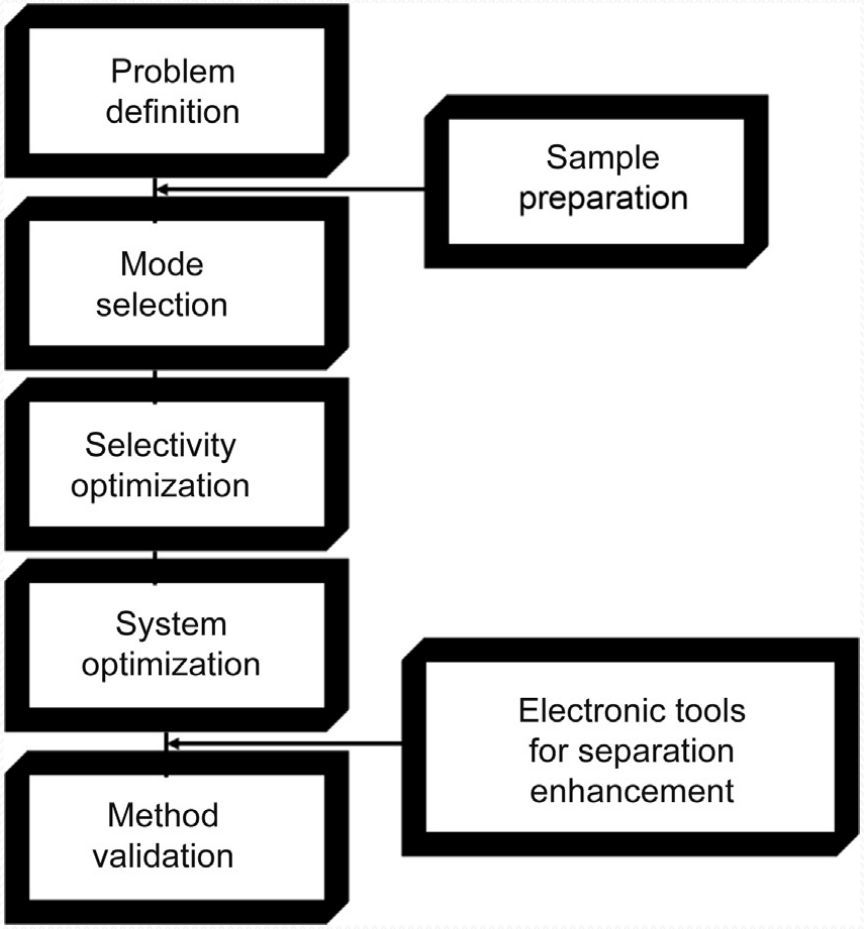
Method Development and Solvent Selection in Liquid Chromatography with Emphasis on Thin-Layer Chromatography

Colin F. Poole
Department of Chemistry
Wayne State University
USA

Method Development Process



Method Development Process

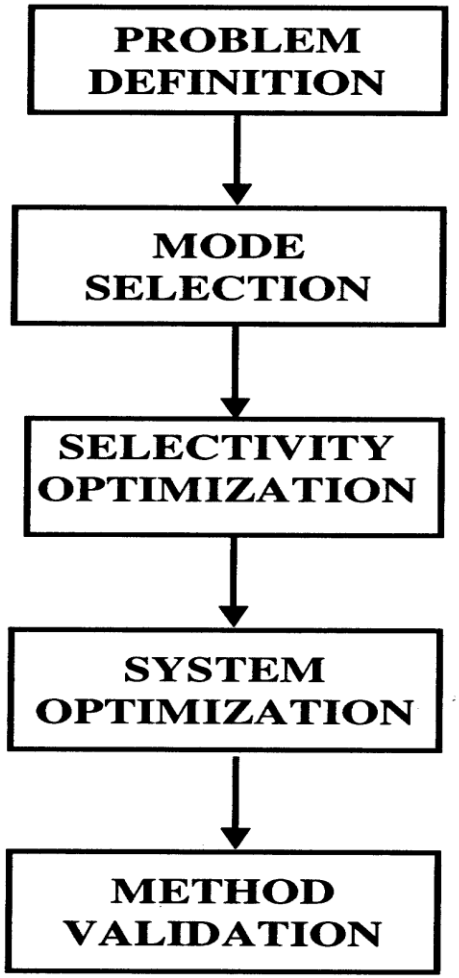
Need to know what to do

Before beginning experiments need to decide how to do it

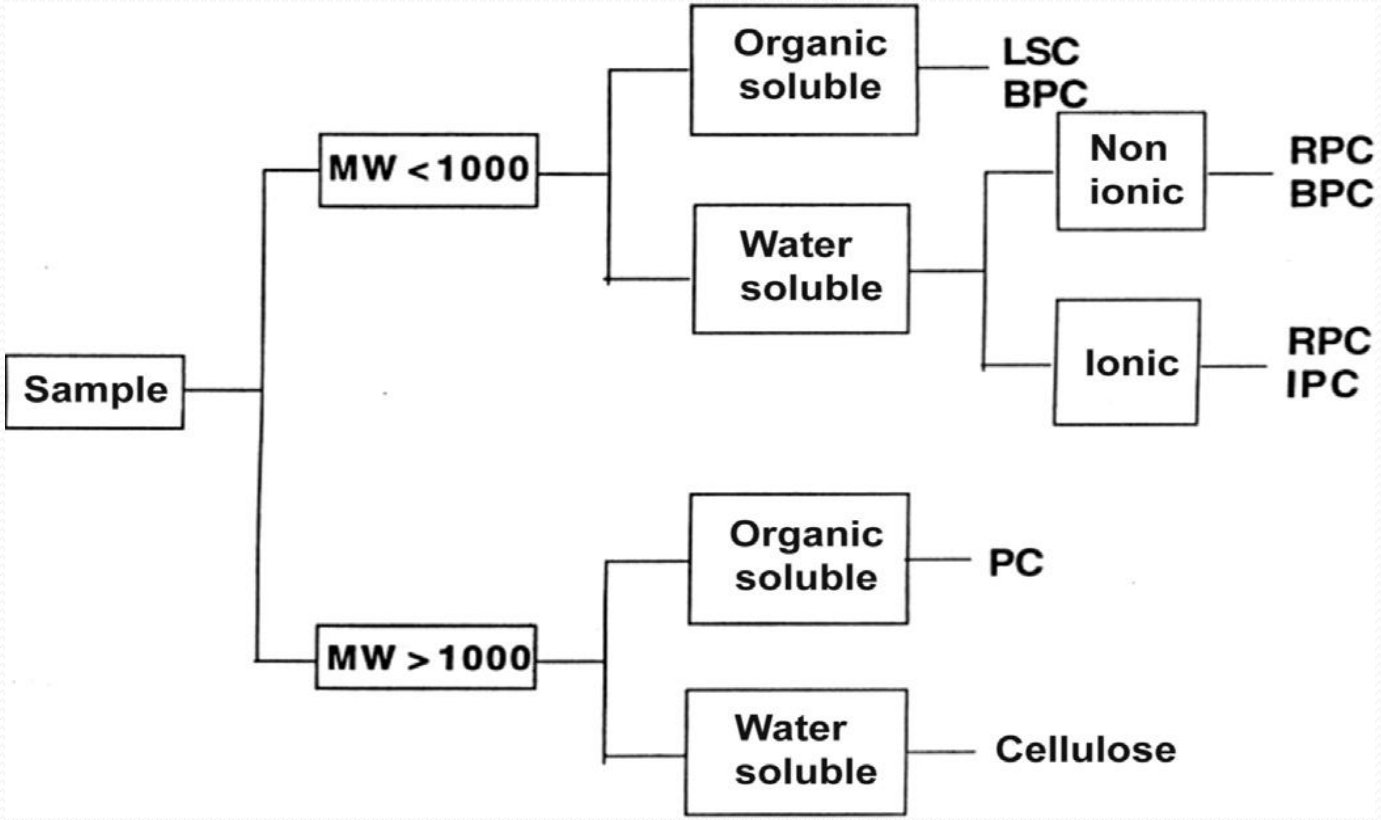
Survey experiments to find conditions compatible with goals

