Method Development and Solvent Selection in Liquid Chromatography with Emphasis on Thin-Layer Chromatography

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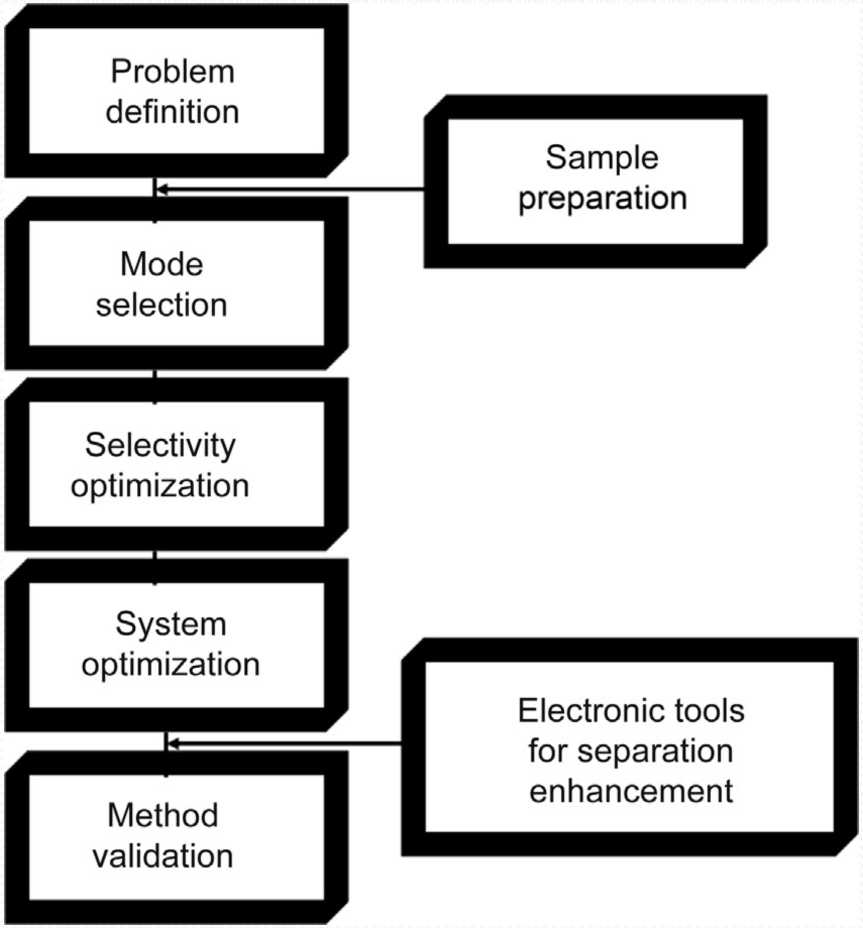
Method Development Process



Method Development Process

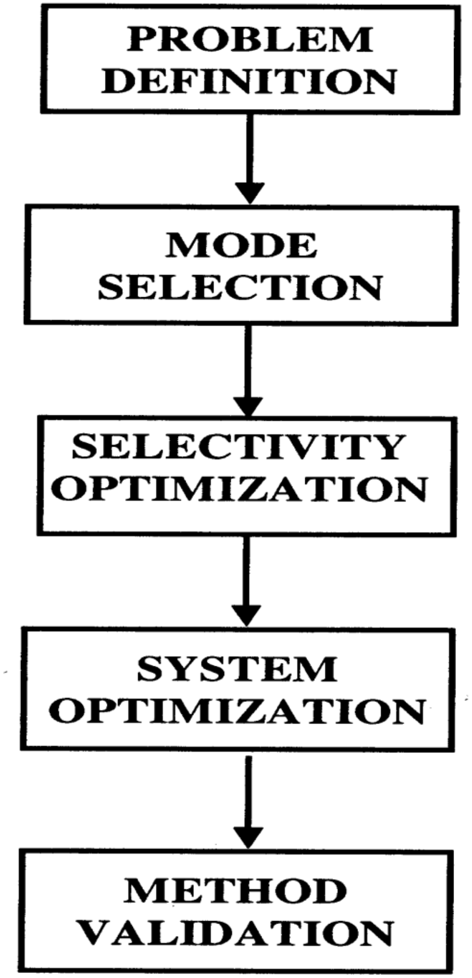
Need to know what to do

Before beginning experiments need to decide how to do it

Survey experiments to find conditions compatible with goals

System properties modified to achieve goals

Confirmation that the method is suitable with respect to goals


PROBLEM DEFINITION

MODE SELECTION

SELECTIVITY OPTIMIZATION

SYSTEM OPTIMIZATION

METHOD VALIDATION

METHOD DEVELOPMENT IN TLC

PROBLEM DEFINITION

- How many detectable compounds in the sample ?

Indicates the complexity of the mixture
Zone capacity in TLC (capillary-controlled flow) = 10-14
(forced flow) = 20-40
(AMD) = 25-40

- Are all compounds equally relevant ?

Compounds not of interest need only be separated from those of interest and not from each other

METHOD DEVELOPMENT IN TLC
PROBLEM DEFINITION

- Are standards available for the relevant compounds ?

Standards simplify zone tracking in method development

Used to establish detection characteristics (possibility of spectroscopic resolution)

Will be required for quantification based on calibration

Needed to construct spectroscopic libraries

METHOD DEVELOPMENT IN TLC

PROBLEM DEFINITION

- What is the concentration range of relevant compounds ?

  Derivatization may be needed

  Minor compounds with similar migration properties to major component require greater zone separation for detection (easier if the minor component is more strongly retained)

- How many samples are to be analyzed ?

Only worth seeking a global optimum separation if the separation is to be in used for some time

METHOD DEVELOPMENT IN TLC
PROBLEM DEFINITION

- Will unknown compounds require identification ?

Hyphenated methods like TLC-MS may be needed

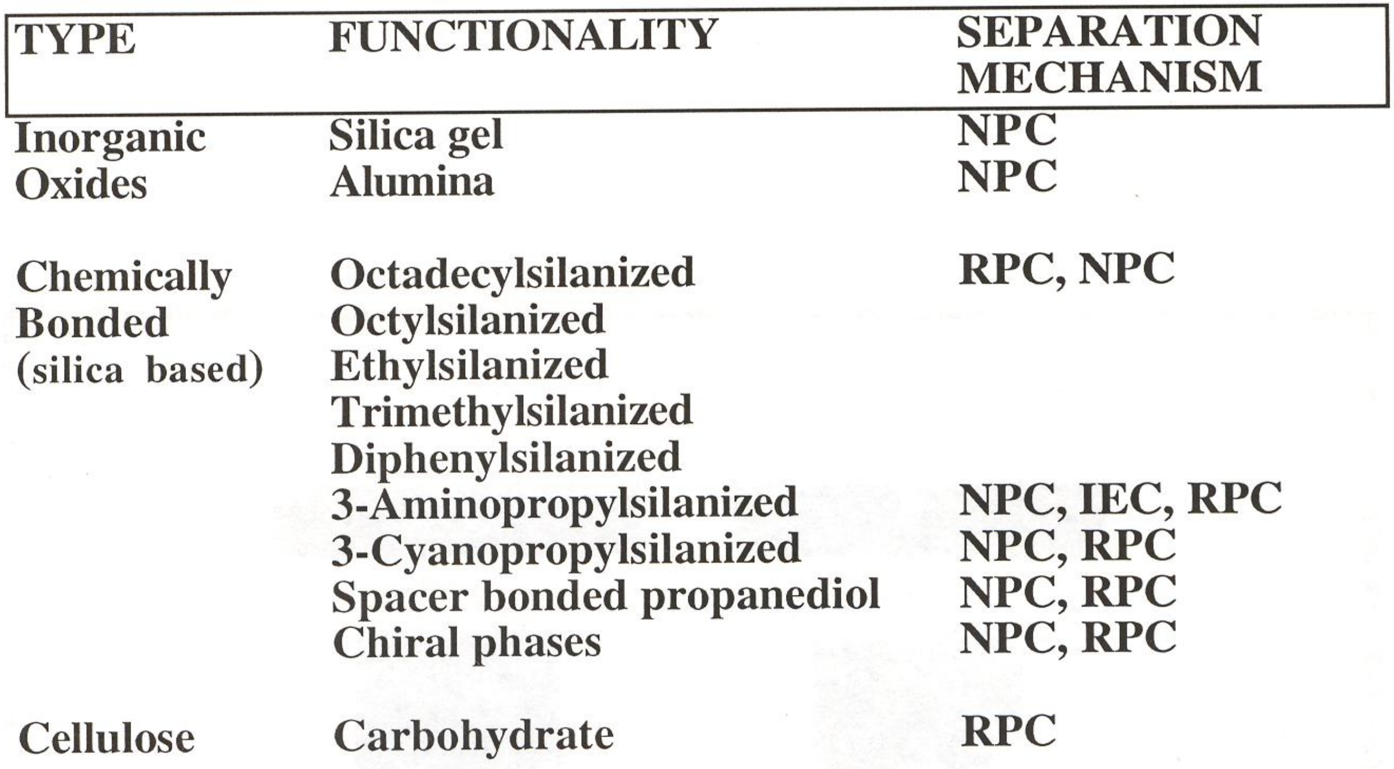
Preparative separation may be required to isolate compounds for subsequent off-line evaluation (e.g., NMR, X-ray crystallography, etc.)

