Rational Method Development in Solid-Phase Isolation Techniques

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Solid-Phase Isolation Techniques

- Solid-Phase Extraction
- Open Column Chromatography
- Low- and Medium-Pressure Chromatography
- High-Pressure Chromatography

Type	Particle size (μm)	Pressure (atm)	Flow Rate (mL/min)	Sample load (g)
Open column	65-200	1	1-5	0.01-100
Flash chromatography	40-63	1-2	5-10	0.01-100
Low pressure	40-63	1-5	1-4	1-5
Medium pressure	15-40	5-20	3-16	0.05-100
High pressure	5-30	> 20	2-20	0.01-1

Operating costs and equipment requirements increase in descending order

Process Considerations

Solid-Phase Isolation Techniques are based on the transfer of compounds from a gas or liquid phase with retention (sorption) on a solid phase

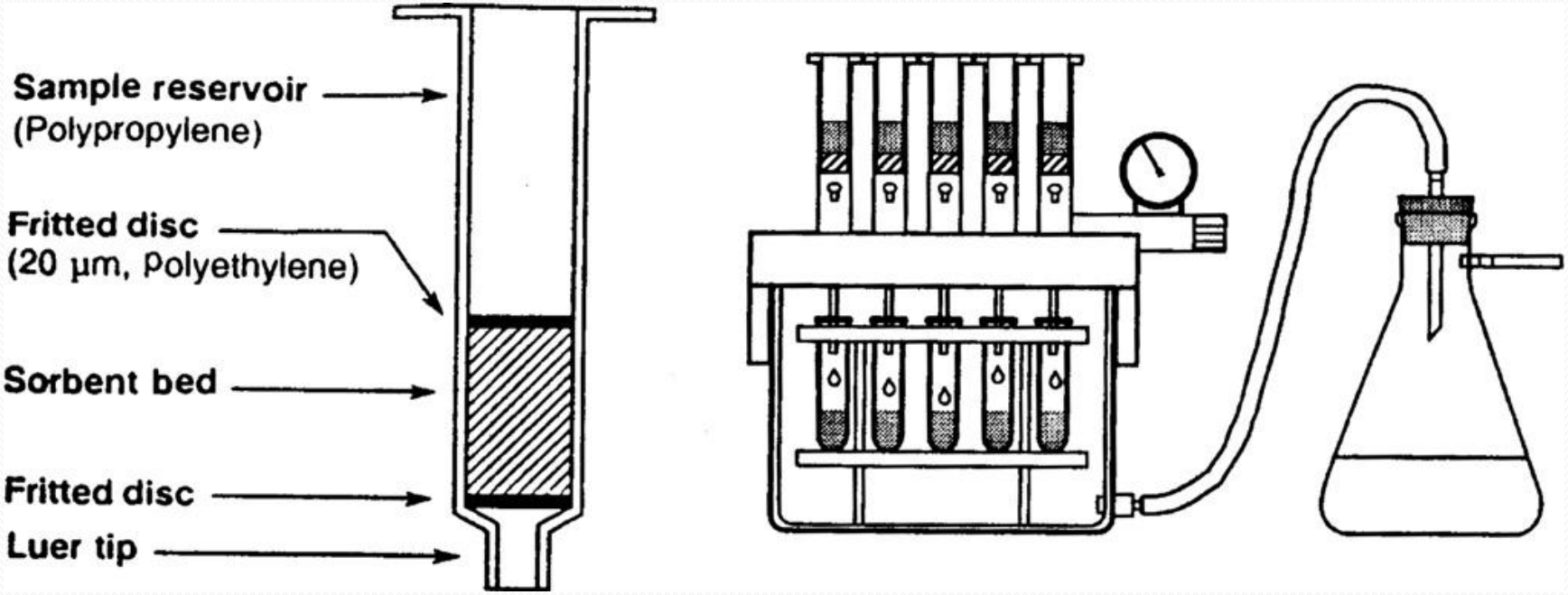
Isolated compounds are recovered by elution with a strong solvent or thermal desorption into the gas phase after separation of the solid-phase from the sample

Purpose

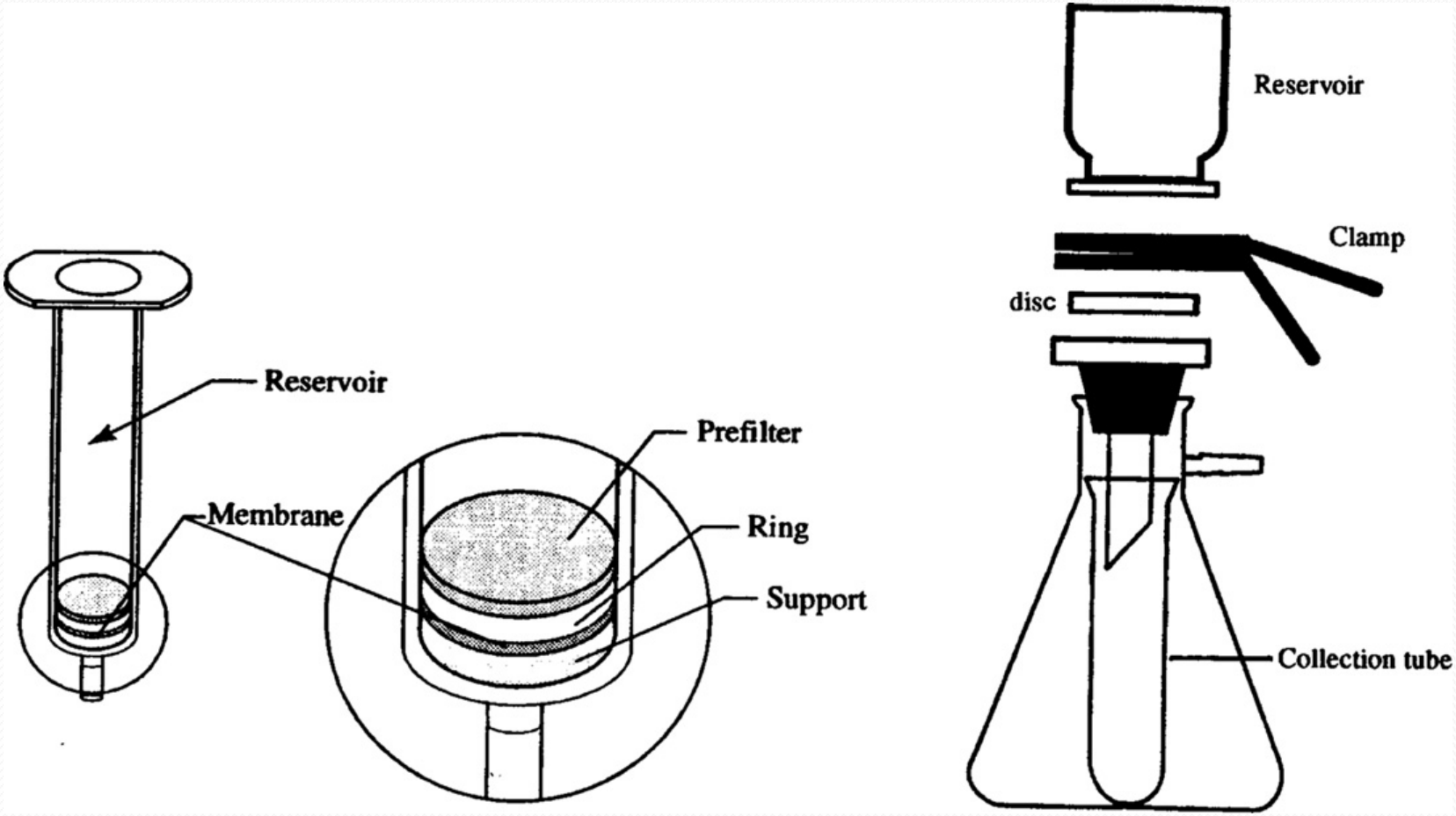
- **Solid-Phase Extraction** (Analytical Technique)
  - Trace enrichment (concentration)
  - Matrix simplification (sample cleanup)
  - Medium exchange

