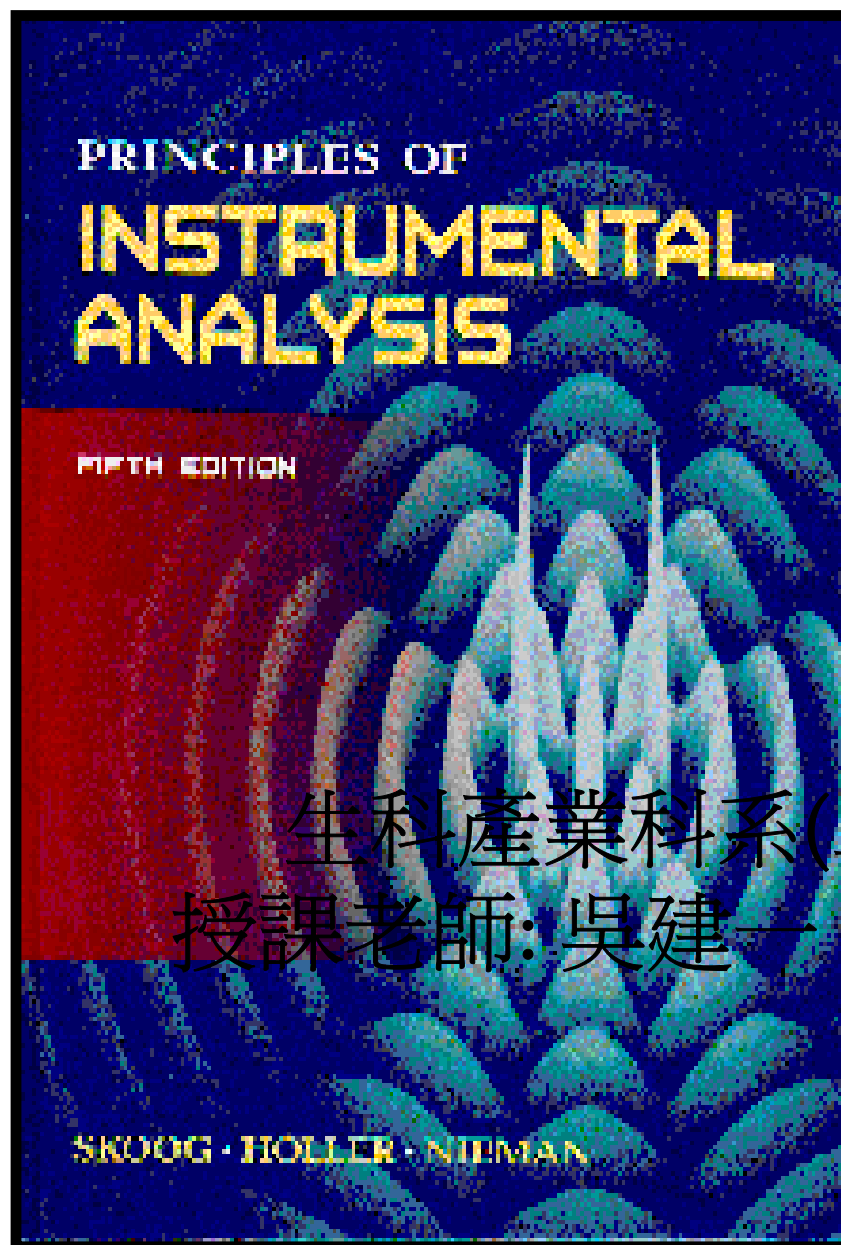




儀器分析

Chapter 28

高效液相層析法

High-Performance Liquid Chromatography

大葉大學

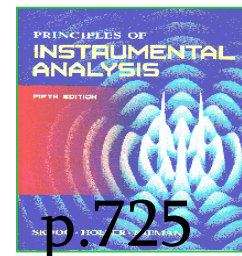
生科產業科系(二)
授課老師: 吳建一

老師 Da-Yeh University



Liquid Chromatography (LC)

液相層析法



★ LC係流動相為液體之層析法，依固定相的類型可分為：

★ **Partition Chromatography** : 分配層析法 (LLC)

- 又稱為液-液層析法(liquid-liquid chromatography, LLC)
- 固定相是吸附在固體上之液體，利用樣品在二個不互溶之液體及液體間之分配 (partition)

★ **Adsorption Chromatography** : 吸附層析法 (LSC)

- 又稱為液-固層析法(liquid-solid chromatography, LSC)
- 固定相是吸附劑，可能是利用吸附、離子交換或大小排除之原理

★ **Ion Exchange Chromatography (IC or IEC)**: 離子交換層析法

- 固定相是填充管柱的離子交換樹脂，吸附劑利用樣品組成與離子交換樹脂表面之親和力的差異以達分離之效果

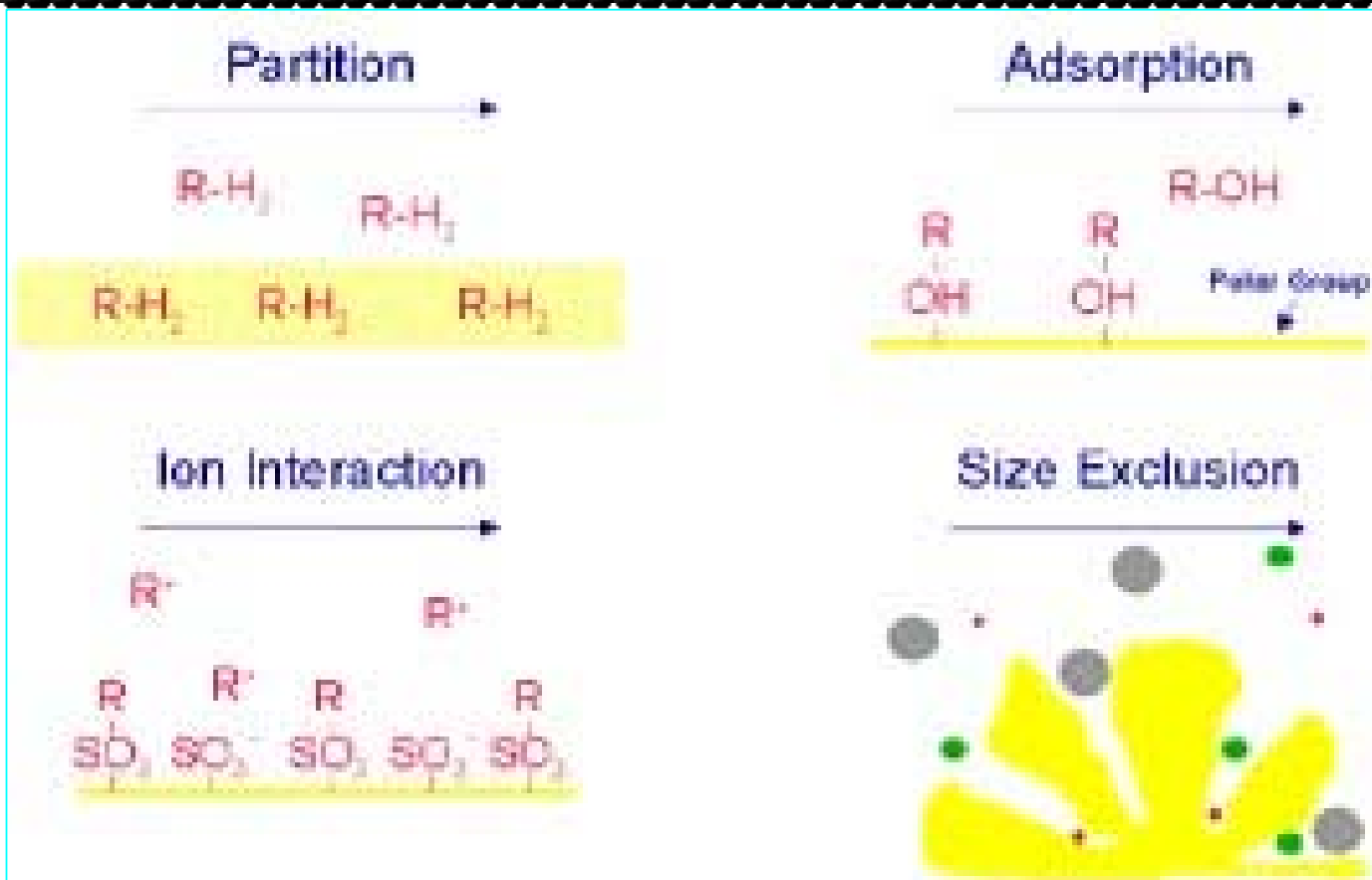
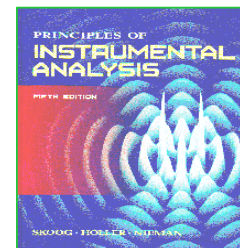
★ **Size Exclusion Chromatography**: 大小排除層析法 (SEC)-固定相為凝膠或多孔無機固體

- 凝膠過濾層析法(gel-filtration chromatography, GFC)
 - 利用水或水溶液作為流動相
- 凝膠滲透層析法(gel-permeation chromatography, GPC)
 - 利用有機溶劑作為流動相



Liquid Chromatography (LC)

液相層析法分類



層析法之分類 - 依固定相的形式來分類

1. 管柱層析法 (column chromatography)

2. 平面層析法(planar chromatography)：

★ 固定相附著在一平板或多孔濾紙上，其流動相則利用毛細管現象或重力作用而流經固定相而分離

★ 又分為二種：

● 薄層層析法(thin-layer chromatography，TLC)：固定相在一平板上

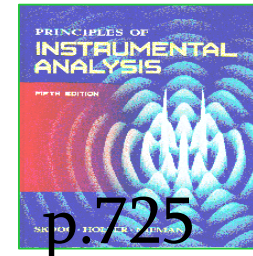
● 在載板上塗佈或燒結一層薄物質做為固定相的平面層析法

● 紙層析法(paper chromatography，PC)：固定相在一濾紙上

★ 液相層析中除了 TLC 及 PC 是在平面上進行之外，其餘均在管柱中進行



Liquid Chromatography (LC) 液相層析法 – 簡介



★ 早期的column：

- ★ 1-5 cm dia., 50-500 cm
- ★ particle : 150-200 μm
- ★ flow rate 0.1 ml/min

★ new column: 1960s 以後

- ★ particle : 3-10 μm
- ★ 高壓條件
- ★ High Performance Liquid Chromatography (HPLC)

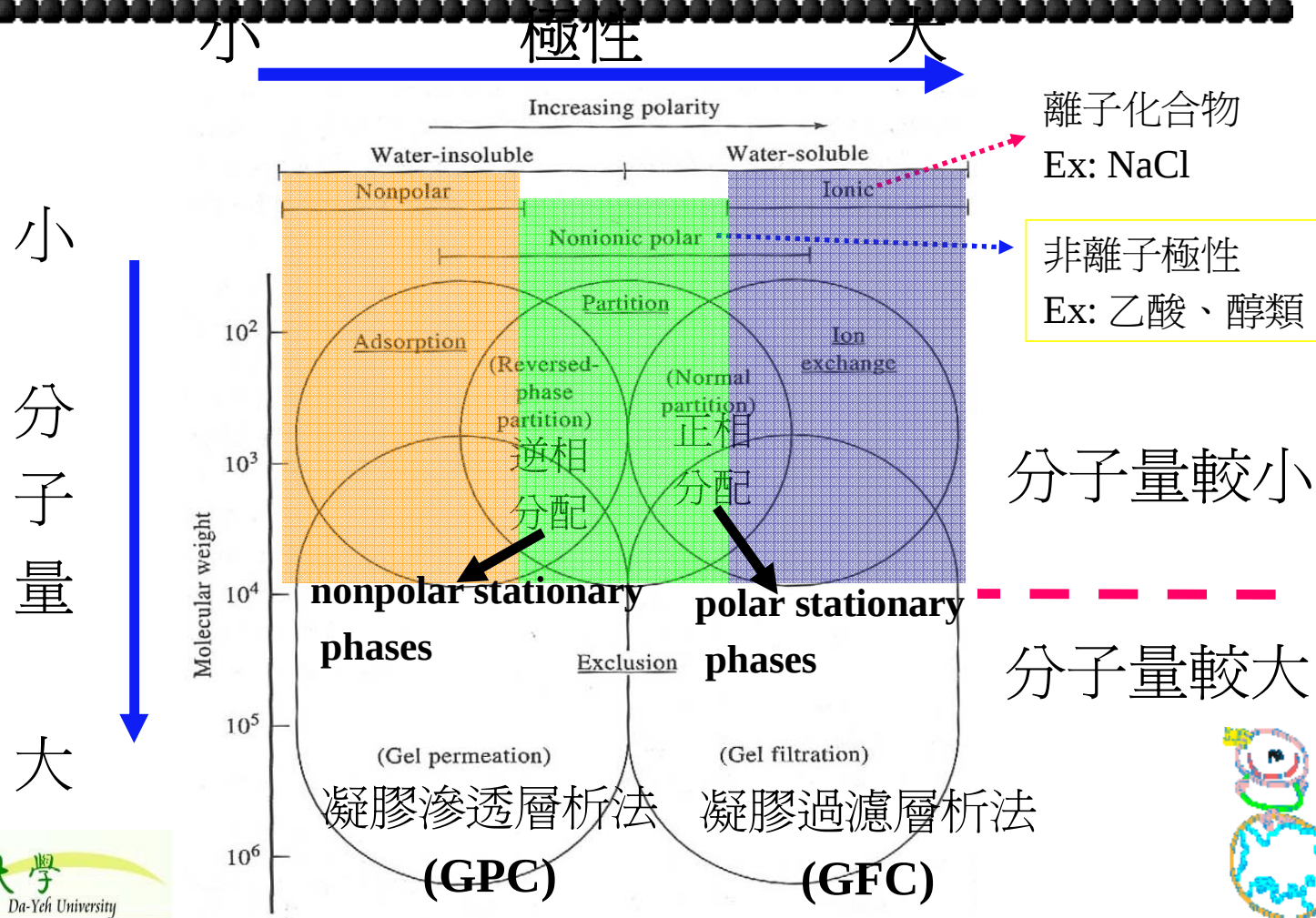
高效液相層析
高壓液相層析
高速液相層析

★ 分析範圍:

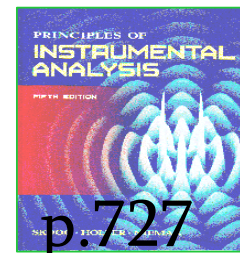
- ★ amino acid, protein, nucleic acid, RH, carbohydrate, drug etc.



