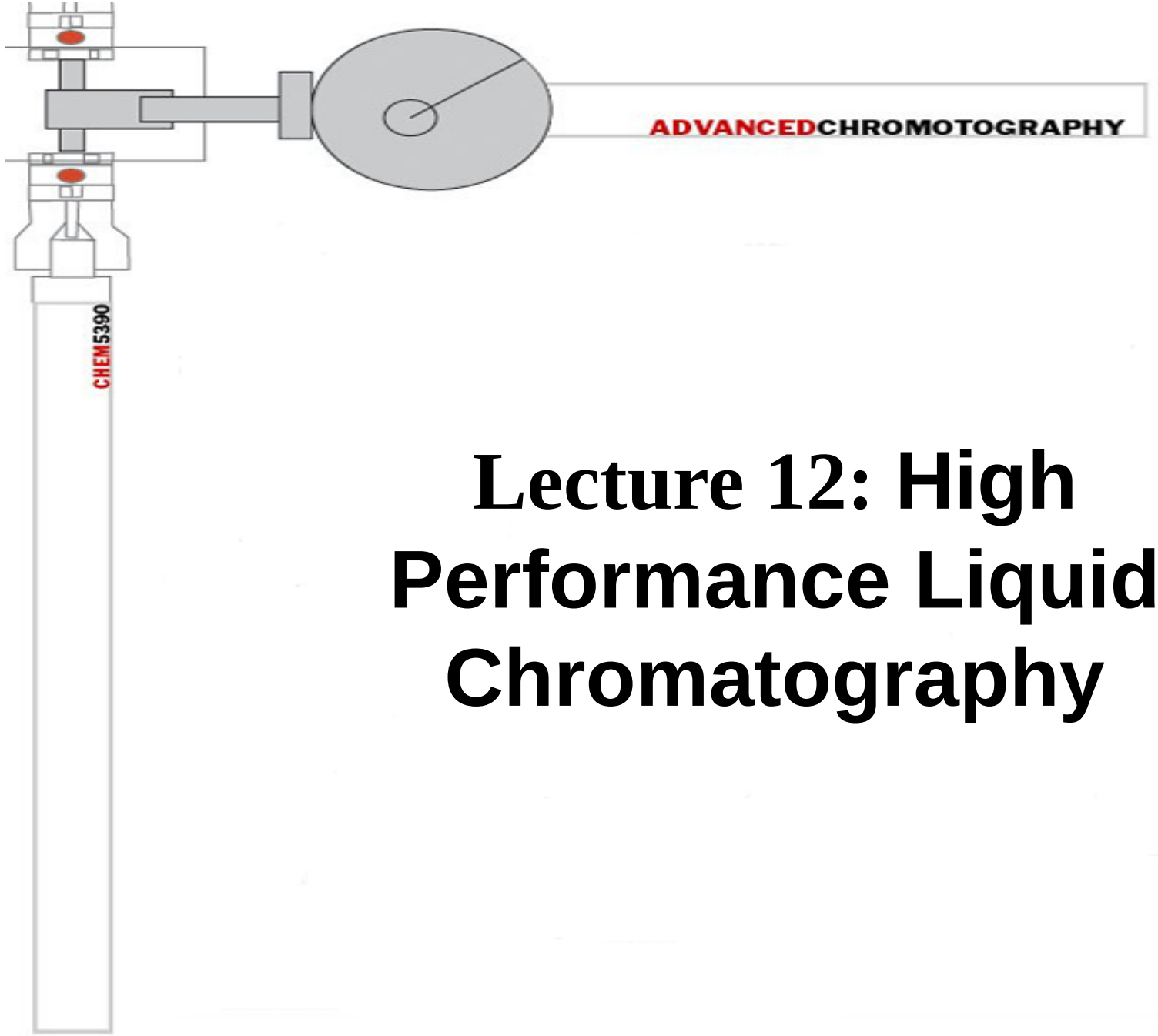
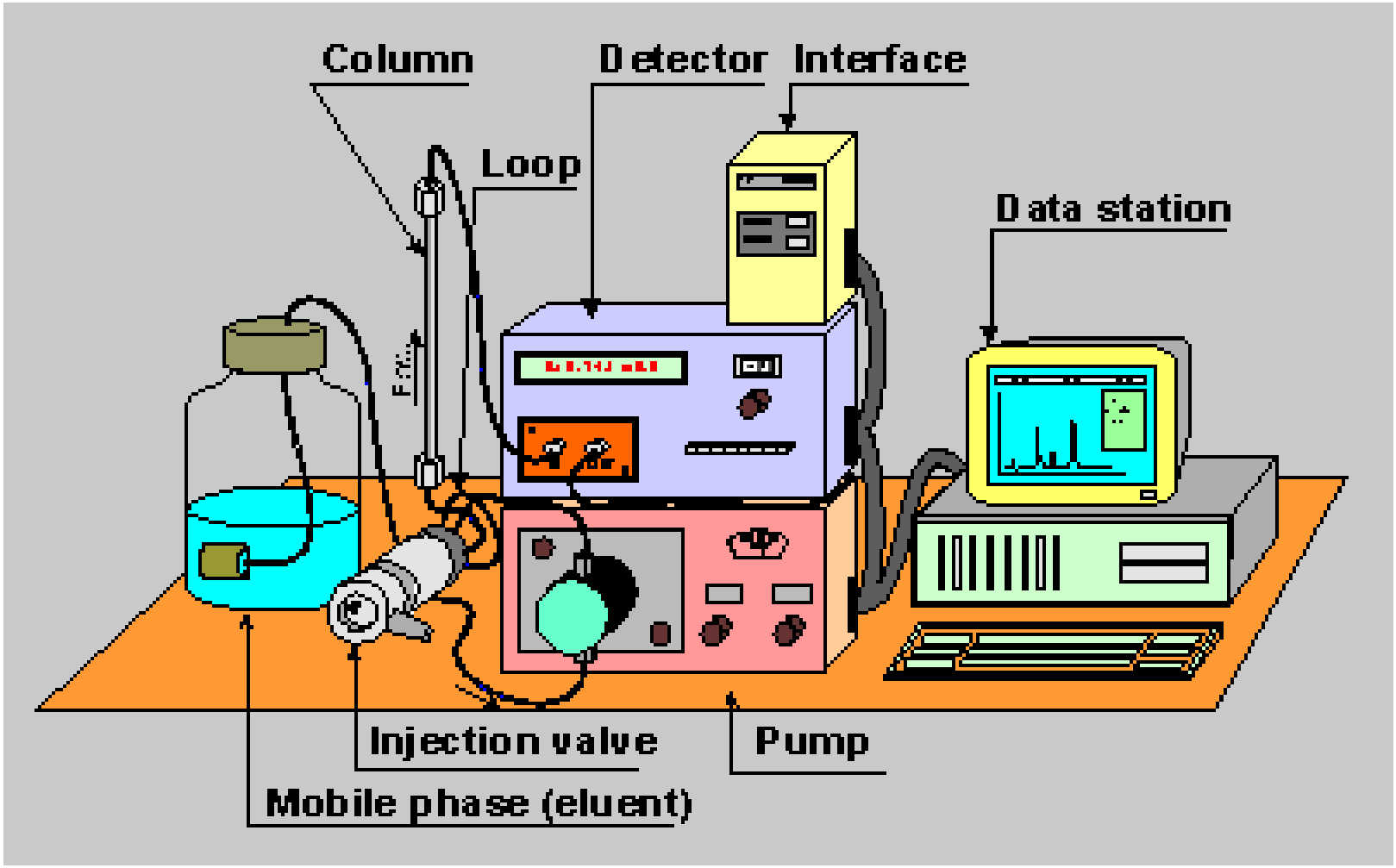
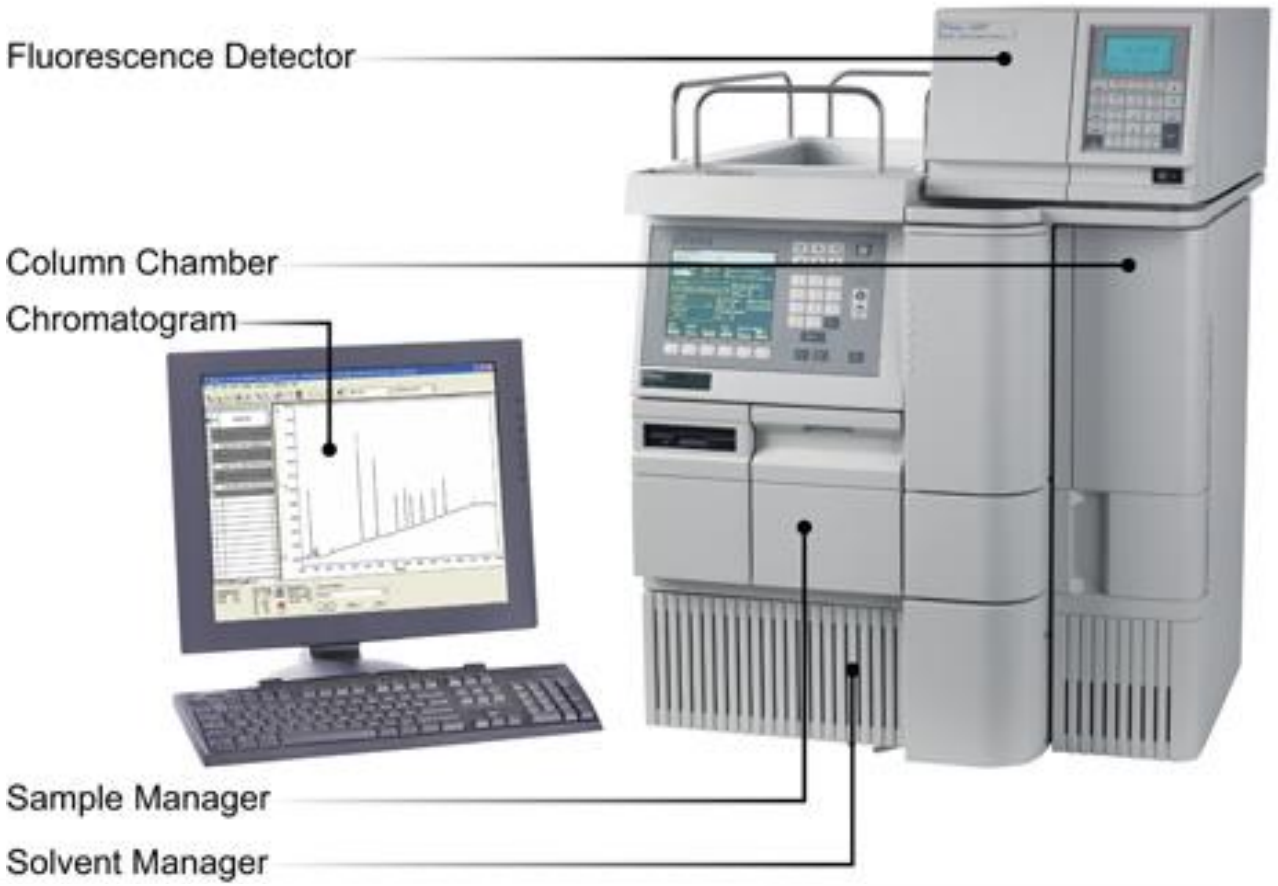


Lecture 12: High Performance Liquid Chromatography

Instrumentation



Instrumentation



History

1938 : REICHSTEIN proposes a theory for the elution and separation of solutes on a column

1952 : application of gradient elution

1967 : beginning of HPLC after the works of HUBER and HUZSMAN, this technique was first named « High Speed Liquid Chromatography » then « High Pressure Liquid Chromatography » and finally « High Performance Liquid Chromatography »

1969 : after the 5th International Symposium International « Advances in Chromatography » the development of HPLC was very fast

HPLC can be combined with MS like GC

CHEM5390

Theory

There are several modes in high performance liquid chromatography - classified according to the mechanism of separation

Each mode corresponds to a given kind of *interaction*:

- ***surface adsorption – 'normal'***
- ***solvent partitioning – 'reverse'***
- ***ion exchange***
- ***size exclusion***
- ***affinity***

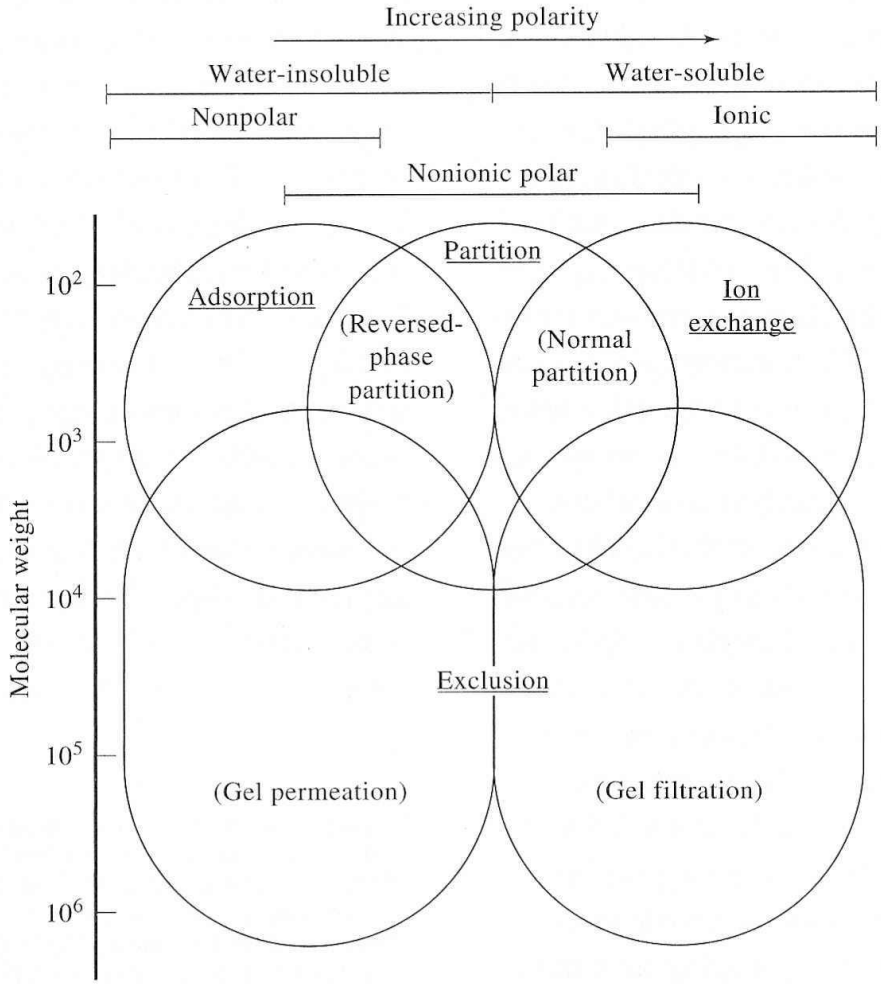
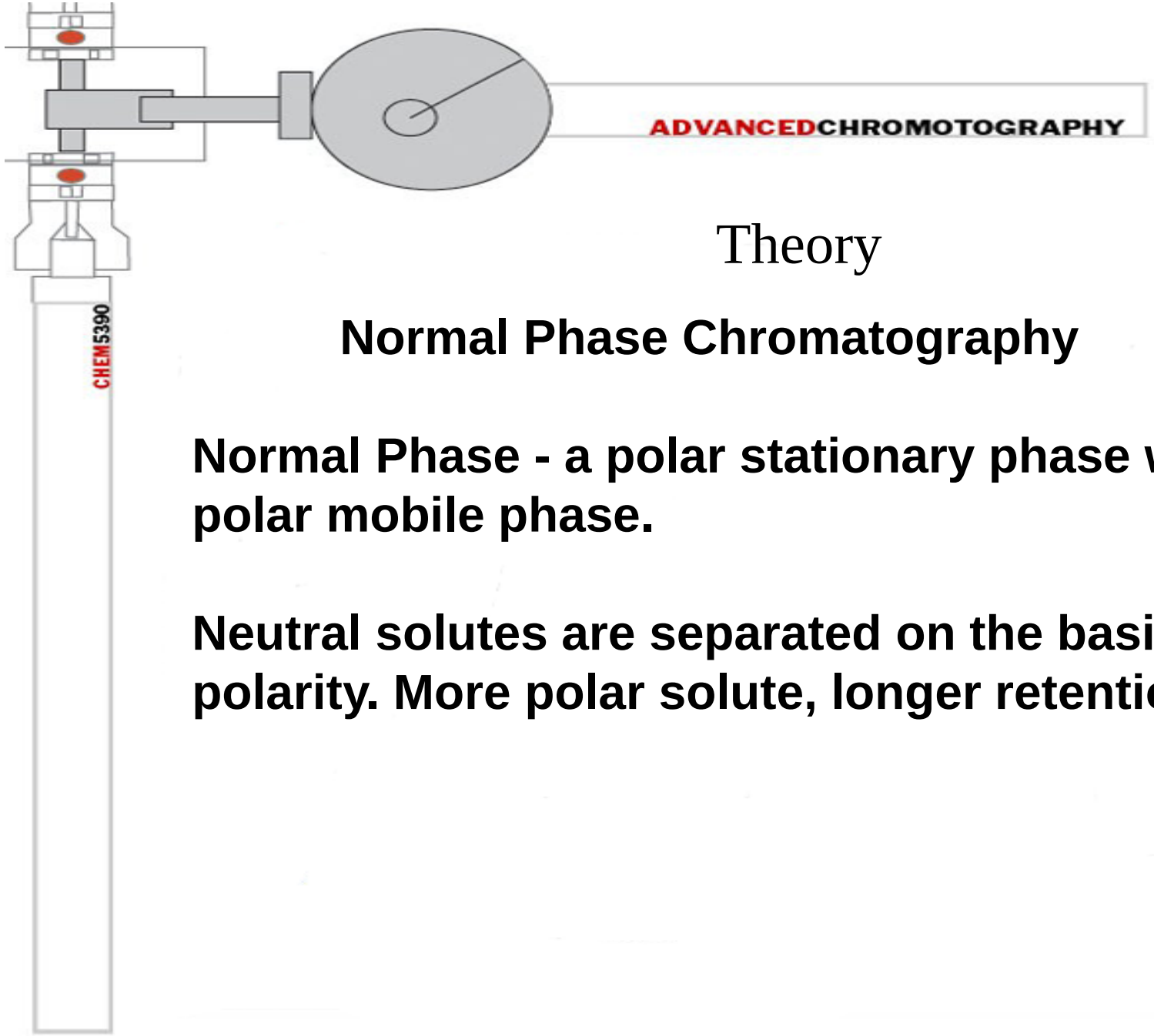