System properties modified to achieve goals

Confirmation that the method is suitable with respect to goals



Mode Selection



Cellulose layers and compounds < 1000 MW

Water soluble polar compounds by partition in reversed-phase mode

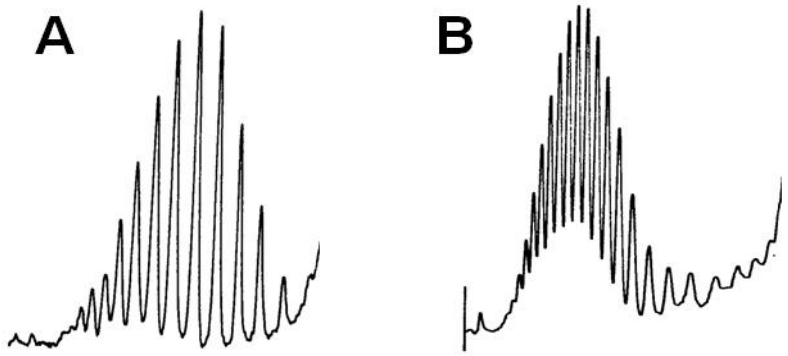
Retention of low-polarity compounds too weak to be useful

Pore Size Selection

Separation of PEG oligomers as 3,5-DNB derivatives on silica gel 60 by AMD

A: average MW 400

B: average MW 1000



60 nm

For compounds with a MW < 1000 (size exclusion effects)

For compounds > 1000 MW precipitation chromatography is a possible option

5000 nm

For compounds that bind strongly to silica gel

Water soluble compounds with MW > 1000

Separation by Adsorption

- 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

Normal-Phase 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
Amides
Carboxylic acids
Sulfonic acids

Difficult to separate because solvent selectivity is too low

Weak

Strong

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.

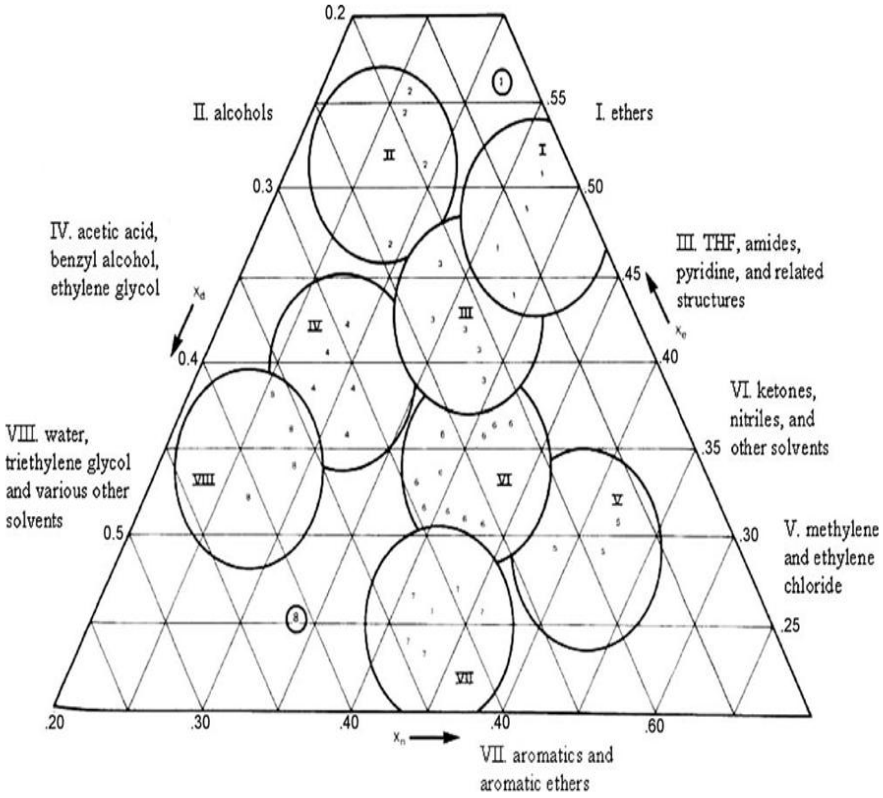
Solvent Selection

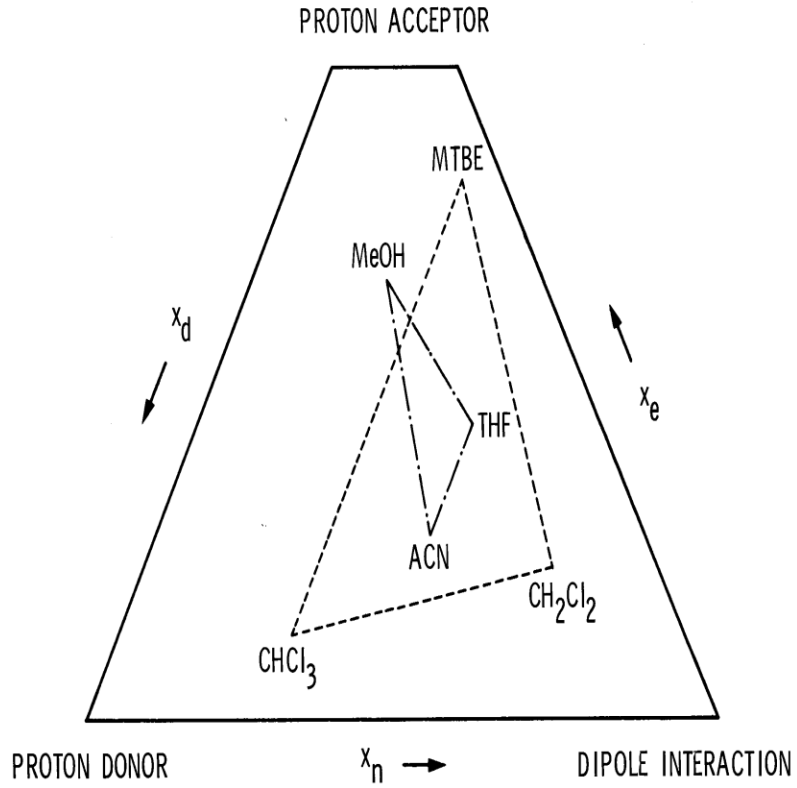
- Solvent strength
  - Estimate of the solvents capability to cause migration in a chromatographic system
  - Depends on the identity of the stationary phase
    - This is both a system and a solvent property
- Solvent selectivity
  - Estimate of the relative ability of the solvent to participate in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

General Strategies for Solvent Classification

- Solubility parameter model
  - Only contemporary use is in polymer science
- Solvatochromic parameters
  - Based on spectroscopic (non-equilibrium properties)
  - Considers only polar interactions and not the cohesive energy of solvents
- Snyder's solvent selectivity triangle
- Solvation parameter model

Snyder's Selectivity Triangle





Prototypical Solutes

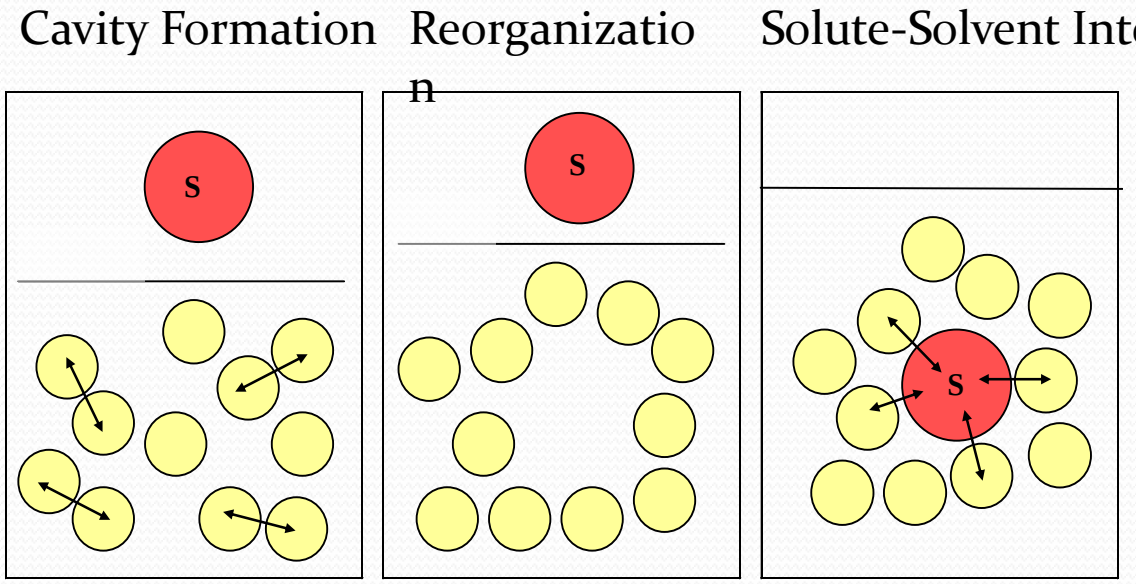
Polar solutes with a single dominant intermolecular interaction are virtually unknown