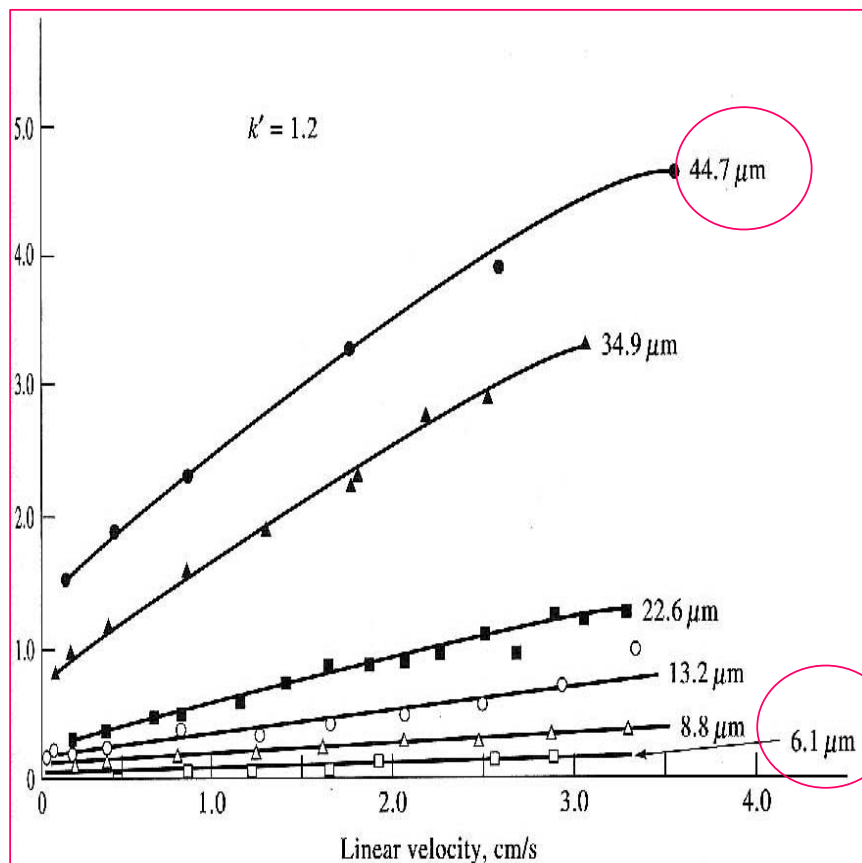
28-A Scope of HPLC Application of Liquid Chromatography



28B-1 Effect of Particle Size of Packings



p.727



凡迪姆特方程式

Van Deemter Equation-

$$H = A + \frac{B}{u} + C \cdot u = A + \frac{B}{u} + (C_s + C_m)u$$

■ LC的管柱效率: 隨填充粒子的粒徑減少而增加

■ dp : 45 μm → 6 μm

➡ H : 降低10 倍或更多

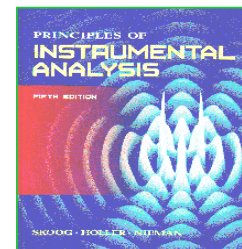


28B-2 Extra- Column Band Broadening in LC 液相層析法的管柱外層析帶變寬現象

- ★ Longitudinal diffusion much less important than in GC
 - ★ $D_{\text{gas}} \approx 1 \text{ cm}^2/\text{s}$
 - ★ $D_{\text{liquid}} \approx 10^{-5} \text{ cm}^2/\text{s}$
- ★ Efficiency improved by going to smaller particles (d_p)
 - ★ limited by pump technology and by methods for making small particles
- ★ Extra-column band broadening (管柱外譜帶變寬) can be important (in connecting tubing):
 - ★ 又稱「柱外效應(extra column effect)」
 - ★ 指從注入點(注射系統)至偵測器之間除了管柱以外的所有死空間引起之譜帶變寬
 - ★ 包括: 進樣器、連接管、接頭、偵測器



HPLC 的特點



★ 高壓

- ★ 流動相為液體，黏度比氣體大很多 ➡ 管柱內填充緊密的微粒(固定相) ➡ 流動相通過管柱時受到很大阻力
- ★ 壓阻高達 $75 \times 10^5 \text{ Pa /m}$ ➡ 必須使用高壓輸液pump系統

★ 高速

- ★ 流動相流經管柱之流速= 3-10 mL/min ➡ 分離加速

★ 高效

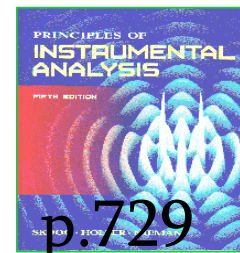
- ★ 管柱填充之微粒均勻且 $d_p < 10 \mu\text{m}$ ➡ 理論平板數可達 10000/m ➡ 管柱效率高

★ 高靈敏度：

- ★ 採用高靈敏的偵測器



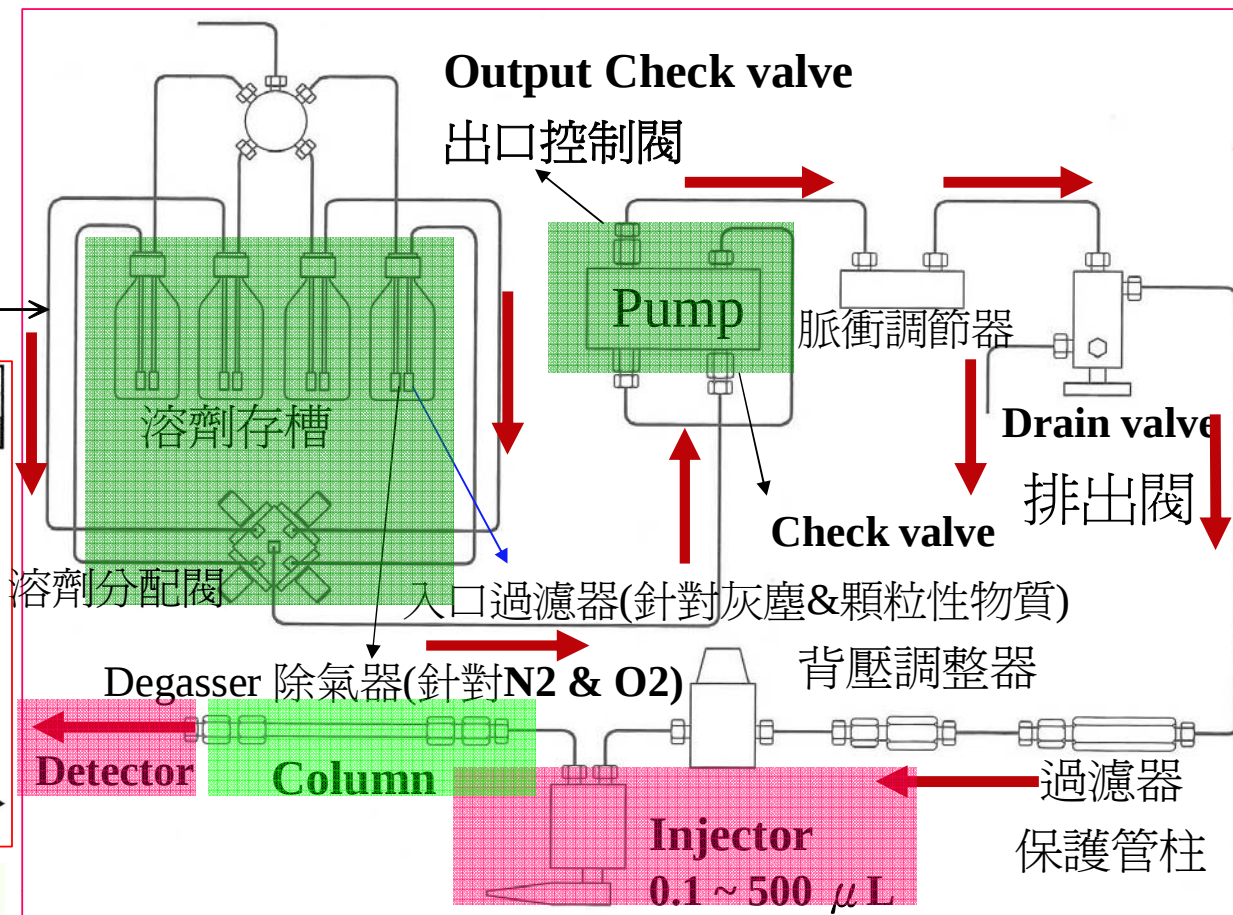
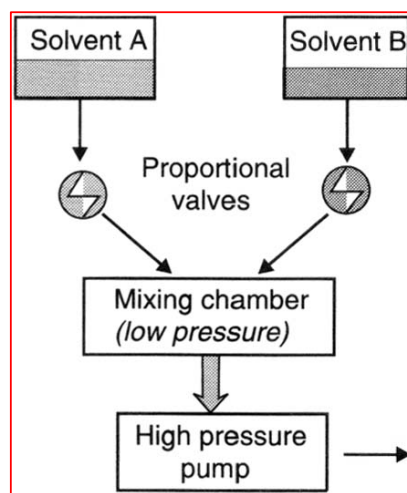
28C Instruments For High Performance Liquid Chromatography



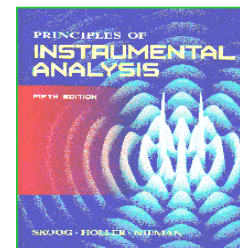
p.729

主要分爲五大部分:

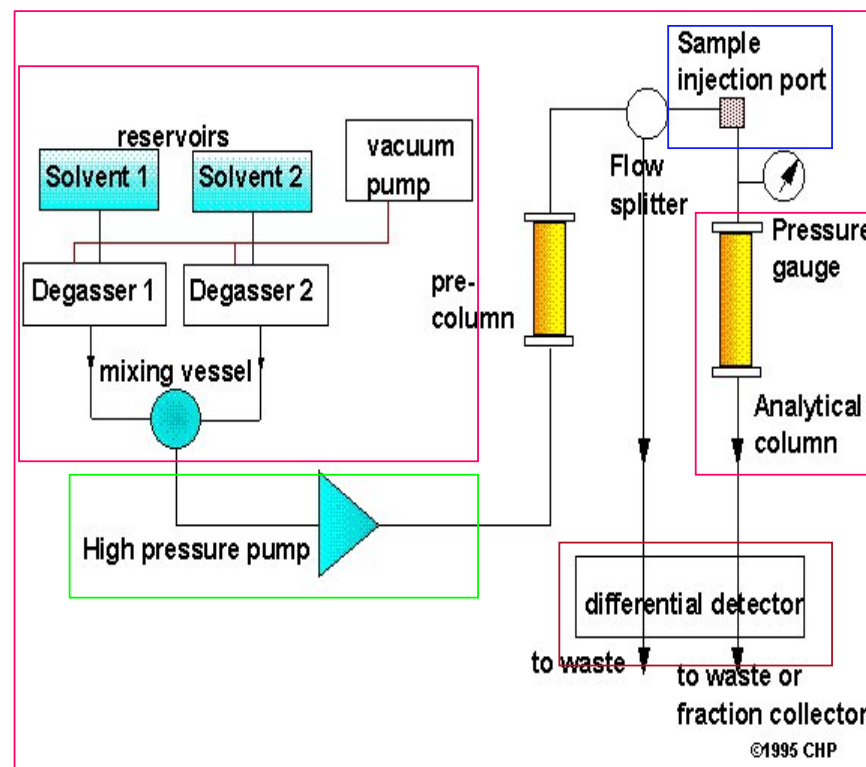
Gradient Elution



HPLC 之主要儀器裝置

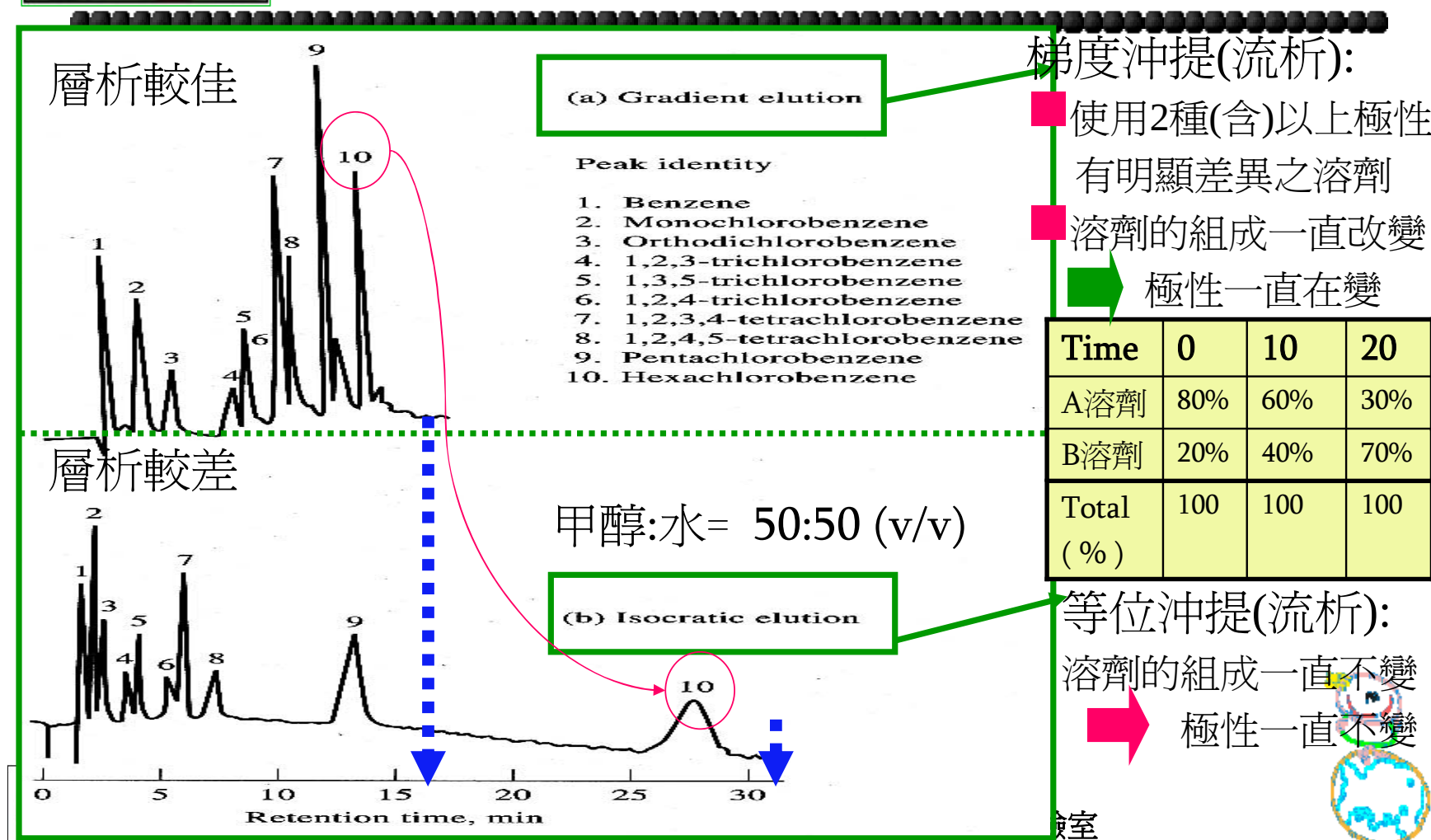


- ✧ Mobile-phase reservoirs (500 mL- 2L)
 - ★ Vacuum pumping system
 - ★ Degasser (脫氣計)
 - ★ Solvent mixer (溶劑混合器)
- ✧ Pumping system (輸液pump)
 - ★ Reciprocating (往復式) pump
 - ★ Displacement pumps = syringe-type pump (置換式)
 - ★ Pneumatic (氣動式) pumps
 - ★ Flow control and Programming systems
- ✧ Sample Injection system
- ✧ Liquid-Chromatographic columns
 - ★ Analytical columns
 - ★ Guard column (保護管柱)
 - ★ Column thermostats (管柱恆溫箱)
- ✧ Detector