Figure 28-1 Applications of liquid chromatography. *(From D. L. Saunders, in* Chromatography, *3rd ed., E. Heftmann, Ed., p. 81. New York: Van Nostrand Reinhold, 1975. With permission.)*

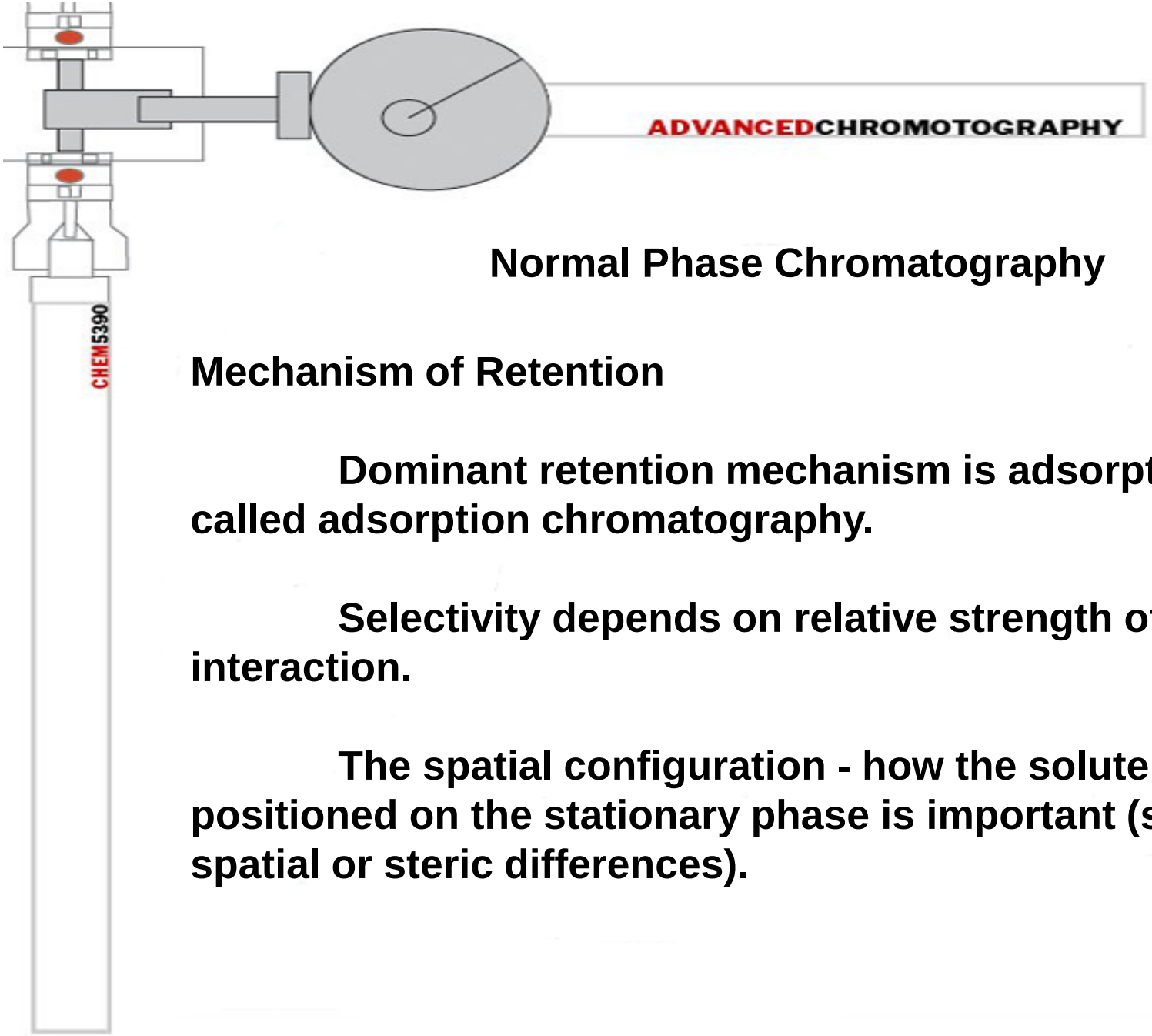


Theory

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.



Normal Phase Chromatography

Mechanism of Retention

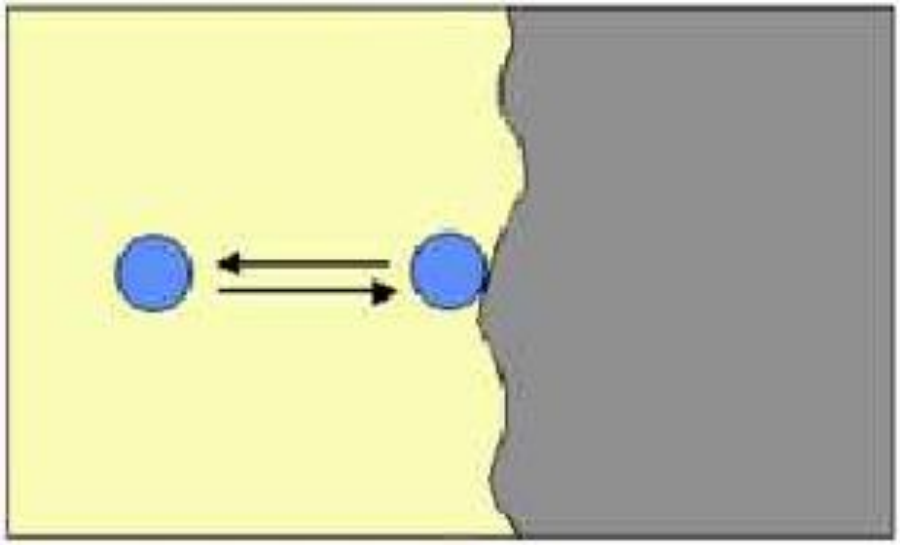
Dominant retention mechanism is adsorption. Also called adsorption chromatography.

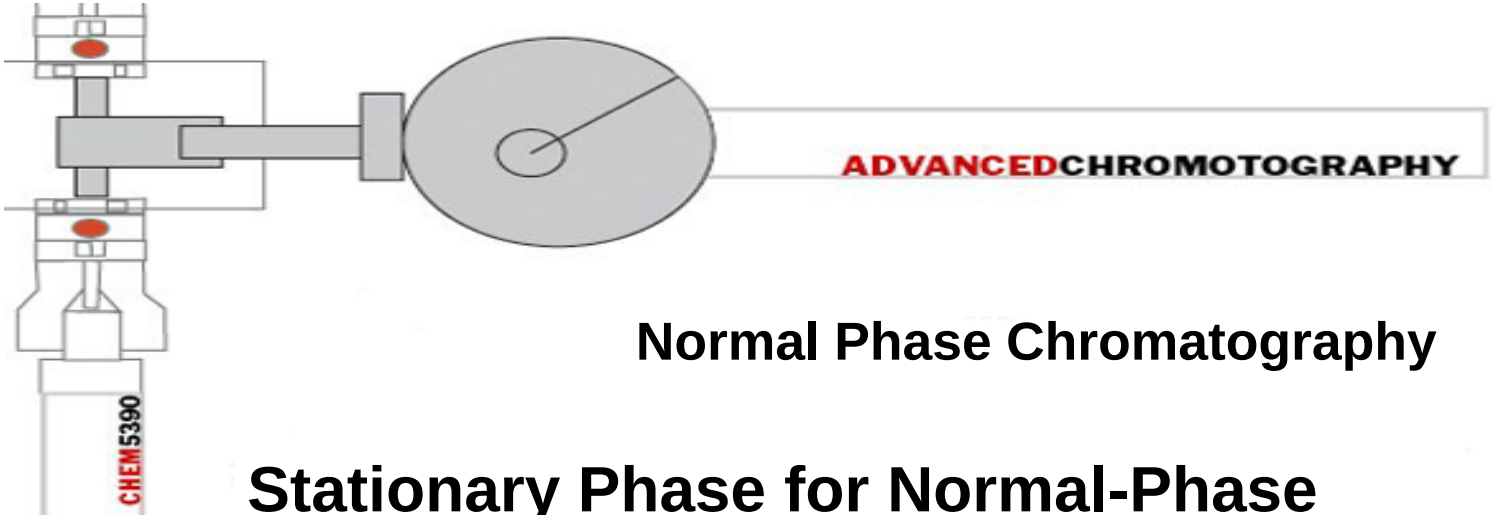
Selectivity depends on relative strength of the polar interaction.

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

Normal Phase Chromatography

- *the stationary phase is a solid adsorbant*
- *retention is due to a series of adsorption / desorption steps*
- *separation is based mainly on differences between the adsorption affinities of the sample components for the surface of the active solid (liquid solid chromatography)*



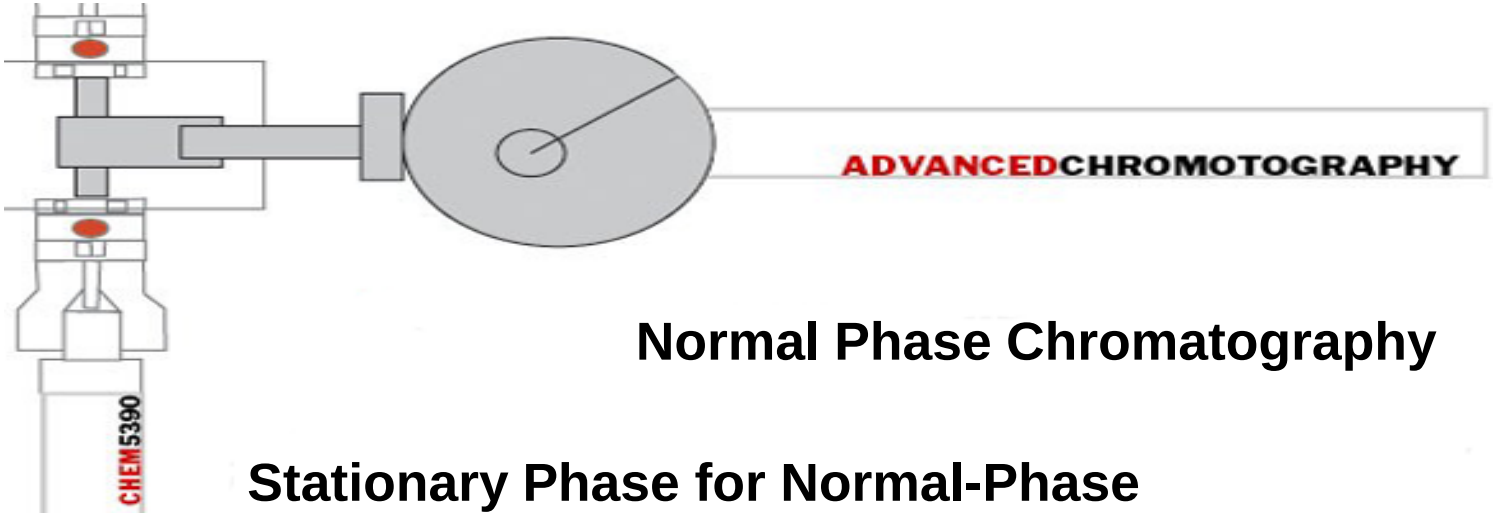


Normal Phase Chromatography

Stationary Phase for Normal-Phase

Wide range of stationary phase materials have been used:

sucrose	**cellulose**
starch	**silica gel**
fluorisil	**charcoal**
magnesium oxide	**alumina**



Normal Phase Chromatography

Stationary Phase for Normal-Phase

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

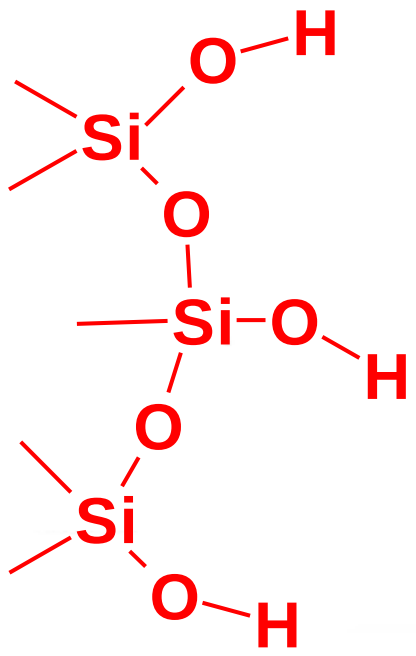
Silica is preferred - available, low cost, allows higher sample loadings, less likely to catalyze decomposition of sample components.

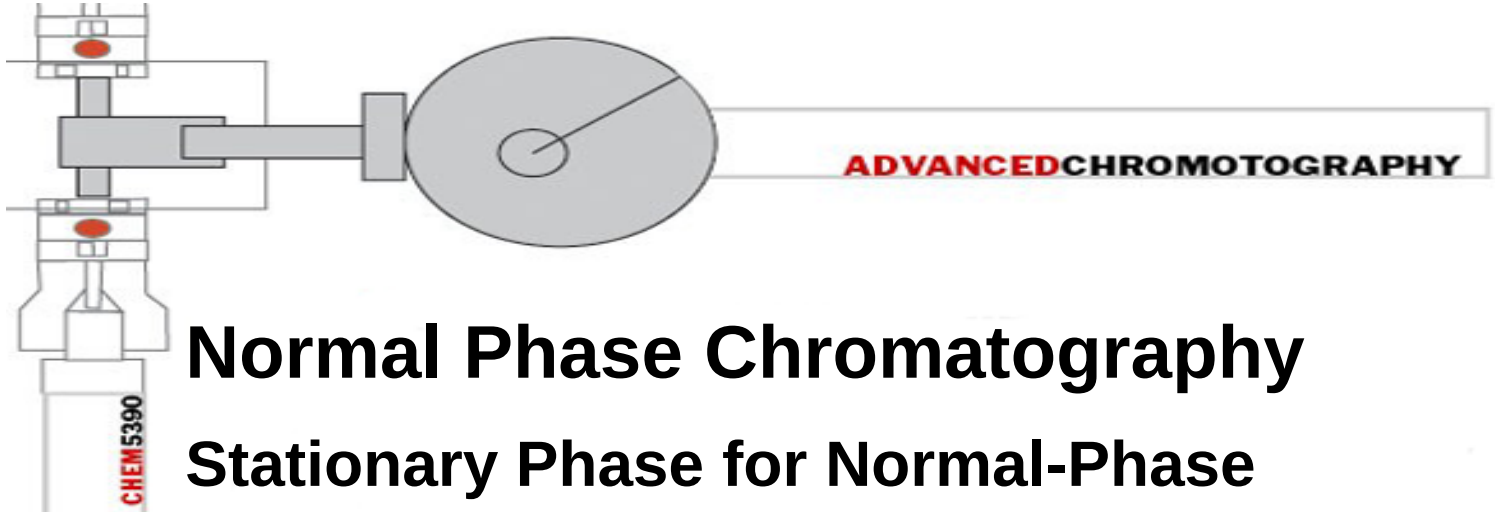
Alumina is used for basic compounds (i.e. amines) which are strongly retained on silica.

In general, both retain more polar compounds strongly.