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



- 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

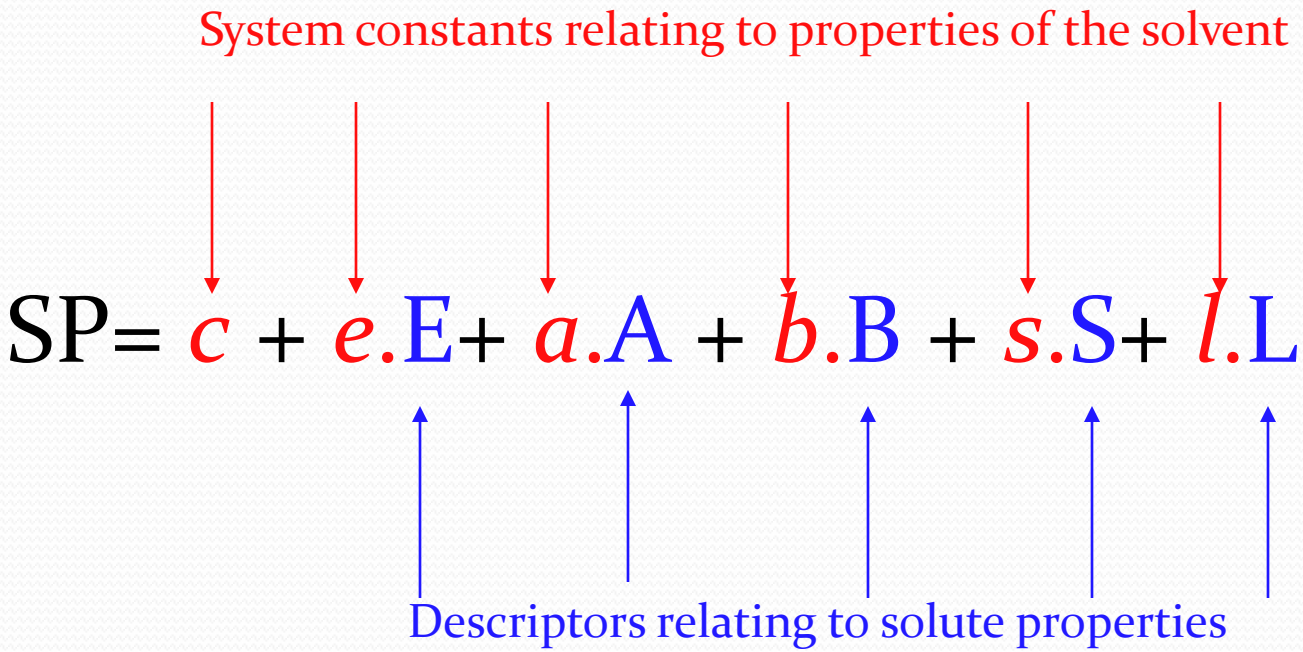
Contains a term to accommodate cavity formation

(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



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

$$\log K = c + eE + sS + aA + bB + lL$$

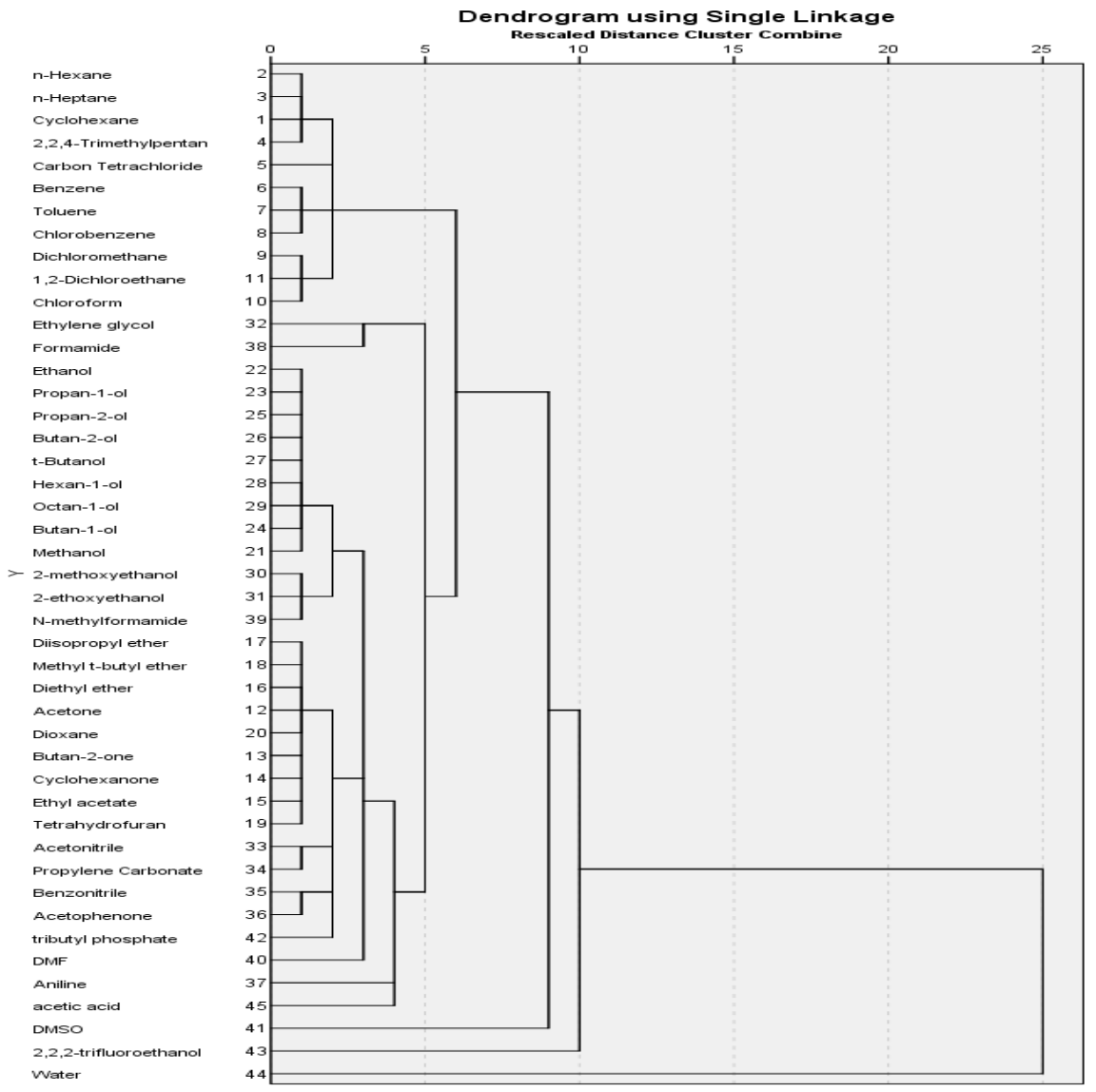
System Constant	Solute Descriptor	Free Energy Contribution
l	L	Ease of cavity formation Residual dispersion interactions
e	E	Electron lone pair interactions
s	S	Dipole-type interactions
a	A	Solvent hydrogen bond base-solute hydrogen-bond acid interactions
b	B	Solvent hydrogen bond acid-solute hydrogen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