- **Flash Chromatography** (Preparative Technique)
  - Purification of synthetic products
  - Isolation of target compounds from natural products
  - Simplification of complex mixtures for high-resolution preparative chromatography

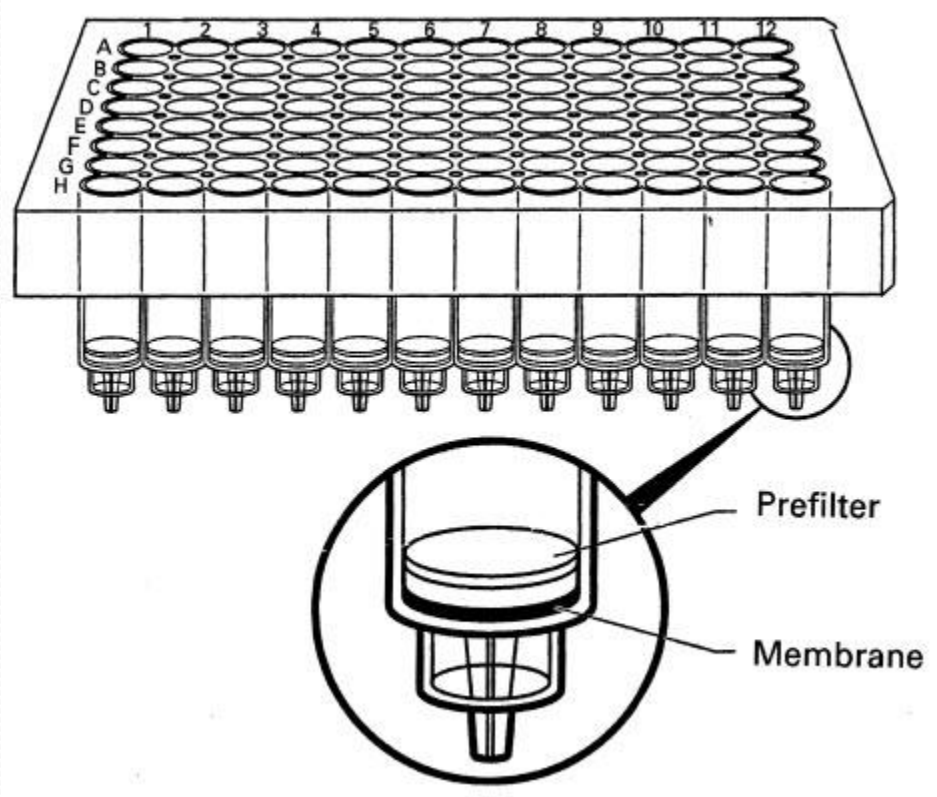
Solid-Phase Extraction Cartridge Format



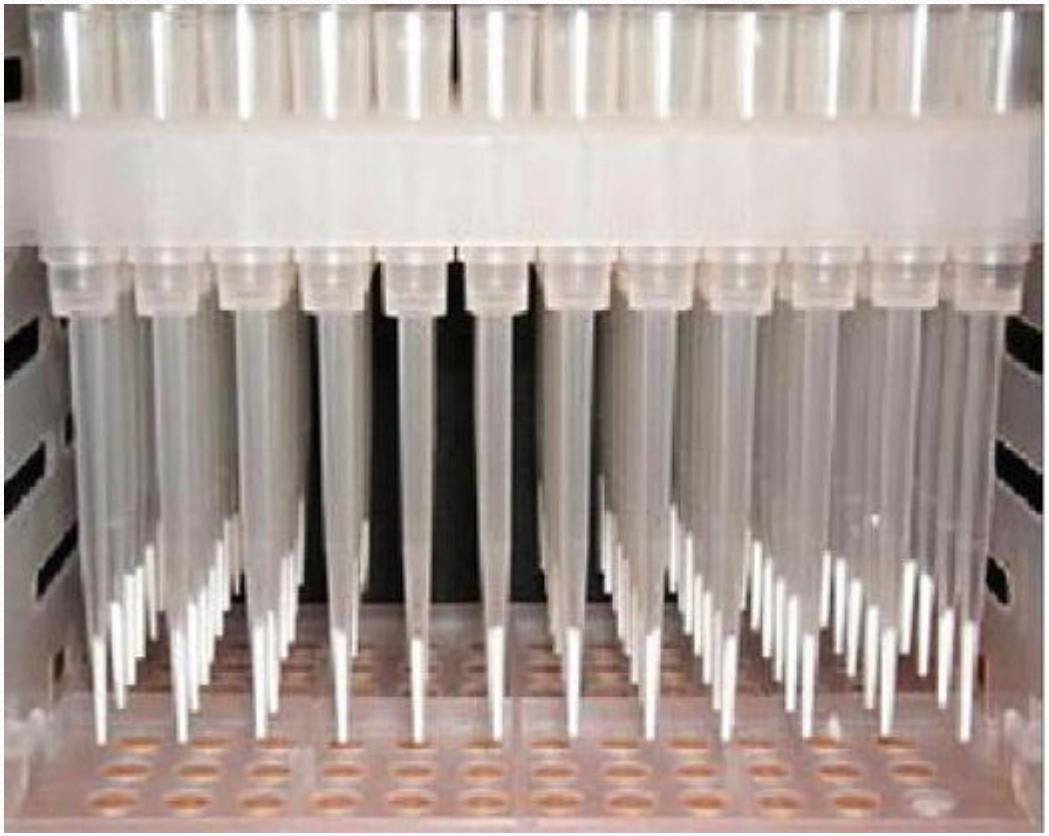
Solid-Phase Extraction Disk Format



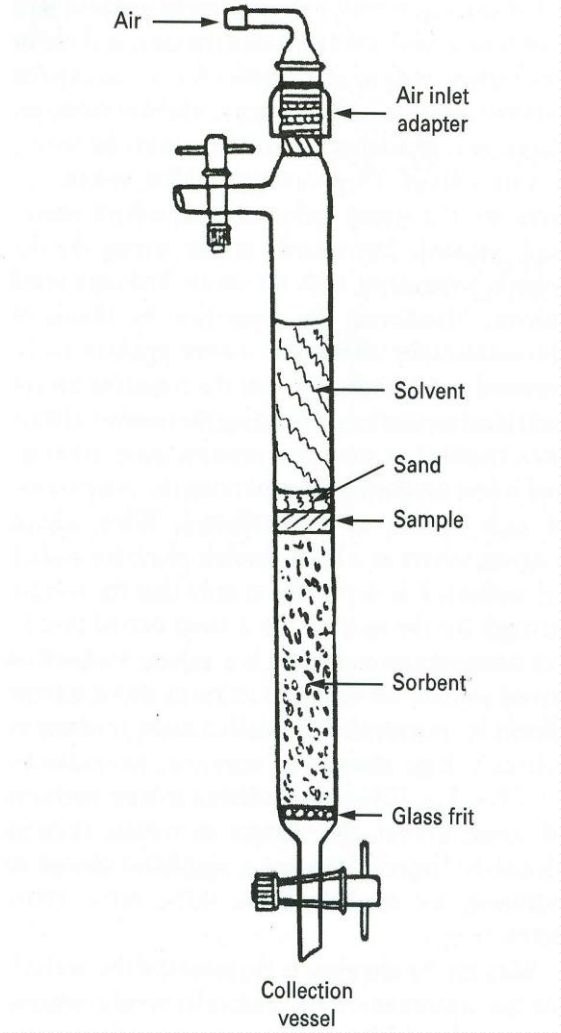
Solid-Phase Extraction Multiwell Plate

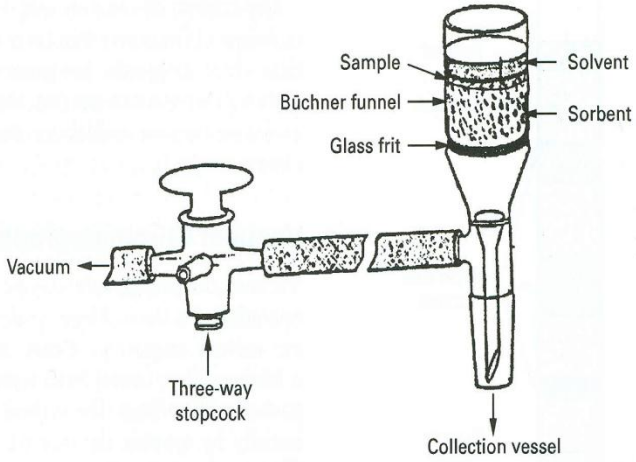


Solid-Phase Extraction Micropipette



Flash Chromatography





Flash Chromatography

- **Stationary Phases**
  - Silica gel (occasionally alumina)
  - Chemically bonded phases (high-value products)
- **Particle Size**
  - Typically 40-63 μm (higher resolution 20-40 μm)
- Bed Height
  - 10-15 cm (rarely > 30 cm)
  - Increase column diameter to increase sample capacity
- **Optimum Mobile Phase Velocity**
  - 5 cm/min
- **Sample Loading**
  - 1-2 g/g sorbent