Normal Phase Chromatography

- ***both* solute and solvent can be attracted to the active sites at the surface of the stationary phase**
- ***the molecules are retained by the interaction of their polar functional groups with the surface functional groups such as silanols of silica***

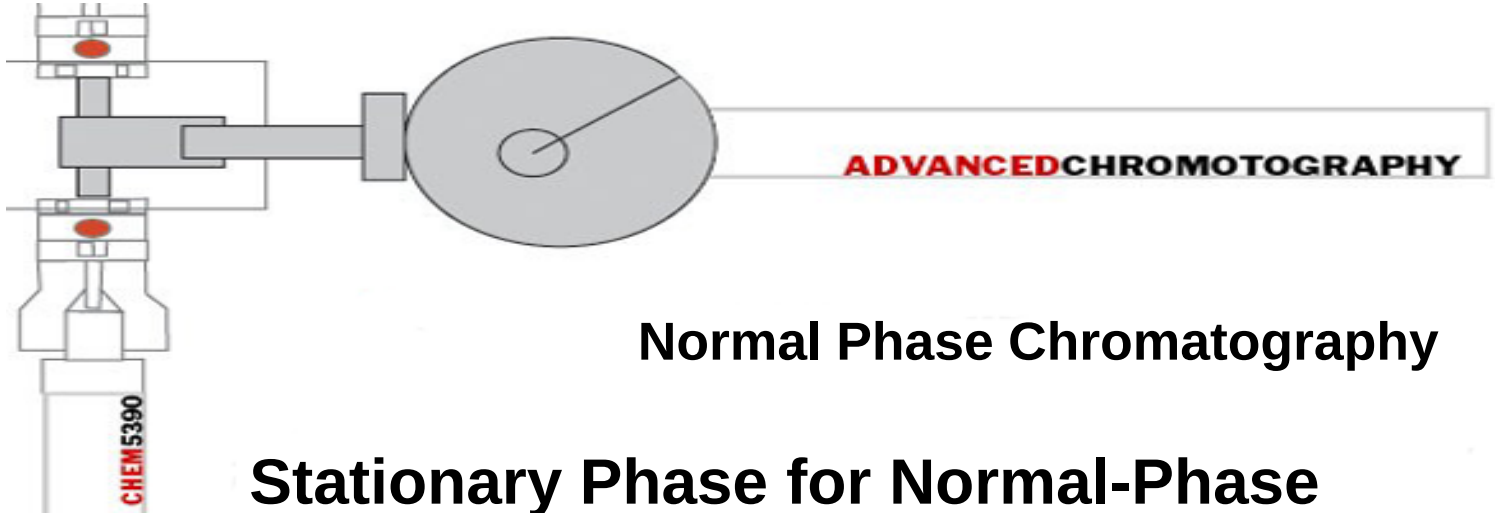




Normal Phase Chromatography

Stationary Phase for Normal-Phase

Order of elution: saturated hydrocarbons < alkenes (olefins) < aromatic hydrocarbons ≈ organic halides < sulfides < ethers (ROR) < nitro compounds < esters (RCO_2R) ≈ aldehydes ≈ ketones < alcohols ≈ amines < sulfones < sulfoxides < amides < carboxylic acids (RCO_2H).



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.

Normal Phase Chromatography

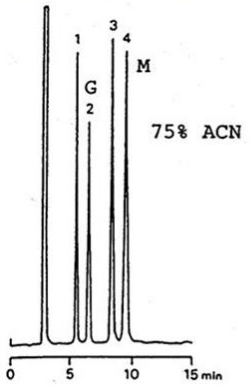
Silica gel type	**: general use**
Cyano type	**: general use**
Amino type	**: for sugar analysis**
Diol type	**: for protein analysis**

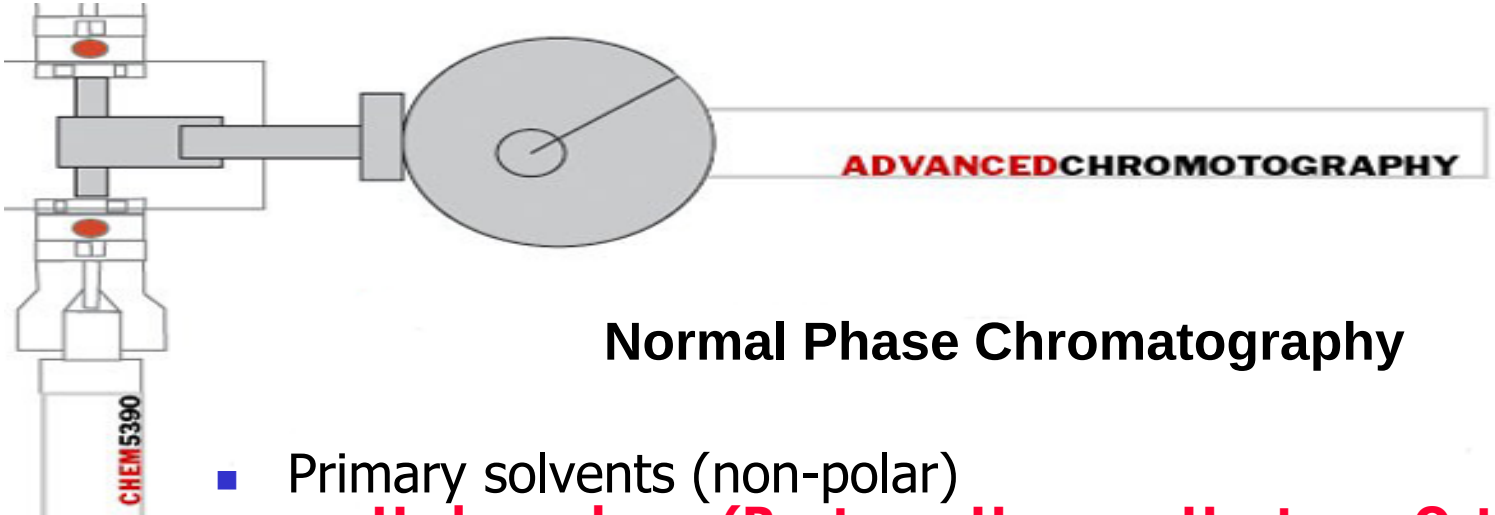
Separation of Very Hydrophilic Molecules

Just as there is non-aqueous reversed-phase chromatography there are also aqueous mobile phases used in normal phase chromatography. In this case, very hydrophyllic samples that are not retained in reversed phase conditions can be chromatographed. Carbohydrates are often separated on an amino column with mobile phases consisting of 60-80% acetonitrile/water.

Normal phase separation of carbohydrates using an amino column and 75% acetonitrile-water as the mobile phase.

1 = fructose
2 = glucose
3 = sucrose
4 = maltose





Normal Phase Chromatography

- Primary solvents (non-polar)
  - **Hydrocarbons (Pentane, Hexane, Heptane, Octane)**
  - **Aromatic Hydrocarbons (Benzene, Toluene, Xylene)**
  - **Methylene chloride**
  - **Chloroform**
  - **Carbon tetrachloride**
- Secondary solvents
  - **Methyl-t-butyl ether (MTBE), Diethyl ether, Tetrahydrofuran (THF), Dioxane, Pyridine, Ethyl acetate, Acetonitrile, Acetone, 2-propaol, ethanol, methanol**
- **A primary solvent is used as mobile phase. Addition of secondary solvents is to adjust retention time.**

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.

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 (ε^o).

Normal Phase Chromatography

Mobile phase - solvent strength

Index of solvent strength is given by the experimental adsorption solvent strength parameter, ε°.

ε° - A measure of adsorption energy per unit area of solvent.
A listing of these values is called an eluotropic series.

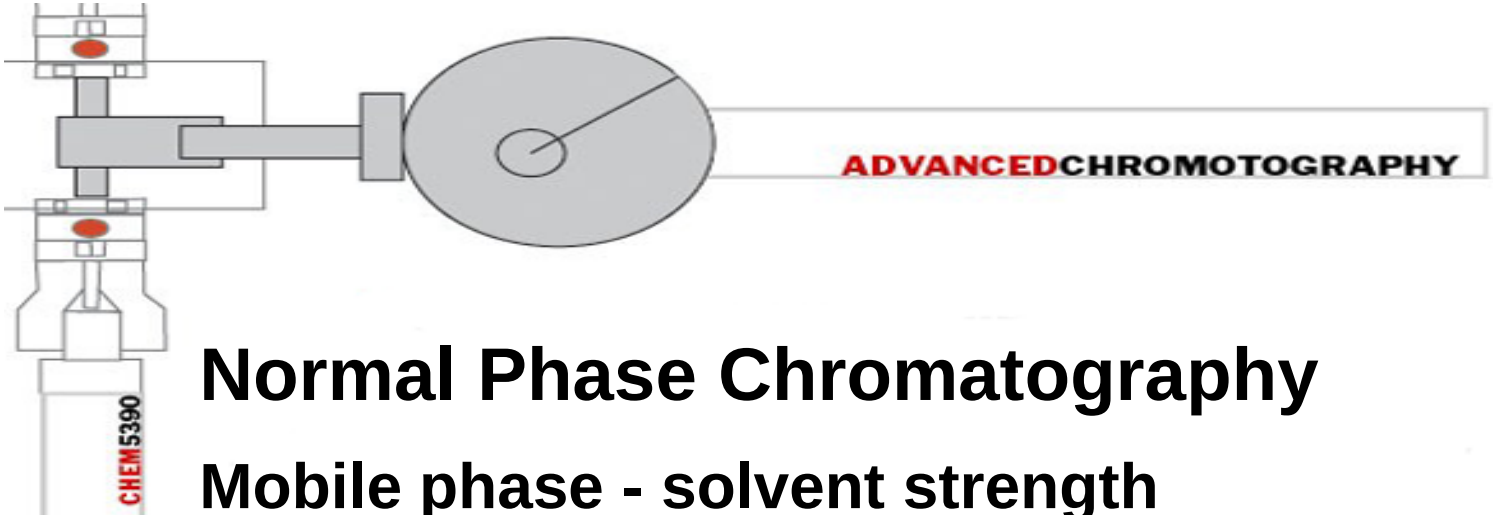
Range from –0.25 to +1.2.

An increase in ε°- means a stronger solvent giving lower retention of a solute. Solvents with high ε° values are strong solvents, and will more easily elute the more polar analytes.

Normal Phase Chromatography

Mobile phase - solvent strength

Solvent	Strength ε^o	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

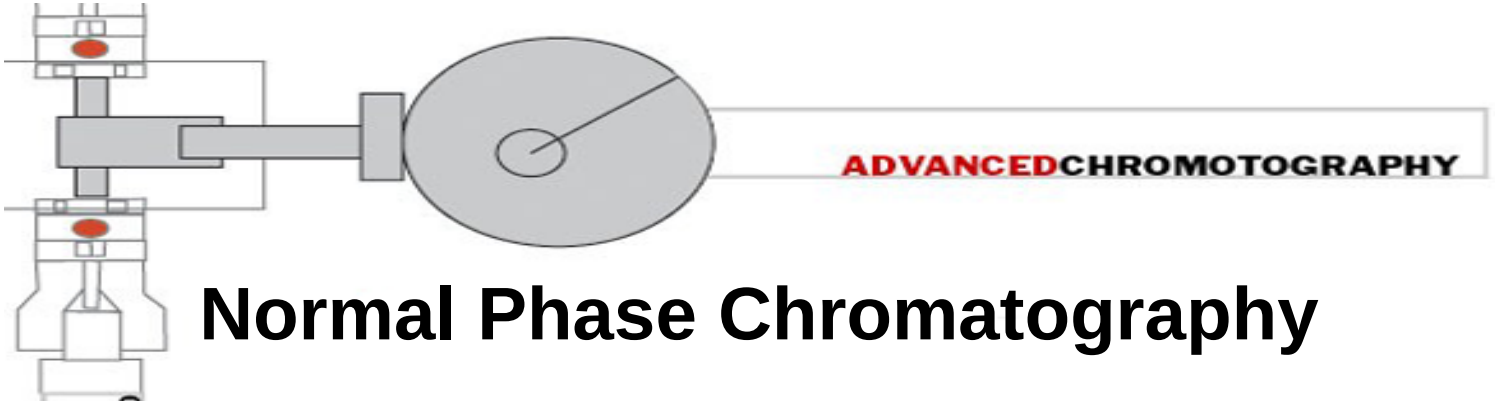


Normal Phase Chromatography

Mobile phase - solvent strength

Sovent	Solvent strength, ε°	
	Silica	Alumina
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 ε° and add solvent with greater ε° until desired separation occurs.



Normal Phase Chromatography

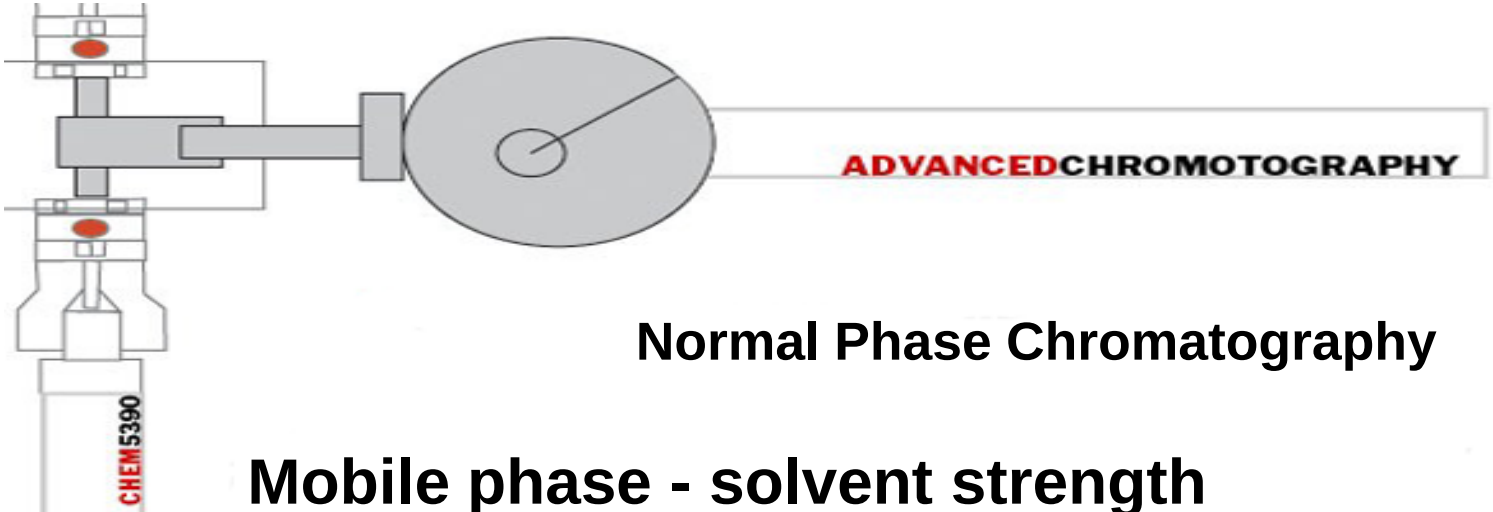
Mobile phase - solvent strength

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.



Normal Phase Chromatography

Mobile phase - solvent strength

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

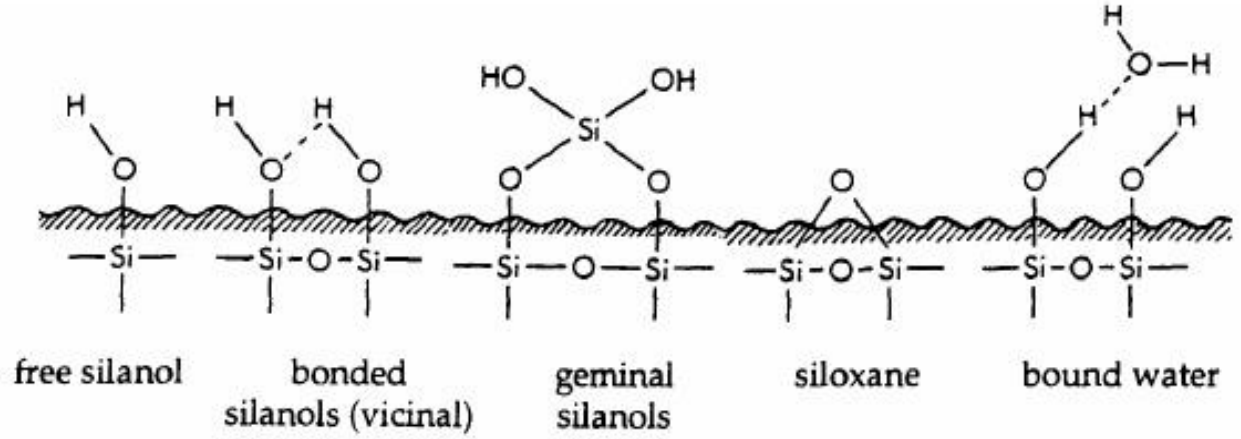
Water adsorbs onto the strongest adsorption sites - leaving weaker sites open to retain the sample – decreasing solute retention.