- What is the composition of the sample matrix ?

A sample preparation strategy may be required

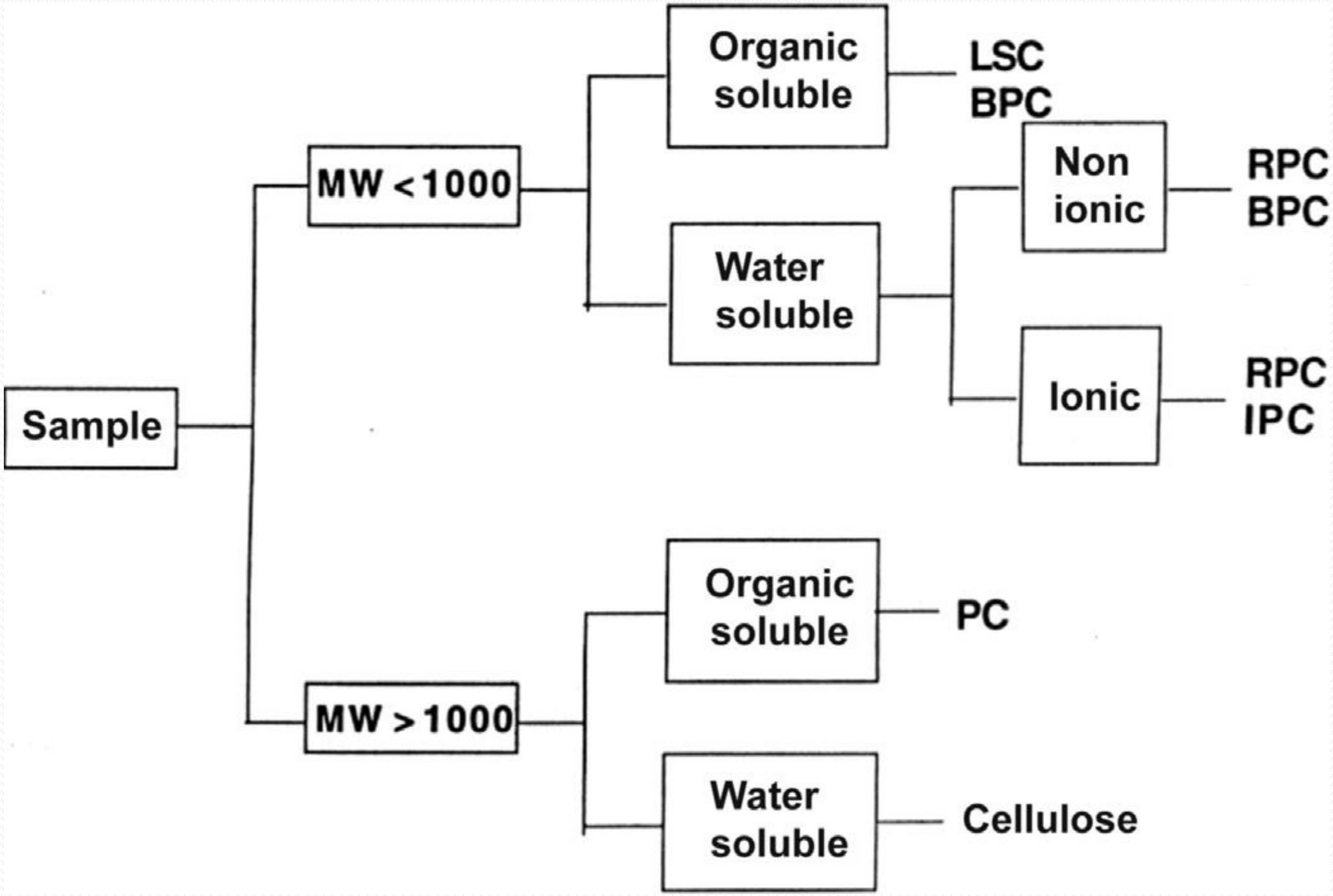
- Is the method to be used primarily for analytical or preparative separations ?

Precoated Layers



TYPE	FUNCTIONALITY	SEPARATION MECHANISM
Inorganic Oxides	Silica gel	NPC
	Alumina	NPC
Chemically Bonded (silica based)	Octadecylsilanized	RPC, NPC
	Octylsilanized	
	Ethylsilanized	
	Trimethylsilanized	
	Diphenylsilanized	
	3-Aminopropylsilanized	NPC, IEC, RPC
	3-Cyanopropylsilanized	NPC, RPC
	Spacer bonded propanediol	NPC, RPC
	Chiral phases	NPC, RPC
Cellulose	Carbohydrate	RPC

Mode Selection



Normal-Phase Chromatography

General adsorption scale for silica gel

Weak ↓ Strong

Difficult to separate because solvent strength is too high

- Alkanes
- Aromatic
- Halogenated Compounds
- Ethers
- Nitro Compounds
- Nitriles
- Carbonyl Compounds
- Alcohol
- Phenols
- Amines
- Amides
- Carboxylic acids
- Sulfonic acids

Difficult to separate because solvent selectivity is too low

Nonpolar Bonded Phases

Normal-Phase Chromatography	low-polarity organic compounds using non-aqueous solvents (strong retention of polar compounds)
Reversed-Phase Chromatography	water soluble compounds using aqueous mobile phases acids and bases after ion suppression or ion-pair formation Separations employing selective complexing agents in the mobile phase

Polar Bonded Phases

Normal-Phase Chromatography

CN	Behaves like a deactivated silica gel with unreacted silanol groups as the dominant active sites. Dipole-type interactions important
Amino	Retention dominated by hydrogen-bonding interactions
DIOL	Stronger hydrogen bond acid and weaker hydrogen bond base than Amino. Weaker retention of dipole-type compounds than CN.

Polar Bonded Phases

Reversed-Phase Chromatography

CN	Similar retention properties to short-chain alkylsiloxane-bonded layers. Dipole-type interactions are not important.
Amino	Weak retention except for hydrogen-bonding compounds. Anion exchanger in acidic mobile phases
DIOL	Stronger hydrogen bond acid and weaker hydrogen bond base than Amino

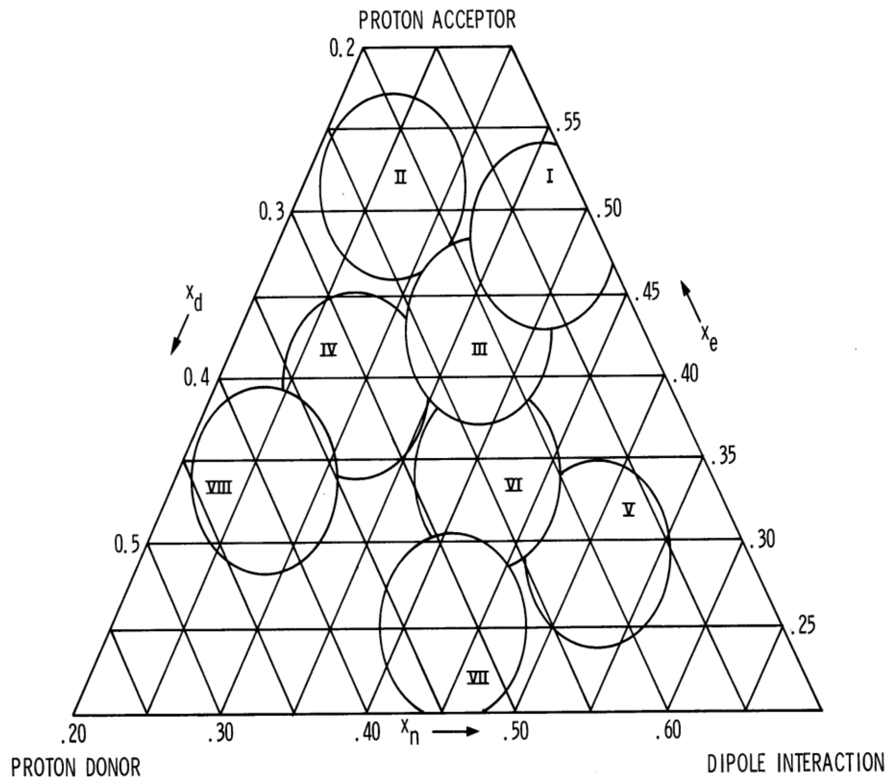
Impregnated Layers

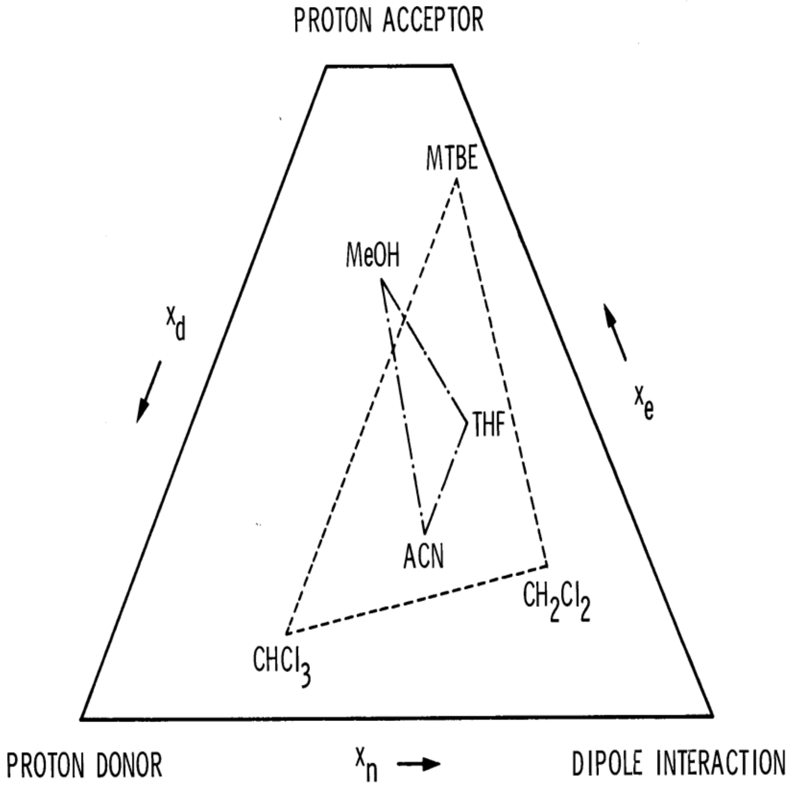
Silver ion	Separation of compounds that differ in the number, configuration or position of double bonds
Boric acid	Separation of compounds with vicinal hydrogen-bonding functional groups
Caffeine	Separation of polycyclic aromatic hydrocarbons by ring number
EDTA	Competes with analytes for formation of metal-analyte complexes