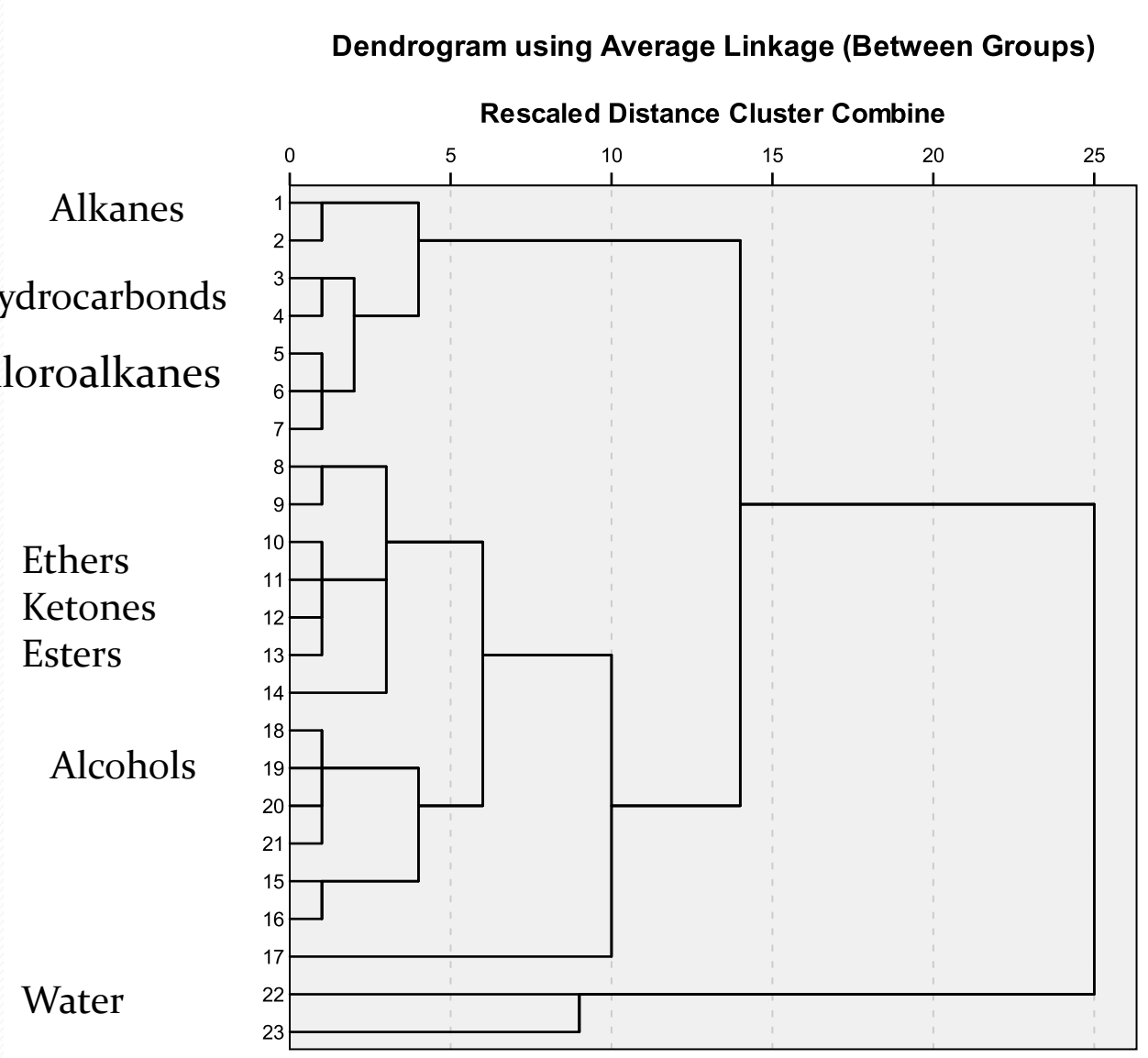
Solvent Properties

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
- Model suitability established by statistical parameters
- Visual classification of solvents requires a reduction in data dimensionality
  - Principal component analysis (PCA)
  - Hierarchical cluster analysis (CA)

Solvent classification

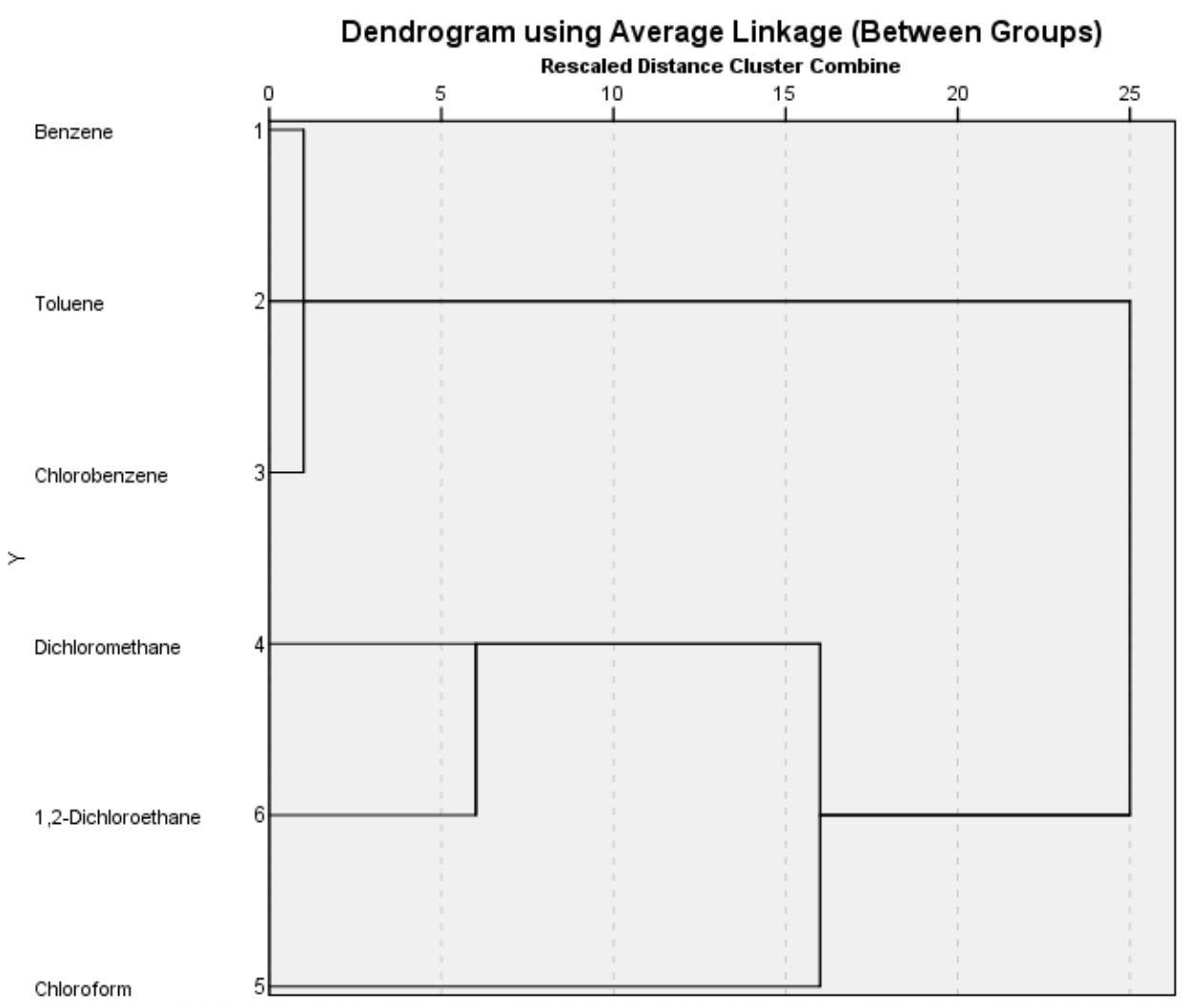



Dendrogram using Average Linkage (Between Groups)
Rescaled Distance Cluster Combine
0
5
10
15
20
25
1
2
3
4
5
6
7
8
9
10
11
12
13
14
18
19
20
21
15
16
17
22
23
Alkanes
Aromatic Hydrocarbonds
Chloroalkanes
Ethers
Ketones
Esters
Alcohols
Water