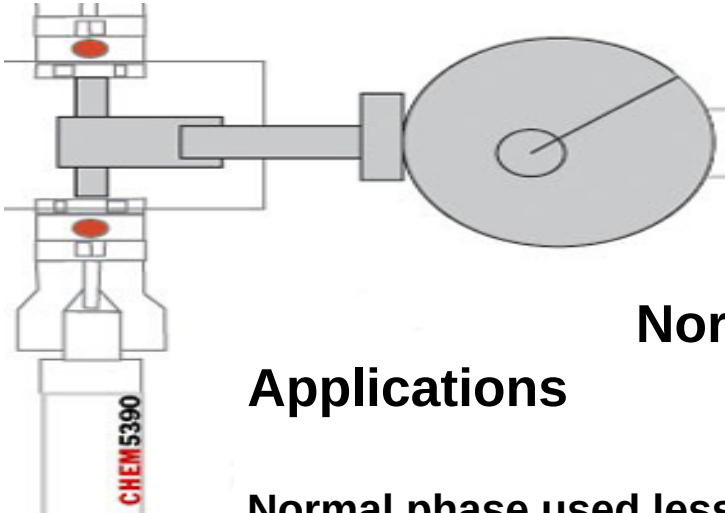
Normal Phase Chromatography

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.





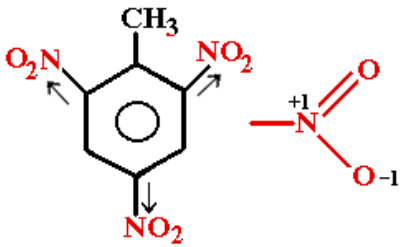
Normal Phase Chromatography

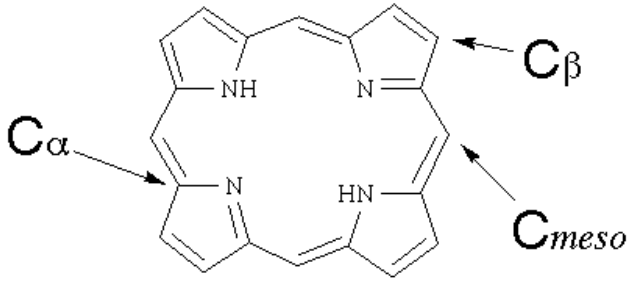
Applications

Normal phase used less than other HPLC techniques.

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

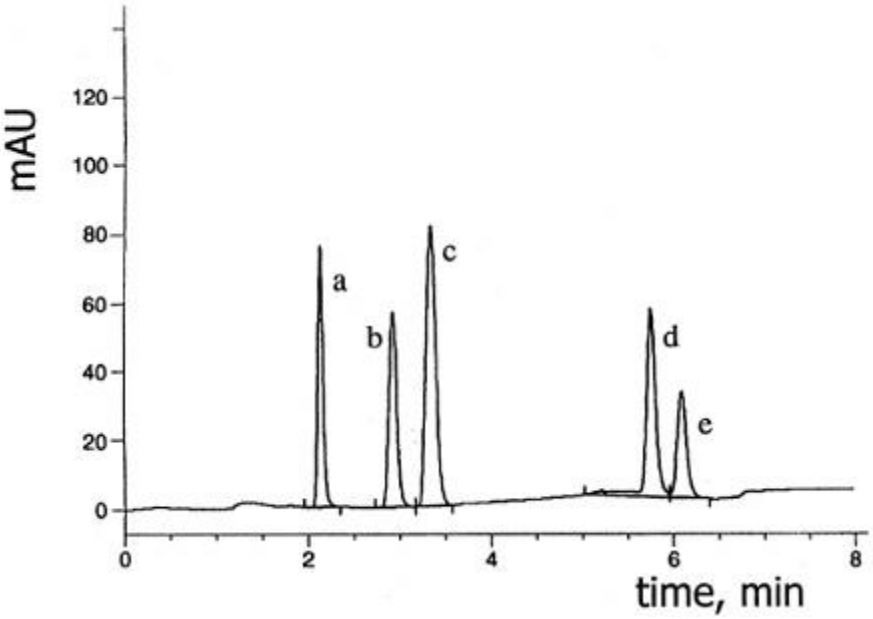
Examples: fat & water – soluble vitamins
Less-polar plant pigments –porphyrins
Pesticides- still better with GC
Separation of isomers (6 isomers of trinitrotoluene)





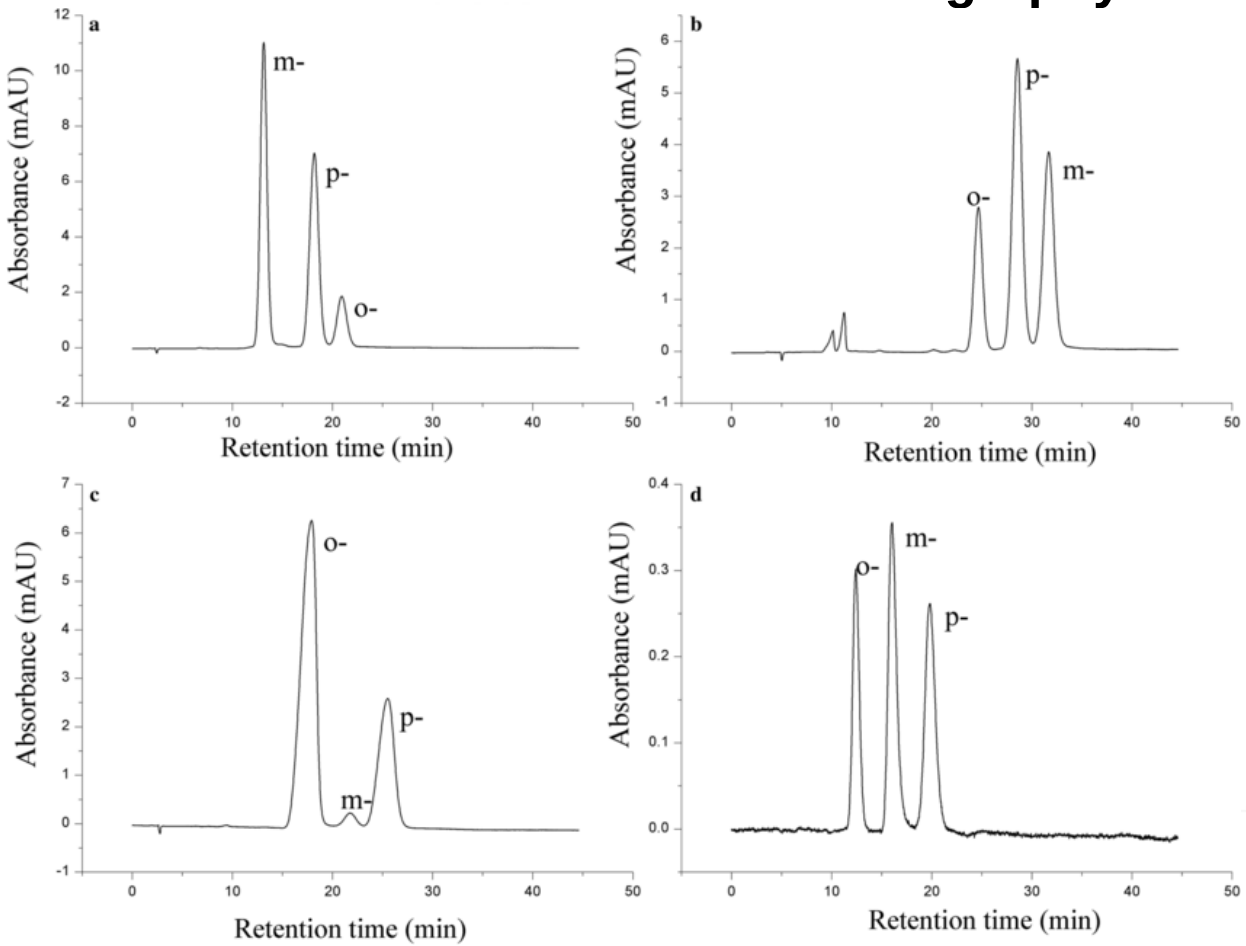
Normal Phase Chromatography

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.

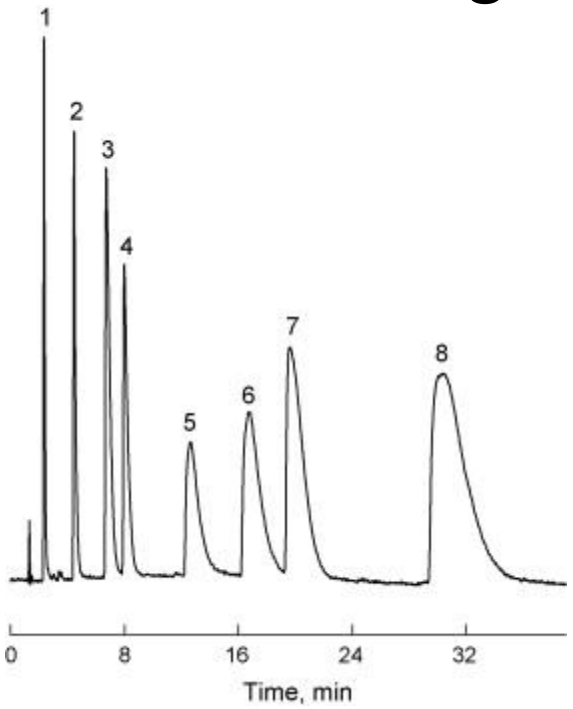
Normal Phase Chromatography



Separation of positional isomers. a Nitroaniline, b bromoaniline, c nitrobromobenzene, d dihydroxybenzene. Mobile phase: water/methanol (80:20 v/v). (o-, m-, p- represent ortho, meta and para of the isomers)

Normal Phase Chromatography

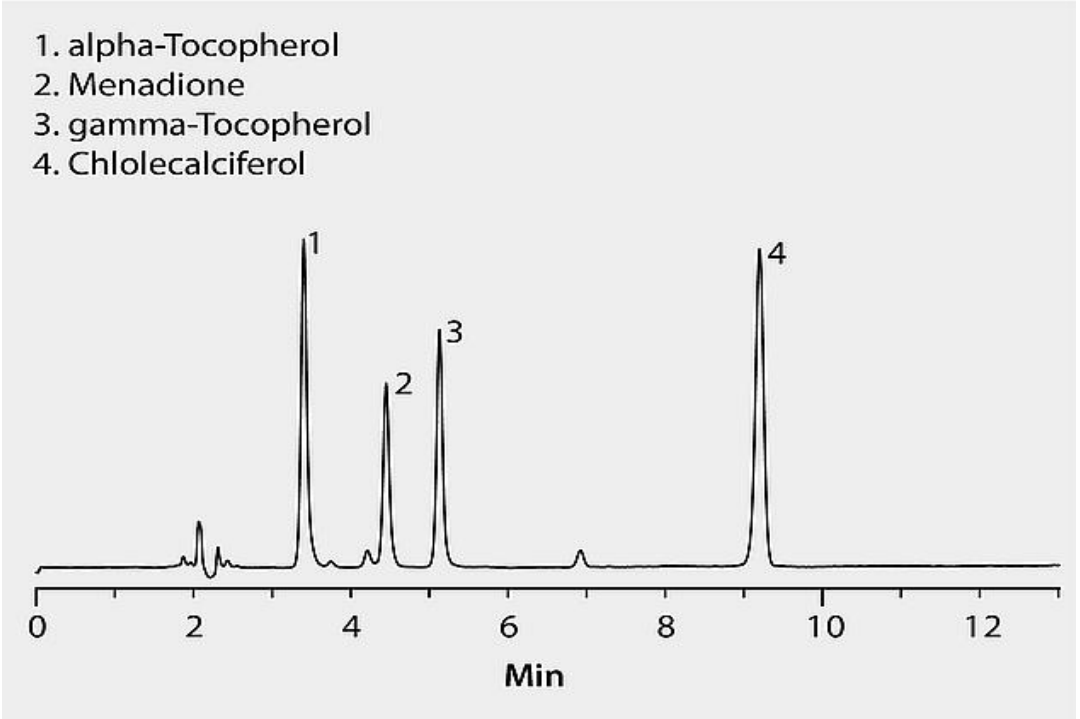
Applications



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-amylbenzene, (8) n-nonylbenzene. Eluent n-pentane.

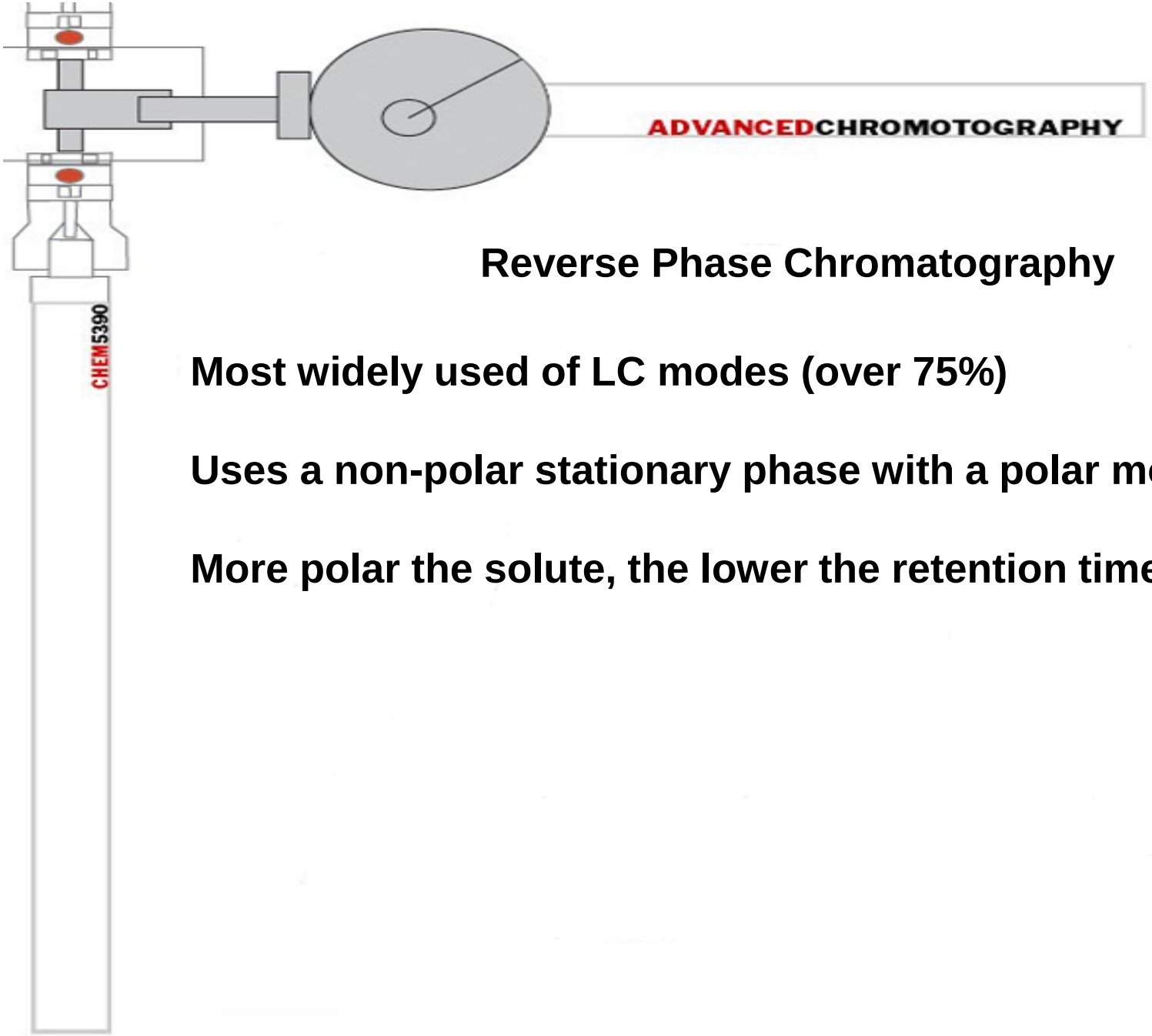
Normal Phase Chromatography

Applications



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 µm 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 µL.



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.