Solvent Selection

- Solvent strength
  - Estimate of the solvents capability to cause migration in a chromatographic system
  - Depends on the identity of the stationary phase
    - This is both a system and a solvent property
- Solvent selectivity
  - Estimate of the relative ability of the solvent to participate in individual intermolecular interactions
  - Solvents can have similar strength and different selectivity
    - Controls band spacing

Snyder’s Selectivity Triangle





Prototypical Solutes

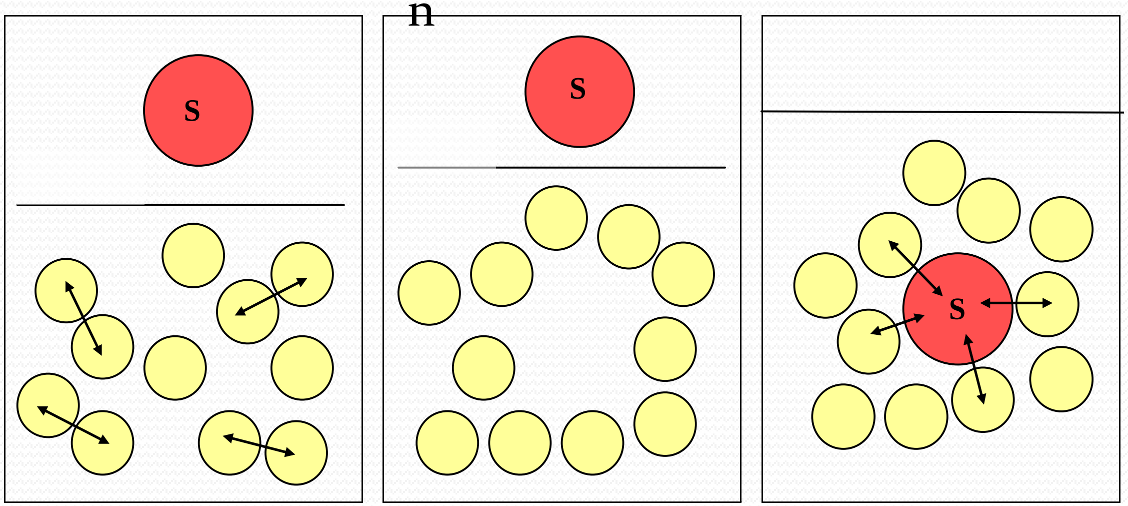
Polar solutes with a single dominant intermolecular interaction are virtually unknown

All solutes that are hydrogen bonding are simultaneously dipolar

Ethanol	Nitromethane	Dioxane
S = 0.42	S = 0.95	S = 0.75
A = 0.37	A = 0.06	A = 0
B = 0.38	B = 0.31	B = 0.64

Solvation Parameter Model

- Parameterization of cavity model of solvation



Cavity Formation Reorganization Solute-Solvent Interactions

- Mass transfer between condensed phases

$$\log \mathrm{SP} = c + e\mathrm{E} + s\mathrm{S} + a\mathrm{A} + b\mathrm{B} + v\mathrm{V}$$

SP = free energy related property such as k, K, or $(1 - \mathrm{R_F} / \mathrm{R_F})$

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

Solvation Parameter Model

Contains a term to accommodate cavity formation

(differences in cohesive energy of solvents)

Assigns general properties to solutes based on their capability for simultaneous multiple interactions

- Dispersion
- Dipole-type (orientation and induction)
- Hydrogen bonding (donor and acceptor properties)

Solvation Parameter Model

System constants relating to properties of the solvent

$$SP = c + e.\mathrm{E} + a.\mathrm{A} + b.\mathrm{B} + s.\mathrm{S} + l.\mathrm{L}$$

Descriptors relating to solute properties

SP = free energy related property

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
- L is the gas-liquid partition coefficient at 25°C with hexadecane as a solvent

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

Solvation Parameter Model

$$R_M = c + eE + sS + aA + bB + vV$$

System Constant	Solute Descriptor	Free Energy Contribution
v	**V**	**Ease of cavity formation** **Residual dispersion interactions**
e	**E**	**Electron lone pair interactions**
s	**S**	**Dipole-type interactions**
a	**A**	**Solvent hydrogen bond base-solute hydrogen-bond acid interactions**
b	**B**	**Solvent hydrogen bond acid-solute hydrogen bond base interactions**

Solvent Properties

Transfer of solutes from the gas phase to a solvent is defined by 5 system constants

- The system constants are independent of solute identity
- System constants are calculated from the experimental properties of a number of varied compounds
- Data requirements established by statistical parameters

