An Interphase Model for Retention in (Thin-Layer) Chromatography
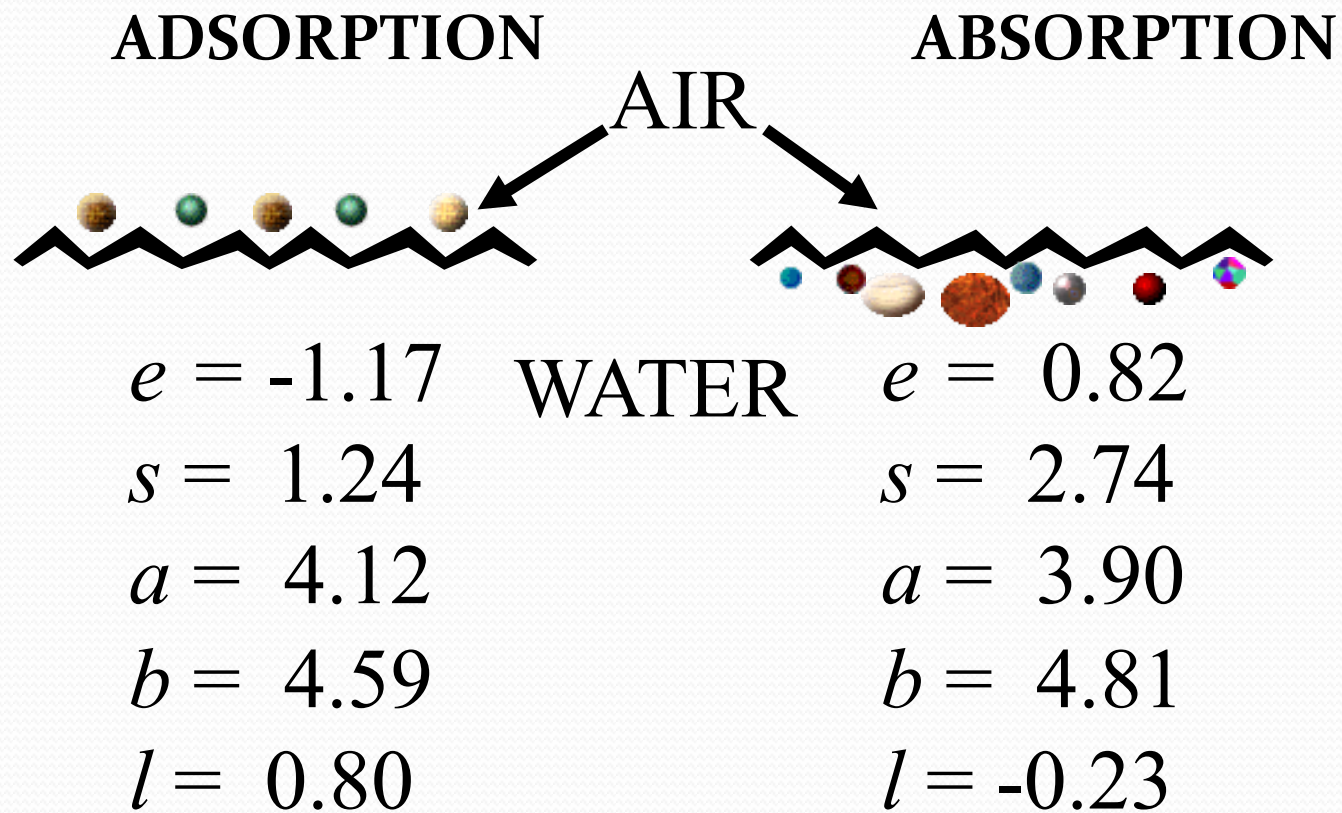
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Distribution Models

- Interface
  - Distinct and microscopically thin dividing plane separating two dissimilar phases
    - Gas-Liquid Chromatography
- Interphase
  - Diffuse and microscopically thick region separating two dissimilar phases
    - Liquid Chromatography
    - Supercritical Fluid Chromatography
    - Micellar Electrokinetic Chromatography

Interface Model

ADSORPTION   **ABSORPTION**

AIR

WATER

$e = -1.17$ $e = 0.82$

$s = 1.24$ $s = 2.74$

$a = 4.12$ $a = 3.90$

$b = 4.59$ $b = 4.81$

$l = 0.80$ $l = -0.23$

Interphase Region and Boundaries

- That region immediately adjacent to the solvated stationary phase surface between the impenetrable region of the stationary phase and the bulk mobile phase
- The boundaries of the interphase region are not well defined nor necessarily fixed

Interphase Model

Interphase Region Characteristics

- The spatial composition of the interphase region is rarely homogeneous

- The interphase region is generally thin and solutes can explore the whole region resulting in an averaging effect for macroscopic properties

- The solvent composition within the interphase region is generally different to the bulk mobile phase composition because of selective solvation of the stationary phase surface