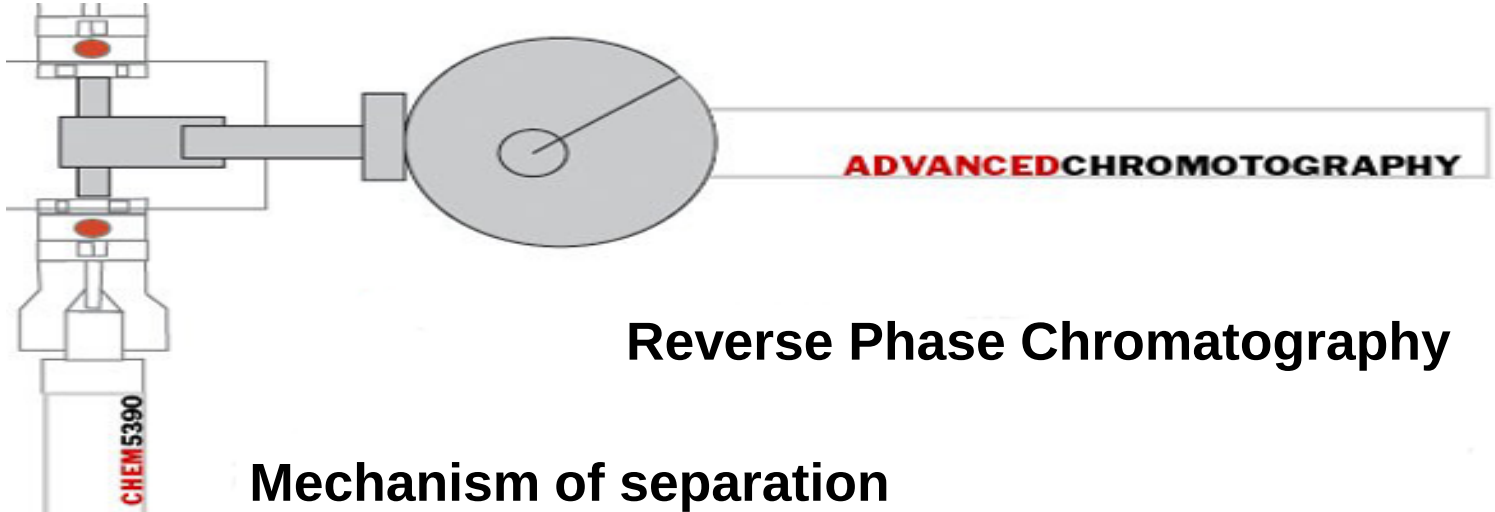
Reverse Phase Chromatography

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.



Reverse Phase Chromatography

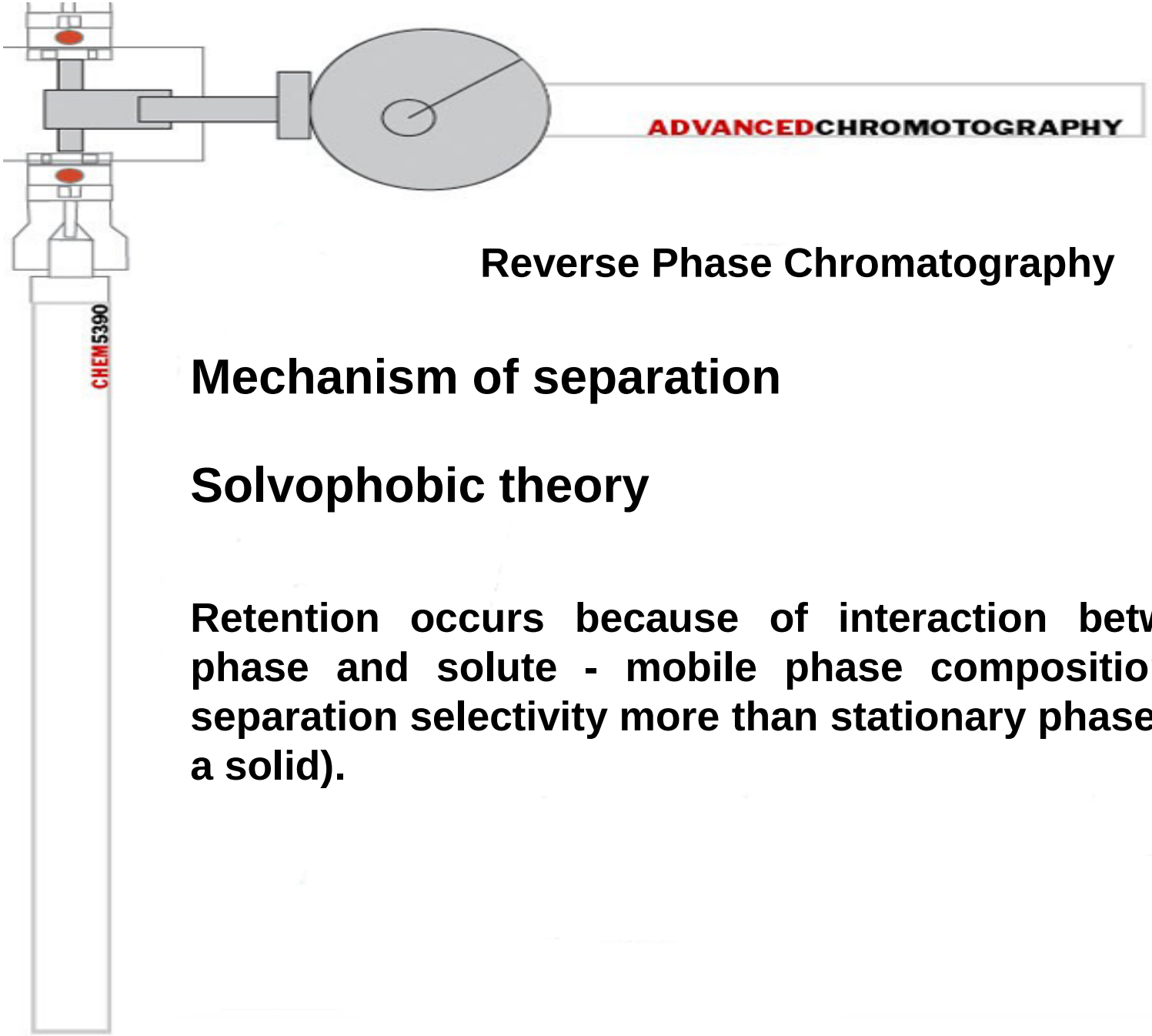
Mechanism of separation

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)

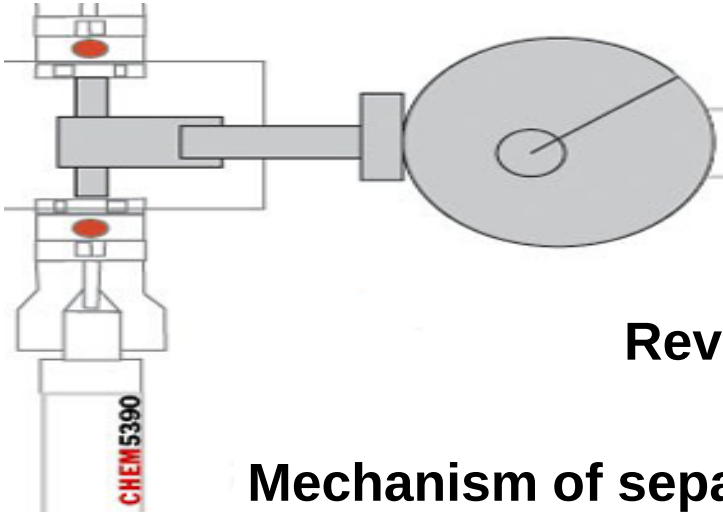


Reverse Phase Chromatography

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).



Reverse Phase Chromatography

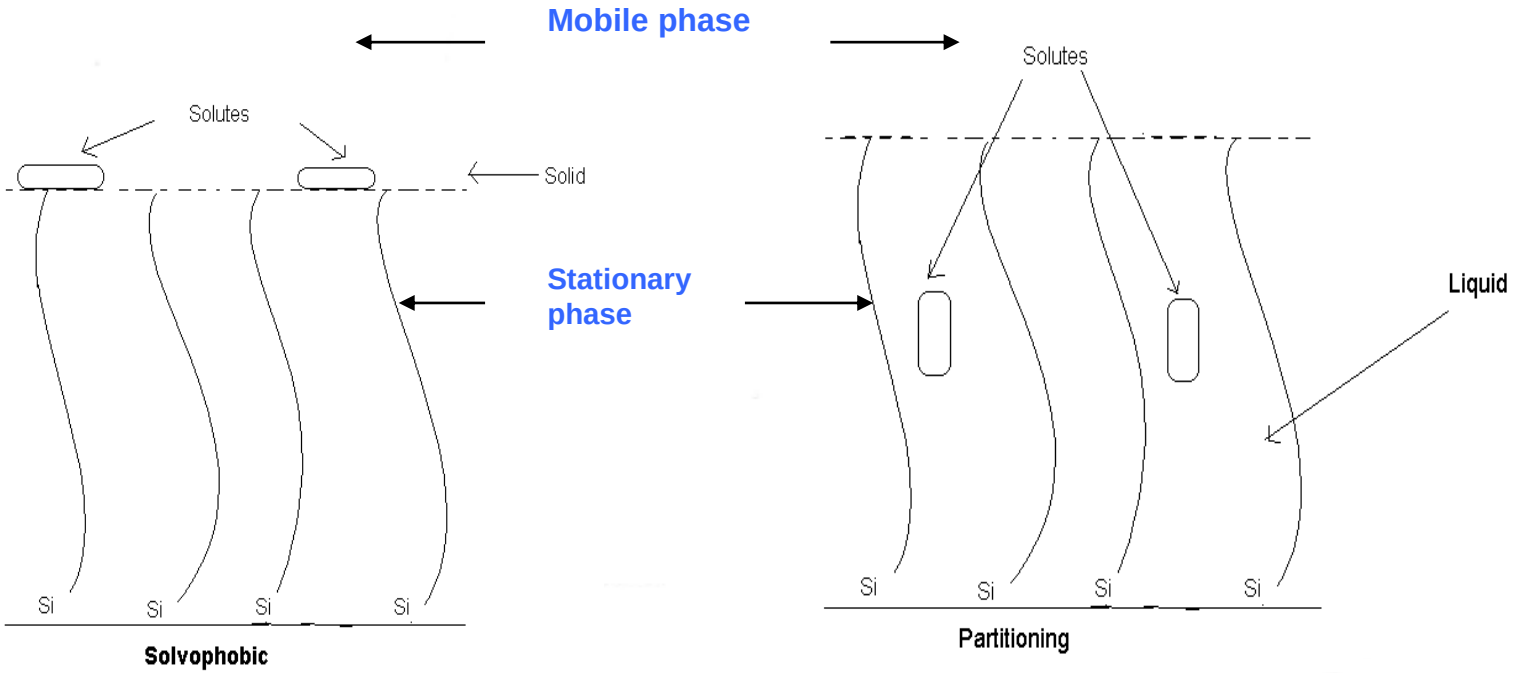
Mechanism of separation

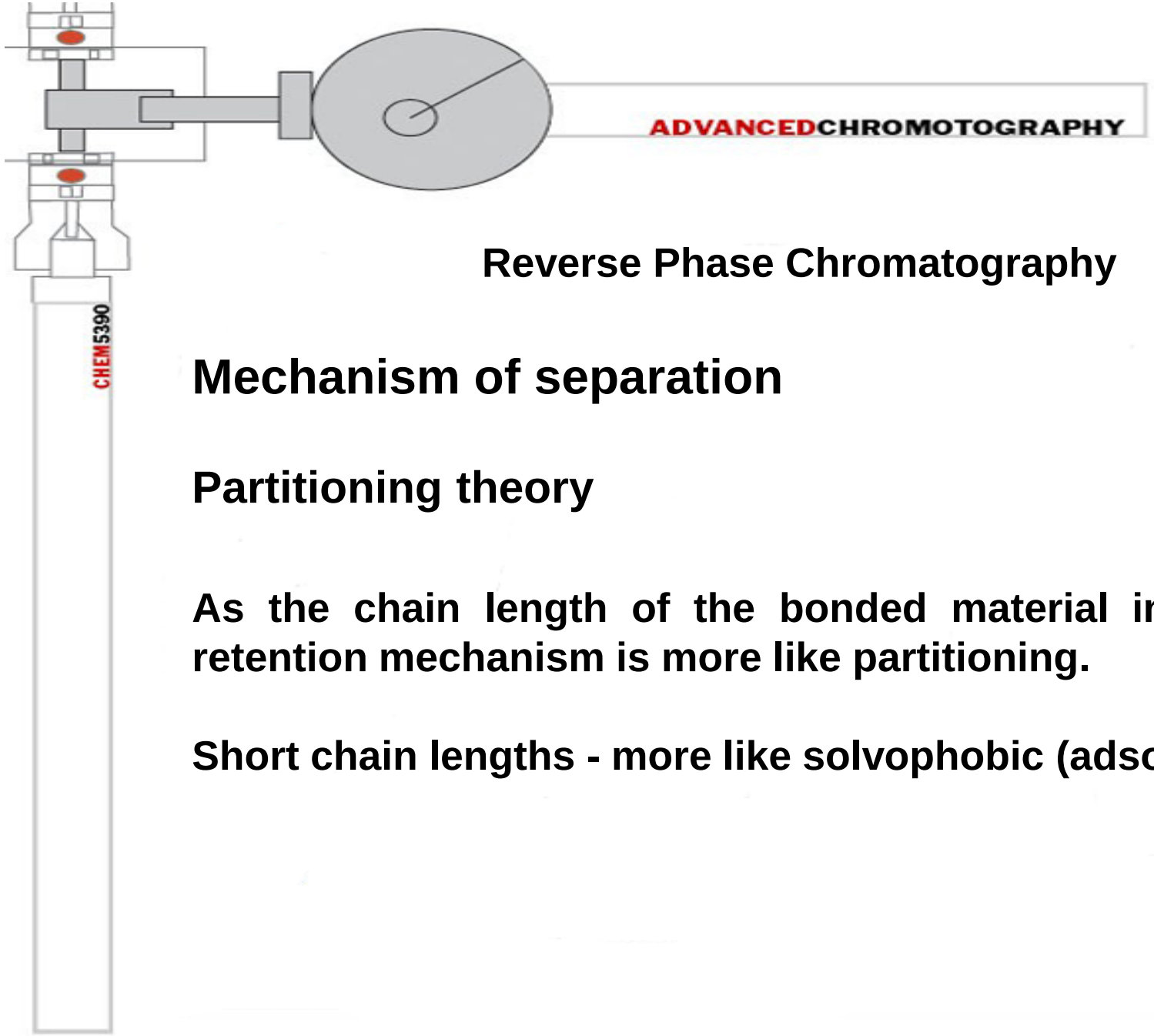
Partitioning theory

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

Reverse Phase Chromatography

Mechanism of separation





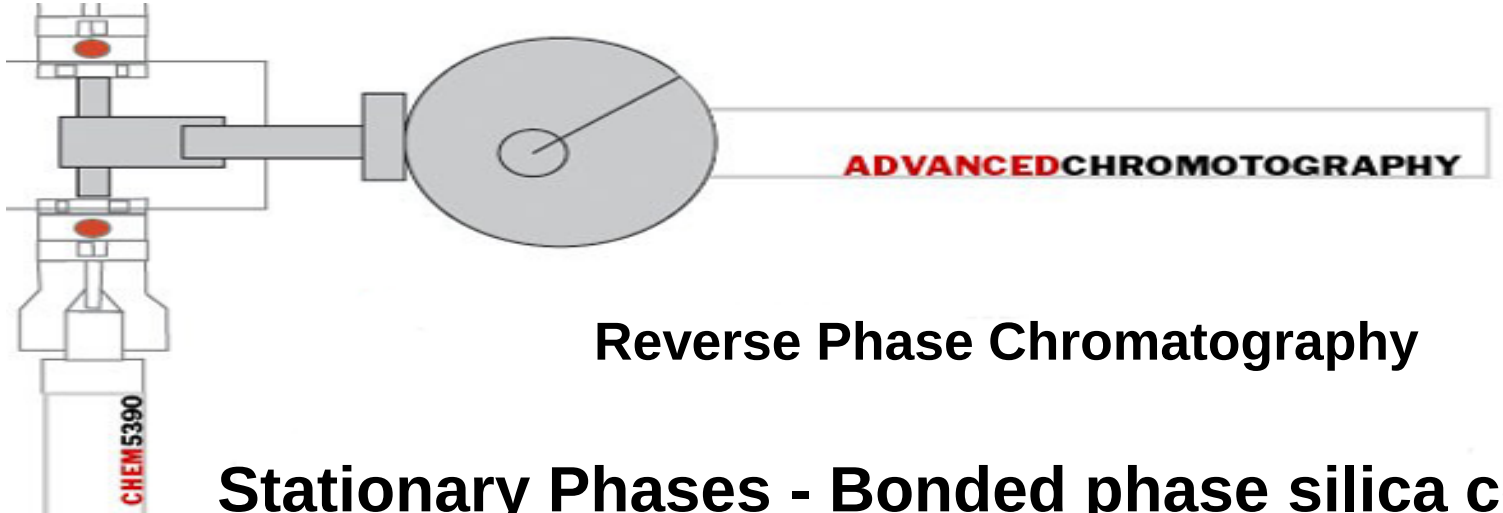
Reverse Phase Chromatography

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).