Alkanes

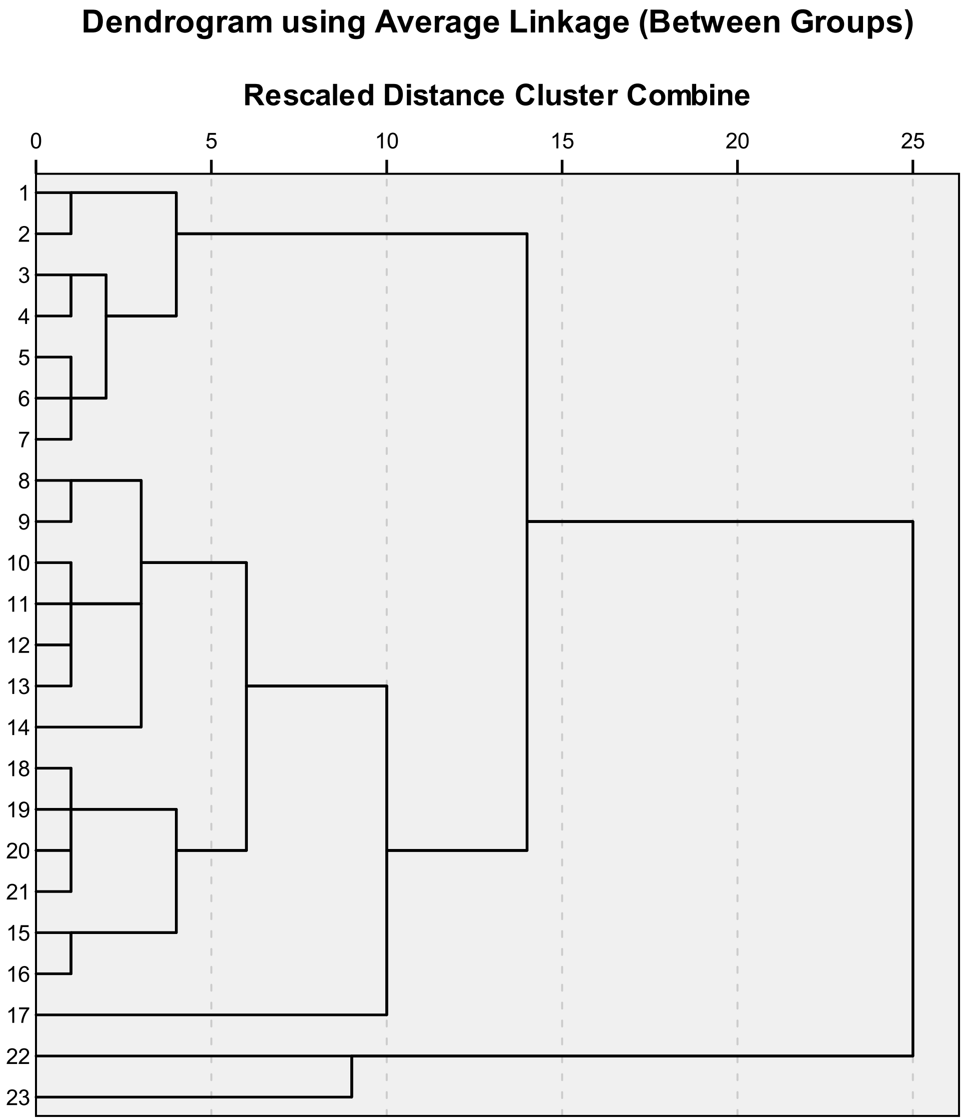
Aromatic Hydrocarbonds

Chloroalkanes

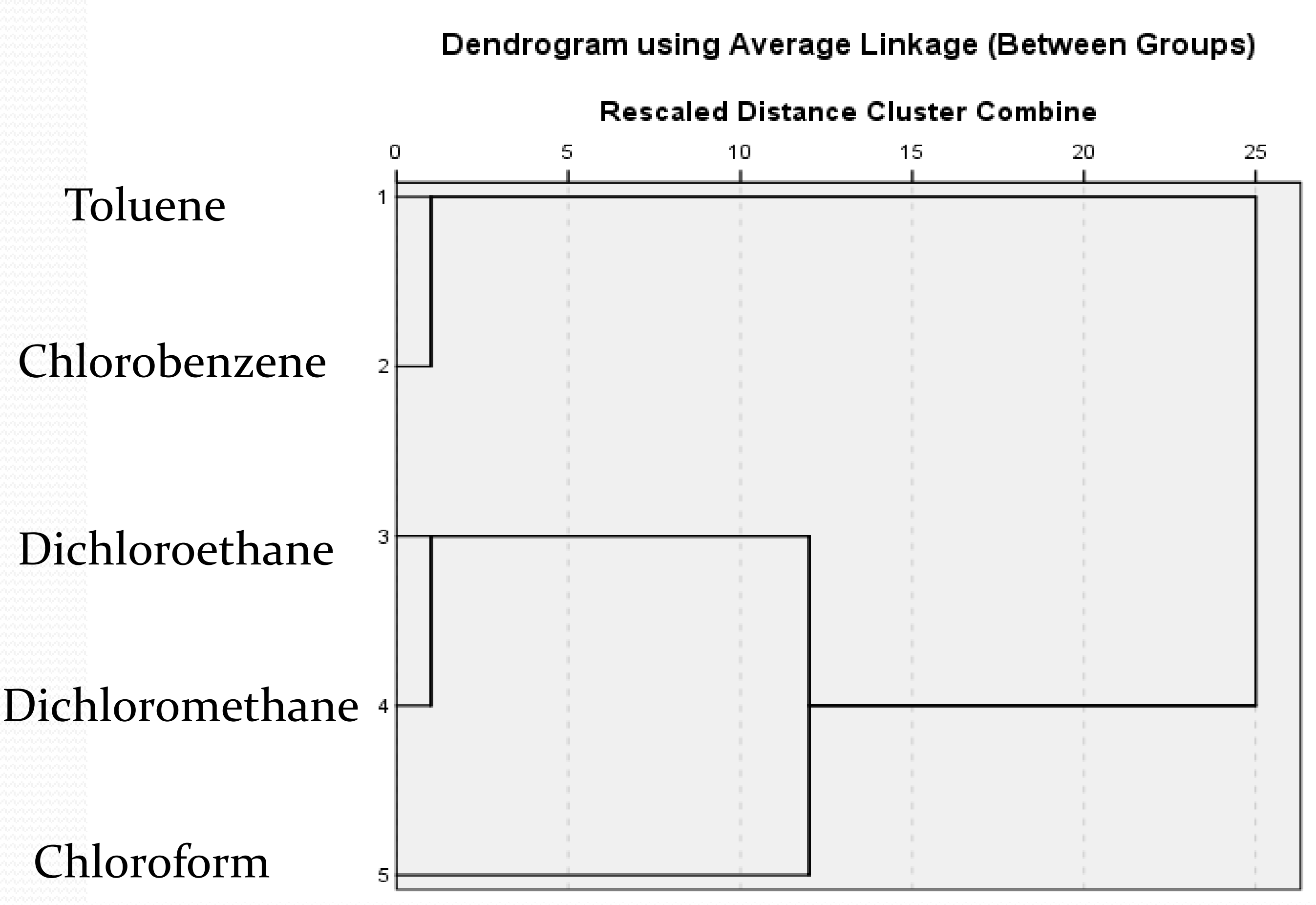
Ethers
Ketones
Esters

Alcohols

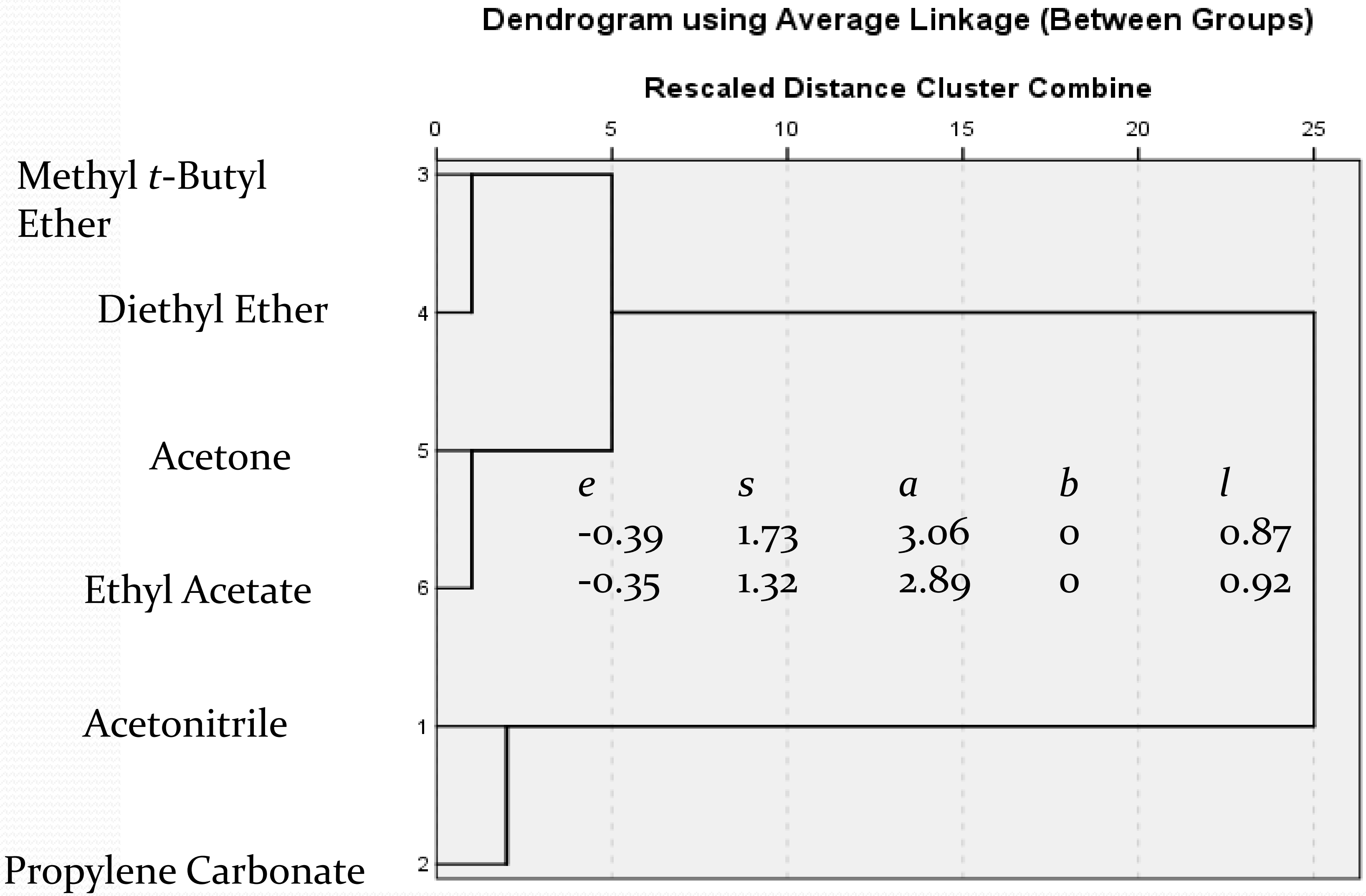
Water



Group 2

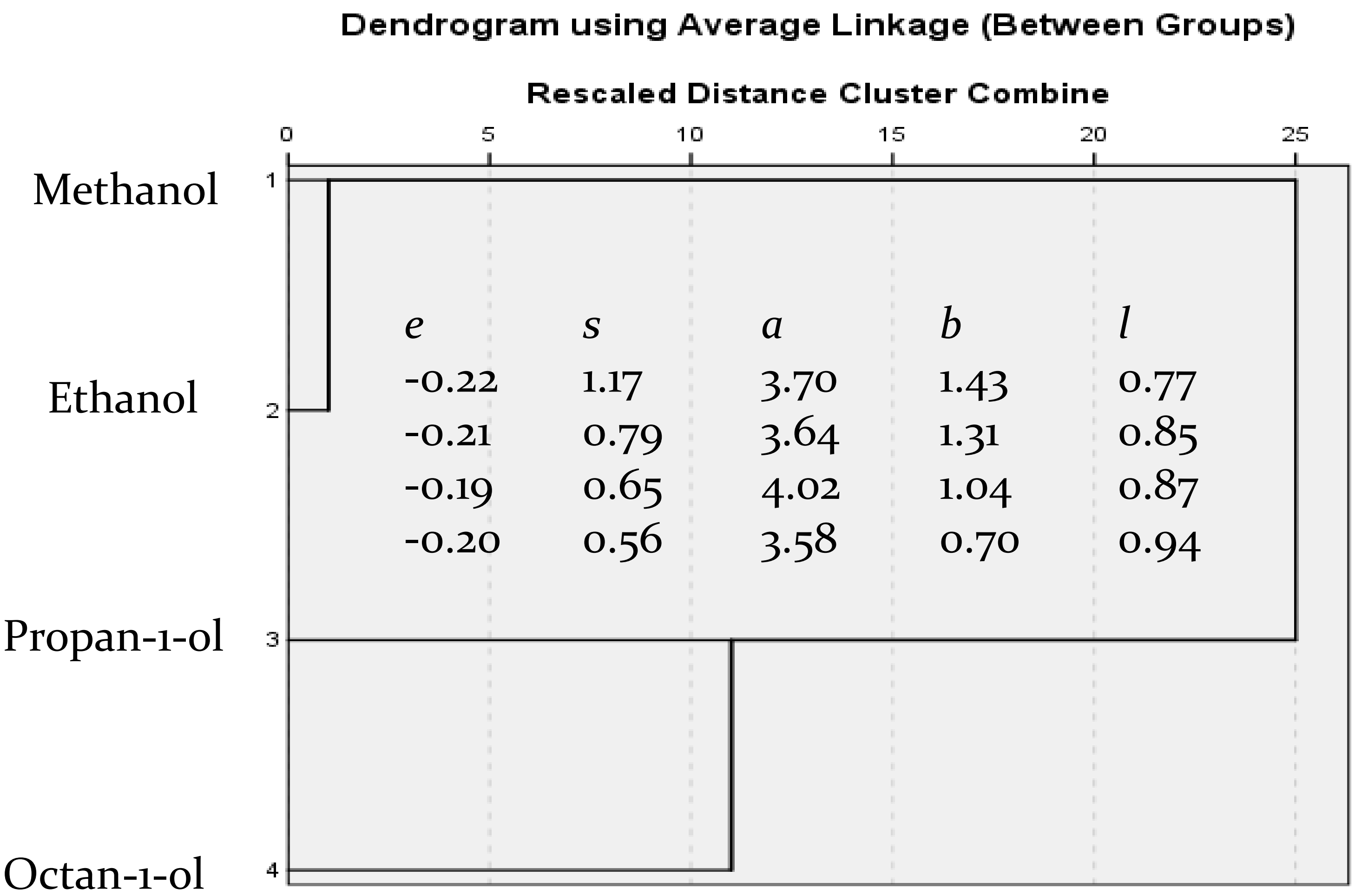

Dendrogram using Average Linkage (Between Groups)
Rescaled Distance Cluster Combine
0
5
10
15
20
25
Toluene
1
Chlorobenzene
2
Dichloroethane
3
Dichloromethane
4
Chloroform
5

Group 3 Ethers, Esters and Ketones



e	*s*	*a*	*b*	*l*
-0.39	1.73	3.06	0	0.87
-0.35	1.32	2.89	0	0.92

Group 4 Alcohols



e	*s*	*a*	*b*	*l*
-0.22	1.17	3.70	1.43	0.77
-0.21	0.79	3.64	1.31	0.85
-0.19	0.65	4.02	1.04	0.87
-0.20	0.56	3.58	0.70	0.94

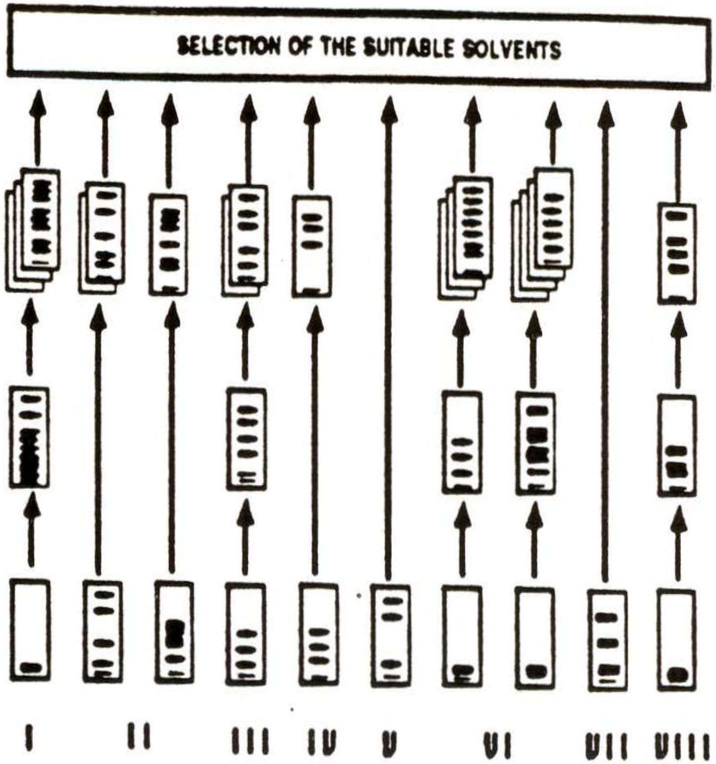
Solvent selection for method development in thin-layer chromatography

- n-Heptane
- Toluene
- Dichloromethane
- Chloroform
- Methyl *t*-Butyl Ether
- Acetone
- Acetonitrile
- Methanol
- Propan-1-ol
- Formamide
- Dimethylformamide
- Trifluoroethanol
- Water

PRISMA MODEL

Identify suitable solvents

Develop TLC plate in 10 select solvents in unsaturated development chambers. Adjust solvent strength as required by dilution with hexane or addition of acetic acid or water so that the substance zones are distributed in R_F range 0.2-0.8. Perform trails in parallel to maximize use of time. Solvents other than the selected solvents evaluated at this point.



Normal or Reversed Phase

Stationary phase	**Cyanopropylsiloxane-Bonded Silica**		**Silica**
Mobile phase	**Water**	**Hexane**	**Hexane**
	(1% v/v methanol)		
v	2.06	-0.61	-0.83
e	0.53	0	0
s	0	0.95	1.06
a	-0.51	1.86	2.23
b	-1.45	1.15	1.56

Normal or Reversed Phase

- The high cohesion and hydrogen-bond acidity of water dictates the separation characteristics in reversed-phase chromatography

- The competition between the sample and mobile phase for polar interactions with the stationary phase dictates separation characteristics in normal-phase chromatography

Strength Adjusting Solvent

- Normal-Phase Chromatography
  - Weak and Moderately Polar Compounds
    - n-Heptane
  - Polar Compounds
    - Strongest solvent that fails to migrate sample