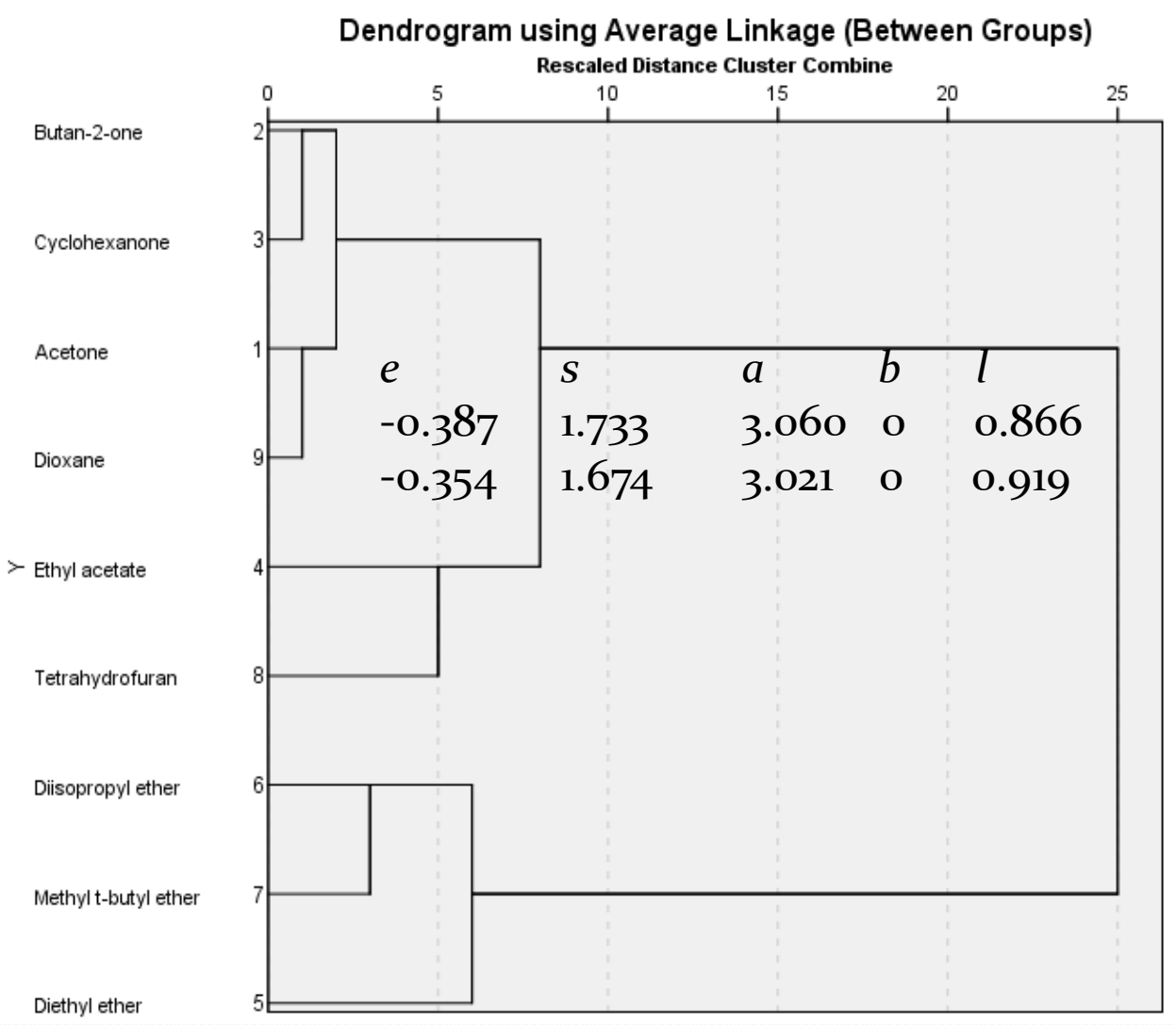
Group 2

2 a

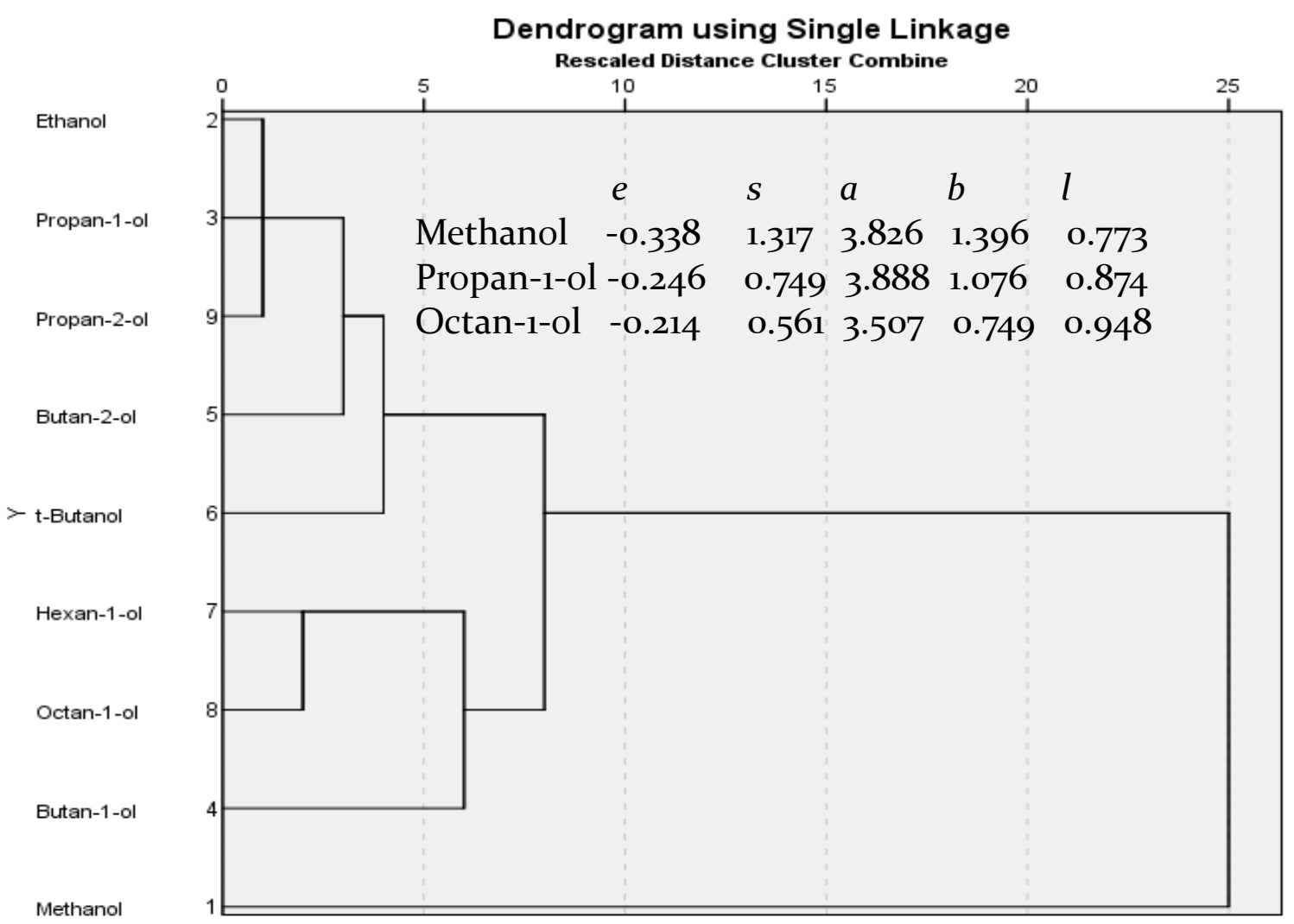
2 b


Dendrogram using Average Linkage (Between Groups)
Rescaled Distance Cluster Combine
0
5
10
15
20
25
Benzene
Toluene
Chlorobenzene
Dichloromethane
1,2-Dichloroethane
Chloroform
1
2
3
4
6
5
Y