Flash Chromatography

Method Development (TLC)

- Solvent strength adjusted to provide $R_F \approx 0.35$
  - Zone of interest or center zone in a mixture

- Find most selective solvent for separation
  - Identified by PRISMA model
  - $\Delta R_F \approx 0.2$ (high sample loading possible)
  - $\Delta R_F < 0.1$ (low sample loading)

Flash Chromatography

Sample Loading Conditions (L = 15 cm)

Column Diameter (cm)	Sorbent Amount (g)	Sample Loading (g) $\Delta R_F > 0.2$	$\Delta R_F \approx 0.1$	Fraction Volume (mL)
1	5	0.1	0.04	5
2	20	0.4	0.16	10
3	45	0.9	0.36	20
4	80	1.5	0.60	30
5	130	2.5	1.0	50

J.D. Fair and C.M. Kormos, Flash chromatography chromatograms estimated From thin-layer chromatograhy data, J. Chromatogr. A 1211 (2008) 49-54

Flash Chromatography

Sample Loading Conditions (L = 10 cm)

Stepwise Gradient

Column Diameter (cm)	Sorbent Amount (g)	Sample Loading (g)	Fraction Volume (mL)
3	30	1-3	50-100
4	55	3-8	100-200
6	125	8-35	200-300
10	350	60-80	300-500

Low- and medium-pressure chromatography

- Pump used to maintain a constant mobile phase velocity
- Samples introduced by valve injection or pump
- On-line detection and automated fraction collection commonly used
- Sorbents of a narrower particle size range employed to improve resolution

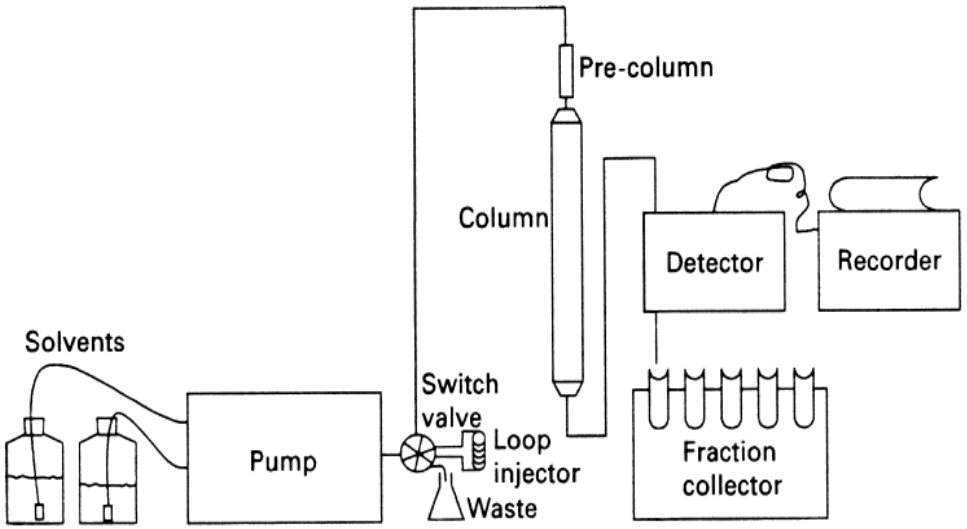
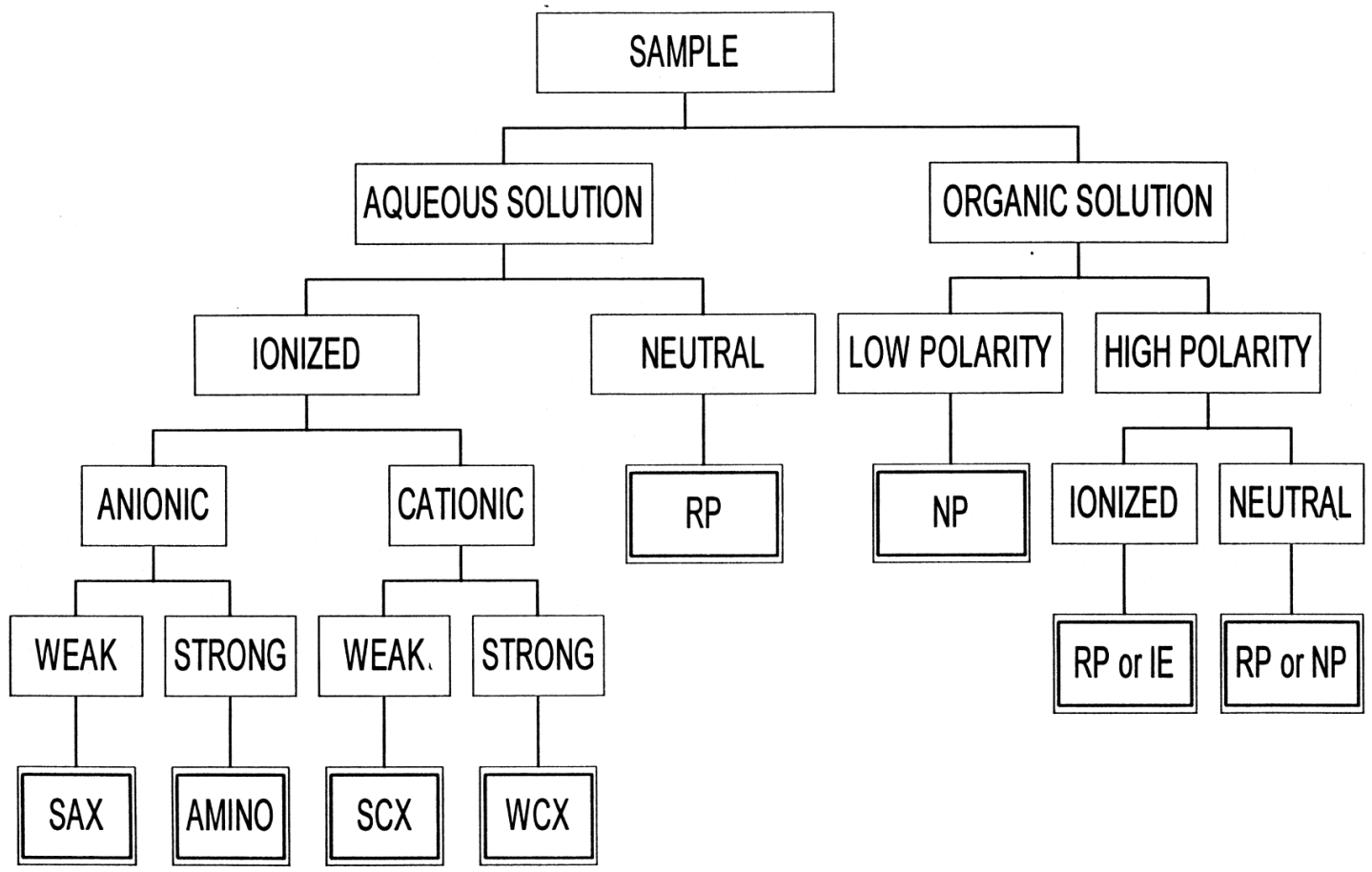


Figure 11.2. Apparatus for low- and medium-pressure preparative-scale liquid chromatography. (From ref. [6]; ©Academic Press).