    - Facilitates incorporation of solvents immiscible with n-Heptane
- Reversed-Phase Chromatography
  - Always water

Relative retention (Selectivity)

$$R_M = c - n\log N_B$$

n = the number of localizing groups in the sample

- rarely a whole number

N_B = mole fraction of strong solvent in a binary mobile phase

Solvent-strength selectivity: Changes in selectivity for compounds with different values of n as the concentration of polar solvent is increased.

Solvent-type selectivity: Arises from differences in the localization of sample and mobile phase components on the adsorbent surface.

- Basic and non-basic solvents exhibit different selectivity for hydrogen-bond acids due to sample-solvent interactions in the interphase region

Localization

- Site-specific interactions of both sample and mobile phase components with the energetically heterogeneous inorganic oxide surfaces results in localization.
  - Localization is the tendency of an adsorbing molecule to become preferentially non-covalently attached to high energy sites on the adsorbent surface
  - Important for polar molecules, particularly those capable of hydrogen bonding to surface adsorption sites.

Expanded Competition Model

- The simple competition model must be modified to handle localizing compounds
  - Restricted-access delocalization of solvent molecules
  - Site-specific delocalization of the sample.

$$\Delta R_M = m\Lambda$$

m = mobile phase property that increases with its localization

Λ = sample property that increases with its localization

  - Modification of the solute's cross-sectional area

Solvent Strength Parameter ε°

- Solvent strength of a pure solvent can be defined by ε° for any adsorbent
- Free energy of adsorption of the solvent per unit surface area with pentane assigned as the zero reference
- Organization of solvents in ascending order of ε° is known as an eluotropic series

Inorganic Oxides

- Solvent strength parameter for silica gel

$\varepsilon° = -0.264V + 0.199S + 0.384A + 0.355B + 0.272$

Can be used to estimate ε° values to about 0.04 units for solvents lacking experimental values