Reverse Phase Chromatography

Stationary Phases - Bonded phase silica columns

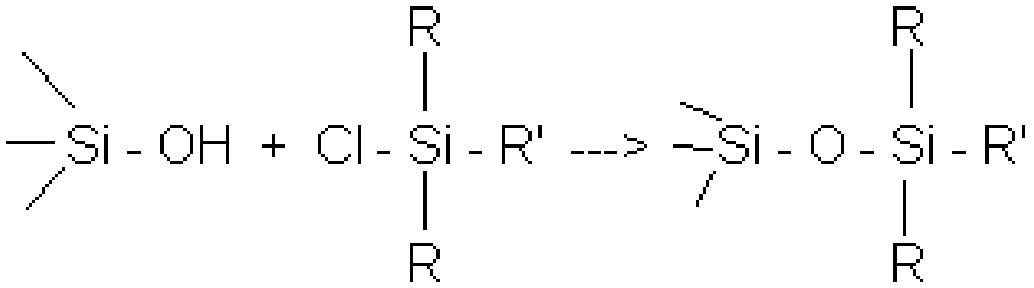
Functional group is chemically attached to a silica support (like used for normal phase).

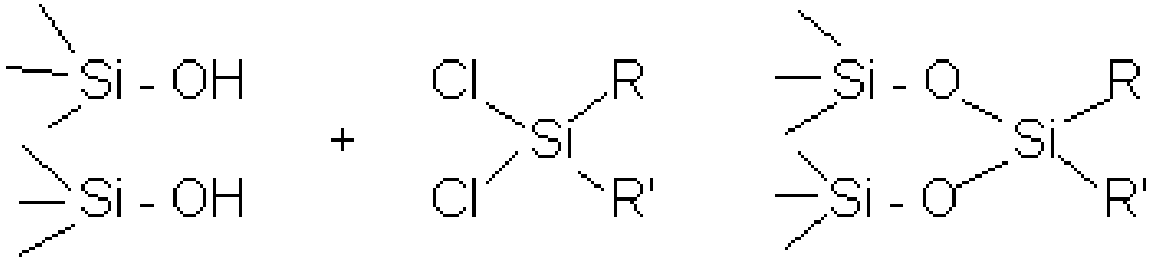
Attaching groups to the silica depends on reaction with the surface silanol groups.

Most common method - reaction of silica with organochlorosilanes to produce siloxane (Si-O-Si-R_3) packings.

Reverse-Phase Chromatography

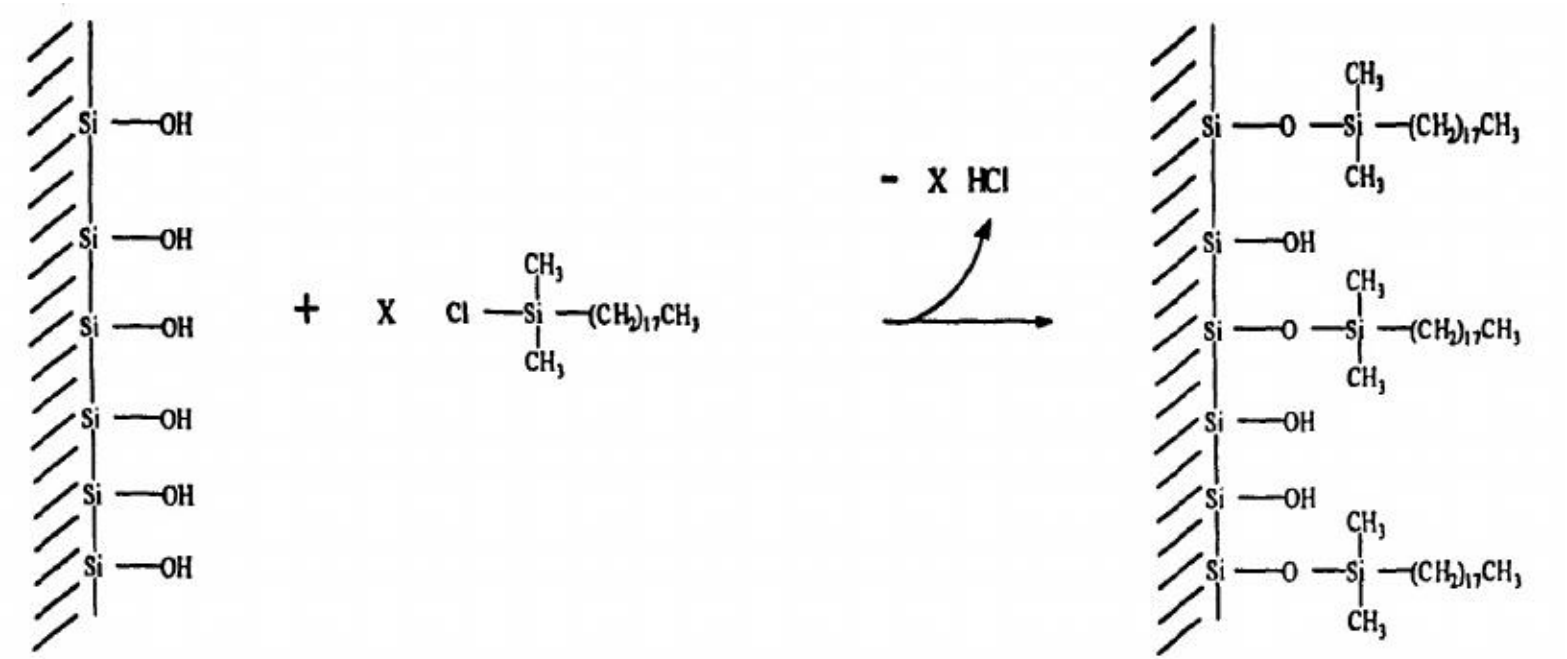
Stationary Phases - Bonded phase silica columns





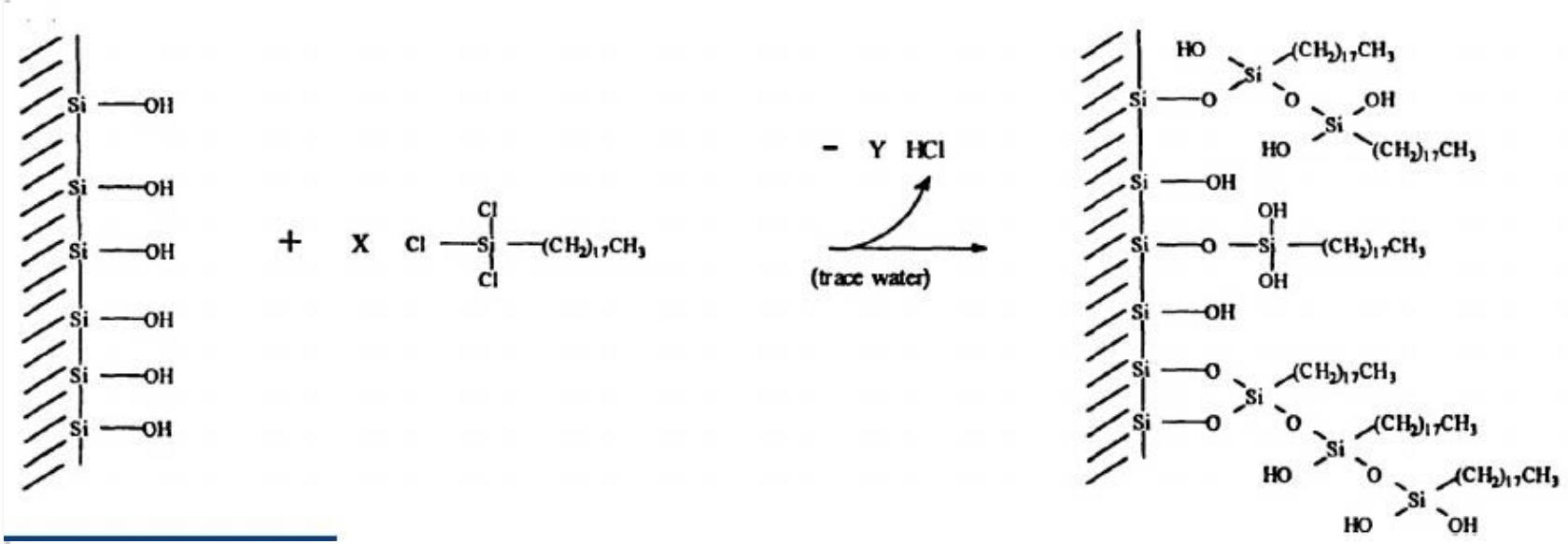
Reverse-Phase Chromatography

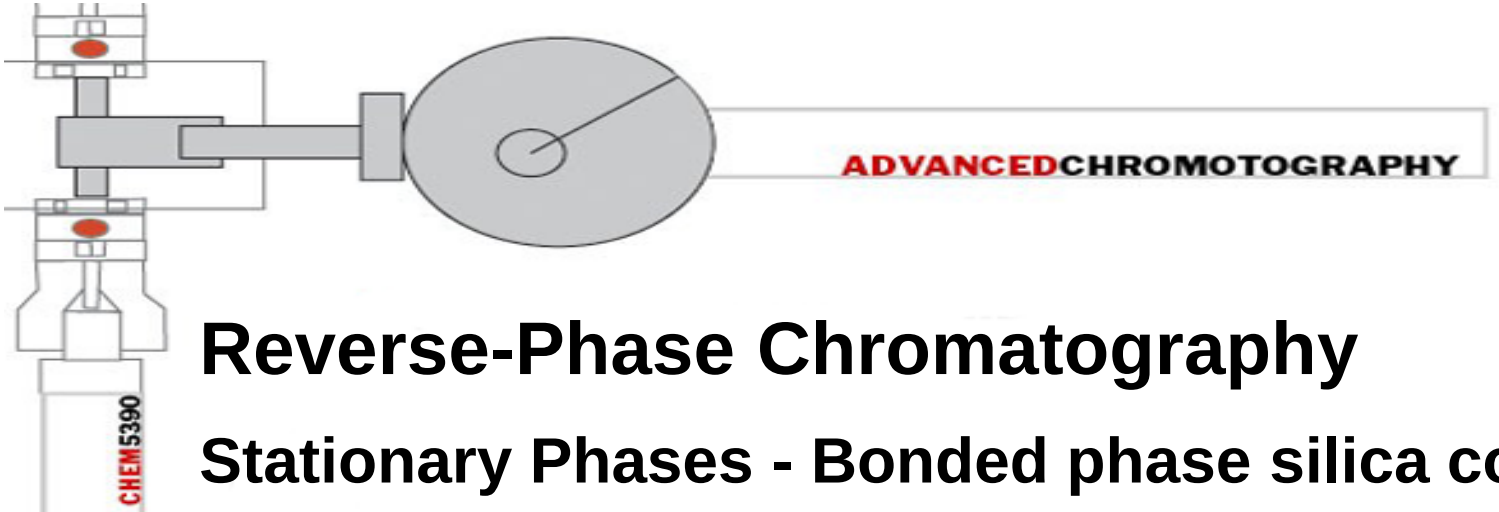
Stationary Phases - Bonded phase silica columns
monomeric type



Reverse-Phase Chromatography

Stationary Phases - Bonded phase silica columns
polymeric type





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_{18} - 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_{18} functionalized column is about 2 mg sample/ g of stationary phase.

Reverse-Phase Chromatography

Stationary Phases - Bonded phase silica columns

Identify the column by the nature of the bonded R' group.
C_{18} - most common.

Others – C_8, phenyl, C_6, C_4, C_2, CN, NH_2, and NO_2.

Retention increases exponentially as alkyl chain length increases.

Reverse-Phase Chromatography

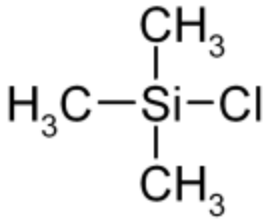
Stationary Phases - Bonded phase silica columns

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.



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.

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.

Reverse-Phase Chromatography

Mobile Phases - Solvent classification

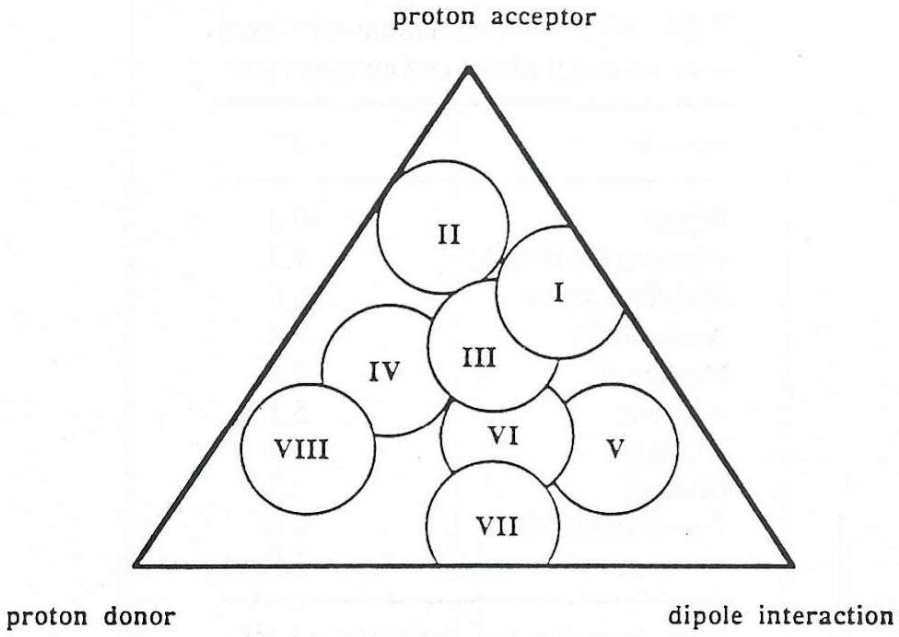


Fig. 6.15. The solvent classification triangle which illustrates the eight selectivity groups determined by polarity and selectivity. Modified with permission from Snyder (1974) *J. Chromatogr.*, **92**, 223.

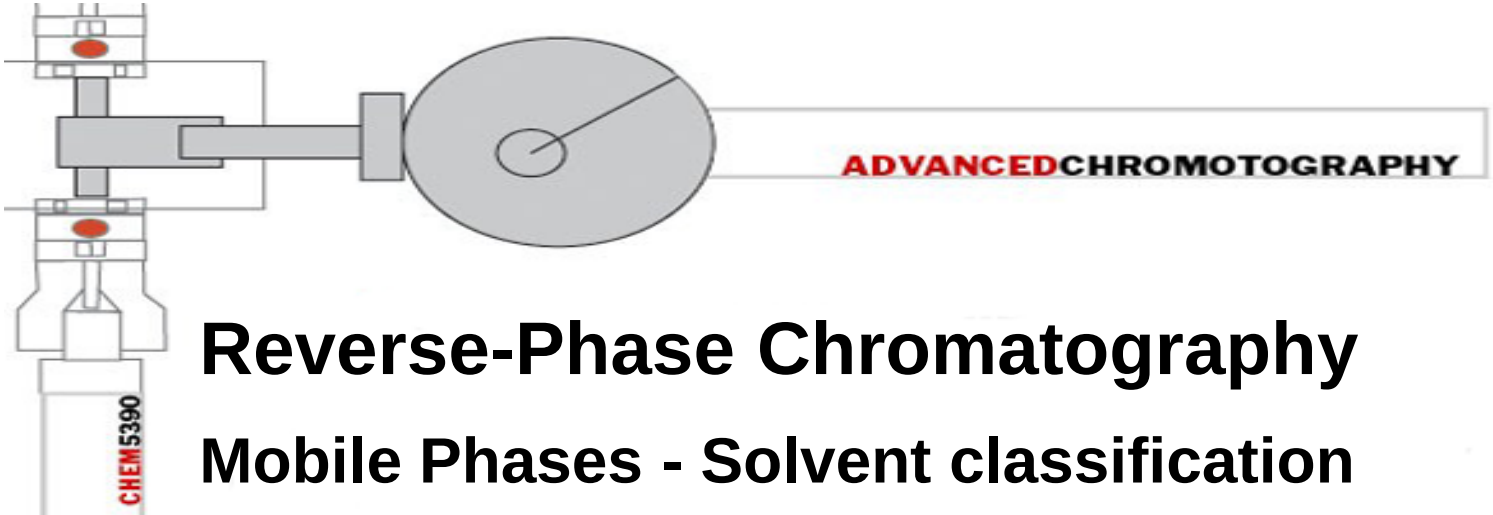
Reverse-Phase Chromatography

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	Pyrine 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, *m*-cresol, chloroform, water

Data from Snyder [17].



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.

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.

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).

Reverse-Phase Chromatography

Mobile Phases - Solvent strength

Eluotropic scale for strength - in general:
methanol, acetonitrile < ethanol = acetone = dioxane < tetrahydrofuran, isopropanol

Reverse-Phase Chromatography

Mobile Phases - Solvent strength

Example:

50:50 H_2O/CH_3OH weaker mobile phase than
50:50 H_2O/tetrahydrofuran.