Interphase Region and Retention

Retention results from the difference in solvation properties of the interphase region
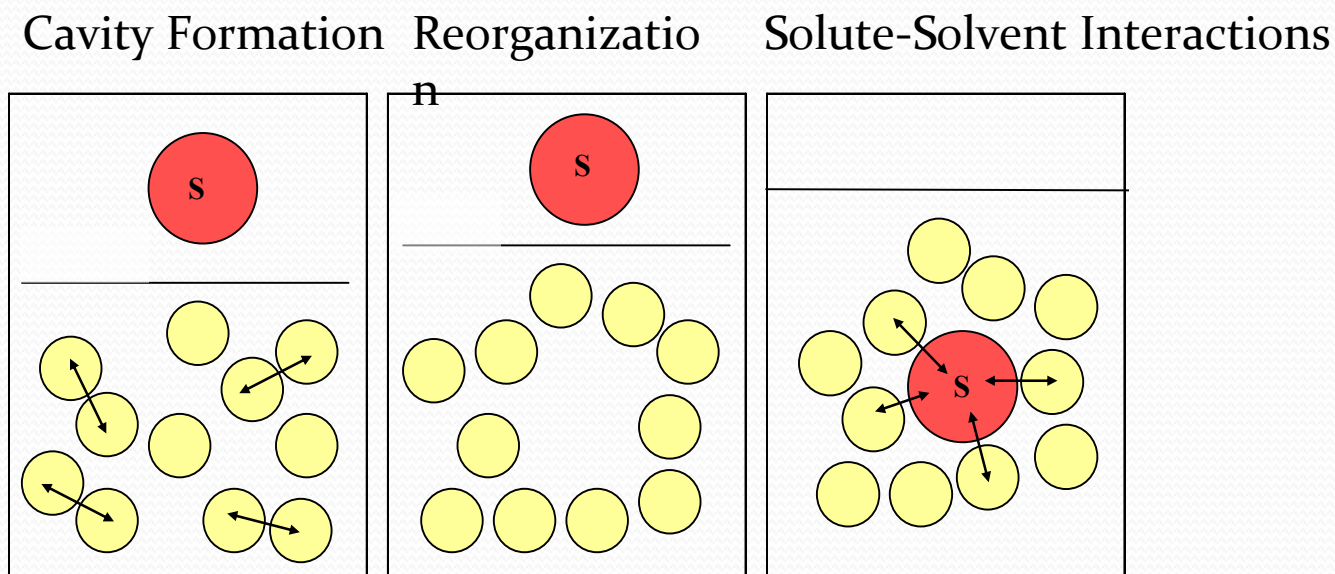
and the bulk mobile phase

Stationary Phase = Interphase Region

Mobile Phase = Bulk Solvent

C.F. Poole, S.K. Poole. J. Chromatogr. A 792 (1997) 59-104

Solvation Parameter Model

➢ Parameterization of cavity model of solvation

Cavity Formation   Reorganization   Solute-Solvent Interactions

➢ Mass transfer between condensed phases

$$\log SP = c + eE + sS + aA + bB + vV$$

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

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

System Map

silica-based octadecylsiloxane-bonded layer
Methanol-Water mobile phase
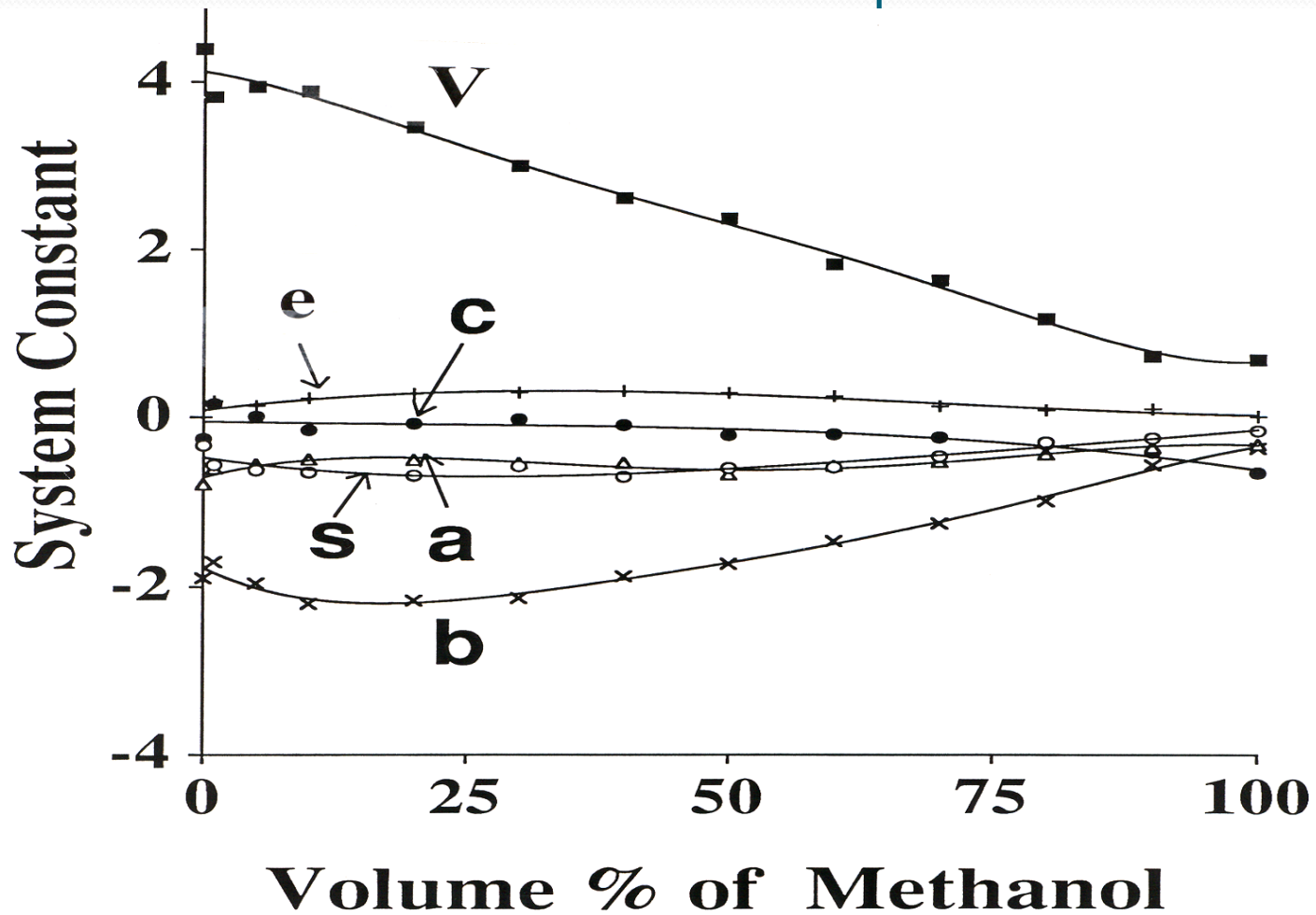
Surface Excess