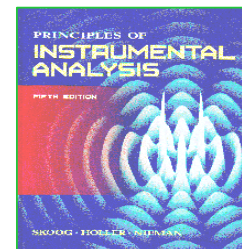


28C-1 Mobile-Phase Reservoirs & Solvent Treatment System

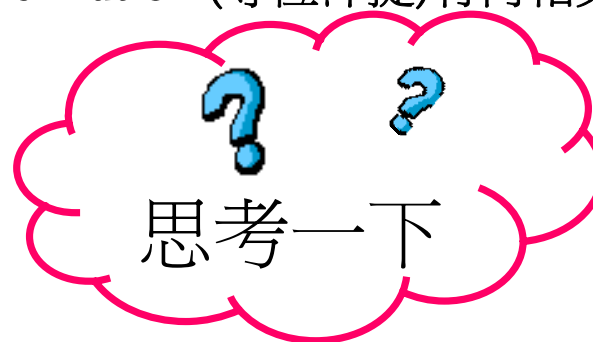
Gradient Elution vs. Isocratic Elution



Gradient Elution (梯度沖提) vs. Isocratic Elution (等位沖提)



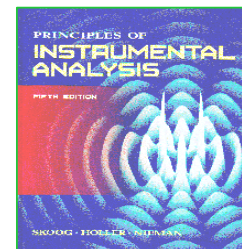
- ★ Gradient Elution (梯度沖提) 比 isocratic Elution (等位沖提) 有何相異之處 (梯度沖提比等位沖提更具有優點)?



- ★ Gradient Elution (梯度沖提) 與 GC 之 溫度程式有異曲同工之意



LC Mobile Phases



★ Normal phase liquid chromatography (NPLC)-正相層析法

★ Hexane (weak solvent) mixed with low polarity organic solvent(s) (strong solvent) ➡ 低極性組成先被沖提出

★ used with polar stationary phases (ex: 氧化鋁, 矽酸鹽)

★ Reversed phase liquid chromatography (RPLC)-逆相層析法

★ Water (weak solvent) mixed with moderately polar organic solvent(s) (strong solvent) (ex: 水, 甲醇, acetonitrile) ➡ 高極性組成先被沖提出

★ used with nonpolar stationary phases

★ Isocratic mode

★ mobile phase with constant composition

★ Gradient mode

★ linear variation of volume percent composition, from weak to strong



28C-2 Pumping System

★符合需求:

- ★輸出壓力可以到達 6000 psi
- ★輸出沒有脈衝現象
- ★產生之流速範圍 = 0.1-10 mL/min
- ★對流速之控制與再現性必須在 0.5%的相對值內
- ★必須採用防腐蝕性之組件(以鐵氟龍或不鏽鋼為主)

★常用之三種pump

- ★ Reciprocating pump (往復式)
 - high pressure
- ★ Displacement pumps (置換式)
- ★ Pneumatic pumps (氣動式)

★ Flow control and Programming systems

液體之壓縮性很小
HPLC產生之高壓力
不會有爆炸的危險性

零件損壞
溶劑外逸
可能引起火災



HPLC 常用之三種 pump (1)

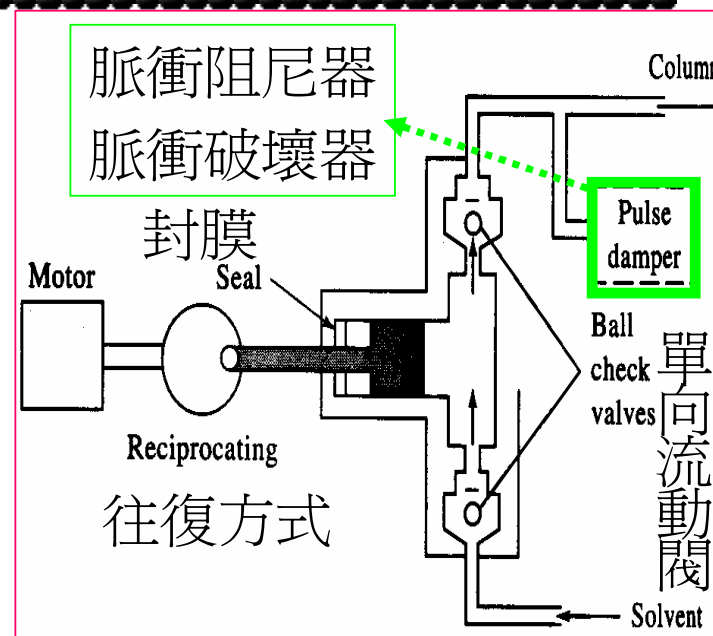
★ 1. Reciprocating pump (往復式)

★ HPLC 90%均採用此pump

★ 缺點:產生脈衝式液流 → 產生
基線雜訊

★ 優點:

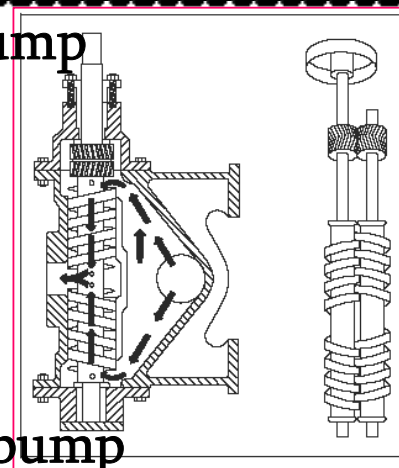
- 內部體積小(35-400 mL)
- 可以進行梯度沖提
- 輸出壓力高(10000 psi)
- 流速穩度(不受管柱逆壓力及溶劑黏性影響)



HPLC 常用之三種 pump (2)

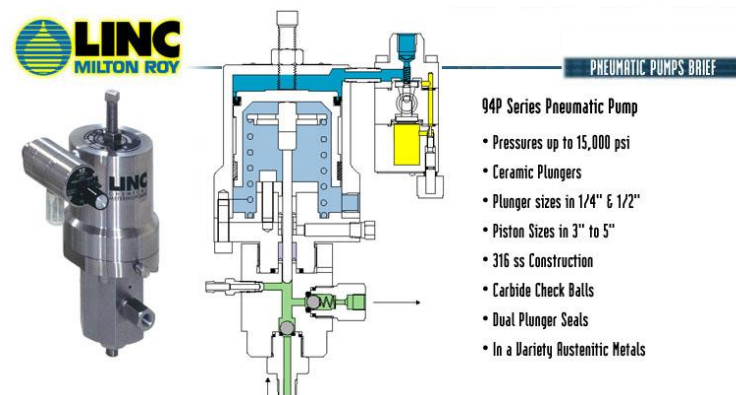
★ 2. Displacement pumps (置換式)=syringe-type pump

- ★ 利用馬達做驅動
- ★ 包含一個大型之類似注射筒形(syringelike)的槽室
- ★ 缺點:
 - 低的溶劑容量 (250 mL)
- ★ 優點:
 - 流速穩度(不受管柱逆壓力及溶劑黏性影響)
 - 無脈衝



★ 3. Pneumatic pumps (氣動式)=constant-pressure pump

- ★ 利用空氣壓縮做輸送
- ★ 優點:
 - 價格低廉
 - 無脈衝現象
- ★ 缺點:
 - 流速受管柱逆壓力及溶劑黏性影響
 - 無法進行梯度沖提
 - 操作壓力小(2000 psi)

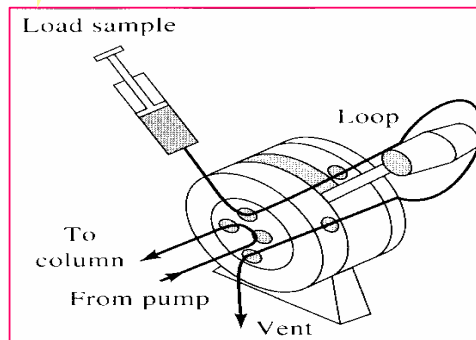
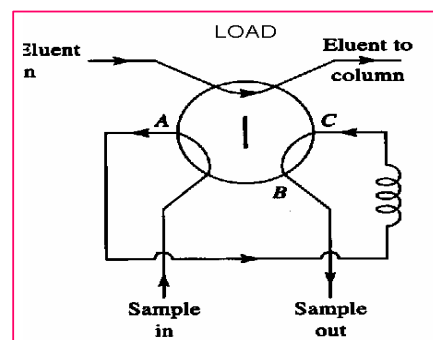


28C-3 Sample Injection System A sample loop for LC

- ◆ Sample 注入量以 μL 為單位 (0.1-500 μL), 壓力可達 7000 psi
- ◆ 載入之量必須 > loop 中的容量 → 以確保能完全填充column

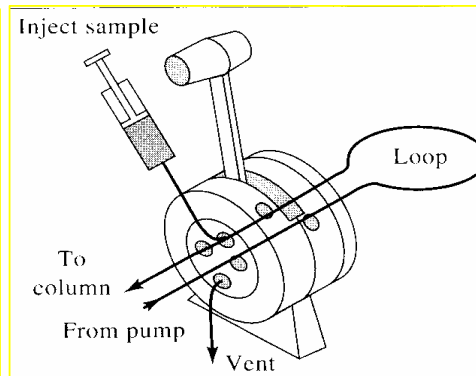
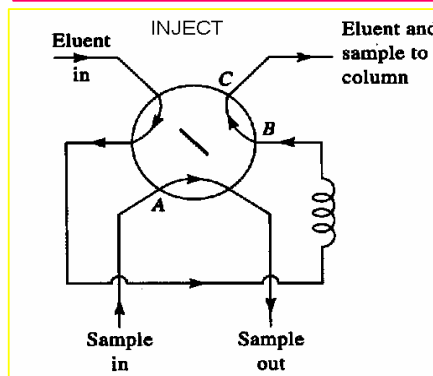


載入樣品



載入樣品

注入樣品



注入樣品

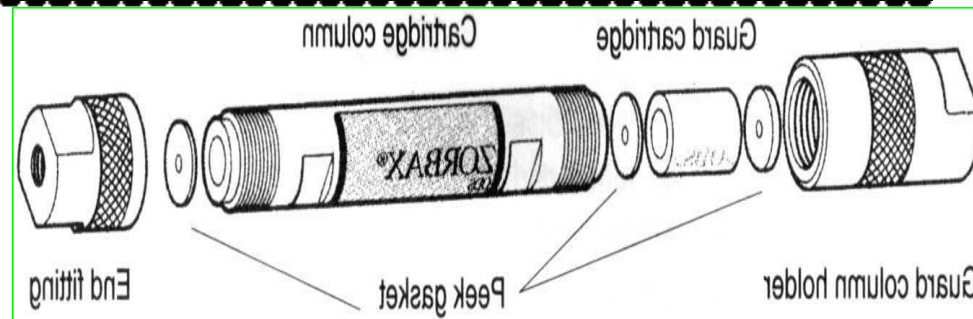


28C-4 LC Columns 分離系統



guard column
保護管柱/前置管柱
(pre-column)

- purpose: column protection
除去雜質避免污染
管柱分析
- 0.4 ~ 1 cm long
- larger stationary phase



填充材料
化學性質相同
大
dp
小

Analytical column
分析管柱

- stainless steel
- 25 cm long (10-30 cm)
- 4.6 mm i.d. (4-10 mm)
- packing 5 μ m dia.
- flow rates: mL ~ μ L/min
- N = 40,000 ~ 60,000 plates/m



High Speed Isocratic Separation 快速之等位沖提分離

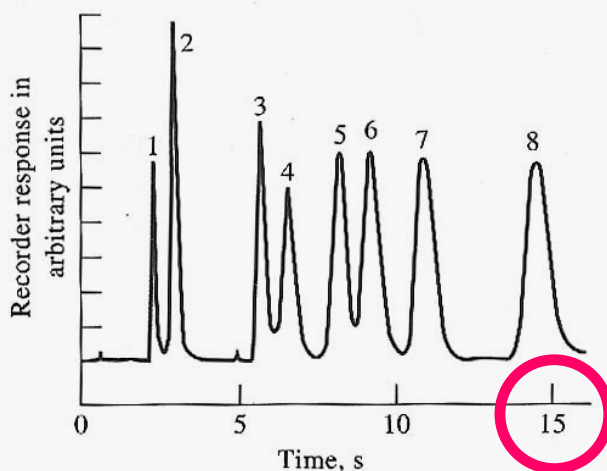


Figure 28-8 High-speed isocratic separation. Column dimensions: 4 cm length; 0.4 cm i.d. Packing: 3- μ m sperisorb. Mobile phase: 4.1% ethyl acetate in *n*-hexane. Compounds: (1) *p*-xylene, (2) anisole, (3) benzyl acetate, (4) dioctyl phthalate, (5) dipentyl phthalate, (6) dibutyl phthalate, (7) dipropyl phthalate, (8) diethyl phthalate. (From R. P. W. Scott, *Small Bore Liquid Chromatography Columns: Their Properties and Uses*, p. 156. New York: Wiley, 1984. Reprinted with permission of John Wiley & Sons, Inc.)