H. Henke, Preparative Gel Chromatography on Sephadex LH-20, Huthig, Heidelberg, 1995

Mode Selection Guide SPE



Solid-Phase Extraction Sorbent Types

- Inorganic Oxides
  - Silica gel, alumina, Florisil
- **Low-Specificity Sorbents**
  - Chemically bonded sorbents
  - Porous polymers
  - Carbon
- **Compound and Class-Specific Sorbents**
  - Ion exchange sorbents
  - Immunosorbents
  - Molecularly imprinted polymers
  - Restricted access materials
  - Surface-bound ligands

Inorganic Oxide Adsorbents

- Isolation of low and medium polarity compounds from non-aqueous solutions
- Isolation of cations (silica or alumina) and anions (alumina) from buffered aqueous solutions
- Matrix simplification by fractionation into groups containing similar number and type of functional groups

Low-Specificity Sorbents Aqueous Solutions

- **Isolation of neutral and ionizable compounds**
  - Weak acids and bases by ion suppression
  - Strong acids and bases by ion pair formation
- **Retention increases with compound size and is reduced by polar interactions (particularly hydrogen-bonding and ionization)**
- **Polar chemically bonded phases provide only weak retention (not particularly useful)**
  - Exception for compounds difficult to elute from non-polar sorbents

Low-Specificity Sorbents Organic Solvents

Retention depends on the number and type of functional groups

- **CYANO**
  - strong dipole-type interactions and weak hydrogen-bond acid
- **AMINO**
  - Strong hydrogen-bond base and acid interactions. Weak dipole-type interactions
- **DIOL**
  - Strong hydrogen-bond acid and weak hydrogen-bond base with significant capacity for dipole-type interactions

Sample Processing Conditions Conditioning Solvent

- **Ensures reproducible flow and retention**
  - Typically 3-5 bed volumes
  - Critical step for particle-loaded membranes (disks)
  - Not essential for water wettable sorbents
- **Minimizes contamination by sorbent impurities**
- **Replace with sample solvent before sample processing**

Sample Processing Conditions

- **Flow Rate**
  - Typical range 0.2-1.5 mm/s
  - Critical for cartridges due to channeling
- **Drying Time**
  - Typically 1-5 min
  - Sufficient to remove all sample solvent trapped in sorbent pores
  - Excessive drying may result in low recovery from poorly solvated regions of the sorbent

Sample Processing Conditions

Rinse Solvent (Optional)

- Small volume of intermediate strength solvent to elute matrix components (analytes immobilized on sorbent)

Eluting Solvent

- Typically 3 bed volumes (or more)
- Strong solvent
- Normally volatile and miscible with the sample solvent

Solid-Phase Extraction: Theoretical Considerations

- Extraction occurs under **Frontal Analysis** conditions
- Sample solutions are generally dilute affording a linear sorption isotherm
- Sample volumes are limited by the breakthrough curve
- Typical sampling devices provide only 5-20 plates / cm of bed height
- Rinse and recovery steps occur in the **Elution** mode
- Most parameters to model the extraction process can be determined by liquid chromatography

Frontal Analysis Model

$$Q = (a_0 + a_1 / N + a_2 / N^2)^{-1/2}$$

Depends on selected breakthrough level

$V_B = 0.1\%$	$a_0 = 0.998$	$a_1 = 29.12$	$a_2 = 57.54$
$V_B = 1\%$	$a_0 = 0.980$	$a_1 = 13.59$	$a_2 = 17.60$
$V_B = 10\%$	$a_0 = 0.810$	$a_1 = 2.88$	$a_2 = 1.94$

Depends on the plate number

N > 100 Q = 1

N < 100 Q is flow rate dependent

- **depends strongly on packing density (channeling) and particle size**

Cartridges provide N ≈ 5-15 plates / cm

P. Lovkvist and J-A. Jonsson, Anal. Chem. 59 (1987) 818-821.

Estimation of Breakthrough Volumes

For sampling devices with low plate numbers

$$V_B = (a_o + a_1 / N + a_2 / N^2)^{-1/2}(1 + k)V_M$$

$$\log V_B = \log QV_M + \log (1 + k)$$

V_M = hold-up volume for the sorbent bed

k = retention factor

N = plate number for the sorbent bed

a_o , a_1 and a_2 = constants that depend on the breakthrough level

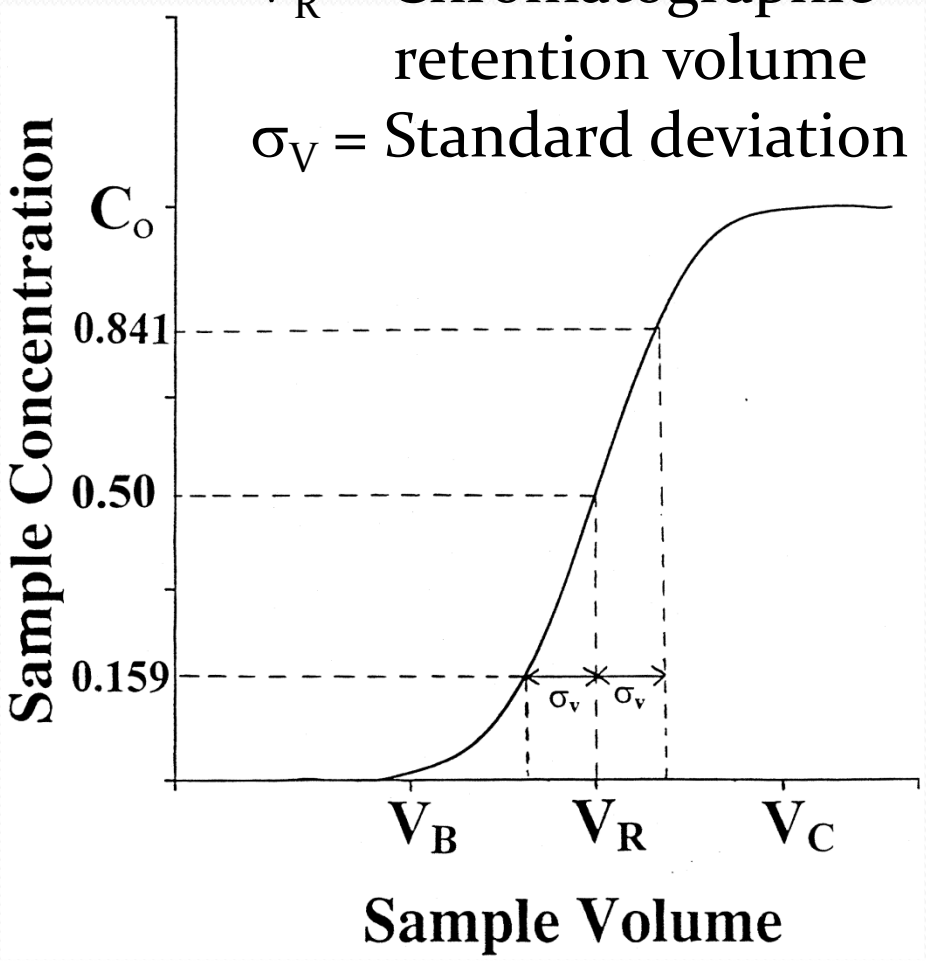
P. Lovkvist and J-A. Jonsson, Anal. Chem. 59 (1987) 818-821.