Group 5 Esters, Ethers and Ketones

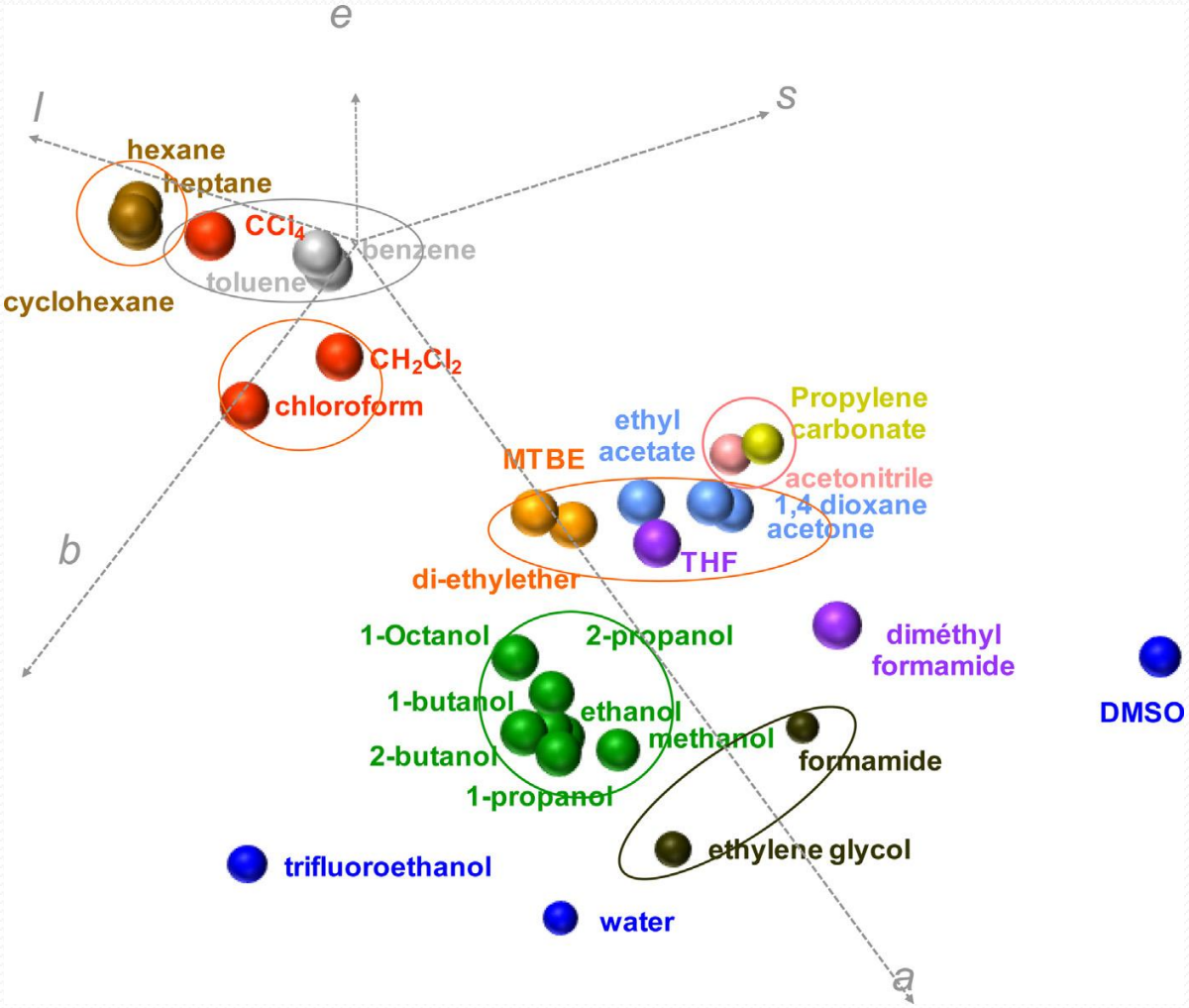


Group 4 Alcohols



	e	*s*	*a*	*b*	*l*
Methanol	-0.338	1.317	3.826	1.396	0.773
Propan-1-ol	-0.246	0.749	3.888	1.076	0.874
Octan-1-ol	-0.214	0.561	3.507	0.749	0.948

Spider Diagram (System Constants)



E. Lesellier, J. Chromatogr. A 1389 (2015) 49-64

Select Solvents from Cluster Analysis

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

Solvents for Reversed-Phase TLC

Solvent	Cluster	Classification
Acetonitrile	4	Dipolar and weakly aprotic
Methanol Propan-2-ol	5	Amphiprotic
Acetone Tetrahydrofuran	6	Polar and non-hydrogen-bond acidic
Trifluoroethanol		Polar and independent
N,N-Dimethylformaide		
Pyridine		

Strength Adjusting Solvent

Single solvents do not allow the simultaneous optimization of solvent strength and selectivity

- 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 Strength Parameter ε°

- Solvent strength of a pure solvent can be defined by ε° for inorganic oxide adsorbents
- Definition
  - Free energy of adsorption of the solvent per unit surface area with pentane assigned as the zero reference
- Organization of solvents
  - Ascending order of ε° is known as an eluotropic series
  - The idea of an eluotropic series is not relevant for polar chemically bonded layers

Solvent Strength Parameter ε°

- Solvent strength parameter for silica gel

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

- Solvent strength parameter for alumina

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

Can be used to estimate ε° values to about 0.04 units for solvents without experimental values

- Silica gel is less hydrogen-bond basic and acidic and dipolar/polarizable than alumina

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
Dimethylformamide	0.51		
Acetonitrile	0.52		
Acetone	0.53		

each CH_2 = -0.05

Solvent Strength Scale For Reversed-Phase Chromatography

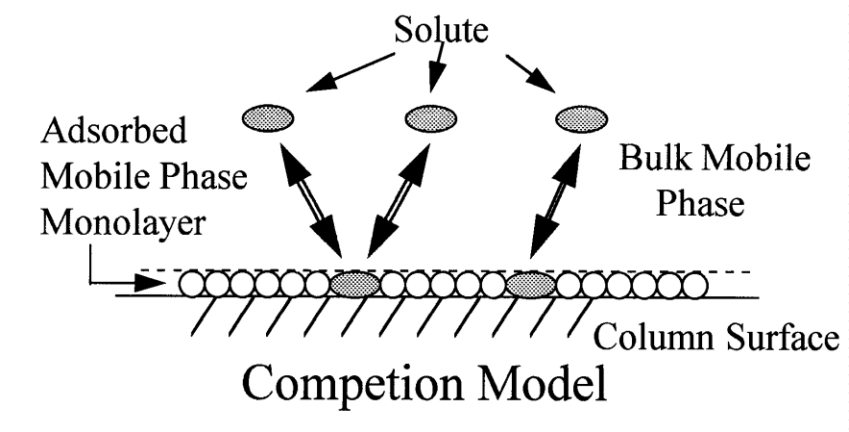
Solvent	**S_i**
Water	0
Acetonitrile	3.1
Methanol	3.0
Acetone	3.4
Dioxane	3.5
Ethanol	3.6
Propan-2-ol	4.2
Tetrahydrofuran	4.4

Solvent mixtures

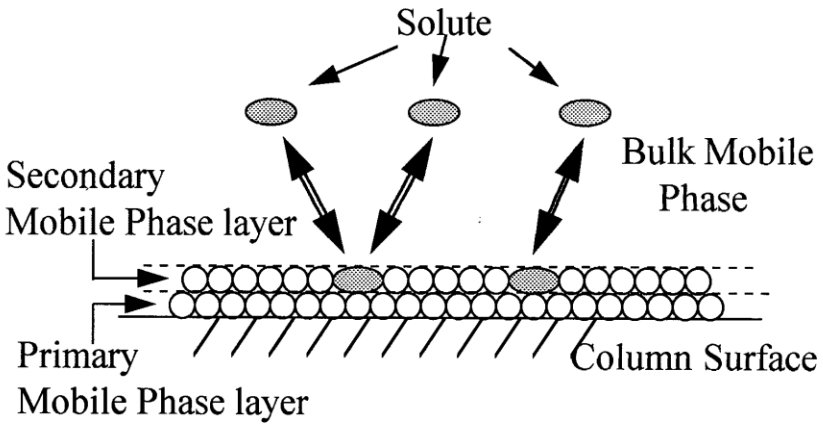
$S_T = \sum_i (S_i \phi_i)$

ϕ_i = solvent volume fraction

Models for Normal-Phase Chromatography



Competion Model



Solvent Interaction Model

Simplified Competition Model

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

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

c = lumped (system constant)

α' = adsorbent activity constant

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

A_S = adsorbent cross-section of the solute

ε° = solvent strength parameter

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 compounds, particularly those capable of hydrogen bonding to surface adsorption sites.