■ LC-column

標準

- 10-30 cm長，
- 4-10 mm i.d.
- Packing: 3-10 μ m
- $N = 40,000-60,000 \text{ m}^{-1}$

高速

- 3-7.5 cm長，
- 1-4.6 mm i.d.
- Packing: 3-5 μ m
- $N = 100,000 \text{ m}^{-1}$



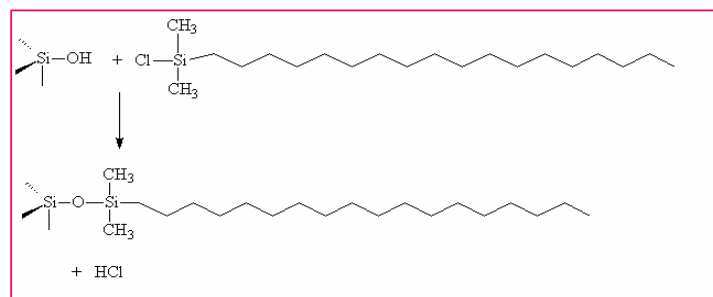
28C-5 Types of Columns Packings 管柱填充物類型

★ 薄層狀 (pellicular):

- ★ 圓球狀, 無孔洞
- ★ dp: 30 - 40 μm 的玻璃或聚合物球珠
- ★ 球珠表面沈積一層薄且多孔的矽酸鹽、氧化鋁、聚乙苯烯-二
乙基苯之合成樹脂

★ 多孔狀 (porous)

- ★ dp: 3 - 10 μm
- ★ 3 - 25 cm long, 2 - 5 mm i.d.
- ★ silica gel : 3 μm - 10 μm
- ★ reacted with groups to form stationary phase



18 烷基 ● octadecyl (reversed phase)

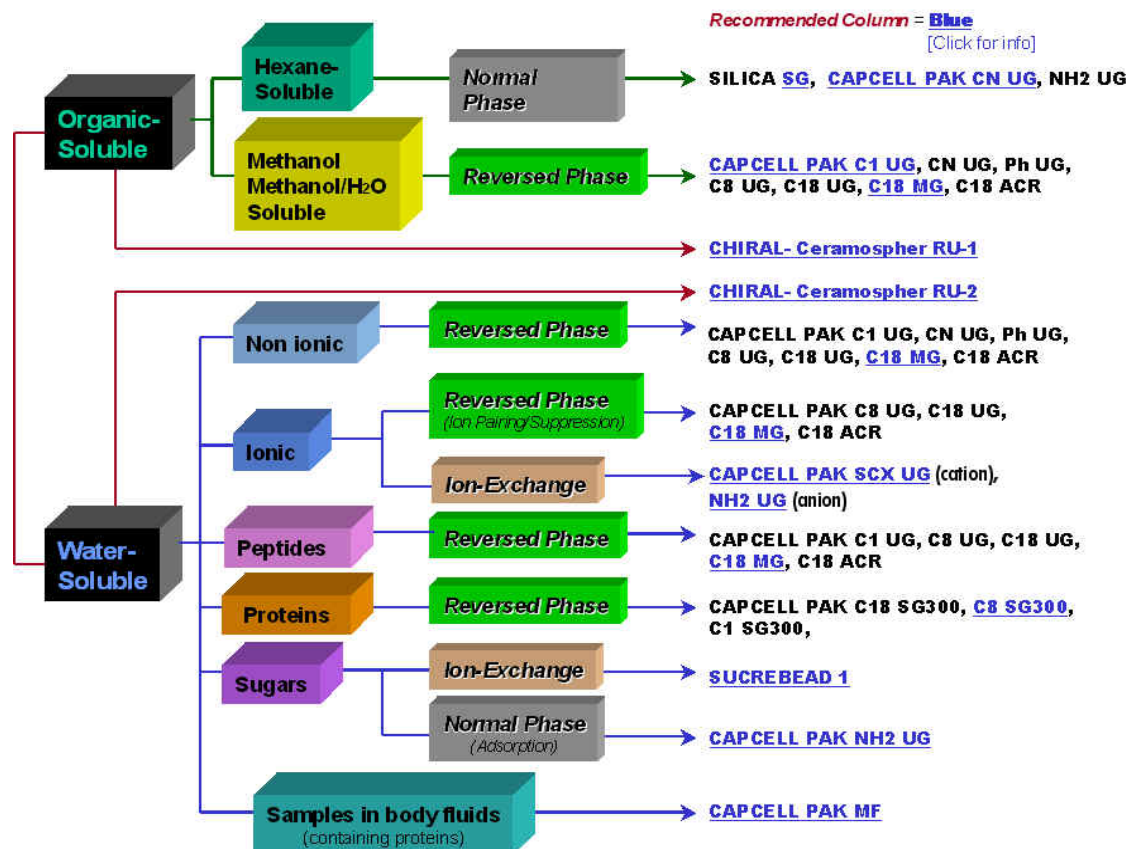
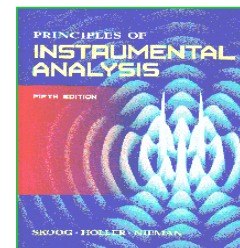
辛烷基 ● octyl (RP)

苯基 C_6H_5 ● phenyl (RP)

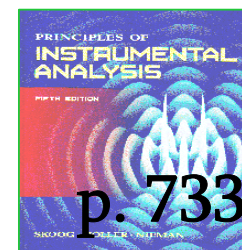
氰基 CN ● cyano (NP)



HPLC Column Selection



28C-6 LC Detectors 偵測器



Types of detectors :

★ 感應流動相的整體性質偵測器 (bulk property detectors)

★ 感應流動相之折射(Refractive)係數

★ 介電常數

★ 密度

★ 溶質性質偵測器 (solute property detectors)

★ 感應溶質之UV吸光度

★ 螢光值 (fluorescence detectors)

★ 擴散電流值



Multi-Dimensional LC Detectors

★ UV-visible

★ 71% :

- 39%利用汞放射譜線為基礎
- 13%利用氙光源之過濾性輻射為基礎
- 48%利用光柵形單光器之放射輻射為基礎

★ diode array detector (二極體列陣型)最有用

★ 紅外線吸收偵測器

★ Fluorescence

★ 15%

★ Refractive index (折射率偵測器)

★ 5.4%

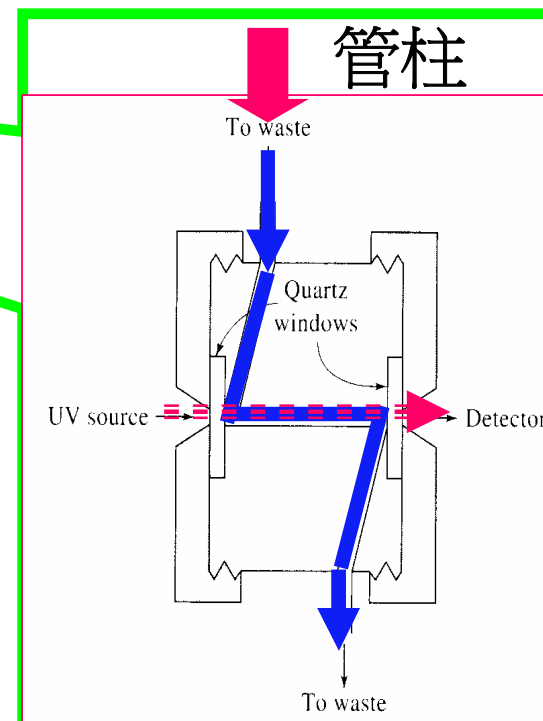
★ Electrochemical: 安培測定法、電導測定法、電量測定法

★ 4.3%

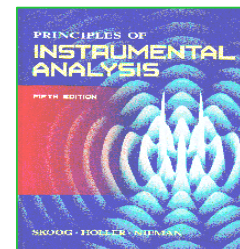
★ mass spectrometry (質譜)

★ electrospray ionization

★ atmospheric pressure ionization



HPLC: why high pressure?



$$H = A + \frac{B}{\bar{u}} + C \cdot \bar{u} = A + \frac{B}{\bar{u}} + (C_s + C_M) \bar{u}$$

B term negligible

C term important

$$C_M = \frac{f_M (k') d_p^2}{D_M}$$

Skoog 表 26-2, -3

$f_M(k')$: retention factor of analyte in mobile phase

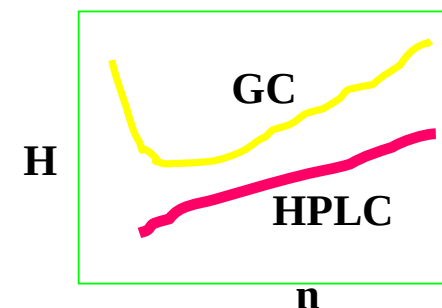
D_M : diffusion coefficient of analyte in mobile phase

d_p : diameter of packing particle

- particle size $d_p \searrow \Rightarrow H \searrow \Rightarrow$ better
- High pressure $\Rightarrow$ High Performance

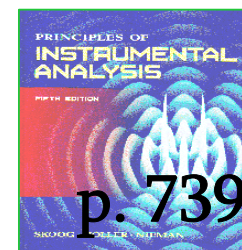
particle size $\searrow \Rightarrow$ resist to solvent flow $\Rightarrow$ need High Pressure
for flow rate of 0.5 ~ 5 mL/min, needs 7 ~ 40 MPa (70 ~ 400 atm)

- more uniform flow $\Rightarrow$ A term $\searrow \Rightarrow H \searrow$
- tighter packing $\Rightarrow$ shorter diffusion force $\Rightarrow C_M \searrow \Rightarrow H \searrow$

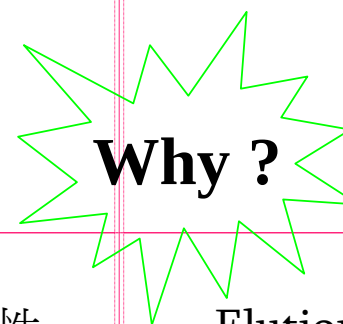


28 D Partition Chromatography

Bonded-phase P.C. vs. Liquid-liquid P.C.



Bonded-phase	固定相 p.739 矽氧烷	固定相	移動相	先出現成分	移動相極性↗時
Normal-phase	Packing: R in siloxane -C ₃ H ₆ -O-CH ₂ CHOH-CH ₂ OH (diol) -C ₃ H ₆ NH ₂ (amino) -C ₂ H ₄ CN (cyano)	極性	非極性 乙醚 二氯甲烷 氯仿 利用正己烷調整極性	非極性	Elution 變快
Reversed-phase	Packing: R in siloxane C ₈ chain (n-octyl) C ₁₈ chain (n-octyldecyl)	非極性	極性 甲醇 Acetonitrile-furan 利用水調整極性	極性	Elution 變慢



Effect of Mobile Phase Polarity in Partition Chromatography

正相層析:

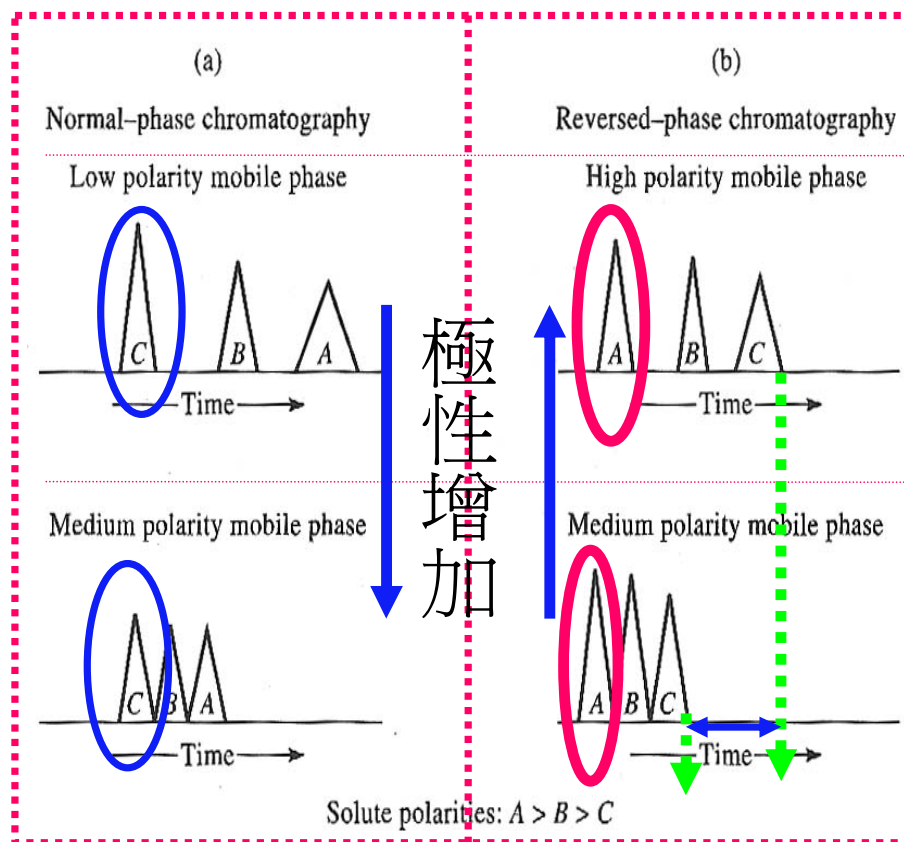
- 1.管柱: 極性
- 2.流動相: 非極性 (低極性)

非極性或低極性物質先離開管柱

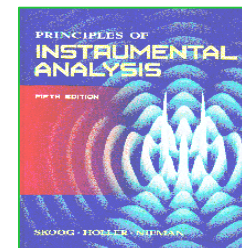
逆相層析:

- 1.管柱: 非極性 (碳氫化合物)
- 2.流動相: 極性

高極性物質先離開管柱



腦力激盪一下

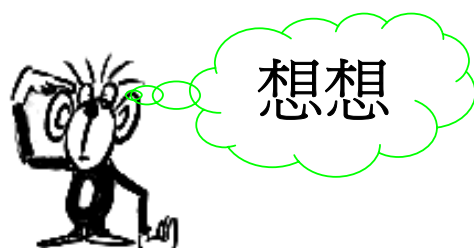


★ 逆相層析:流動相之極性增大

低極性
 C_M 極性
 低極性
 C_S

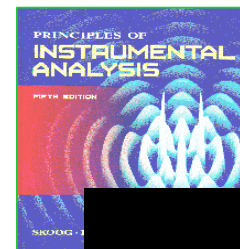
流動相極性增加 → 從固定相溶析出之非極性分子減少
 → C_M 減少 & C_S 增加 → $K = C_S/C_M$ 增加 → $k'_A = K_A \frac{V_S}{V_M}$
 k' 增加 → $k'_A = \frac{(t_R)_A - t_M}{t_M} = \frac{(t'_R)_A}{t_M}$ → t_R 增加

★ 正相層析:流動相之極性增大????