Breakthrough Curve

V_B = Breakthrough volume
V_C = Saturation capacity
V_R = Chromatographic retention volume
σ_V = Standard deviation

V_B is the position on the curve at which some arbitrary amount of sample (e.g. 1%) is observed at the outlet of the sampling device

V_C = the sample volume at which the concentration leaving the sampling device is the same as the concentration entering it. Corresponds to the sample volume for maximum isolation of sample but with a lower overall recovery

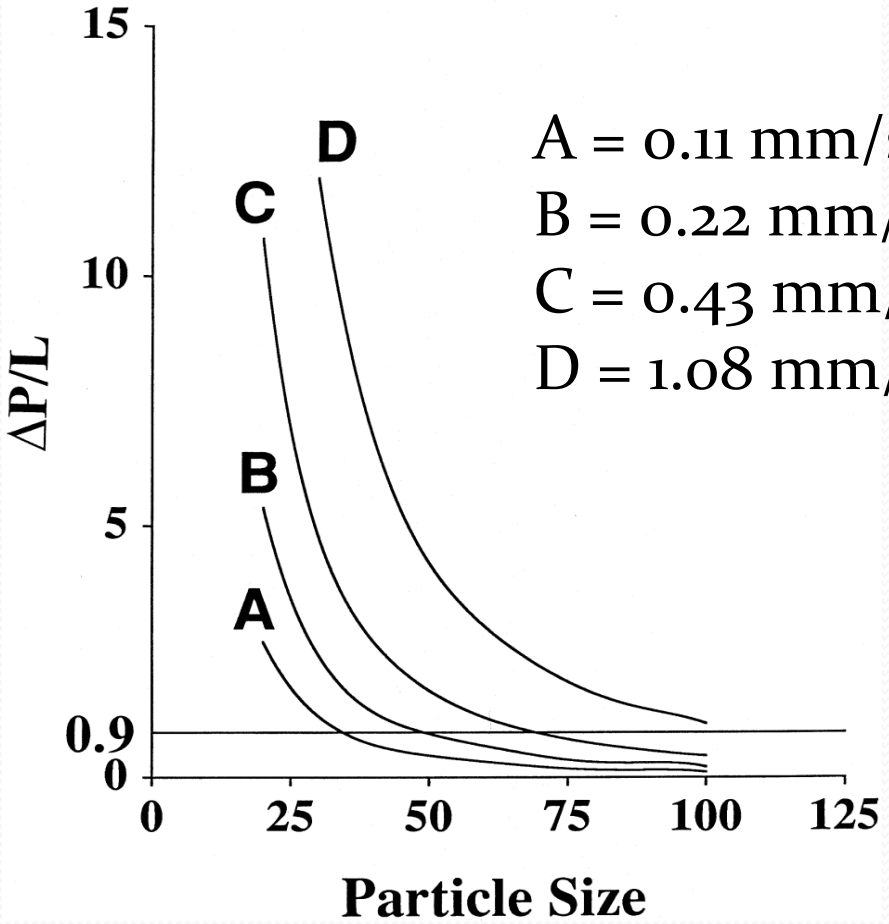


Early Breakthrough in Frontal Analysis

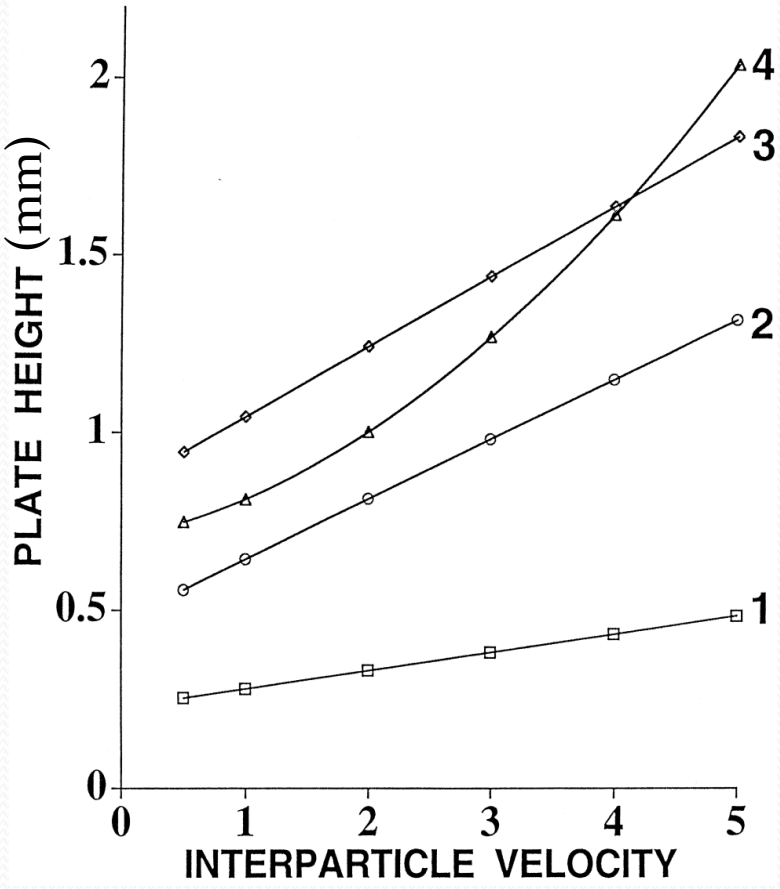
- **A high concentration of analyte or matrix diminishes the retention capacity of the sorbent**
  - Overloading leads to non-linear isotherms
- **The sorbent bed fails to retain analytes due to an inadequate plate number**
  - Breakthrough volumes depend on the plate number for the sampling device

Selection of Particle Size

Particle size is limited by the available pressure drop ΔP per unit bed length L required to transport the sample through the sampling device at an optimum velocity



Cartridge Plate Height Curves



Sorbent Type

1 = octadecylsiloxane (LL)