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

Example:

50:50 H_2O/ CH_3OH	**nitrobenzene**	**t_r= 2 min**
45:55 H_2O/ CH_3OH	**nitrobenzene**	**t_r= 1 min**
55:45 H_2O/ CH_3OH	**nitrobenzene**	**t_r= 4 min**

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.

Reverse-Phase Chromatography

Mobile Phases - Solvent strength

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.

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].

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.

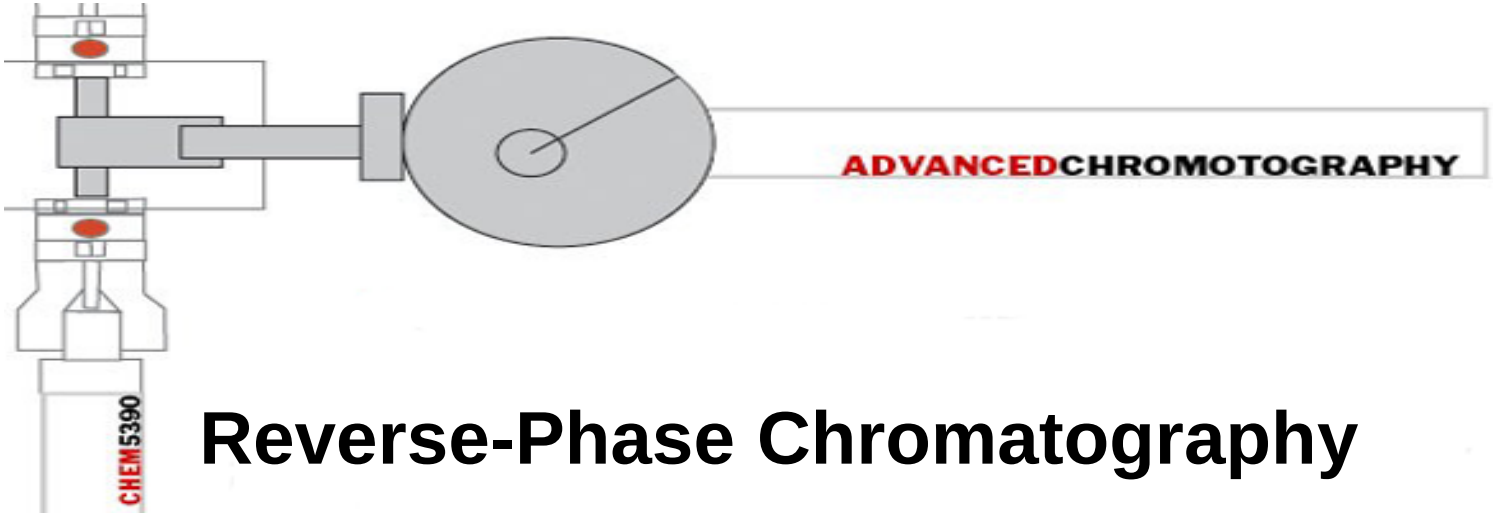
Example: 3 mobile phases: 30:70 CH_3OH/H_2O or 22:78 ACN/H_2O or 16:84 THF/H_2O -all give equal retention but different selectivity.

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

$\phi ACN = 0.77\ \phi CH_3OH$

$\phi THF = 0.66\ \phi CH_3OH$

ϕ_M – represents the mobile phase volume fraction.



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

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.

Reverse-Phase Chromatography

Applications

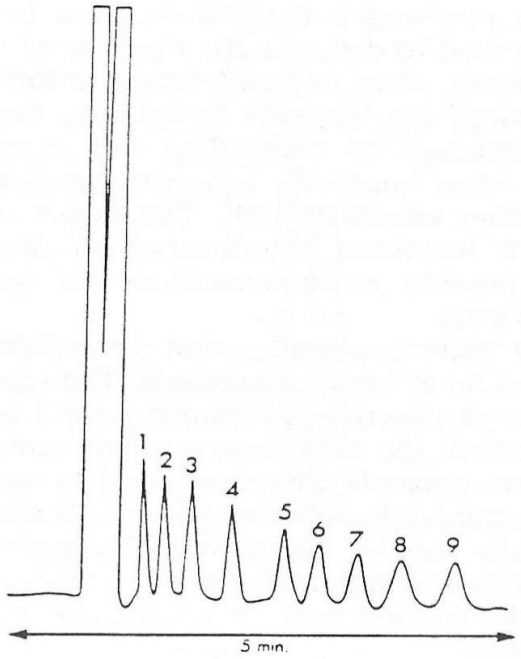
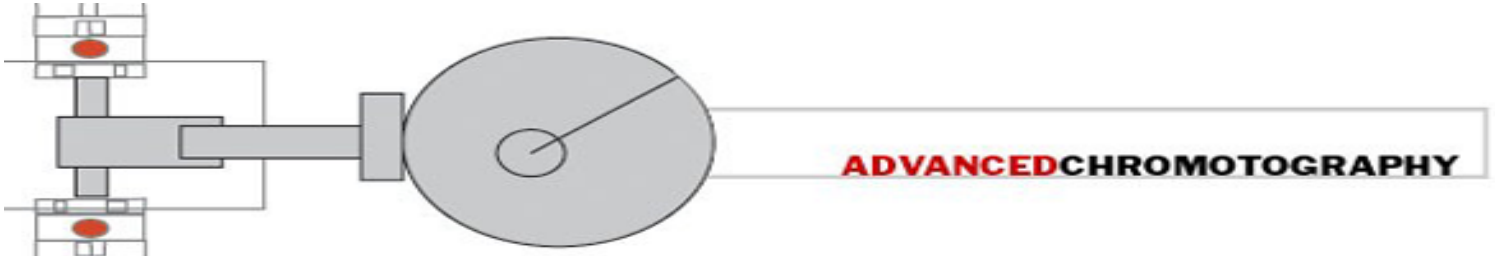


Fig. 6.18. Separation of fatty acids by reversed phase chromatography. A Waters Free Fatty Acid Analysis Column was used with a mobile phase of 45/20/35 ACN/THF/water at 1.5 ml min^{-1}. RI detection was used. Solute identities: 1, capric acid; 2, lauric acid; 3, myristic acid; 4, palmitic acid; 5, stearic 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.



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, sweetners.

Environmental samples - pesticides, pollutants

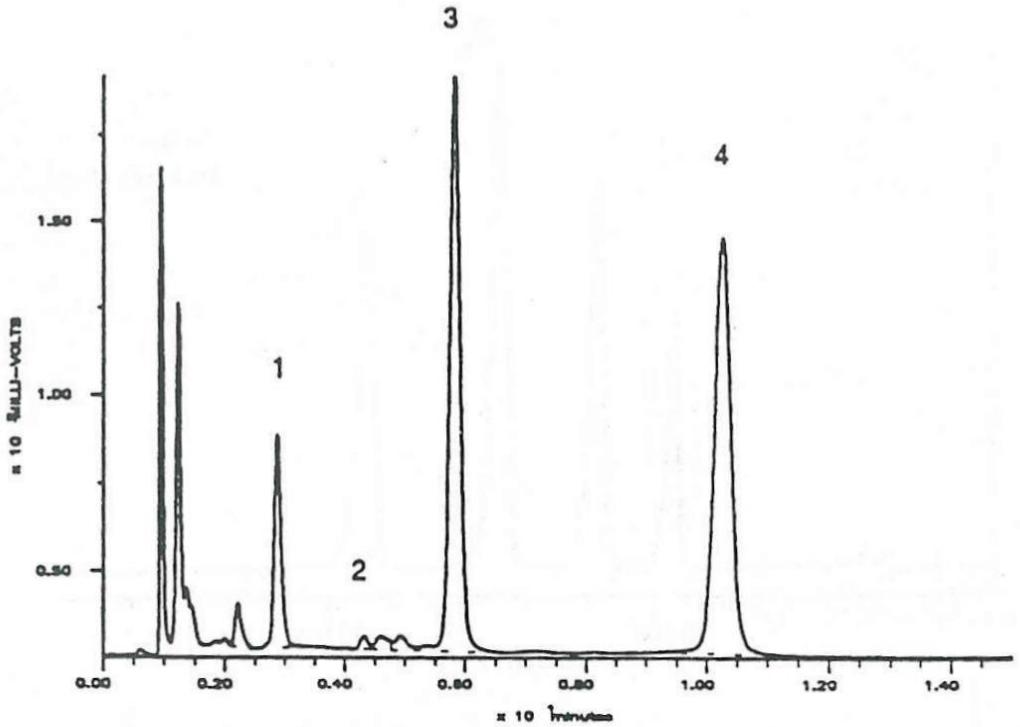
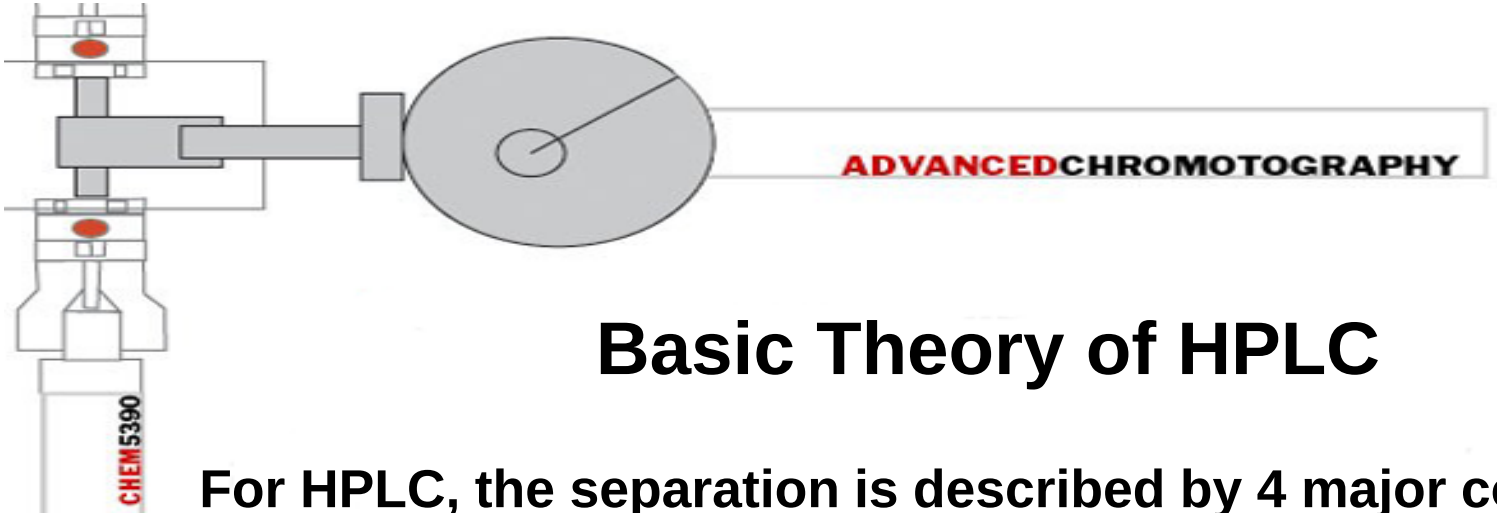


Fig. 6.19. Separation of catecholamines in urine by RPLC. A Waters Nova-Pak column was used with a mobile phase of 50 mM sodium acetate, 1 mM octane sulfonate, 0.1 mM EDTA in 96:4 water:MeOH. Amperometric detection was used. Solute identities: 1, noradrenaline; 2, adrenaline; 3, dihydroxybenzylamine;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



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.

Basic Theory of HPLC

Capacity Factor

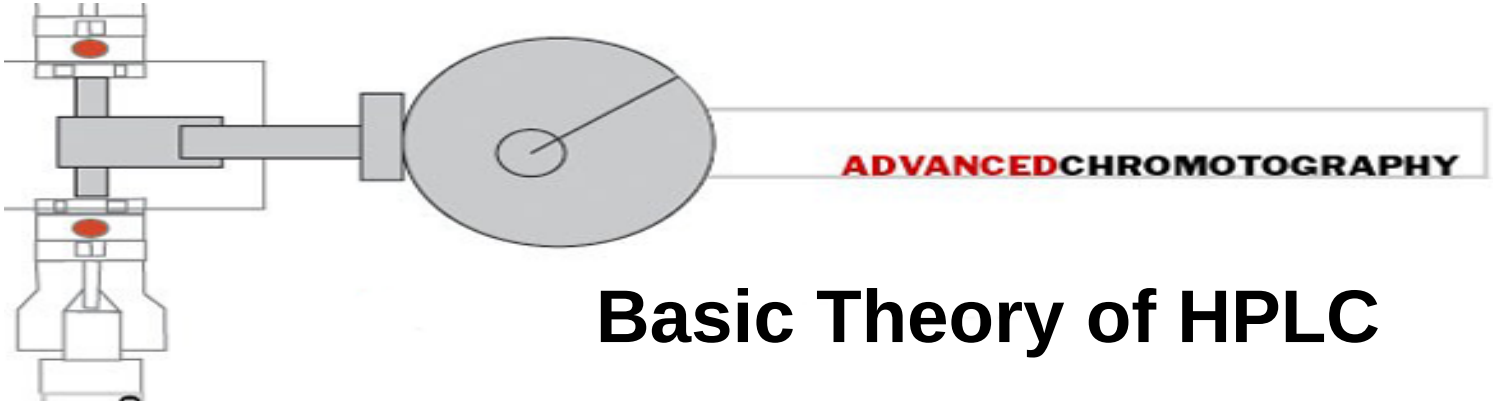
For effective LC separations, a column must have the capacity to retain samples.

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)



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.

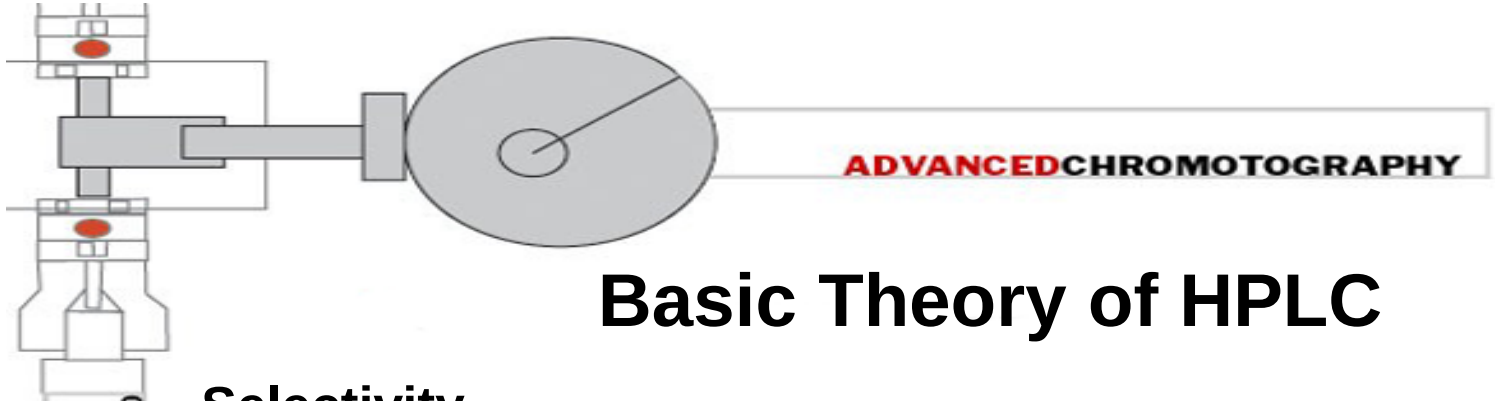
Higher k′ - the greater the ability of the column to retain solutes, longer analysis time.

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



Basic Theory of HPLC

Selectivity

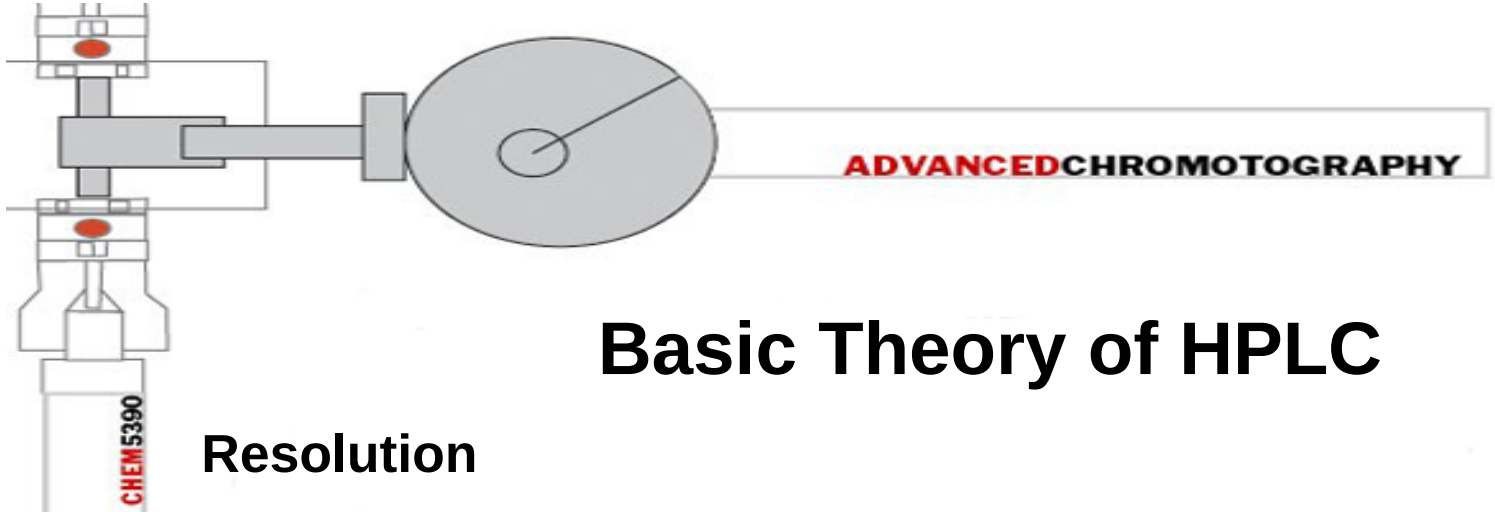
Selectivity or separation factor, α , describes the separation of two peaks relative to each other.