容量因子或滯留因子(k_A')

Capacity Factor or Retention Factor

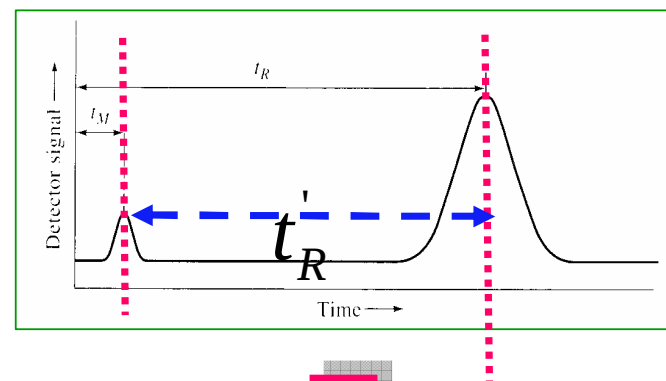


- ✳ 層析法中，容量因子 k_A' 是一個很重要之參數
- ✳ 容量因子 k_A' 是表示分析物種在管柱中的移動速率

對任何一管柱而言
此比值是固定

$$k'_A = K_A \left(\frac{V_S}{V_M} \right) \quad \text{for species A, unitless}$$

$$k'_A = \frac{t_R - t_M}{t_M} = \frac{(t'_R)_A}{t_M}$$

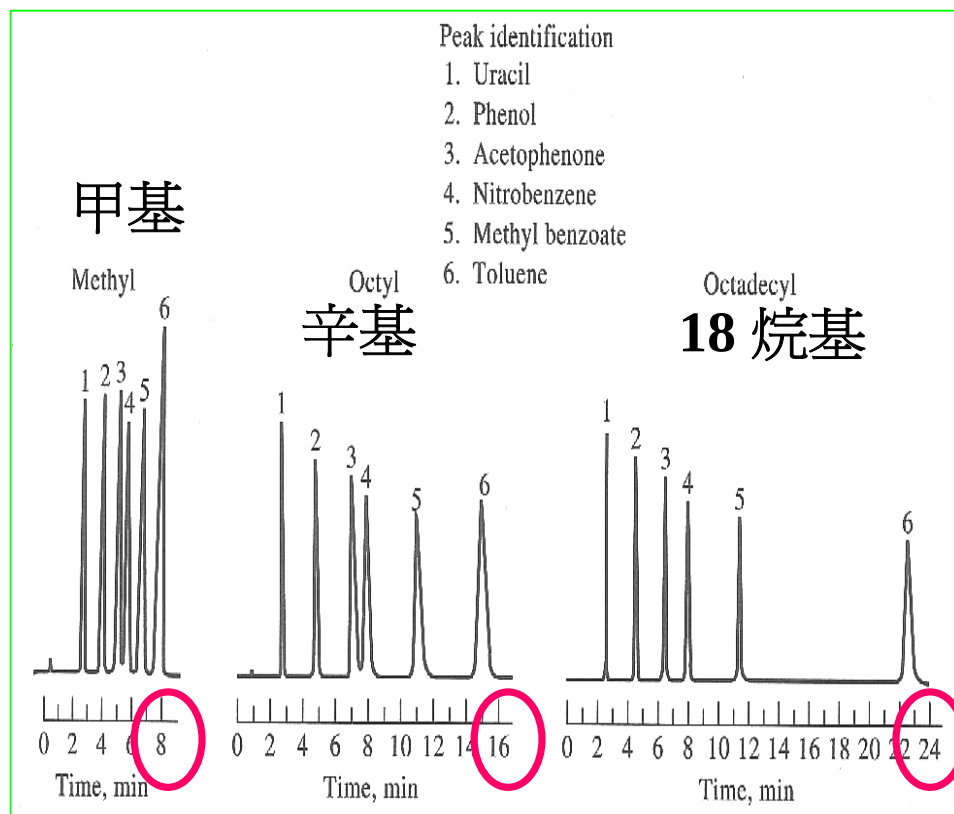


≤ 1.0 poor separation
 $2 \sim 10$ optimum
 > 30 too slow

t_M 及 t_R 可由層析圖得知



取代基鏈之長短 對逆相管柱效能之影響



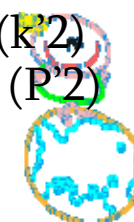
■ Reversed-Phase C.
管柱填充物質之
Longer chain compound
➡ t'_R ↗



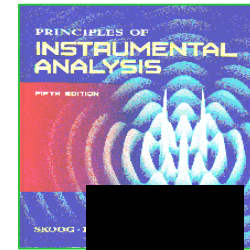
28 D-2 Method Development in Partition Chromatography 分配層析法的方法開發

HPLC固定相與移動相極性之選擇

- ★ $T'_R = f(N, k', \alpha)$ [先調整 k' ➡ 若peak仍發生重疊 ➡ 調整 α]
 k' : 移動相的組成比; α : 移動相的組成種類; 固定相的選擇
- ★ 相對極性大小: $RH < \text{ether (醚類)} < \text{ester (酯類)} < \text{ketone (酮類)} < \text{aldehyde (醛類)} < \text{amide (醯胺類)} < \text{amine (胺類)} < \text{alcohol (醇類)} < \text{water}$
 (Table 28-2)
- ★ Snyder: Polarity index (極性指數)
 - ★ P' = 一種定量溶劑極性之指標
 - ★ 可視為不同溶劑之相對極性的數值化測量
- ★ $P'_{AB} = \Phi_A P'_A + \Phi_B P'_B$ (Φ : mixed solvent 容積比)
 ★ P'_{AB} : A 溶劑與 B 溶劑混合後之極性指數
- ★ Normal-phase separation : $k'_2/k'_1 = 10^{(P'_1 - P'_2)/2}$
- ★ Reversed-phase separation : $k'_2/k'_1 = 10^{(P'_2 - P'_1)/2}$
 - ★ k'_1 與 k'_2 分別代表溶質(欲測物質)之起始 k' 值 (k'_1) 與最後之 k' 值 (k'_2)
 - ★ P'_1 與 P'_2 分別代表溶質(欲測物質)之起始 P' 值 (P'_1) 與最後之 P' 值 (P'_2)



26 B-5 相對移動速率：選擇因子 Relative Migration Rates: The Selectivity Factor



✧ 選擇性因子 α ：層析法中，判斷分析物種A及B在管柱中的分離效果。不同物種 $\rightarrow$ 不同 t_R $\rightarrow$ 達分離效果

✧ 又稱為相對滯留(relative retention)或分離因子(separation factor)

✧ 定義：
$$\alpha = \frac{K_B}{K_A} > 1$$

★ K_B ：較強滯留物種B(移動速率較慢)之分配係數

★ K_A ：較弱滯留物種A(移動速率較快)之分配係數

✧ 根據容量因子：
$$k'_A = \frac{K_A V_S}{V_M} \quad \text{同理 For } B \text{ 物種} \Rightarrow k'_B = \frac{K_B V_S}{V_M}$$

$$\therefore \alpha = \frac{k'_B}{k'_A} \quad \text{又已知} \quad k'_A = \frac{(t_R)_A - t_M}{t_M} \quad \& \quad k'_B = \frac{(t_R)_B - t_M}{t_M}$$

$$\Rightarrow \alpha = \frac{(t_R)_B - t_M}{(t_R)_A - t_M} = \frac{(t'_R)_B}{(t'_R)_A}$$



EXAMPLE 28 - 1

In a reversed-phase column, a solute was found to have a retention time of 31.3 min, while an unre-
tained species required 0.48 min for elution when the
mobile phase was 30% (by volume) methanol and
70% water. Calculate (a) k' and (b) a water/methanol
composition that should bring k' to a value of about
5.

(a) Application of Equation 26-8 yields

$$k' = (31.3 - 0.48)/0.48 = 64 \quad k'_1$$

(b) To obtain P' for the mobile phase we substitute
polarity indexes for methanol and water from
Table 28-2 into Equation 28-2 to give P'_1

$$P' = 0.30 \times 5.1 + 0.70 \times 10.2 = 8.7$$

Substitution of this result into Equation 28-4
gives **Reversed-phase separation** :

$$\frac{k'_2}{k'_1} = 10^{(P'_2 - P'_1)/2}$$

$$\frac{5}{64} = 10^{(P'_2 - 8.7)/2}$$

Taking the log of both sides of this equation
gives

$$-1.11 = \frac{P'_2 - 8.7}{2} = 0.5 P'_2 - 4.35$$

$$P'_2 = 6.5$$

Letting x be the volume fraction methanol in the
new solvent mixture and substituting again into
Equation 28-2, we find

$$6.5 = x \times 5.1 + (1 - x)10.2$$

$$x = 0.73 \text{ or } 73\%$$

Thus, a 73% methanol/27% water mixture
should provide the desired value of k' .

TABLE 28-2 Properties of Common Chromatographic Mobile Phases

Solvent	Refractive Index ^a	Viscosity, cP ^b	Boiling Point, °C	Polarity Index, P'	Eluent Strength, ϵ^b
Fluoroalkanes ^d	1.27-1.29	0.4-2.6	50-174	<-2	-0.25
Cyclohexane	1.423	0.90	81	0.04	-0.2
<i>n</i> -Hexane	1.372	0.30	69	0.1	0.01
1-Chlorobutane	1.400	0.42	78	1.0	0.26
Carbon tetrachloride	1.457	0.90	77	1.6	0.18
<i>i</i> -Propyl ether	1.365	0.38	68	2.4	0.28
Toluene	1.494	0.55	110	2.4	0.29
Diethyl ether	1.350	0.24	35	2.8	0.38
Tetrahydrofuran	1.405	0.46	66	4.0	0.57
Chloroform	1.443	0.53	61	4.1	0.40
Ethanol	1.359	1.08	78	4.3	0.88
Ethyl acetate	1.370	0.43	77	4.4	0.58
Dioxane	1.420	1.2	101	4.8	0.56
Methanol	1.326	0.54	65	5.1	0.95
Acetonitrile	1.341	0.34	82	5.8	0.65
Nitromethane	1.380	0.61	101	6.0	0.64
Ethylene glycol	1.431	16.5	182	6.9	1.11
Water	1.333	0.89	100	10.2	Large

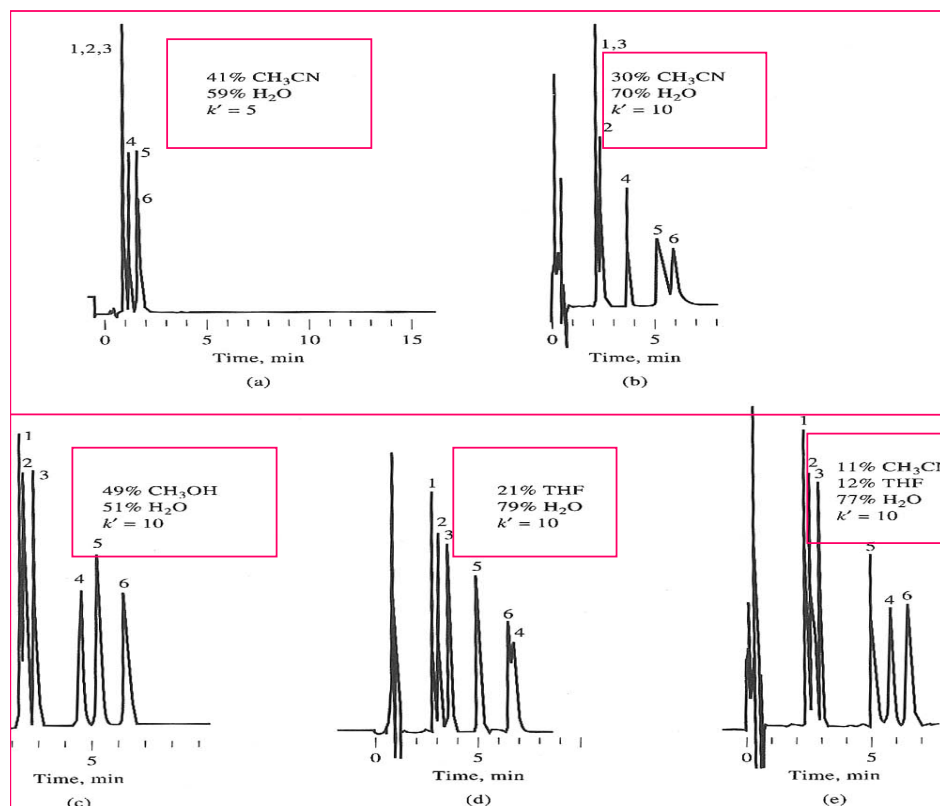


溶劑能力對滯留因子之影響

流動相對選擇因子之影響

6 種固醇類之分離操作

逆相之操作



k' : 移動相的組成比

溶劑能力對滯留因子(k')之影響

■ 改變水的量

改變流動相之極性

α : 移動相的組成種類

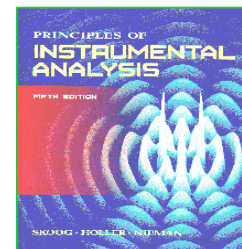
流動相對選擇因子(α)之影響

■ 流動相之極性改變了溶質 t_M

➤ 不同流動相之組成層析
結果不同



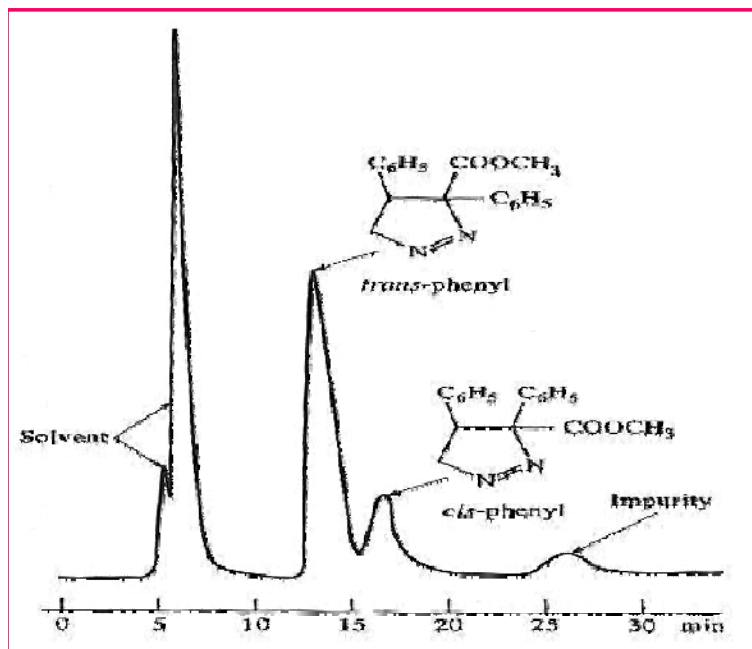
Liquid Chromatography



- Adsorption Chromatography
- Ion Chromatography
- Size Exclusion Chromatography



28E Adsorption Chromatography 吸附層析法

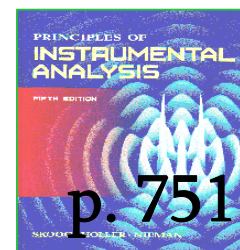


由 Tswett 提出:

- 固定相：silica (較常用) 或 氧化鋁
- 移動相：溶劑之能力
 - P' (極性指標)
 - Eluent strength (沖提劑能力): ϵ^0
 - 單位溶劑面積之吸附能量
 - 隨吸附物不同而改變
- 溶劑系統之選擇: 類似分配層析法
- 適於分離：
isomeric mixture (異構物)

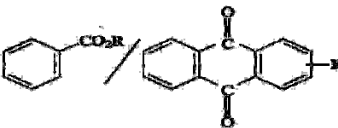
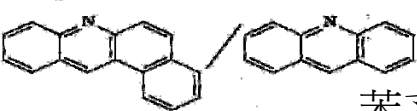
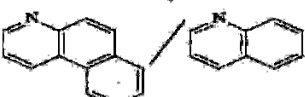
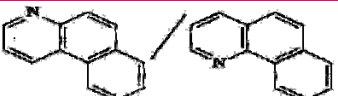
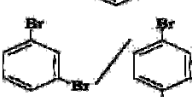
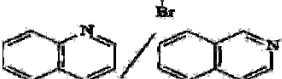


吸附層析法 與 分配層析法之應用選擇



p. 751

TABLE 28-5 Comparison of Selectivities of Adsorption and Reversed-Phase Chromatography*

Separation of	Compound	Adsorption R _f	Reversed-Phase R _f
Homologs	 同一系列化合物		
	R = C ₁	4.8	3.3
	C ₂	4.1	6.5
	C ₄	3.6	17
	C ₁₀		
Benzoate	 苯系列化合物	1.2	1.4
		1.1	1.8
Isomers		12.5	1.06
異構物		1.8	
		3.4	
	1,2,3,4, dibenzanthracene, C ₂₂ H ₁₄ /Picosin C ₂₂ H ₁₄	20	

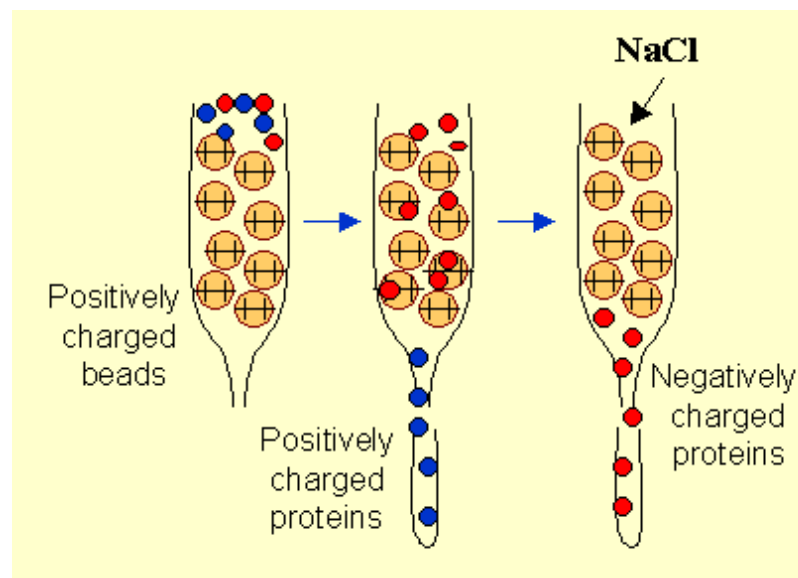
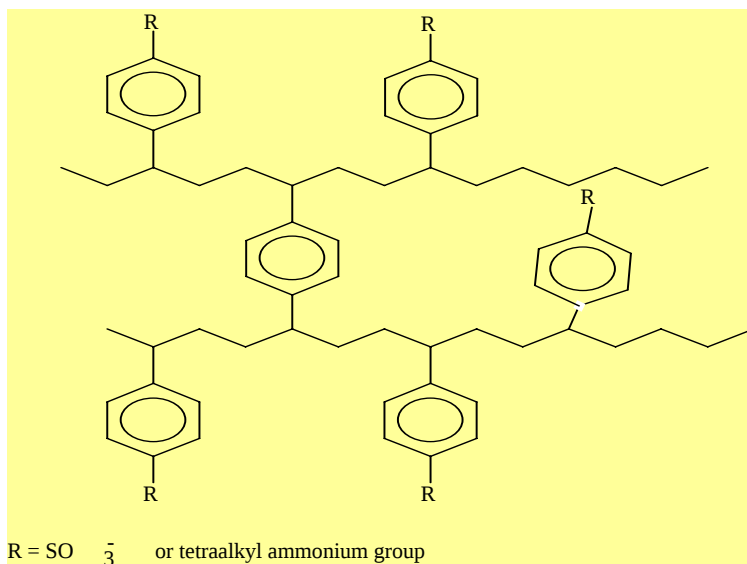
*Data from: L. R. Snyder and J. J. Kirkland, *Introduction to Modern Liquid Chromatography*, 2nd ed., pp. 357-358, New York: Wiley, 1979.

Adsorption
Chromatography
for Isomeric
Mixture



Ion Exchange Chromatography

- 一般以水作為流動相，離子交換樹脂為固定相，來分離及鑑定離子的一種層析法
- Cross-linked styrene divinyl benzene - Percentage of divinyl benzene controls degree of cross-linking, where 4 - 16 % is typical



Ion Exchange Chromatography

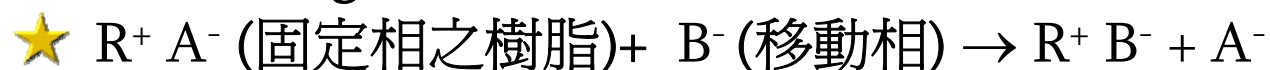
★ Cation Exchange:

- ★ $R^- A^+ (\text{固定相之樹脂}) + B^+ (\text{移動相}) \rightarrow R^- B^+ + A^+$
- ★ 陽離子交換樹脂主要有:
 - sulfonate (strongly acidic): 磺酸基型 $R-SO_3^- H^+$
 - carbonate (weakly acidic, used for pH's > 6): 羧基型 $R-COO^- H^+$
- ★ Relative retention: $3^+ \gg Ba^{2+} > Pb^{2+} > Sr^{2+} > Ca^{2+} > Ni^{2+} > Cd^{2+} > Cu^{2+} > Co^{2+} > Zn^{2+} > Mg^{2+} > UO_2^{2+} \gg Tl^+ > Ag^+ > Cs^+ > Rb^+ > K^+ > NH_4^+ > Na^+ > H^+ > Li^+$
- ★ separate organic bases based on relative K_b values - use an increasing pH gradient.



Ion Exchange Chromatography

★ Anion Exchange:



★ 陰離子交換樹脂主要有:

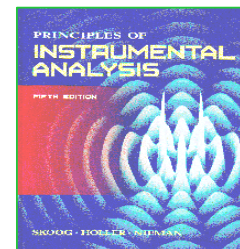
● trialkyl ammonium (strongly basic): 四級胺基型 $R-N(CH_3)_3^+ OH^-$

● dialkyl ammonium (weakly basic, used for pH's < 8):
一級胺基型 $R-NH_3^+ OH^-$

★ Relative retention: citrate > sulfate > ^{草酸}oxalate > chromate
> Br^- > SCN^- > Cl^- > formate > acetate > OH^- > F^- ^{鉻酸}
蟻酸



Mobile Phase: H₂O+electrolytes

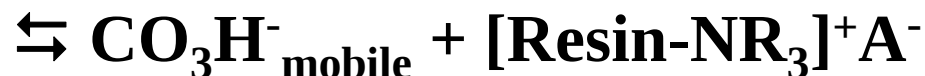


- **Cation Exchanger**



mobile phases: acidic, aqueous or MeOH

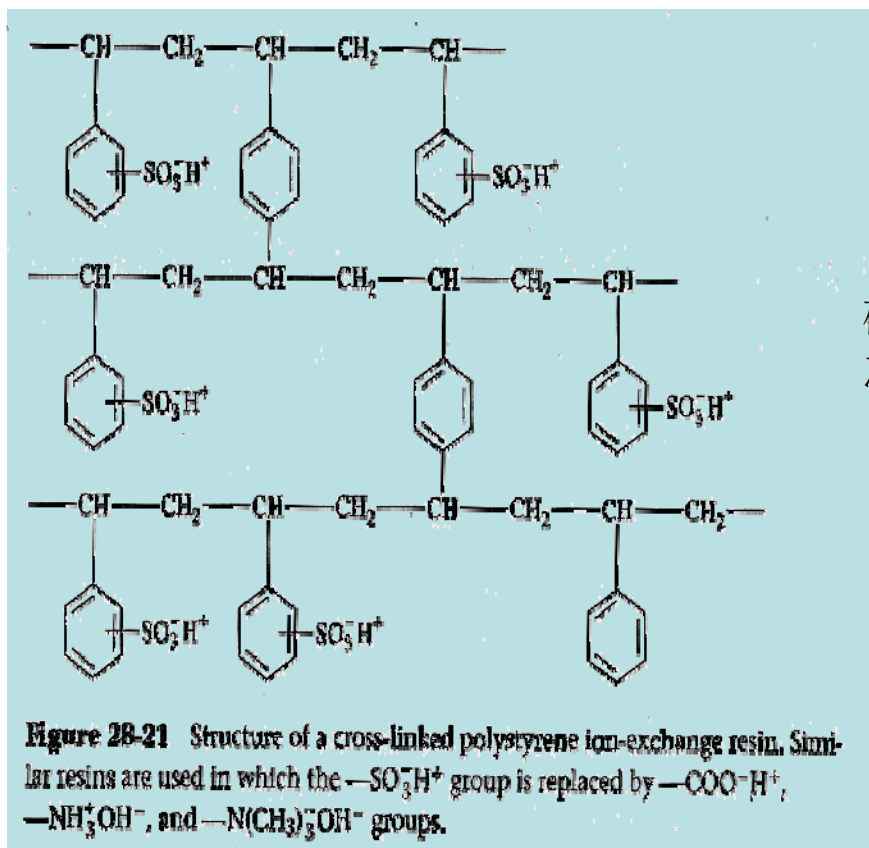
- **Anion Exchanger**



mobile phases: Na₂CO₃/NaHCO₃



Ion Chromatography



■ 固定相：

➤ Natural ion exchanger:

□ Clay (黏土)

□ Zeolite (矽藻土)

矽藻土係為火山矽藻熔岩沉積於海底所形成之地層土，其本身為有多孔質物

➤ Synthetic ion-exchange resin:

□ Cation :

✓ $-\text{SO}_3^-\text{H}^+$, $-\text{COO}^-\text{H}^+$

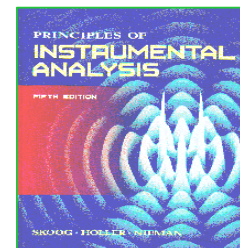
□ Anion :

✓ $-\text{N}(\text{CH}_3)_3^+\text{OH}^-$,

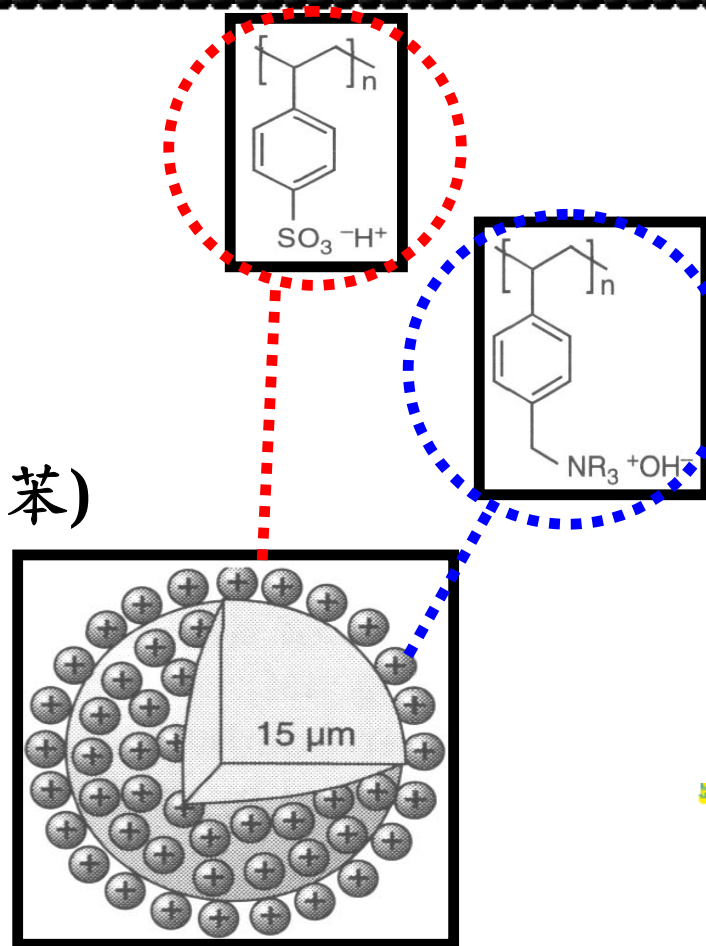
NH_3^+OH^-



Stationary Phase

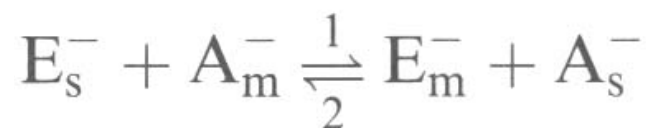


- **Modified silica:**
covalent bonding
 $(\text{silica-R}'\text{-NR}_3)^+\text{OH}^-$
- **Film Resin: Latex**
- **Synthetic copolymer**
 - polystyrene (聚苯乙烯)
 - divinylbenzene (二乙烯苯)
- **chemically modified to carry charges**