In Reversed-Phase separations with mixed mobile phases an excess (μmol/m²) of one (or more) components of the mobile phase exists at the stationary phase surface (a major component of the interphase region) which depends on the relative affinity of the various components of the mobile phase for the stationary phase surface.
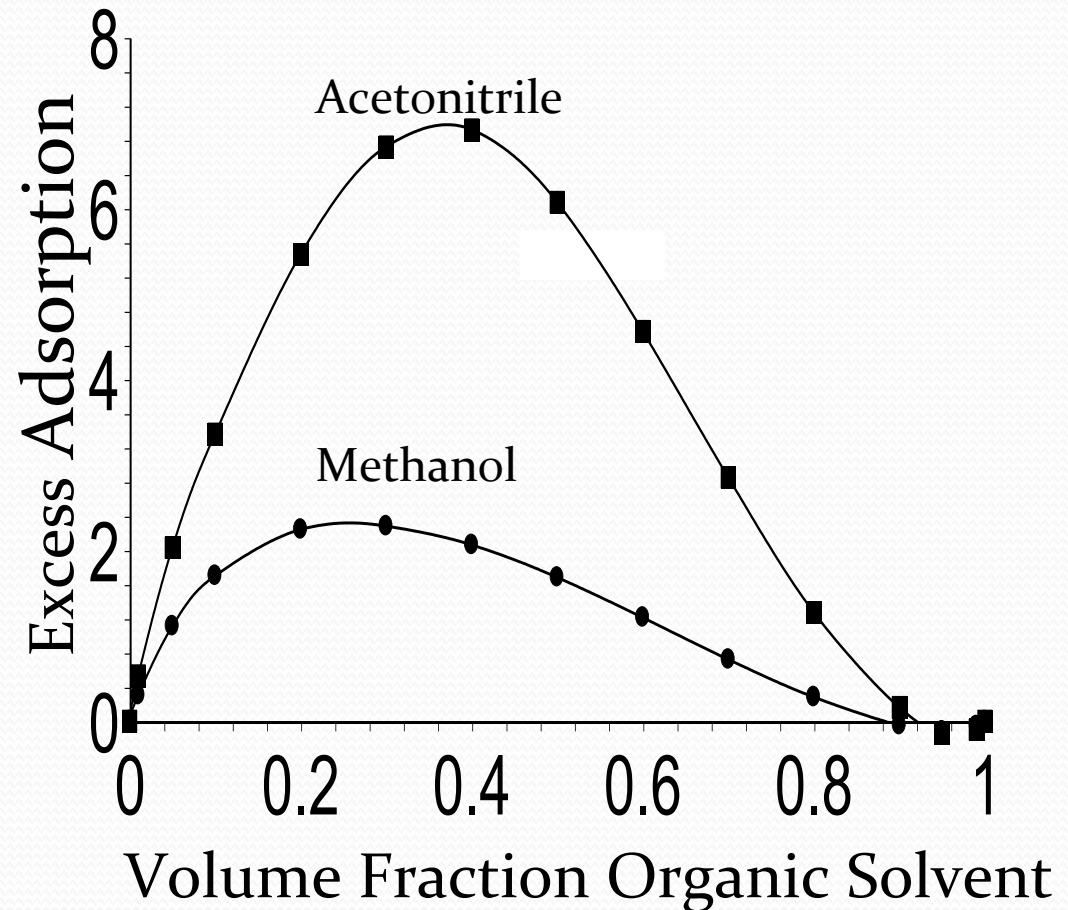
Consequences of solvation of the interphase region on retention

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

Steric resistance

Inability of solutes to penetrate freely into the interphase region because of their size and/or shape

- Angular compounds
- Rigid planar compounds
- Compounds with long alkyl chains
- Bulky compounds in general

C.F. Poole, S.K. Poole, J. Chromatogr. A 1216 (2009) 1530-1550
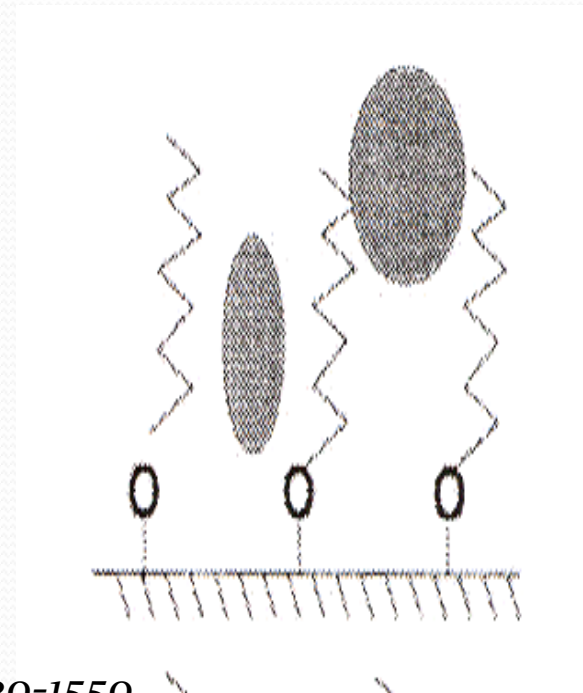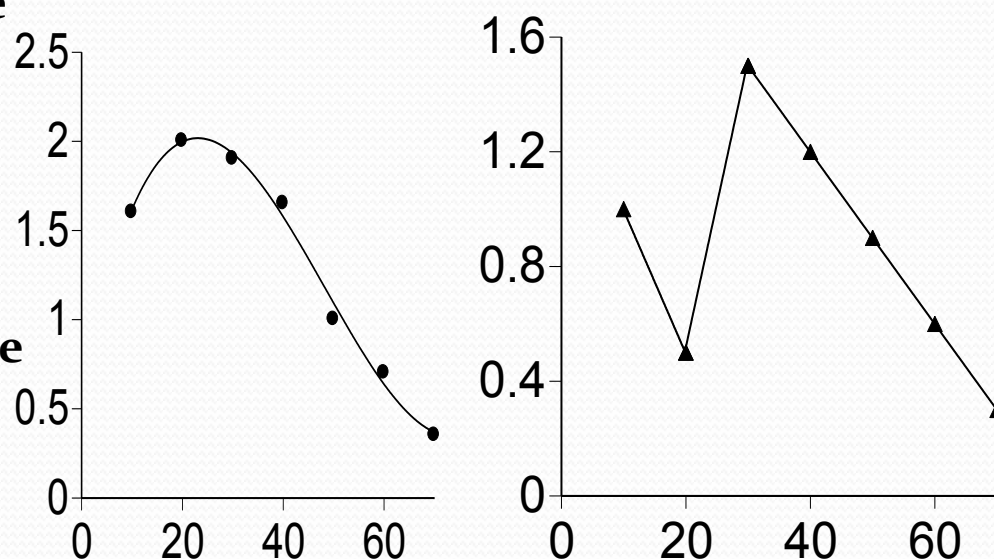
Retention plot types (steric resistance)