Localization and Solvent Strength

Solvent	Type	Solvent strength parameter	
		$\varepsilon^{\circ}_{(silica)}$	$\varepsilon^{\circ}_{(alumina)}$
Hexane	non-localizing	0	0
Toluene	non-localizing	0.22	0.30
Chloroform	non-localizing	0.26	0.36
Dichloromethane	non-localizing	0.30	0.40
Acetonitrile	localizing	0.52	0.65
Methanol	localizing and basic	0.70	0.95
2-Propanol	localizing and basic	0.60	0.80
Acetone	localizing	0.53	0.58
Diisopropyl ether	minor localizing	0.32	0.28
Methyl *t*-butyl ether	localizing and basic	0.32	0.31
Ethyl acetate	localizing	0.48	0.60
Tetrahydrofuran	localizing and basic	0.48	0.51
Dioxane	localizing and basic	0.51	0.61
Formamide		0.55	1.45
2,2,2-Trifluoroethanol		0.61	0.99
N,N-Dimethylformaide		0.76	0.91
Water		0.82	1.29

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

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)

Normal or Reversed Phase

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

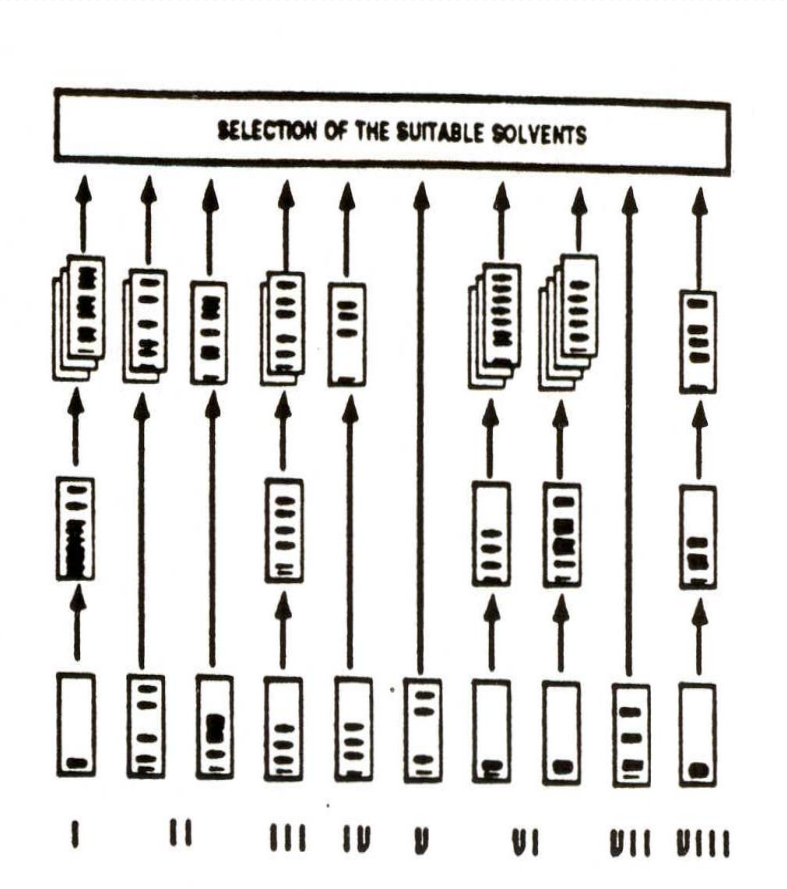
- The competition between the sample and mobile phase for polar interactions with the stationary phase dictates separation characteristics in normal-phase chromatography

Parameter Space

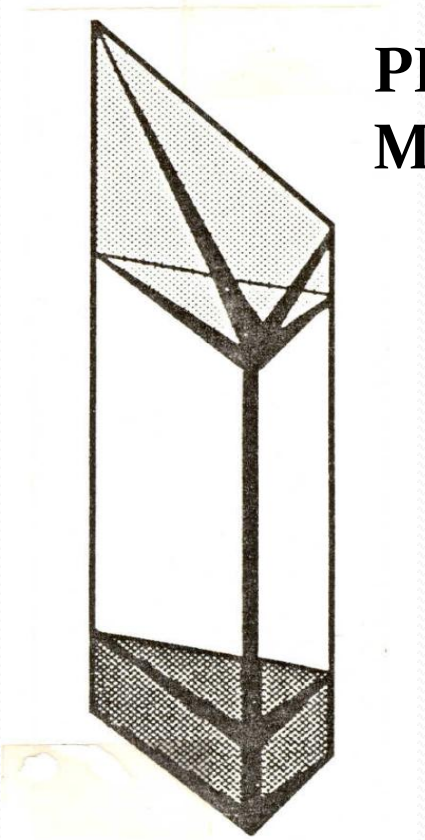
The parameter space is that combination of experimental variables and their limiting values that define the search area