Ion Exchange Mechanism

e.g. anion exchange



- exchange constant and partition constant

$$\alpha = f(K)$$

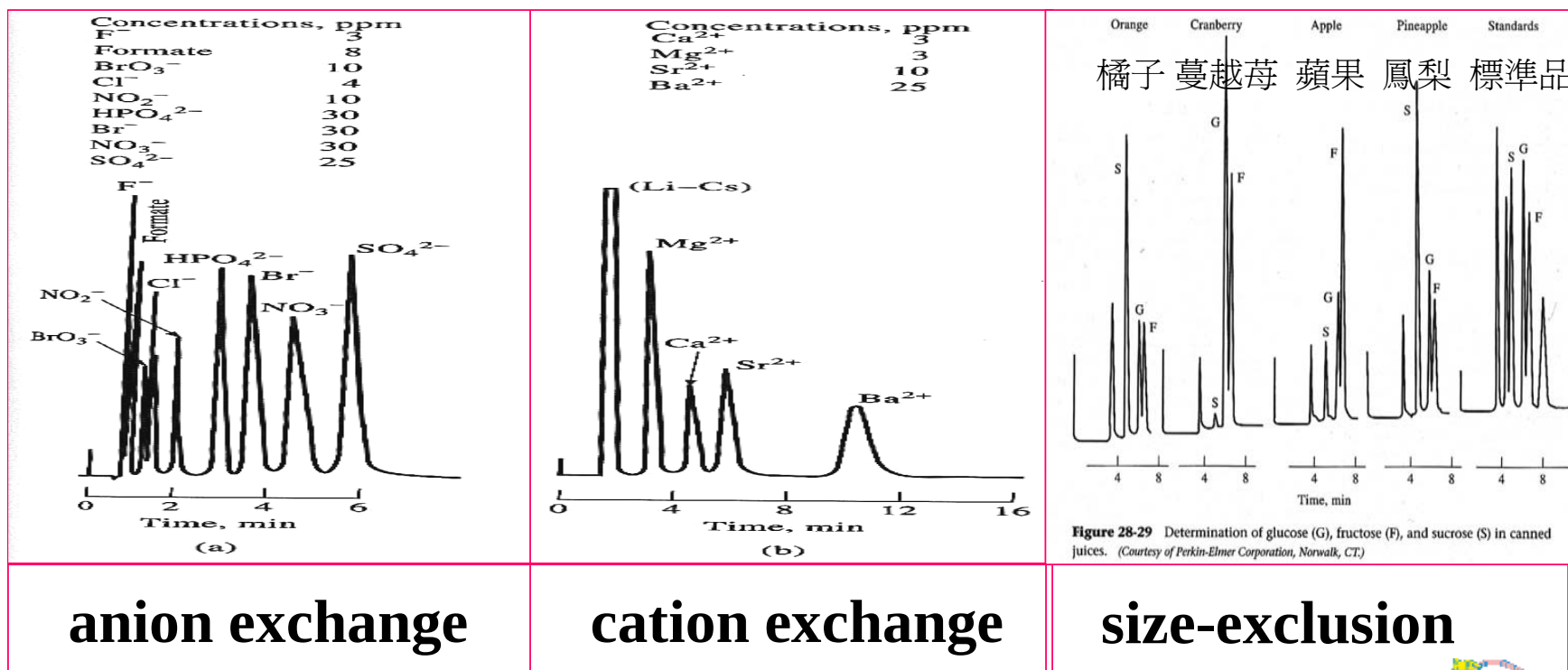
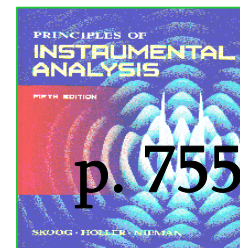
$$\alpha = \frac{[E^-]_m \cdot [A^-]_s}{[E^-]_s \cdot [A^-]_m} = \frac{1}{K_E} \cdot K_A = \frac{K_A}{K_E}$$

$$K_X = \frac{\text{moles of X per g of stationary phase}}{\text{moles of X per ml of mobile phase}}$$

- $K_A \uparrow \Rightarrow \alpha_A \uparrow \Rightarrow \text{retention time} \uparrow$

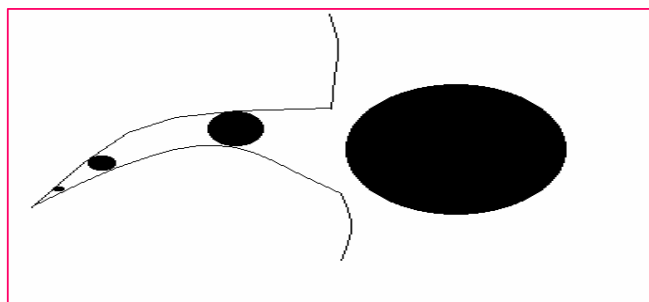


Ion Chromatography

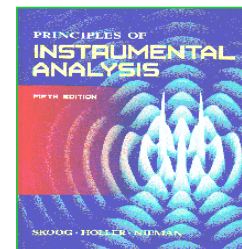


28G Size Exclusion Chromatography 大小排除層析法

- ✴ based on the partial exclusion of large molecules from some fraction of the pore volume
- ✴ surface of the solid support is assumed to not interact with the solutes
- ✴ gel filtration (膠體過濾) or gel permeation (膠體穿透)
- ✴ big molecules come out 1st; little molecules come out last



Size-Exclusion Chromatography



■ 固定相： $10\ \mu\text{m}$ silica or polymer particles
with pore size $10^2\text{-}10^6\text{\AA}$

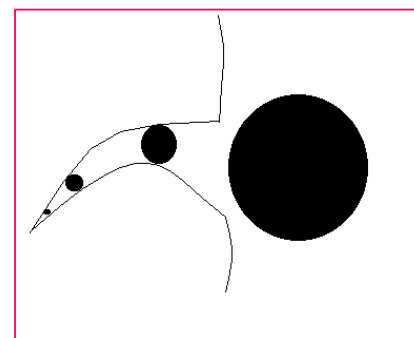
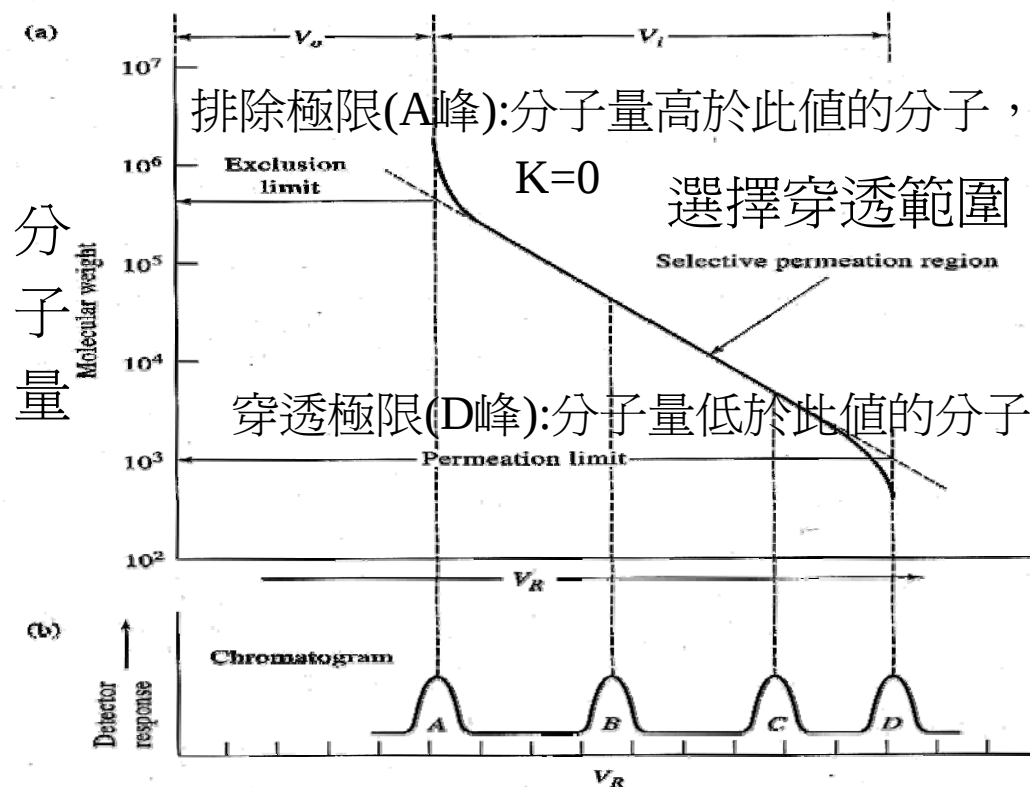
■ Type：

- Gel Filtration (膠體過濾)
stationary phase: hydrophilic (親水性)
mobile phase: aqueous
適用: protein, amino acid, peptide.
- Gel Permeation (膠體穿透)
stationary phase: hydrophobic (疏水性)
mobile phase: nonpolar solvent
適用: oligomers

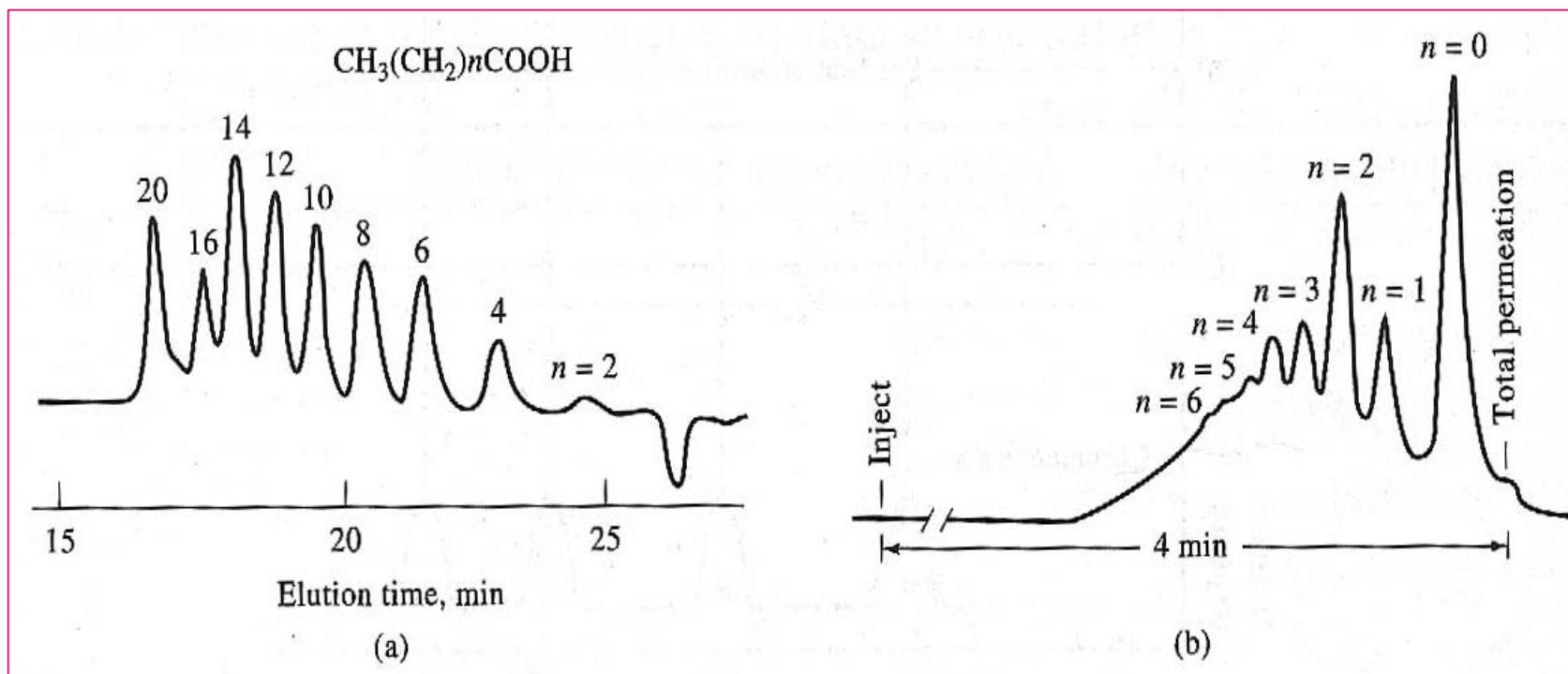


Size-Exclusion Chromatography

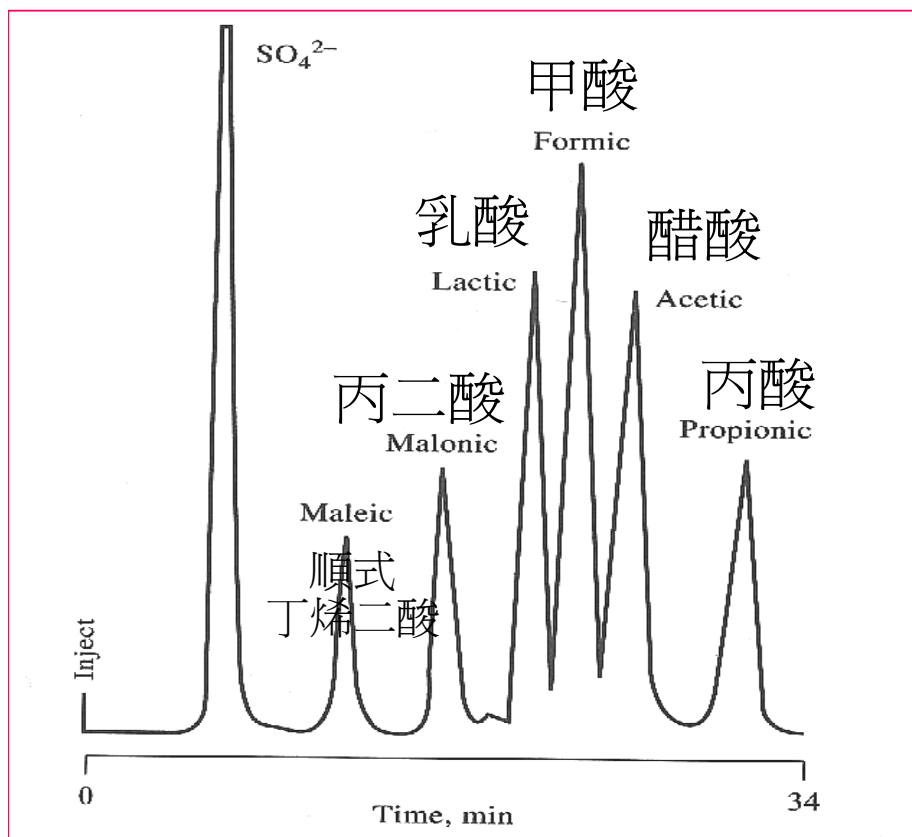
大小排除管柱之校正曲線



Size-Exclusion Chromatography 大小排除層析法之應用



Ion-Exclusion Chromatography 離子排除層析法



離子排除層析法

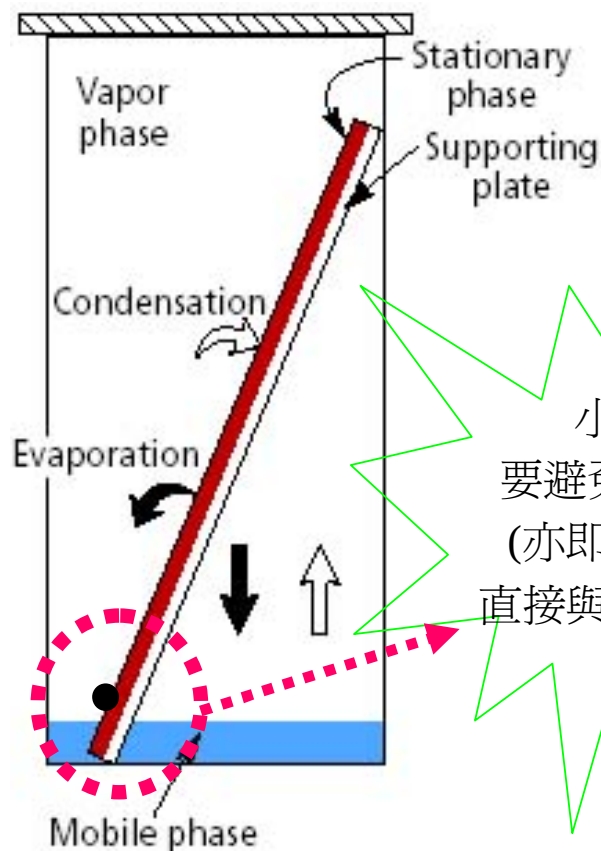
- 分離的是電中性物質，而非離子，不屬於離子層析
- 使用離子交換管柱
- 適合分離簡單的羧酸類