Inorganic Oxides

- Solvent strength parameter for alumina

$$\varepsilon° = -0.226V + 0.359S + 0.938A + 0.475B + 0.230$$

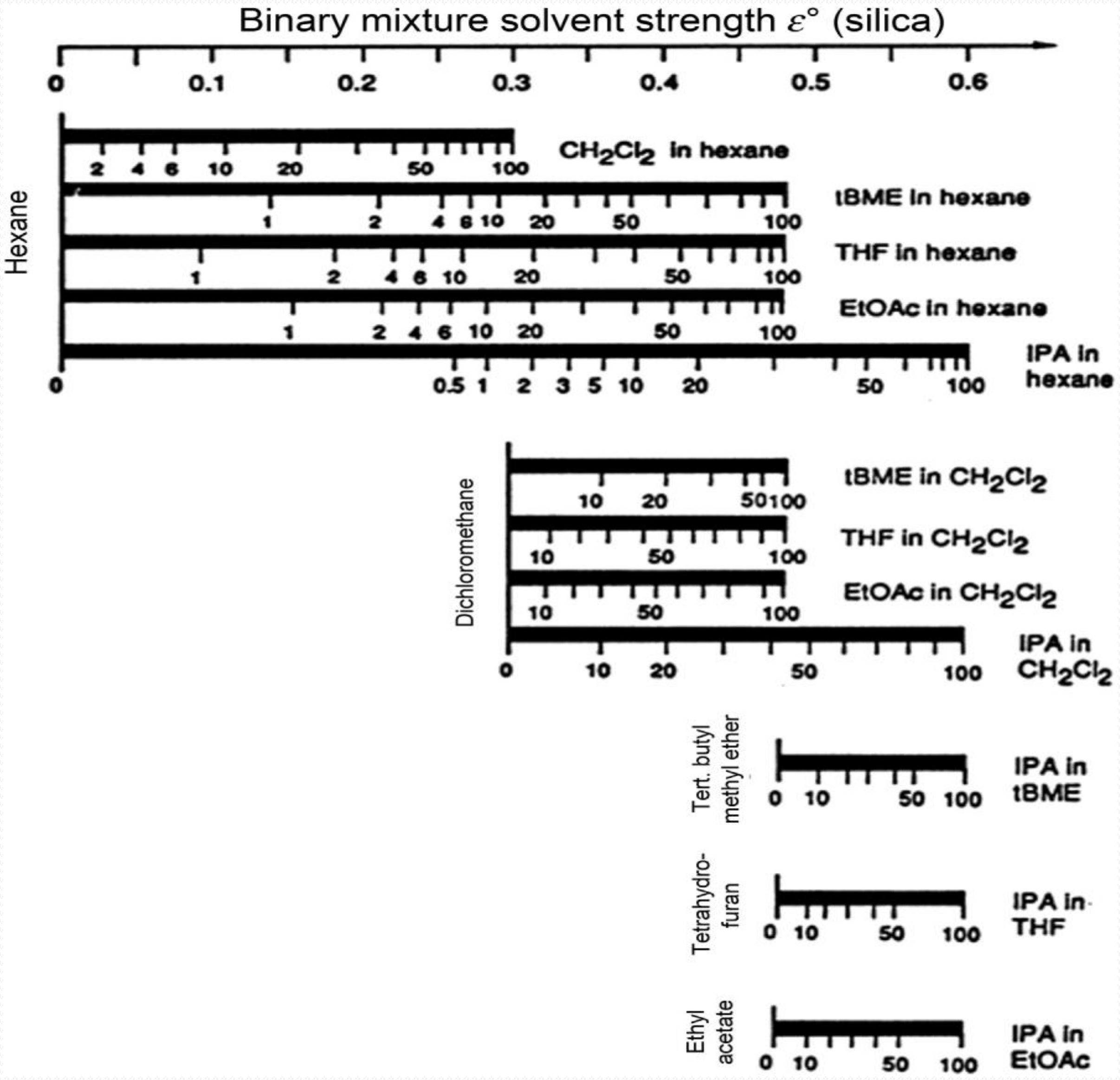
- Significantly more hydrogen-bond basic and dipolar/polarizable than silica gel
- Less hydrogen-bond acidic than silica gel

S. K. Poole and C. F. Poole, Chromatographia 53 (2001) S-162-166

Eluotropic series for silica gel

Solvent	ε°	Solvent	ε°
n-Heptane	-0.02	Formamide	0.55
Toluene	0.22	Propan-1-ol	0.60
Chloroform	0.26	Trifluoroethanol	0.62
Methyl t-butyl ether	0.29	Methanol	0.70
Dichloromethane	0.30	Water	0.72
Acetonitrile	0.52		
Acetone	0.53		
Dimethylformamide	0.51		

each CH_2 = -0.05


Binary mixture solvent strength $\varepsilon°$ (silica)
0 0.1 0.2 0.3 0.4 0.5 0.6
Hexane
CH_2Cl_2 in hexane
2 4 6 10 20 50 100
tBME in hexane
1 2 4 6 10 20 50 100
THF in hexane
1 2 4 6 10 20 50 100
EtOAc in hexane
1 2 4 6 10 20 50 100
IPA in hexane
0 0.5 1 2 3 5 10 20 50 100
Dichloromethane
tBME in CH_2Cl_2
10 20 50 100
THF in CH_2Cl_2
10 50 100
EtOAc in CH_2Cl_2
10 50 100
IPA in CH_2Cl_2
0 10 20 50 100
Tert. butyl methyl ether
IPA in tBME
0 10 50 100
Tetrahydro-furan
IPA in THF
0 10 50 100
Ethyl acetate
IPA in EtOAc
0 10 50 100

Limitation of Models for Retention on Inorganic Oxides

- Models fail to separate independent solvent and solute interactions with the solvated adsorbent (R_M is a composite parameter)
- The simple competition model ignores contributions from solute-solvent interactions in the mobile phase (mobile phase interactions are important)
- Active sites on the adsorbent surface have a heterogeneous energy distribution (site-specific interactions)
- Steric access to active sites is variable due to their non uniform distribution (steric repulsion)

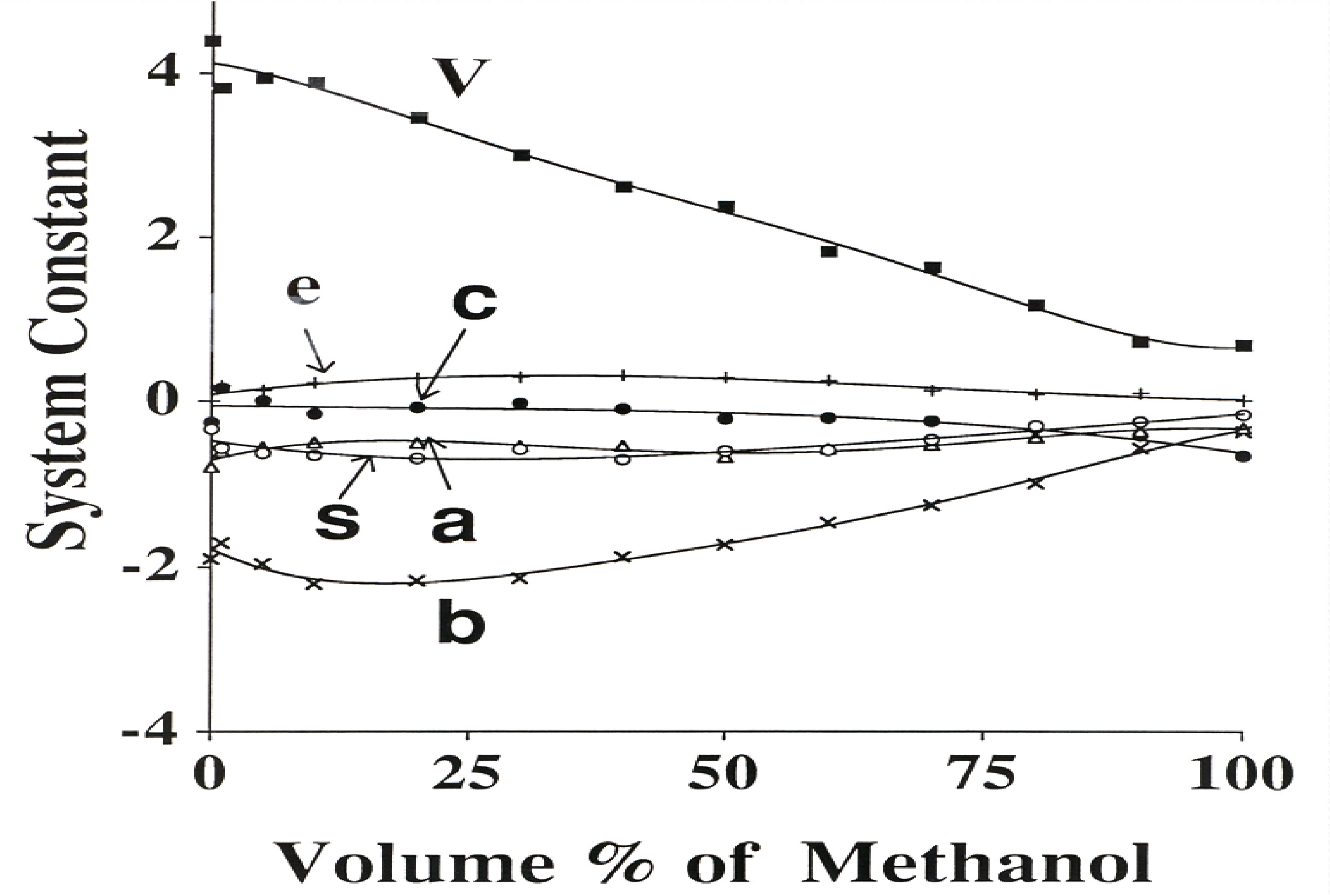
Solvents for Reversed-Phase TLC

Solvent	Cluster	Classification
Acetonitrile	4	Dipolar and weakly aprotic
Methanol Propanol	5	Amphiprotic
Acetone Tetrahydrofuran	6	Polar and non-hydrogen-bond acidic
Trifluoroethanol *N*,*N*-Dimethylformaide Pyridine		Polar and independent