2 = DIOL

3 = CN

4 = butylsiloxane

Typical cartridges provide < 20 N/cm
At practical sampling flow rates

Apparatus for Characterization of Particle-Loaded Membranes

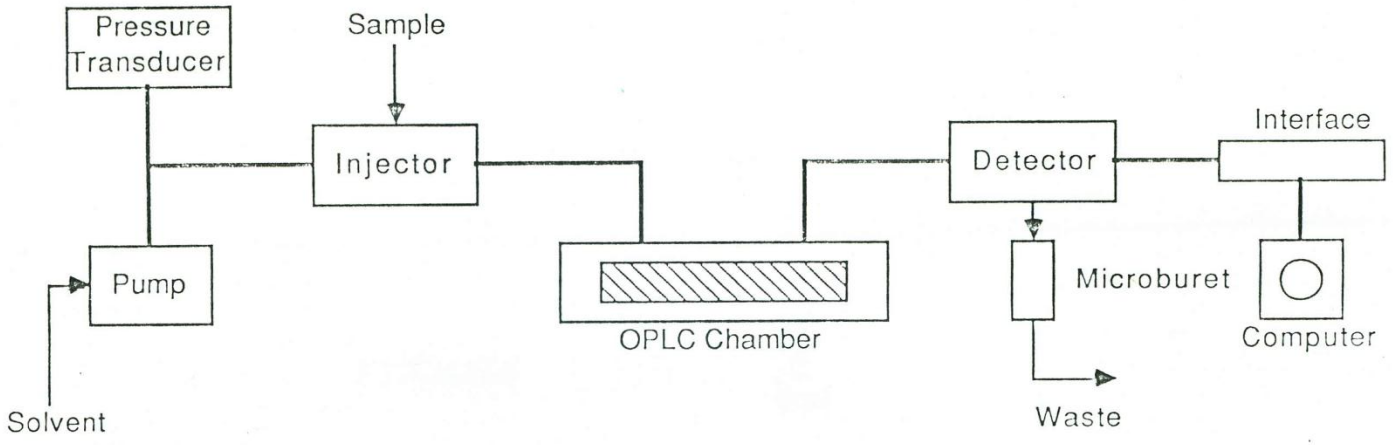
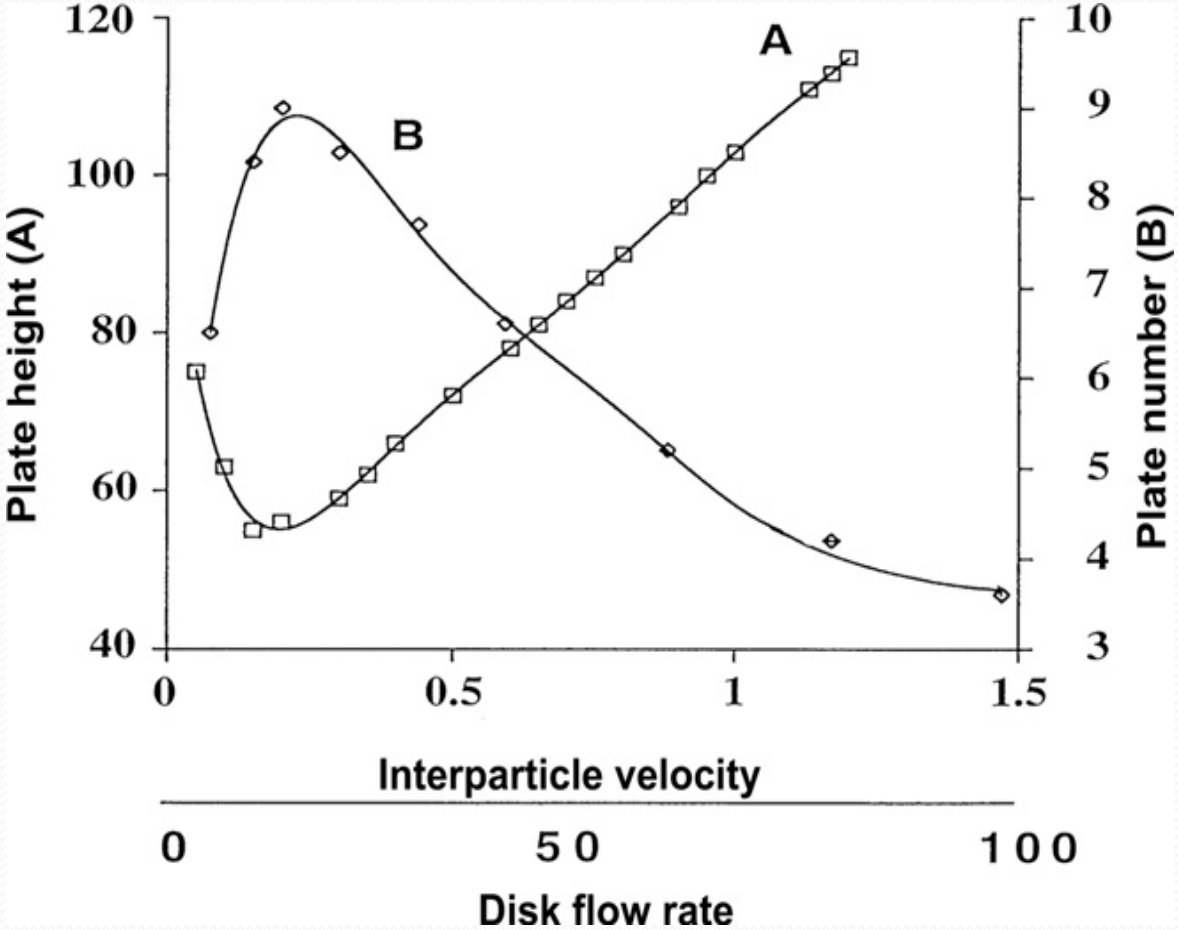
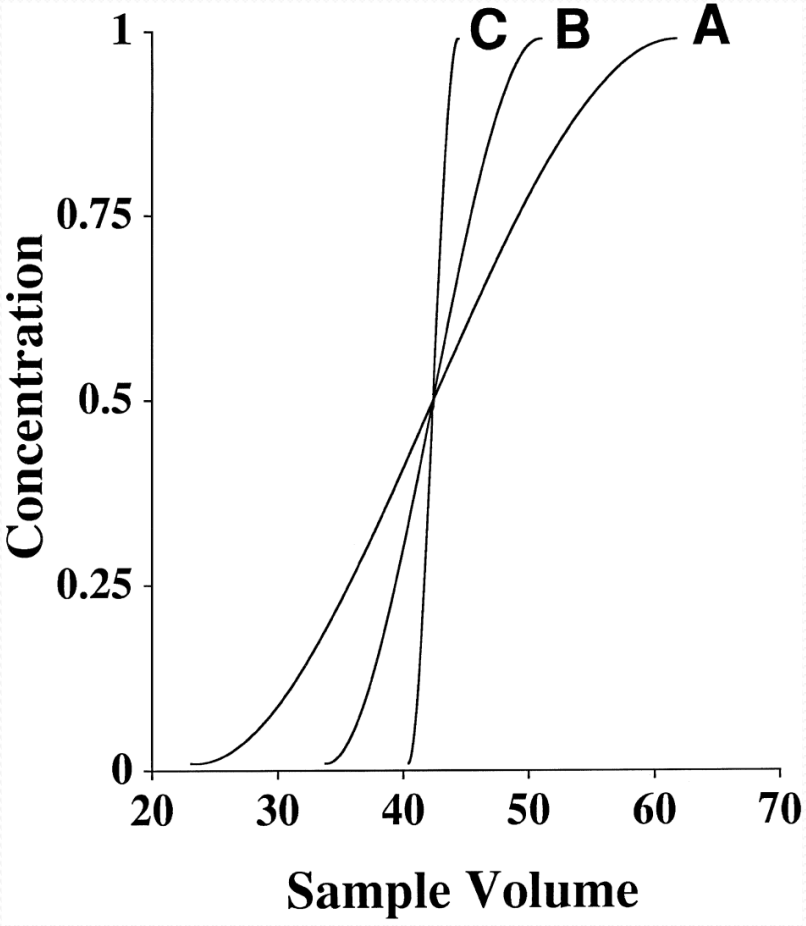


Plate Height for a Particle-Loaded Membrane



H_m = 50 µm
U_{opt} = 0.20 mm/s
N ≈ 4-9

Effect of the Limited Plate Number on Breakthrough Curves



Properties of Sampling Device
A = 5 plates and $V_B \approx$ 23 mL
B = 20 plates and $V_B \approx$ 34 mL
C = 100 plates and $V_B \approx$ 40 mL
k = 100
V_M = 0.42 mL

Elution volume for Rinse and Recovery Steps

For a sorbent trap with a low plate number

$$V_E = V_M[1 + k][1 + (2.3/\sqrt{N})]$$

To minimize V_E

- use a small sorbent bed (small V_M)
- strong solvent ($k < 3$ and preferably $k < 1$)
- large value of N (sharper desorption front requiring less solvent for quantitative elution)

General Model for Solid-Phase Extraction (Retention Factor)

$$\log V = \log QV_M + \log (1 + k_s)$$

For a specific sampling device with a limited range of flow rates QV_M is approximately constant (numerical value)

log V depends on k_s

Determine k_s:

- LC (make column from sorbent packing)
- Estimate by TLC $k_s = (1 - R_F) / R_F$
- Calculate using the solvation parameter model

General Model for Solid-Phase Extraction (Solvent Volume)

- Adequate breakthrough volumes require a large k_s value for the sample processing conditions
  - $k_s > 100$
- Rinse solvent volume requires identification of solvent conditions that preserve a sufficiently large value for k_s
  - $k_s > 20$
- Elution solvent volume requires identification of solvent conditions that minimizes k_s
  - $k_s < 1$