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

Selectivity is a function of packing material.

Value of α ranges from unity (1) when $t_{r2} = t_{r1}$, $\alpha \geq 1$.

Easy way to increase α is to change the composition (components) of the mobile phase.



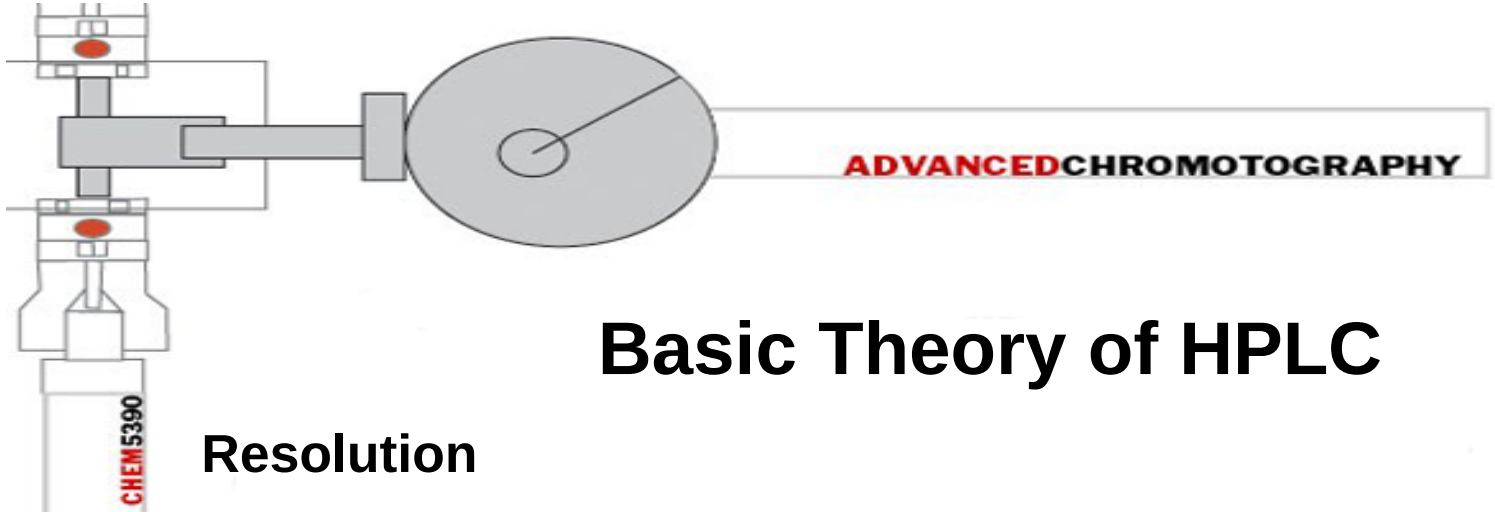
Basic Theory of HPLC

Resolution

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

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

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



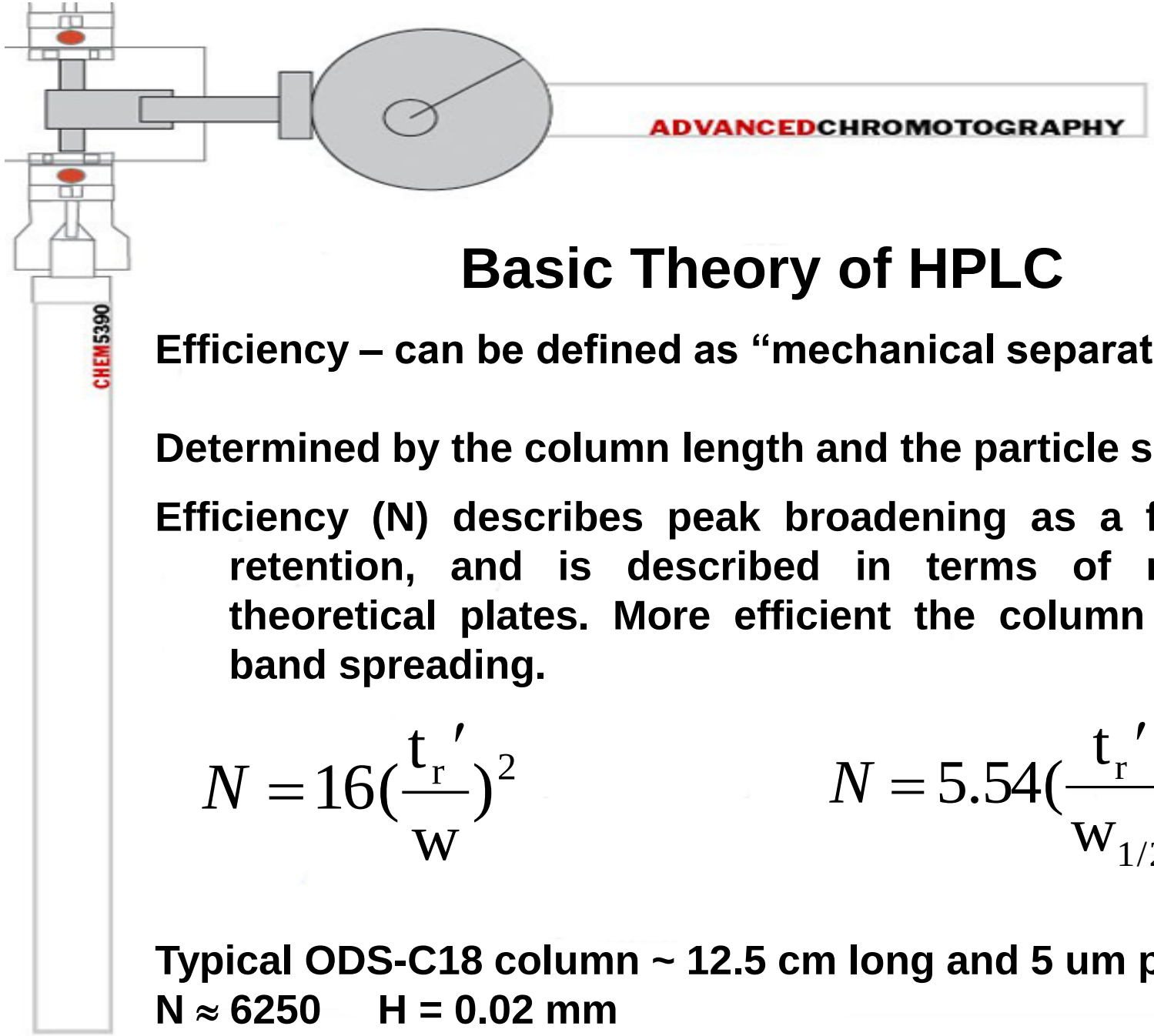
Basic Theory of HPLC

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, α, by varying the mobile phase or capacity factor, k' by varying mobile phase strength.



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.

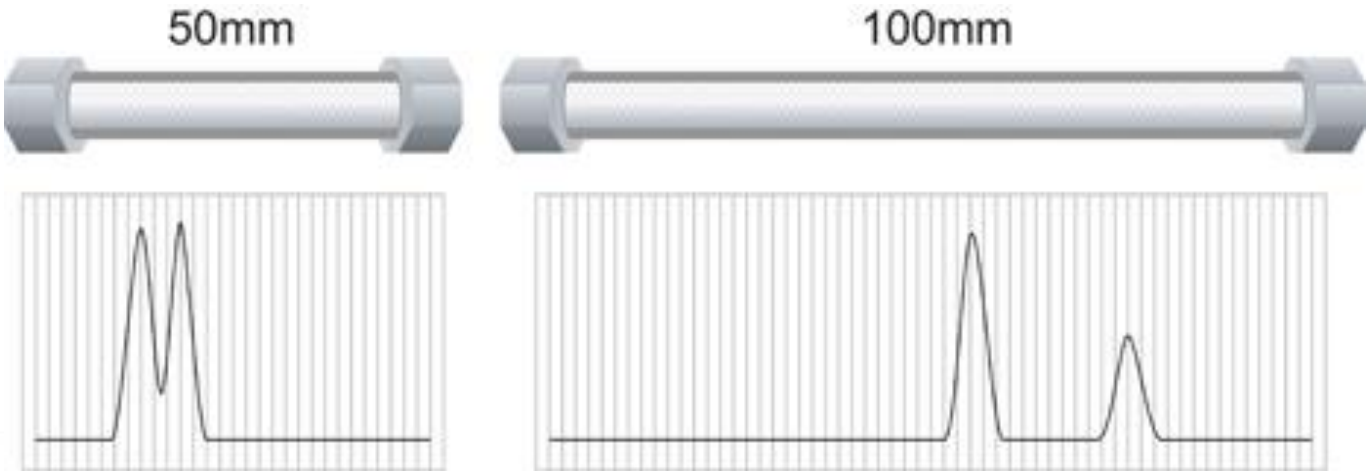
$$N = 16\left(\frac{t_r'}{w}\right)^2 \qquad N = 5.54\left(\frac{t_r'}{w_{1/2}}\right)^2$$

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

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.

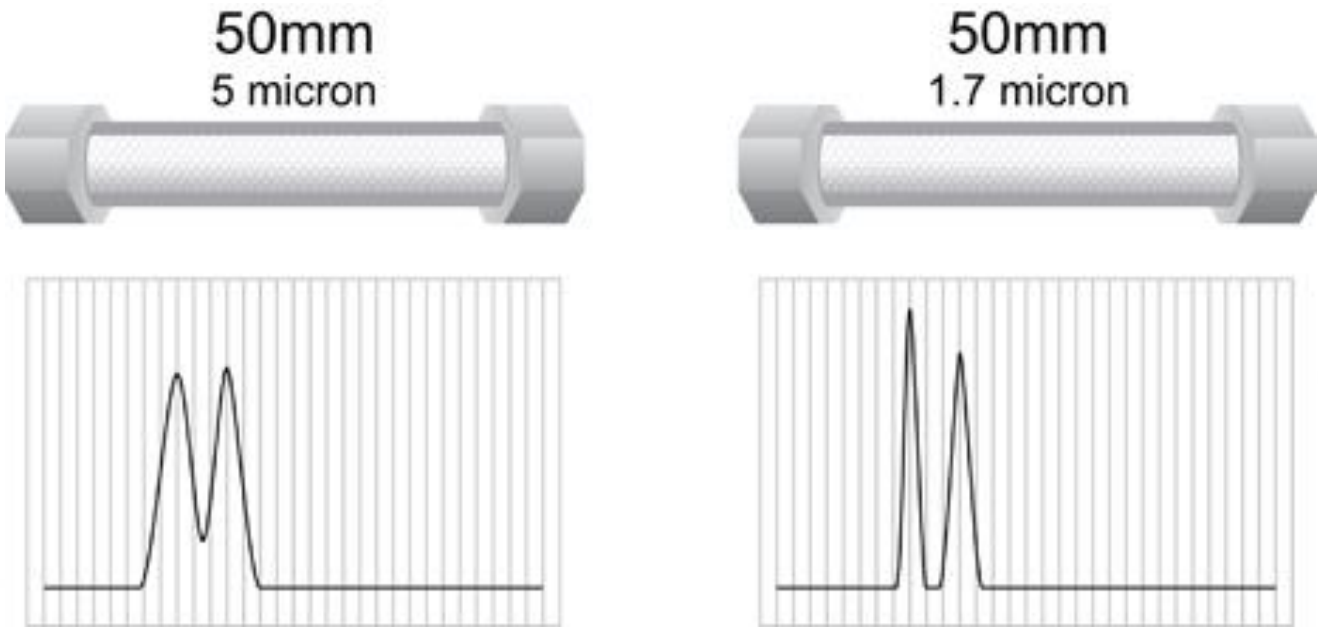


Basic Theory of HPLC

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.



Basic Theory of HPLC

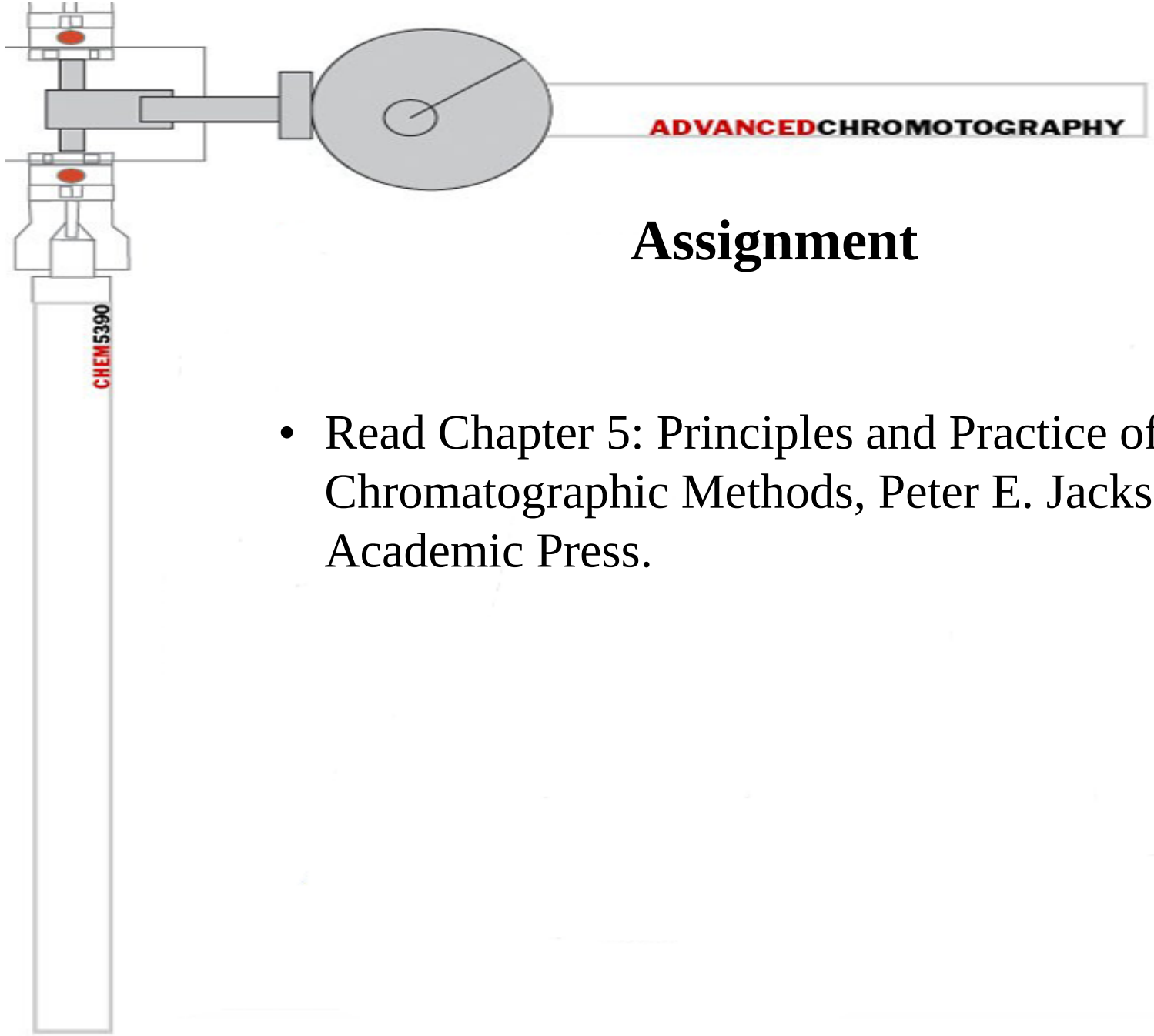
Fundamental Resolution Equation

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

α = **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

- Read Chapter 5: Principles and Practice of Modern Chromatographic Methods, Peter E. Jackson, Academic Press.