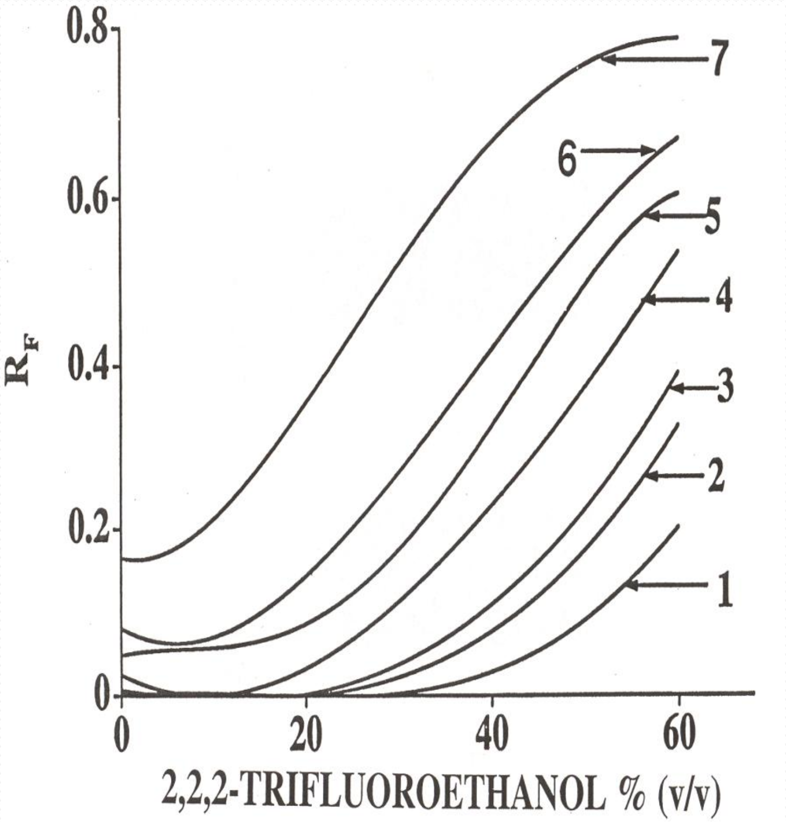
System Map

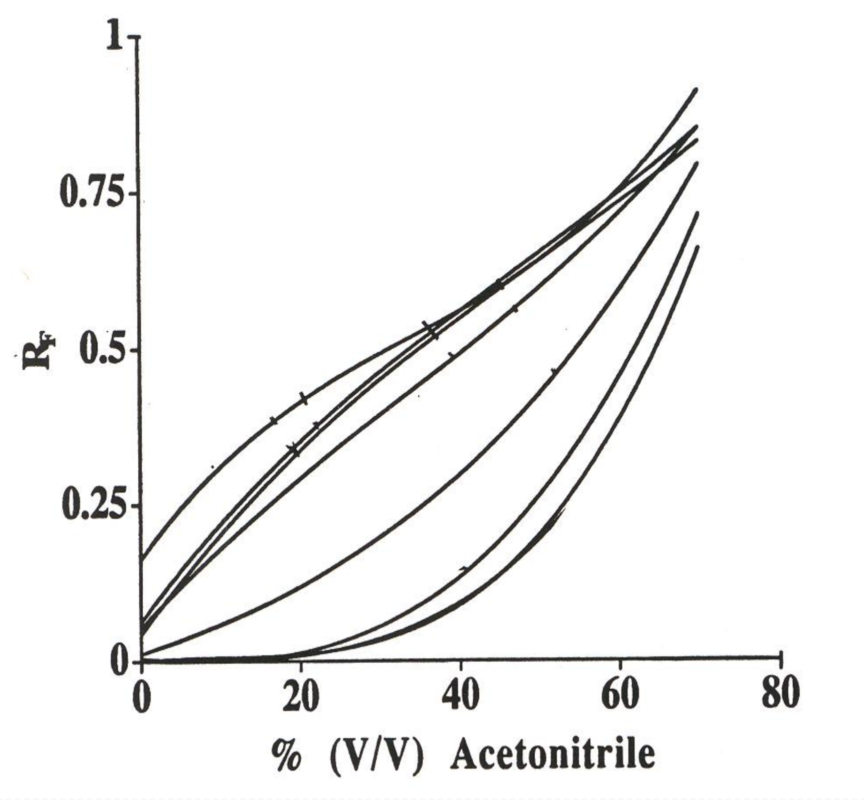
silica-based octadecylsiloxane-bonded layer

Methanol-Water mobile phase



Solvent Selection for Reversed - Phase Chromatography





Consequences of solvation of the interphase region on retention

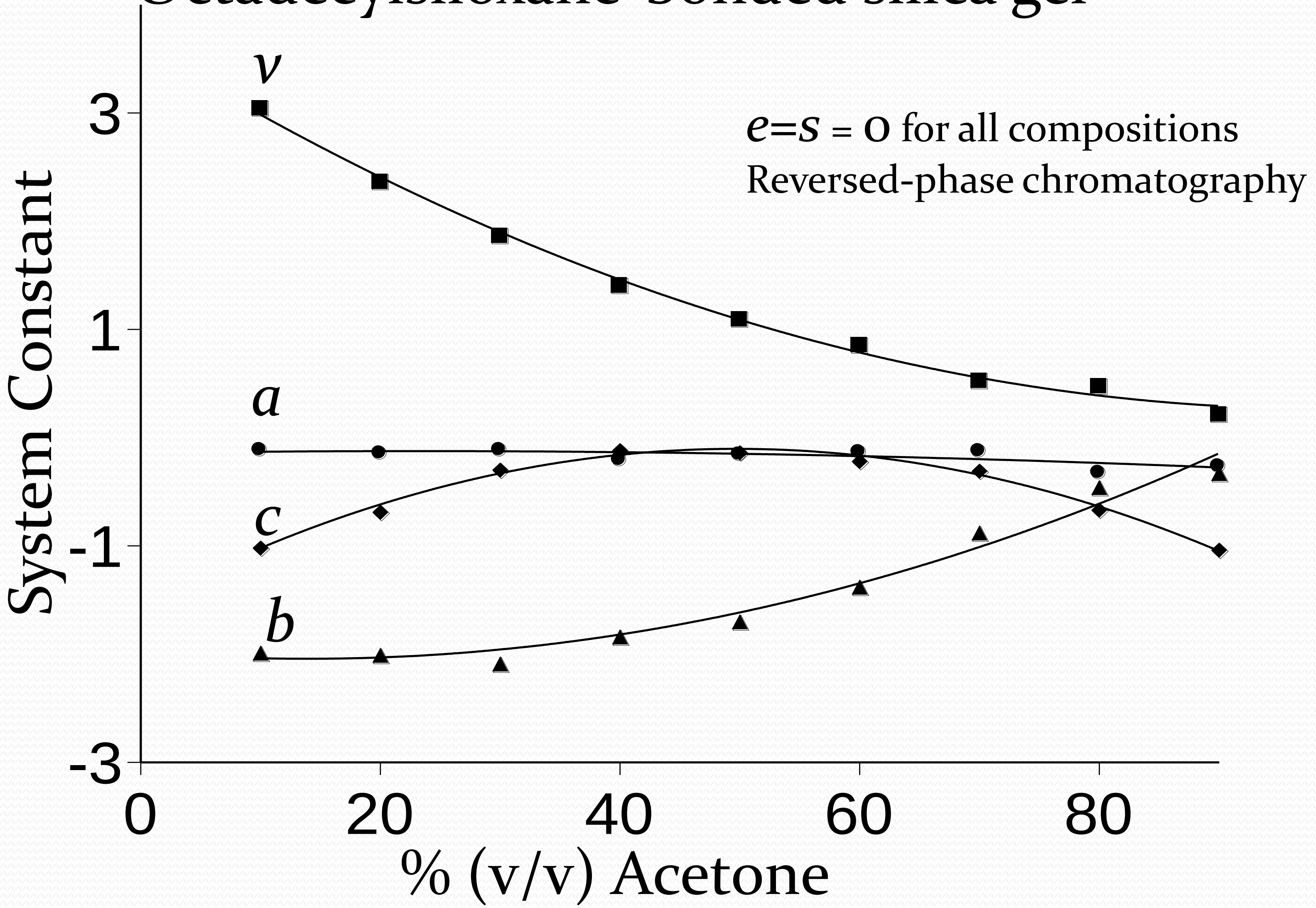
Reversed-Phase Chromatography
Mobile Phase = Methanol-Water (1:9)

Stationary Phase		System constants				
		v	*e*	*s*	*a*	*b*
Octadecylsiloxane	C18	3.64	0.29	-0.58	-0.59	-1.99
3-Aminopropyl	NH_2	0.38	0.37	0	0	-0.52
3-Cyanopropyl	CN	0.50	0.26	0	-0.17	-0.51
Spacer bonded propanediol	DIOL	0.49	0.89	-0.37	-0.26	-0.50

Polar Chemically Bonded Layers

- Method translation is difficult because retention on C18 layers is much larger than for polar chemically bonded layers
  - Low retention results from higher cohesion of the interphase region and an unfavorable phase ratio (smaller interphase volume)
- More dipolar and hydrogen-bond acidic than C18
  - Hydrogen-bond acidity does not correlate with the identity of the polar group
  - Dipole-type interactions are not significant for retention on CN layers
  - Selectivity differences among NH_2, CN, and DIOL layers are small compared to the difference with C18