28H Thin-Layer Chromatography 薄層分析法

(Planar Chromatography)

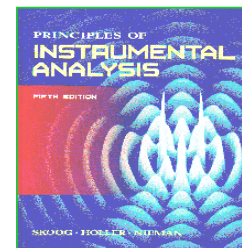
- Capillary action
- Coating: $100\ \mu\text{m}$
- Particle: $5\ \mu\text{m}$
- $N = 2000 / 12\ \text{cm}$



小心！
要避免展佈劑
(亦即流動相)
直接與樣品接觸



Sample Application 樣品之施用



★樣品濃度範圍: 0.01-0.1%

★對於濃度較稀之樣品，在一次點完乾燥後可重複施加

★樣品點的位置: 離平板邊緣 1-2 cm

★點的最小直徑:

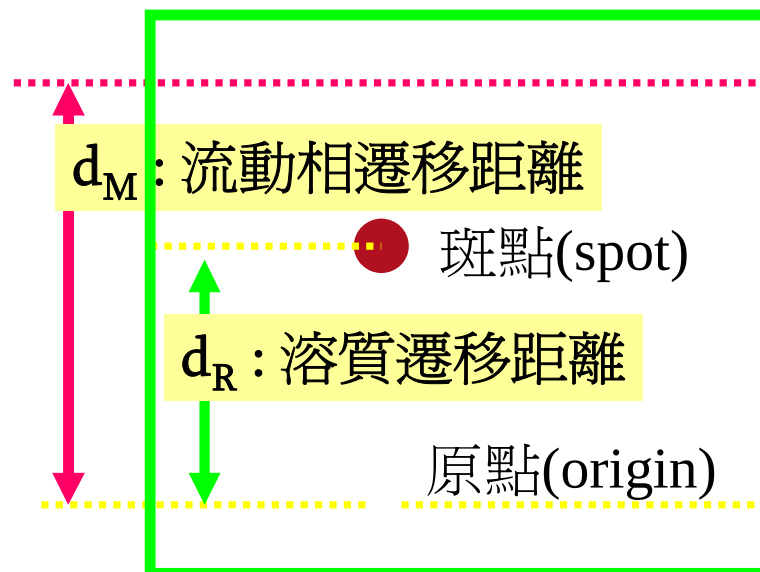
★定性: 約 5 mm

★定量: < 5 mm

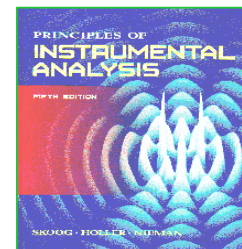
★樣品點的方式

★毛細管

★皮下注射針



Performance Characteristics of Thin-Layer Plates



★ 阻滯因子 R_F (Retardation factor)

★ $R_F = d_R / d_M$ (由 1 開始變化, 最佳值 0.4-0.5)

★ 有效數字只有一位

★ 影響因子:

- 溶質與流動相(展開相)之性質
- 固定相性質
- 溫度、水分
- 展開方式與距離

★ 滯留因子(Retention factor)

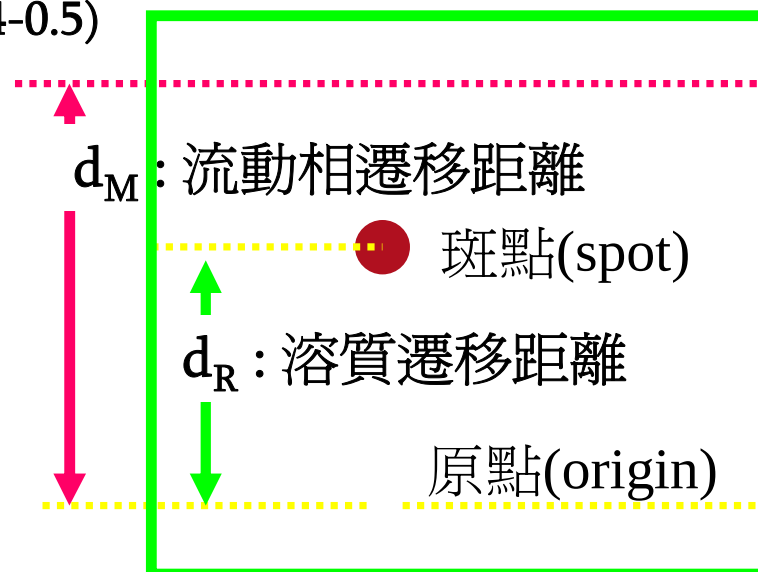
★ t_M (溶劑時間) = d_R / u

★ t_R (溶質時間) = d_M / u

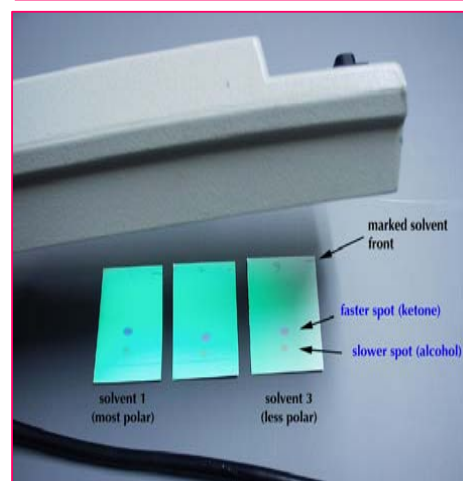
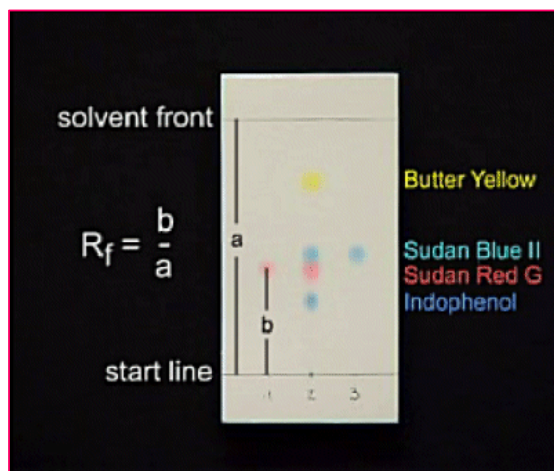
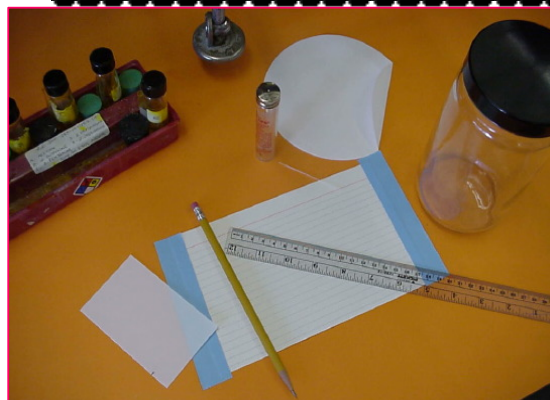
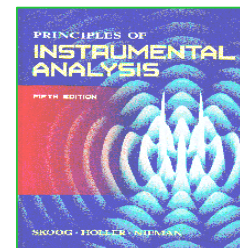
★ $k' = (t_R - t_M) / t_M = (d_M - d_R) / d_R$
 $= (1 - R_F) / R_F$

★ 相對滯留因子(relative Retention factor), R_x

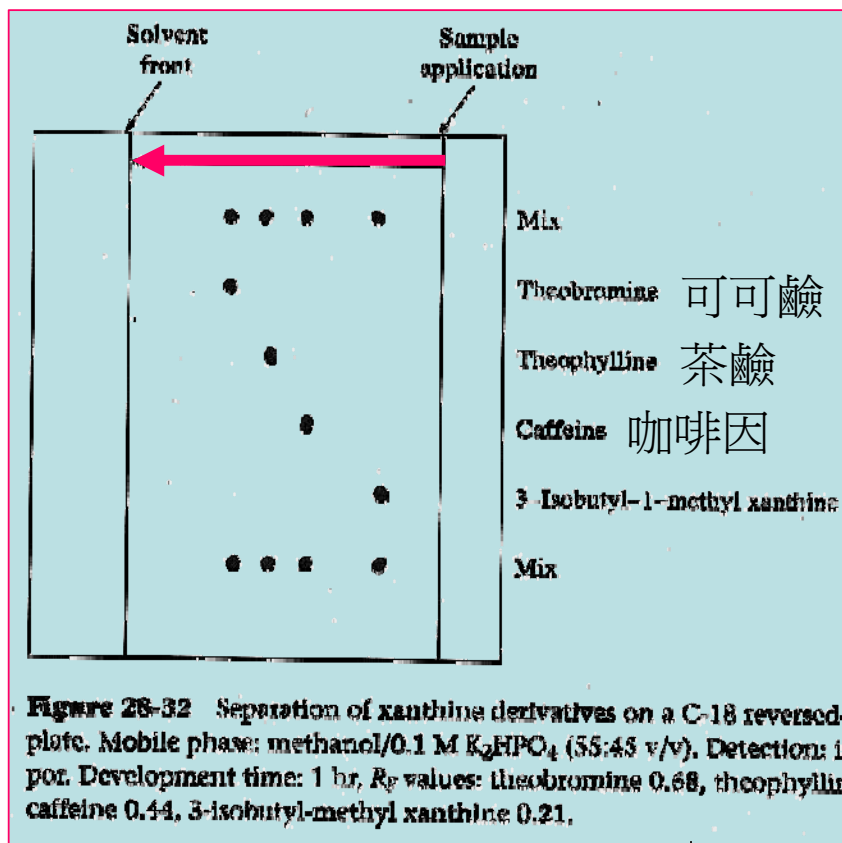
★ $R_x = \text{分析物之移動距離} / \text{標準物之移動距離}$



Thin-Layer Chromatography 薄層分析法



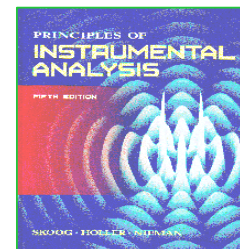
Thin-Layer Chromatography 薄層分析法



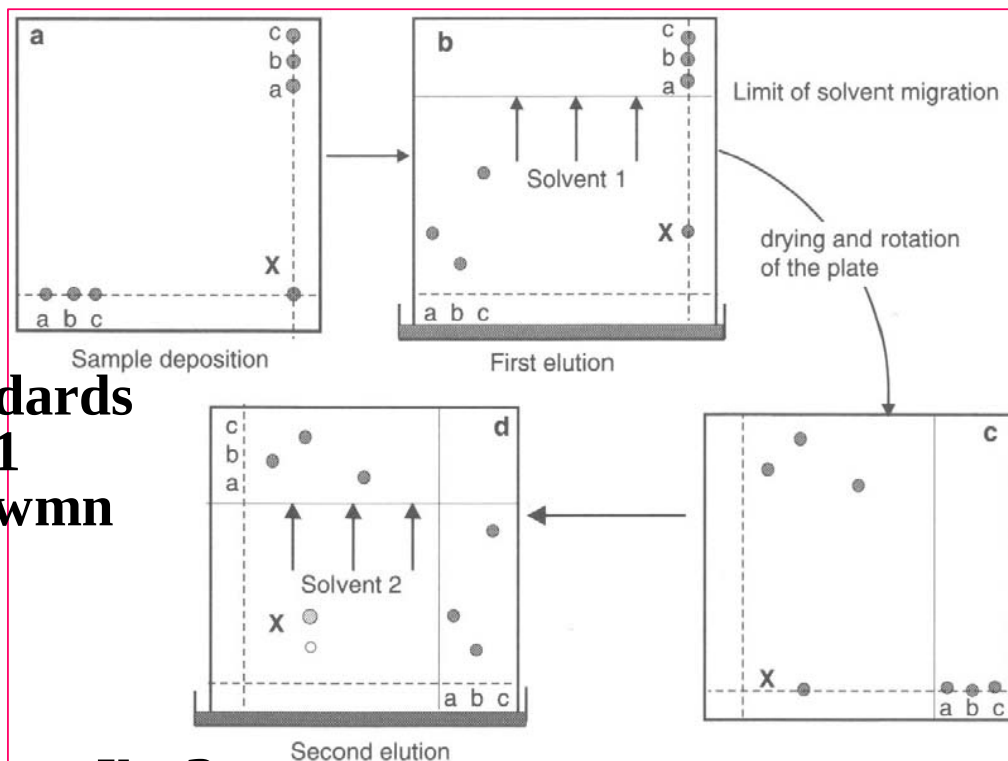
3-異丁基-甲基黃腺呤



Two-Dimensional Thin-Layer Chromatography 二維薄層分析法



3 standards
+1
unkown



$x \sim a$

impurity $\neq b$ or c



Two-Dimensional Thin-Layer Chromatography 二維薄層分析法