Solvation Parameter Model

System constants describe sorbent properties

$$\log V_x = c + e.E + a.A + b.B + s.S + v.V$$

Descriptors define analyte properties

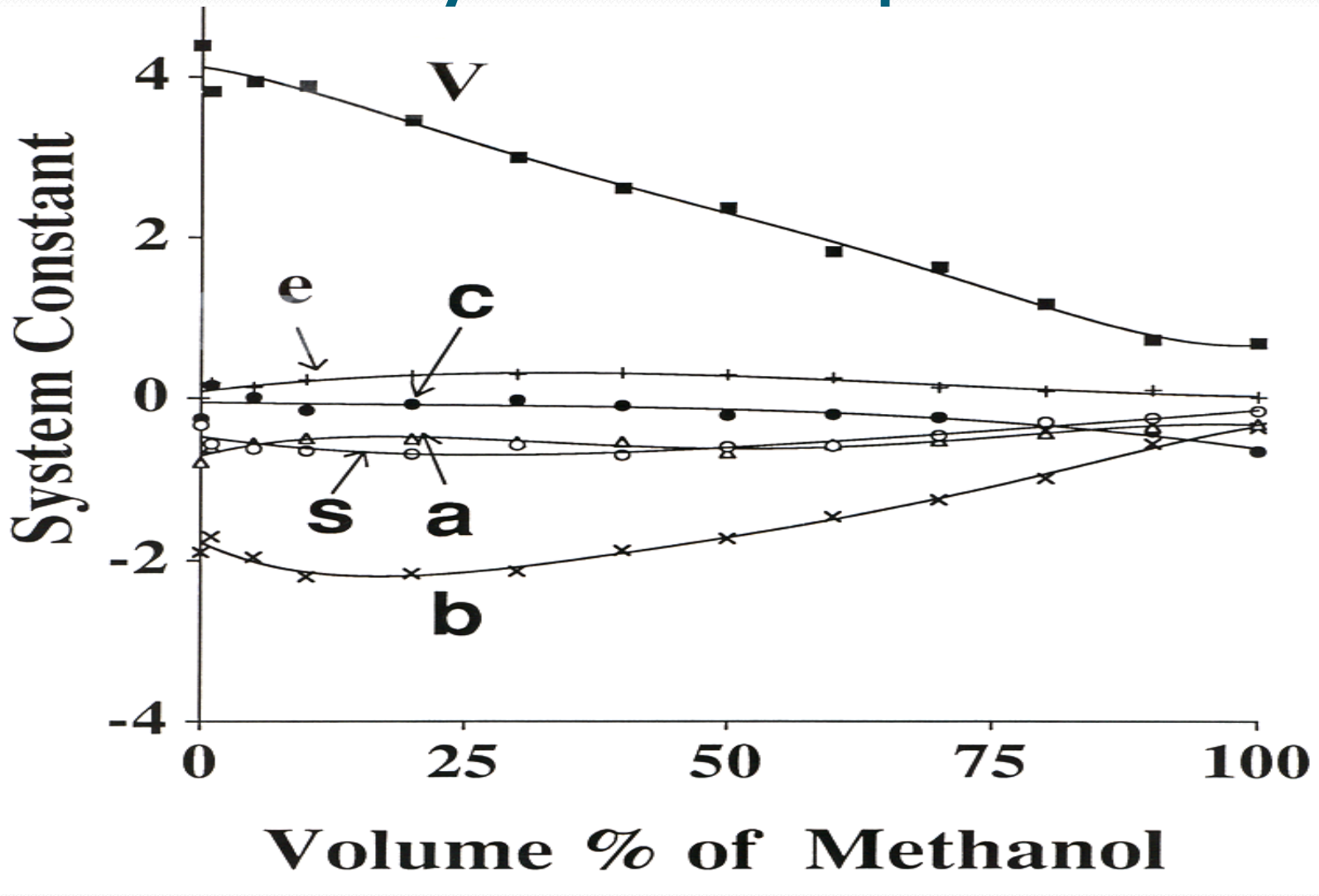
System Map illustrates the variation of the system constants with solvent composition

Solute descriptors

- V is McGowan's Characteristic Volume
- E is the excess molar refraction
- S is the solute dipolarity/polarizability
- A is the effective solute hydrogen-bond acidity
- B is the effective solute hydrogen-bond basicity

C.F. Poole, S.N. Atapattu, S.K. Poole, A.K. Bell, Anal. Chim. Acta 652 (2009) 32-53.

System Map

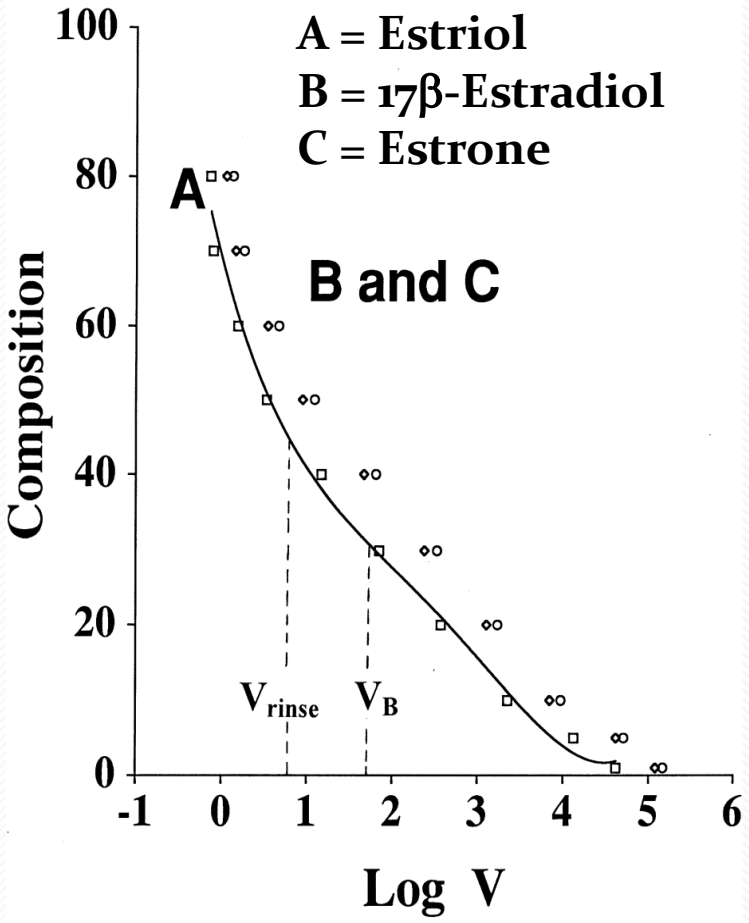

System Constant
4
2
0
-2
-4
V
e
c
s
a
b
0
25
50
75
100
Volume % of Methanol

Sorbent System Constants

Sample solvent (water + 1% v/v) methanol)

Sorbent	***v***	***e***	***s***	***a***	***b***	***c***
IST C18 (HL)	4.39	0	0	-0.79	-1.90	-0.27
IST C18 (LL)	3.92	0	-0.11	-0.54	-1.55	-0.90
IST C4	3.36	0	0	-0.46	-1.53	-1.38
OASIS HLB	3.32	1.62	0.36	-0.66	-2.47	-0.13
Carbon	5.62	0	1.35	0	-3.54	-2.78
JTB CN	2.06	0.53	0	-0.51	-1.45	-0.88
JTB DIOL	1.57	0.61	0	-0.45	-0.80	-1.05

Simulation of Sampling Conditions



Isolation of estrogens from urine

Sorbent = Octadecylsiloxane-Bonded Silica

Solvent = Methanol-Water Mixtures

V_B = 45 mL (sufficient analyte for determination)

- Any sample composition containing < 25% (v/v) methanol

V_{rinse} = 6 mL (matrix simplification)

- Any solvent composition containing < 40% (v/v) methanol

$V_E \approx 4V_M$ methanol for elution of estrone (most retained analyte)