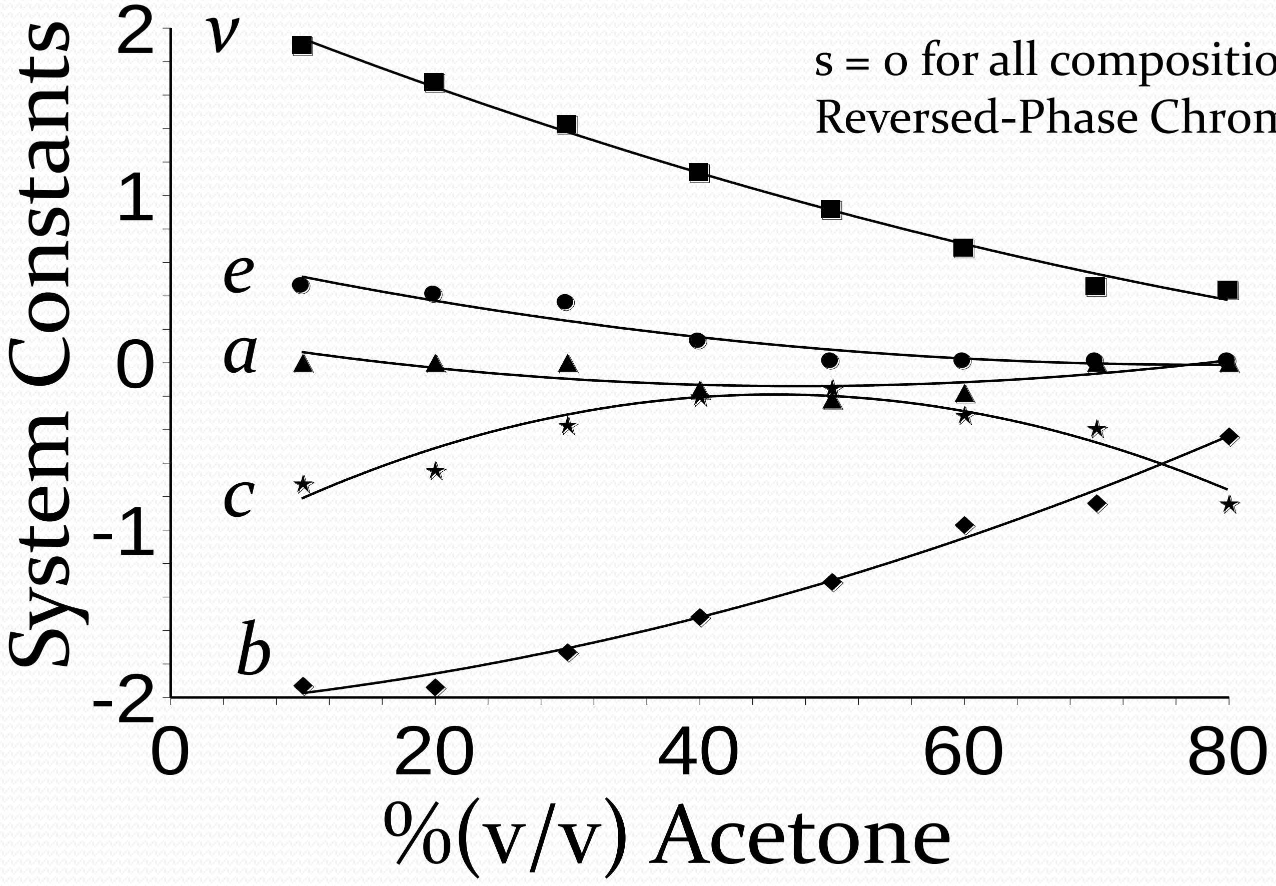
Octadecylsiloxane-bonded silica gel



e=*s* = 0 for all compositions

Reversed-phase chromatography

Cyanopropylsiloxane-bonded silica



s = 0 for all compositions
Reversed-Phase Chromatography

Limitation of the R_M value

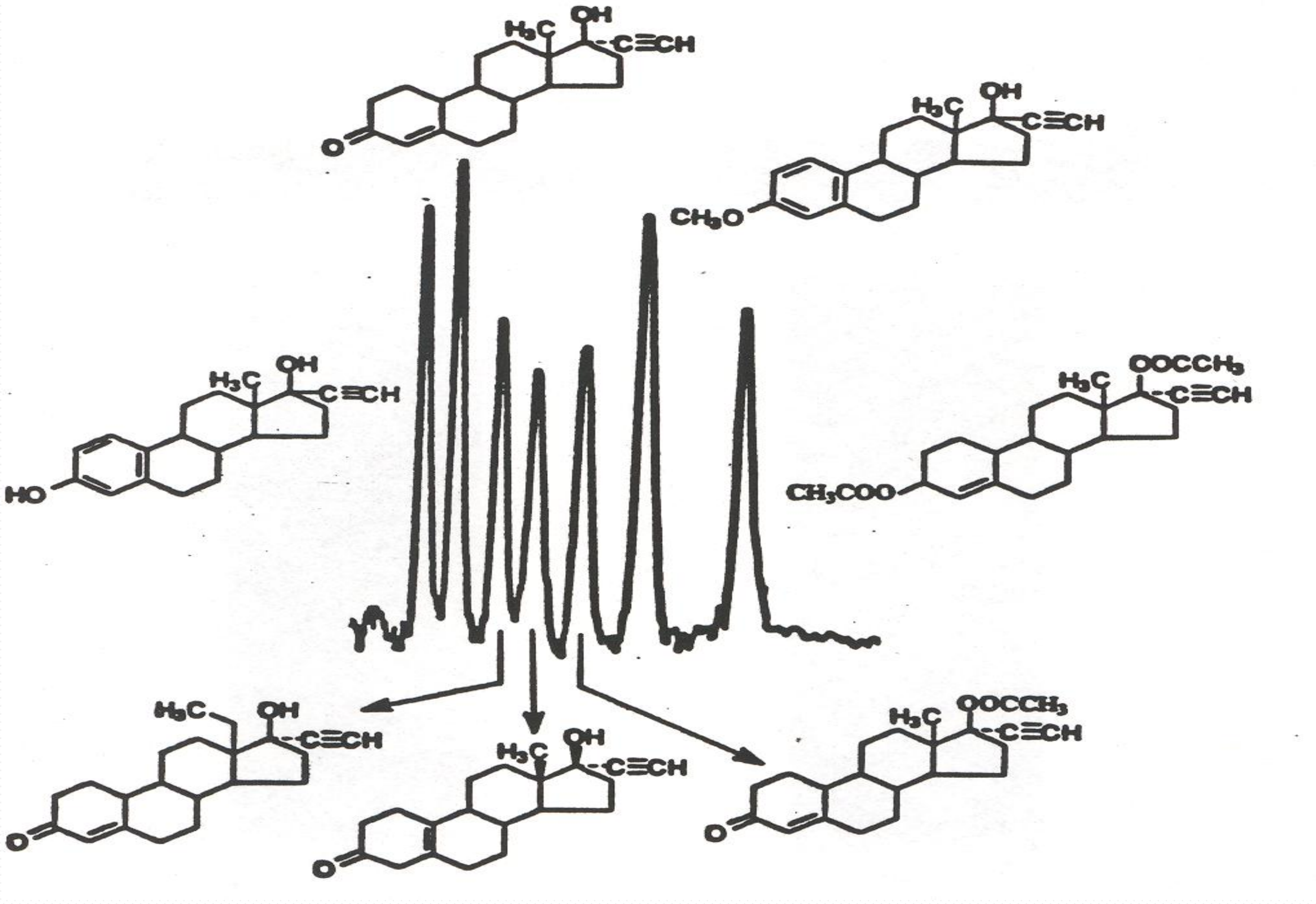
- For inorganic oxide adsorbents the R_M value is a composite term determined by independent sample and mobile phase interactions with the adsorbent surface.
- For weakly localizing solvents the competition model handles this problem by describing retention in terms of:
  - a solute-independent solvent adsorption term $\varepsilon°$
  - a solvent-independent solute adsorption term, $S°$

Liquid-solid chromatography

- Suitable for non-ionic compounds soluble in organic solvents
- Samples are separated based on their competition with the mobile phase for adsorption on fixed or mobility restrained surface sites on the stationary phase
- Retention depends on the type, number and position of polar functional groups

- Stationary phases
  - Inorganic oxides
  - Polar chemically bonded phases
- Significant difference in steric fitting facilitates the separation of isomers and diasteromers

Ethenyl Estrogens



Polar Chemically Bonded Phases

Retention dominated by polar interactions while size generally reduces retention

CN Sorbent behave like a deactivated (weak) silica gel with unreacted silanol groups as the dominant active sites

◆ small quantities of moderators (< 1% alkylamines, alcohols, etc) used to optimize retention and peak shapes

Amino Complementary to CN with the amino group imparting strong hydrogen-bonding properties to the stationary phase

DIOL Generally less retentive of dipolar compounds than CN and less retentive of hydrogen-bonding compounds than Amino stationary phases

Parameter Space

The parameter space is that combination of experimental variables and their limiting values that define the search area

- An acceptable separation must be within the parameter space
- If the parameter space is set artificially large the search will be inefficient
- Include only those variables that have a significant effect on selectivity

Solvents for Normal-Phase TLC

Solvent	**Cluster**	**Classification**
Heptane	1	Apolar
Toluene	2	Apolar aromatic
Dichloromethane	3	Haloalkane
Chloroform		
Acetonitrile	4	Dipolar and weakly aprotic
Methanol	5	Amphiprotic
2-Propanol		
Acetone	6	Polar and non-hydrogen-bond acidic
Methyl *t*-butyl ether		
Formamide	7	Polar and cohesive
Trifluoroethanol		Polar and independent
N,N-Dimethylformamide		
Dimethyl sulfoxide		
Water		