Solute properties of importance
- Size
- Conformational rigidity
- Presence of hydrogen-bonding functional groups

**System properties of importance**
- Type and volume fraction of organic solvent
- Bonding density of stationary Phase
- Ligand chain length

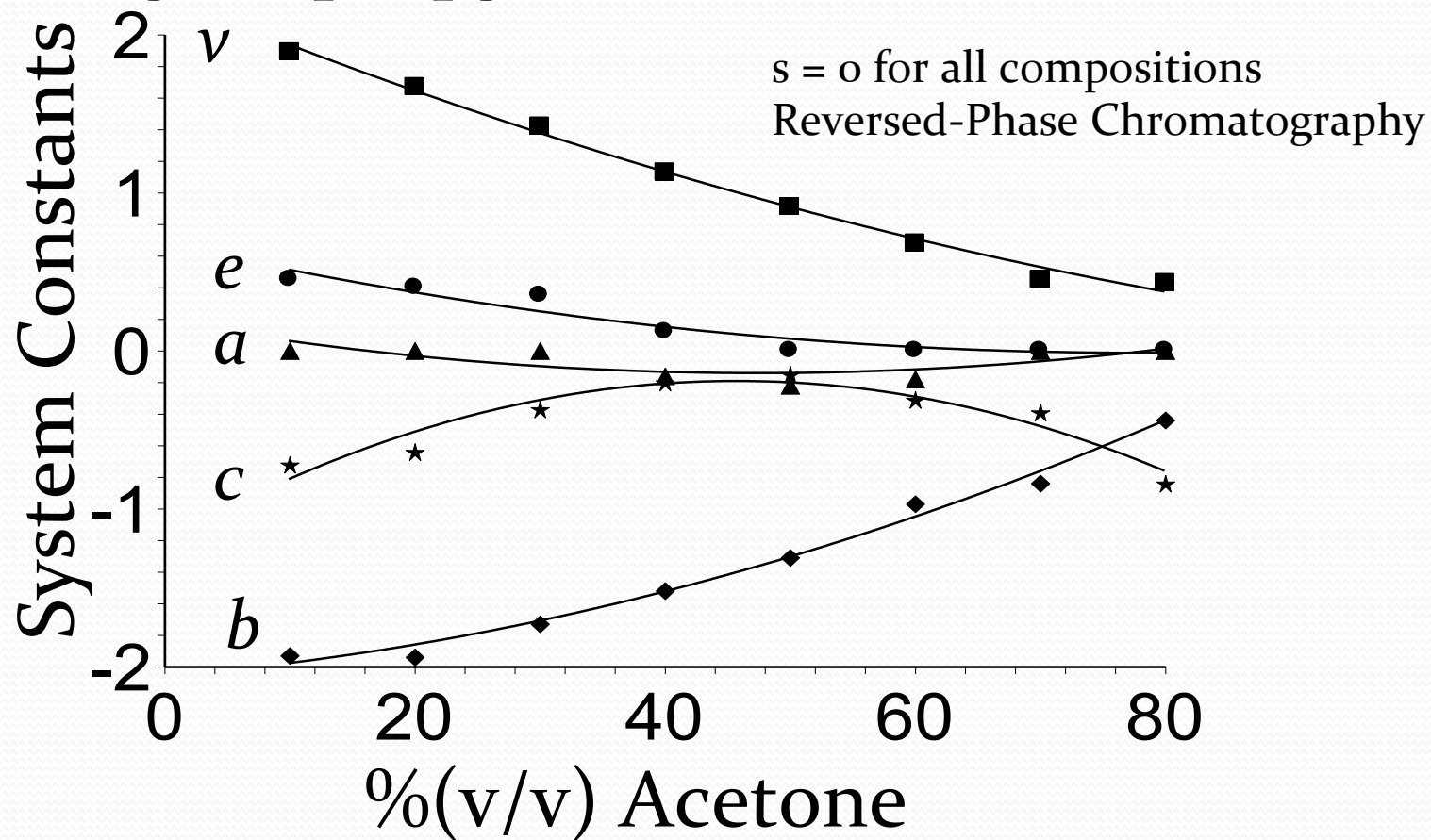R_M vs mobile phase composition

Steric resistance is usually enhanced by methanol-water mobile phases and by water-rich mobile phases in general