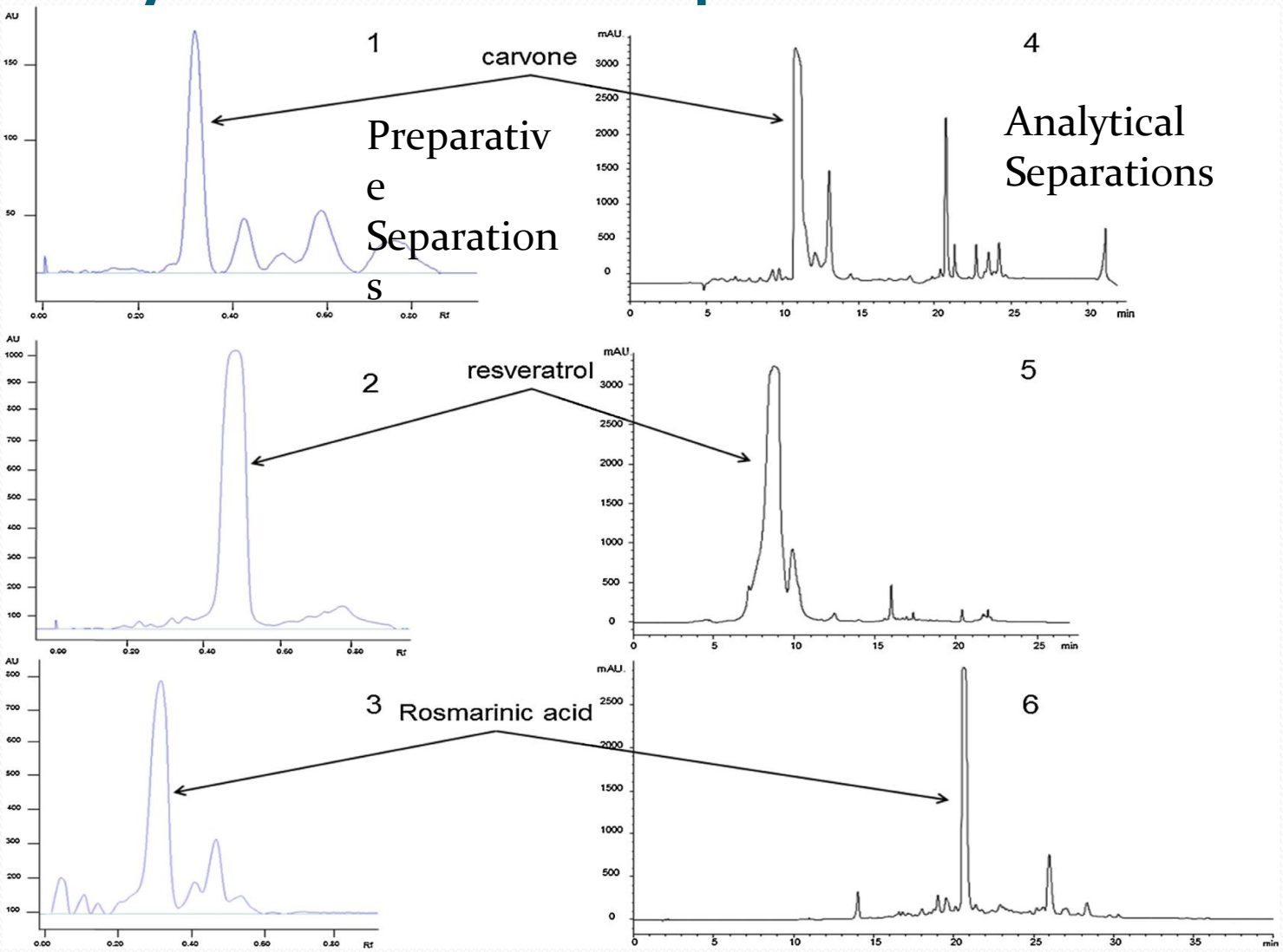
Sample Properties

- Reduce sample processing times by diluting viscous samples with a weak low viscosity solvent
- Remove particle matter (filter or centrifuge) to maintain constant flow
- Add small amount of organic solvent (1-3 % v/v) to water samples to maintain constant sample flow rates
- Deproteination of biological fluids recommended for consistent recovery of analytes
- Maintain constant ionic strength for samples and standards

Sorbent Shortcommings

- Sorbents properties are not as reproducible as solvent properties
- Contamination of extracts with sorbent impurities
- Variable packing density of cartridges
  - General reason for flow rate dependent recovery
  - Larger bed mass required to ensure quantitative recovery
- Competitive processes affect analyte recovery
  - Overlaoding
  - Displacement
  - Irreversible adsorption at active sites
  - Blocking of pores

Analytical and Preparative LC view



T.K.T. Do et al, J. Chromatogr. A 1325 (2014) 256-260

Non-Linear Chromatography

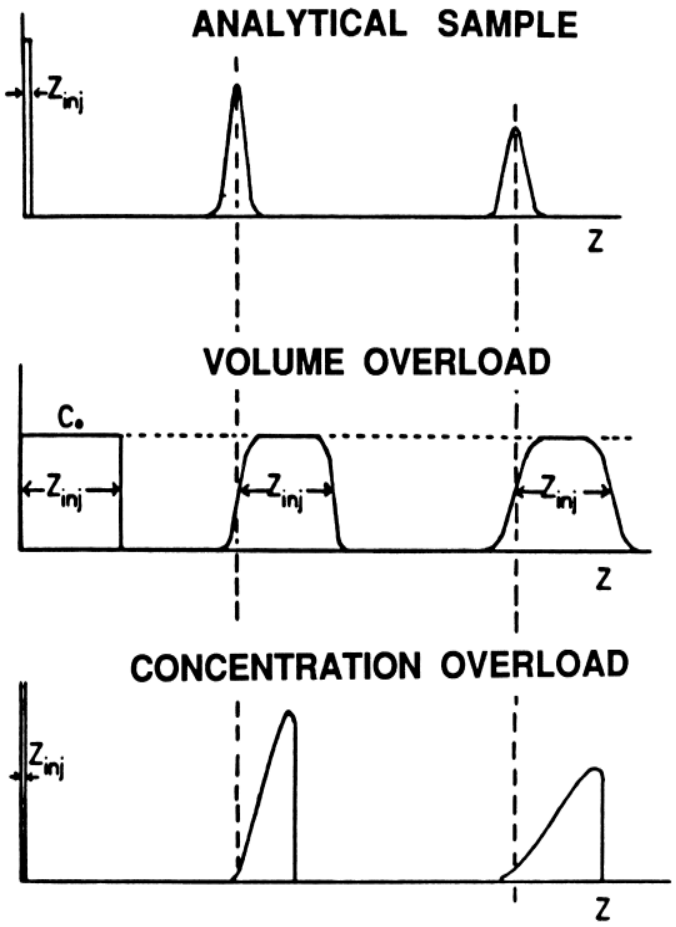


Figure 11.8. Development of peak profiles during migration along the column for analytical and overload samples. (From ref. [65]; ©Elsevier).

The properties of chromatographic bands under nonlinear conditions are difficult to

Peak Shaving

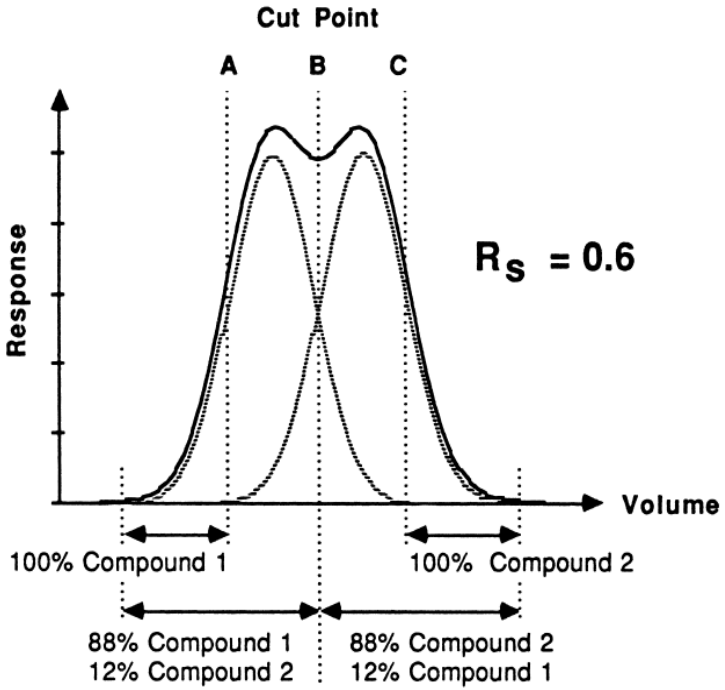


Figure 11.6. Peak shaving technique for collection of pure product when the separation selectivity is inadequate for acceptable sample throughput.

TLC to Monitor Fractions