Octadecylsiloxane-bonded silica gel

Cyanopropylsiloxane-bonded silica

s = 0 for all compositions
Reversed-Phase Chromatography
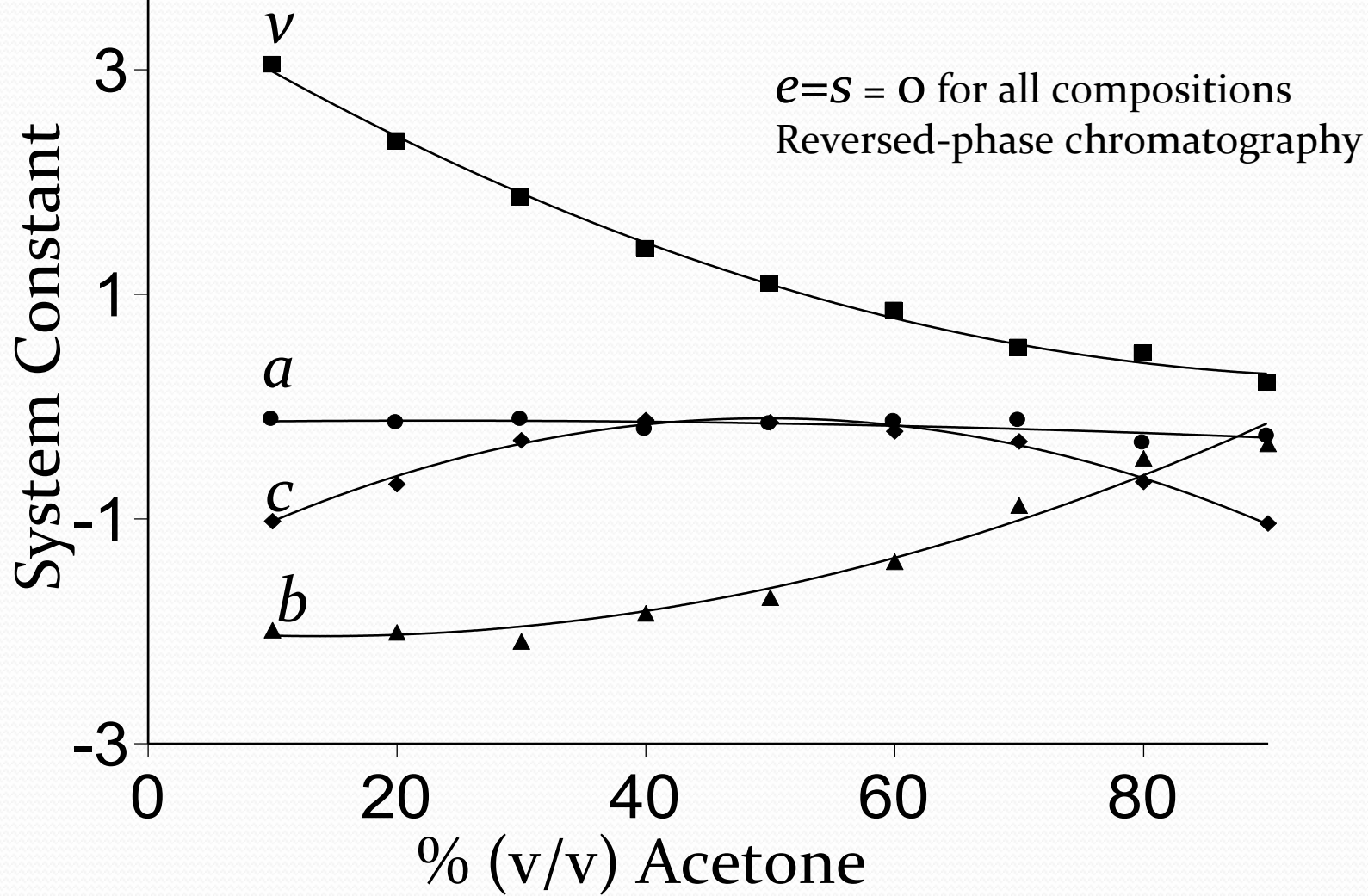
Polar Chemically Bonded Layers

- 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

Normal or Reversed Phase

Stationary phase	Cyanopropylsiloxane-Bonded Silica		Silica
Mobile phase	Water	Hexane (1% v/v methanol)	Hexane
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

Models for Normal-Phase Chromatography

Competion Model

Solvent Interaction Model
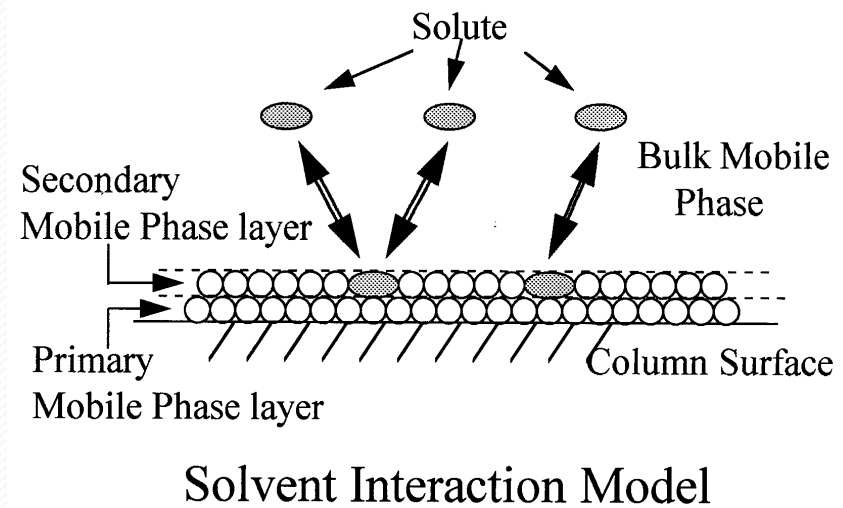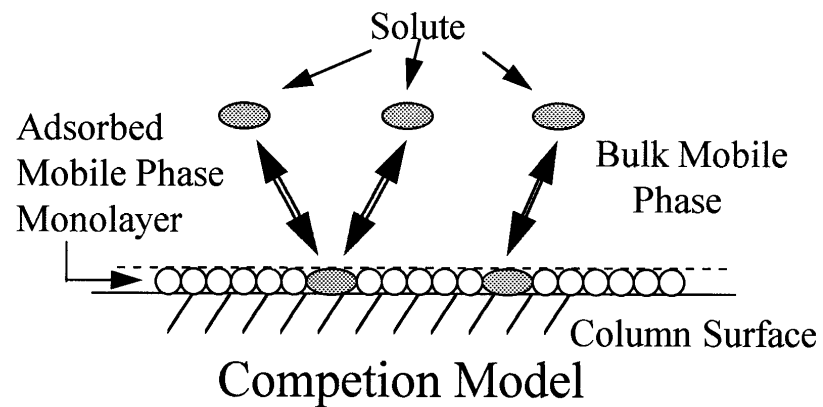
Simplified Competition Model

$$X_m + nM_a \leftrightarrow X_a + nM_m$$

$$R_M = c + \alpha'(S° - A_S \varepsilon°)$$

c = lumped (system constant)

α' = adsorbent activity constant

S° = free energy of solute adsorption on a standard adsorbent (α' = 1)

A_S = adsorbent cross-section of the solute

$\varepsilon°$ = solvent strength parameter

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, $S°$

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

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

Solute Adsorption Cross-Section

$$A_s = 1.81 - 2.40E + 8.67S + 3.74A + 11.6B$$

- Average error of prediction similar to the experimental error

- $v = 0$

- Main contribution from polar interactions

- A_s is a chemical term rather than a physical property describing a molecular dimension

Free Energy of Solute Adsorption

- Analysis of the S° term
  - dependent on the solvent identity for weakly localizing solvents
  - physical significance ambiguous
- Modeling of the A_s and S° parameters of the competition model suggests some co-mingling of solute and solvent properties
  - explains some of the uncertainty in retention predictions

Solvent Strength Parameter $\varepsilon°$

- Solvent strength of a pure solvent can be defined by $\varepsilon°$ 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 $\varepsilon°$ 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 $\varepsilon°$ 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	each CH_2 = -0.05	
Dimethylformamide	0.51		

Inorganic Oxides

The R_M value is a composite term reflecting independent solvent and solute interactions with the stationary phase

Competition model handles this problems by describing retention in terms of:

- solute-independent solvent adsorption term $\varepsilon°$
- solvent-independent solute adsorption term $S°$

Reasons for failure to model retention

A_S has characteristics of polar interaction term and is more than a size-dependent property

$S°$ is not independent of solvent identity

At least secondary interactions involving either solute interactions in the mobile phase or solvent modification of the absorbent surface are included in $S°$

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)

Solvent strength

Single parameter estimate of a solvent's ability to cause migration in a chromatographic system

- Not a fundamental solvent property since it depends on the system
- Determined by experiment

# 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

Solvent selectivity

- The parameter that distinguished the ability of a solvent to provide separation as well as migration
  - Determined by the solvent's capability to enter into specific intermolecular interactions
    - Dispersion
    - Orientation
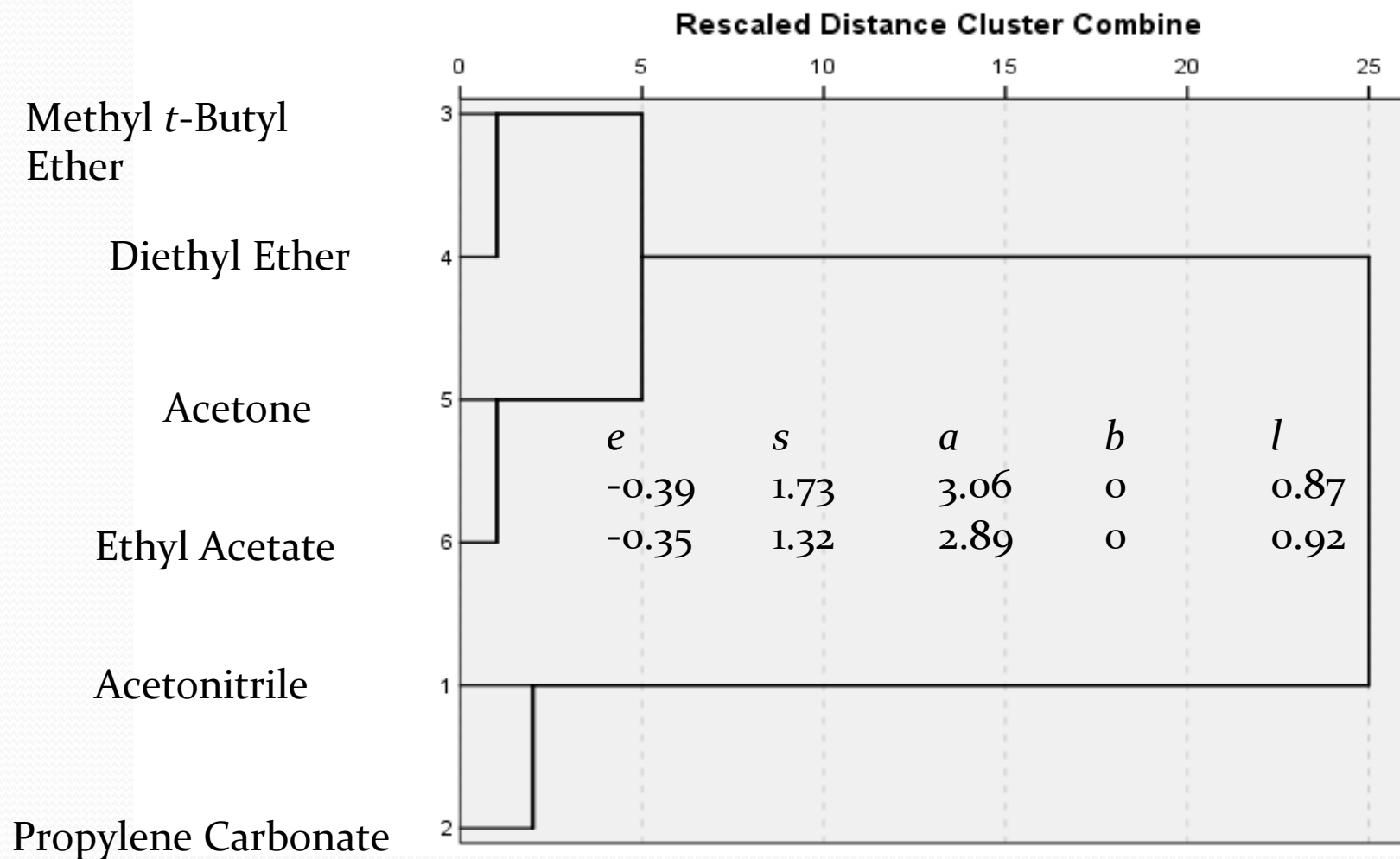   - Induction
    - Hydrogen bonding

Dendrogram using Average Linkage (Between Groups)
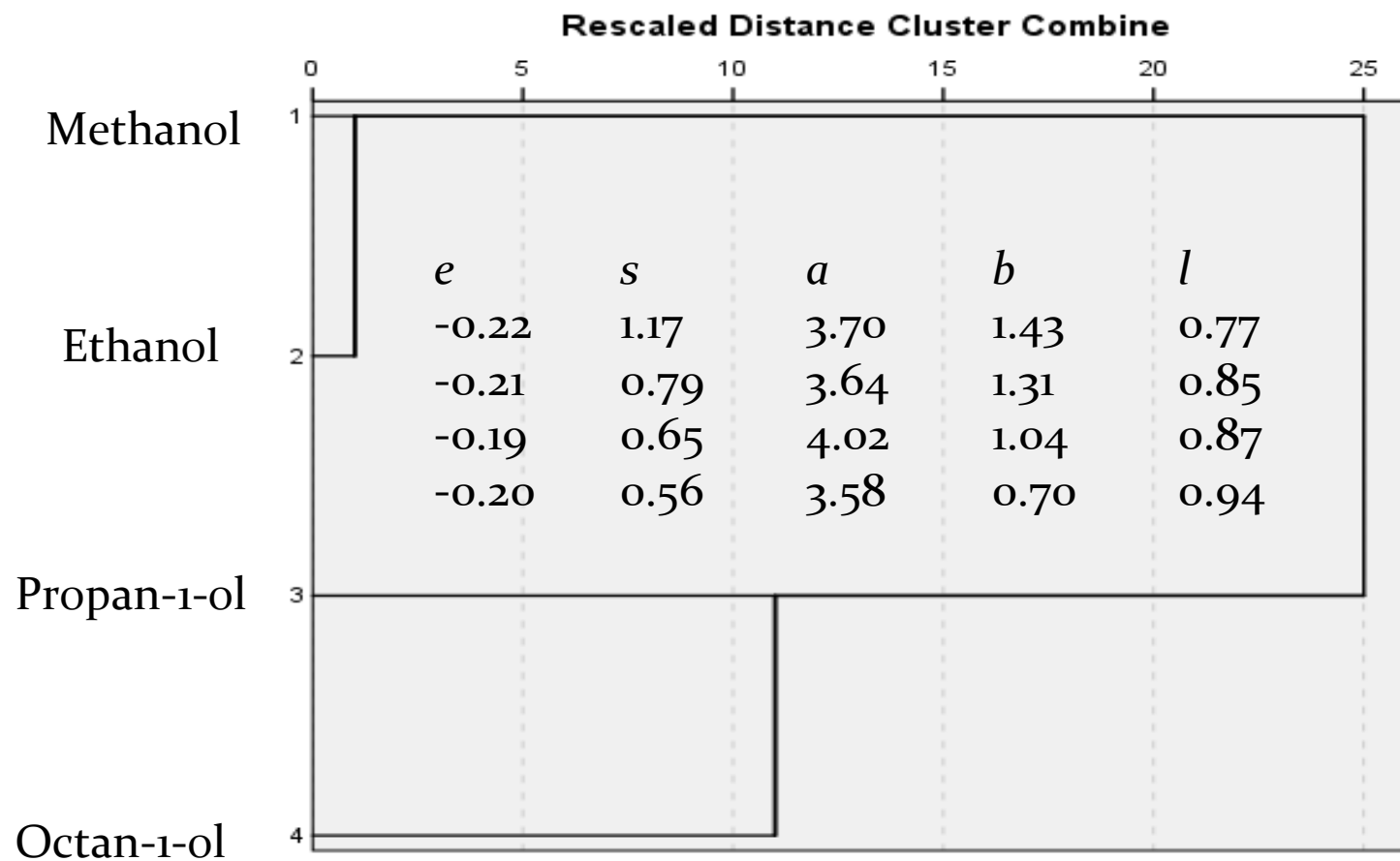
Rescaled Distance Cluster Combine
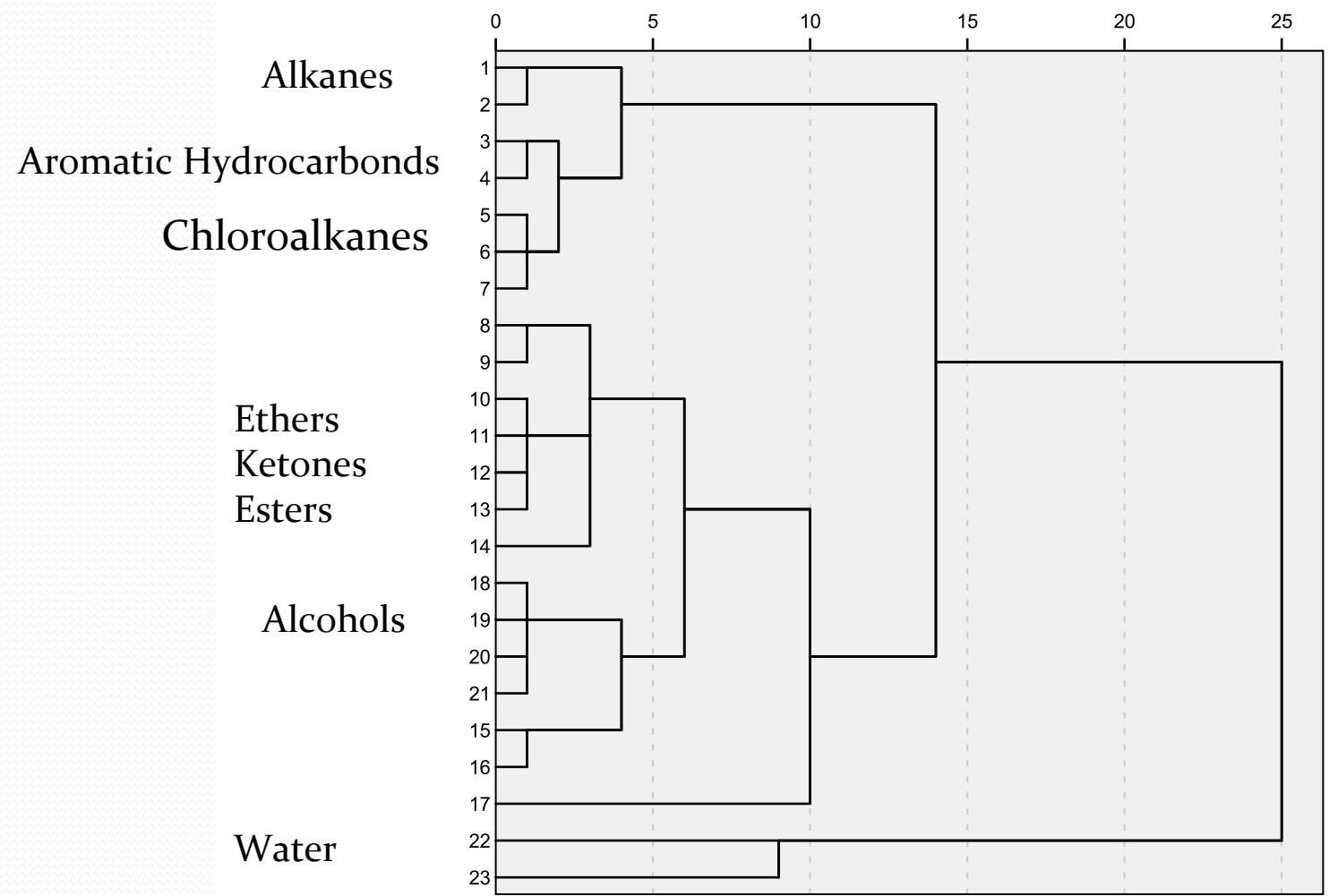
Group 3 Ethers, Esters and Ketones

Dendrogram using Average Linkage (Between Groups)

Rescaled Distance Cluster Combine

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

Methyl *t*-Butyl Ether

Diethyl Ether

Acetone

Ethyl Acetate

Acetonitrile

Propylene Carbonate

Group 4 Alcohols

Dendrogram using Average Linkage (Between Groups)

Rescaled Distance Cluster Combine

	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

Methanol

Ethanol

Propan-1-ol

Octan-1-ol

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
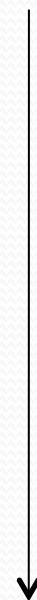- Trifluoroethanol
- Water

Liquid-solid chromatography

General adsorption scale for silica gel

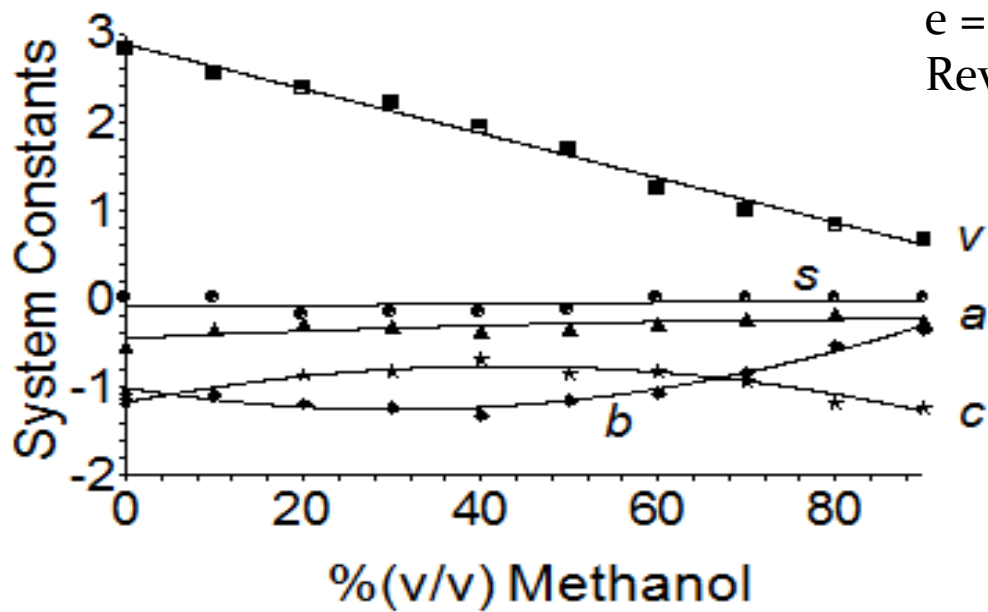|		
Difficult to separate because solvent strength is too high	Alkanes Aromatic Halogenated Compounds Ethers Nitro Compounds Nitriles Carbonyl Compounds Alcohol Phenols Amines	**Weak**
Difficult to separate because solvent selectivity is too low	Amides Carboxylic acids Sulfonic acids	**Strong**

System Map

Octadecylsiloxane-Bonded Silica

e = o for all compositions
Reversed-Phase Chromatography