- 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

Solvent Selection and Optimization for Normal-Phase Chromatography



Screen solvents from different selectivity groups

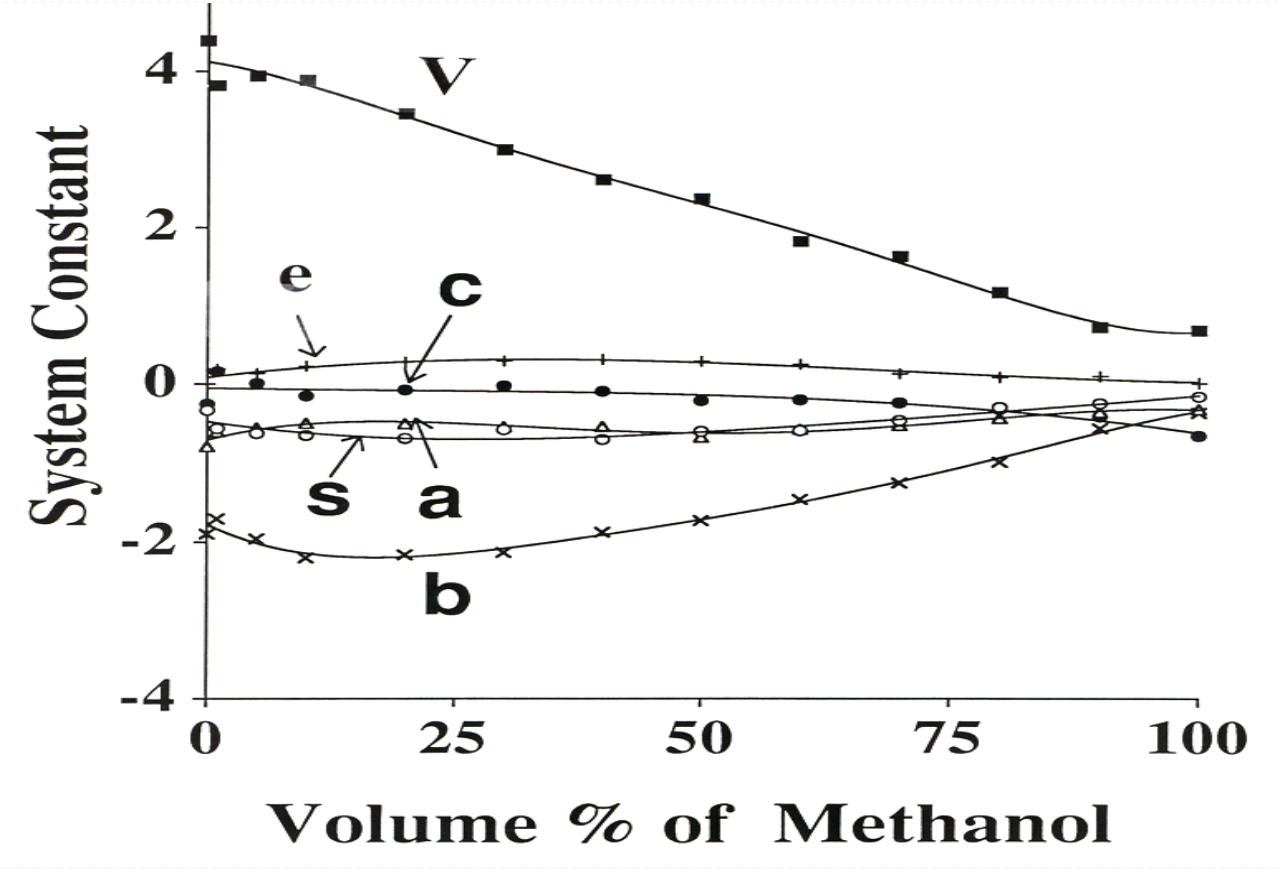


PRISMA MODEL

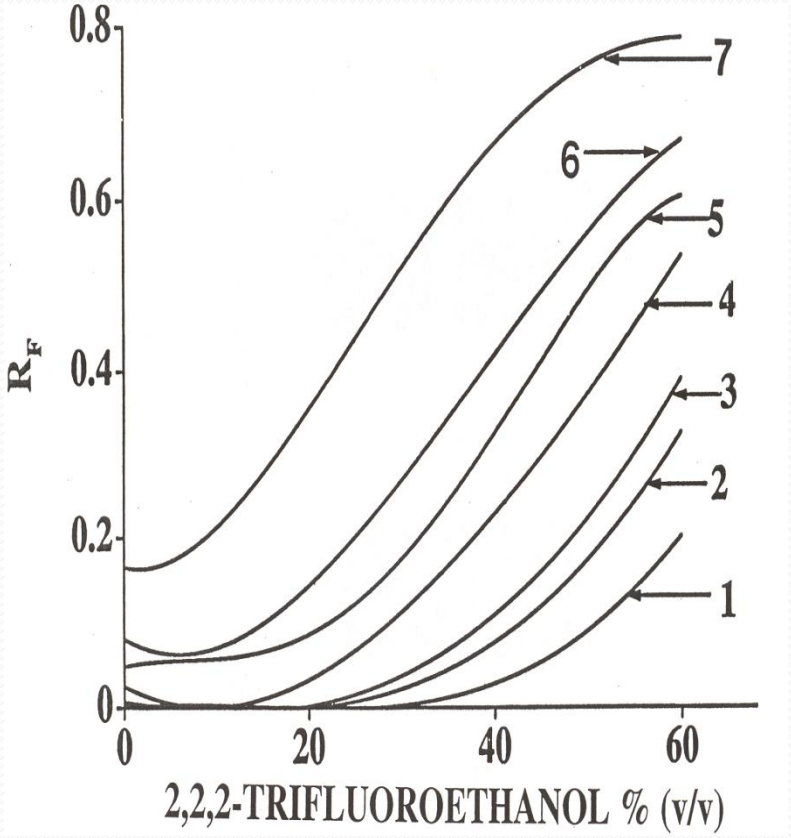
Optimize selected solvents

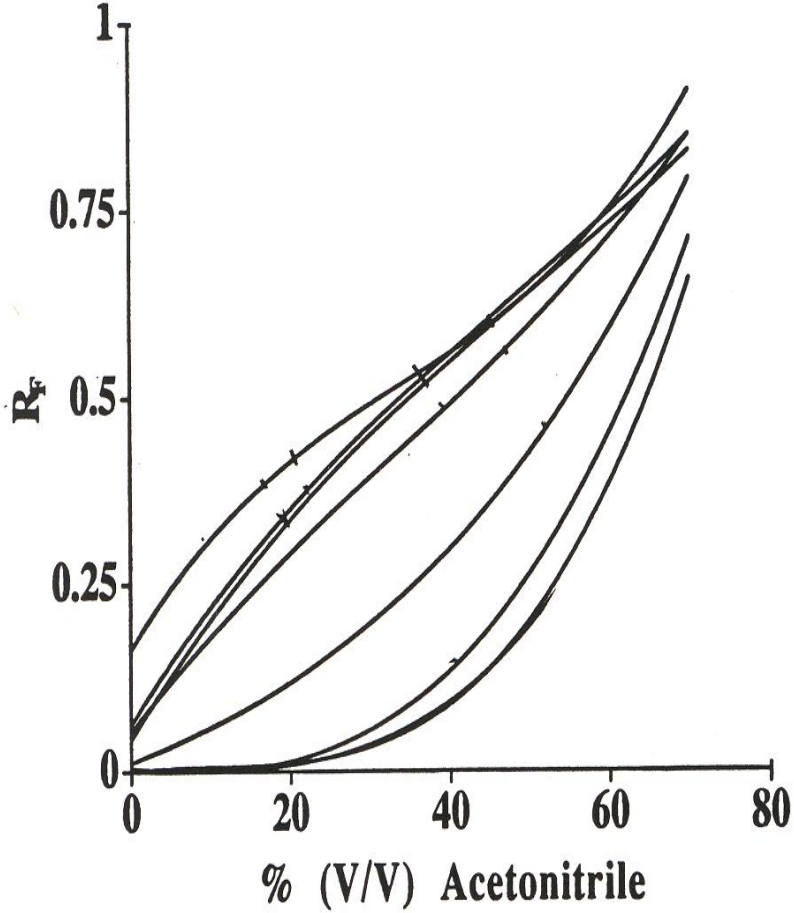
System Map for Reversed Phase Chromatography

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

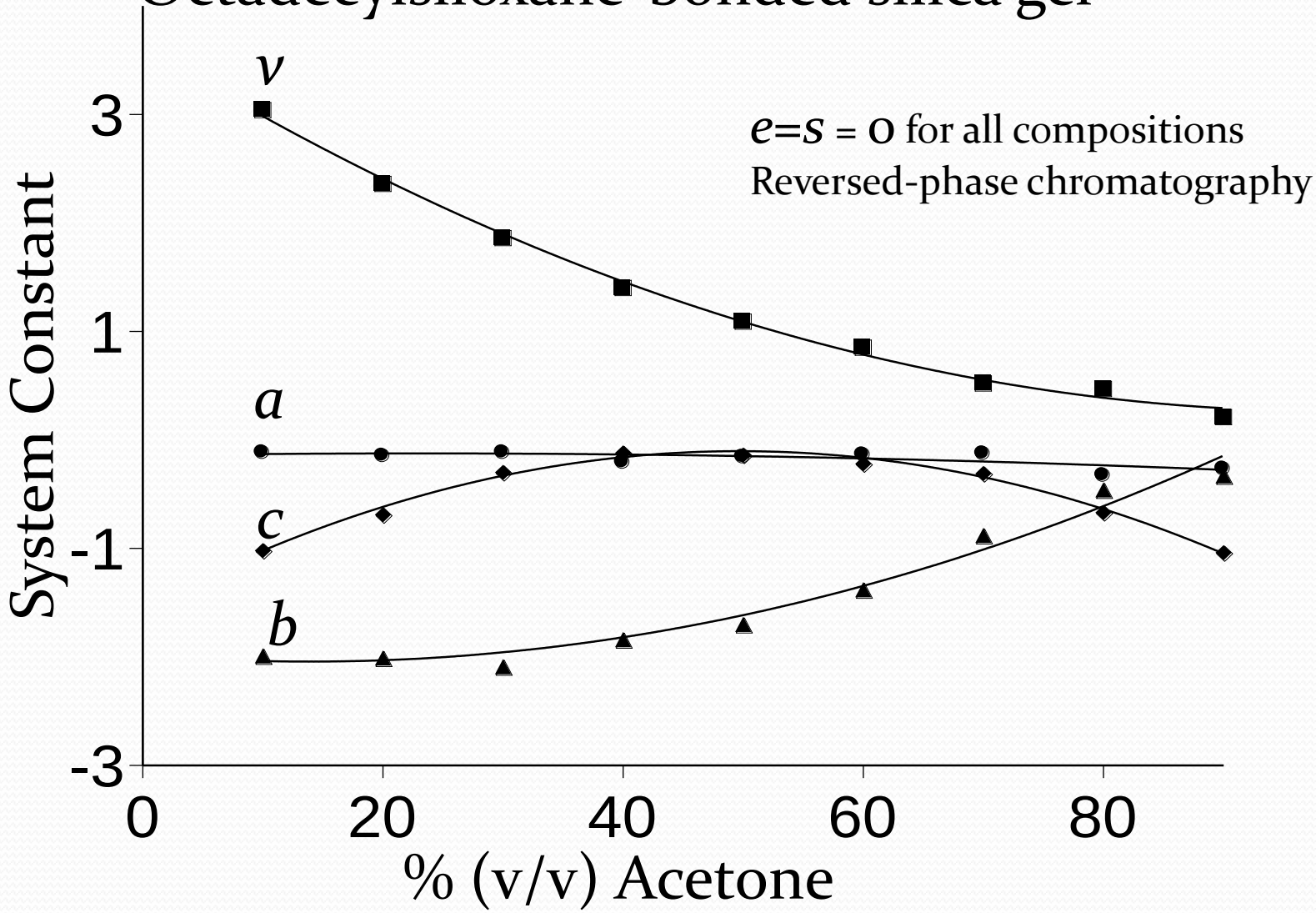


Retention Maps Calculated From System Maps for Reversed -Phase Chromatography

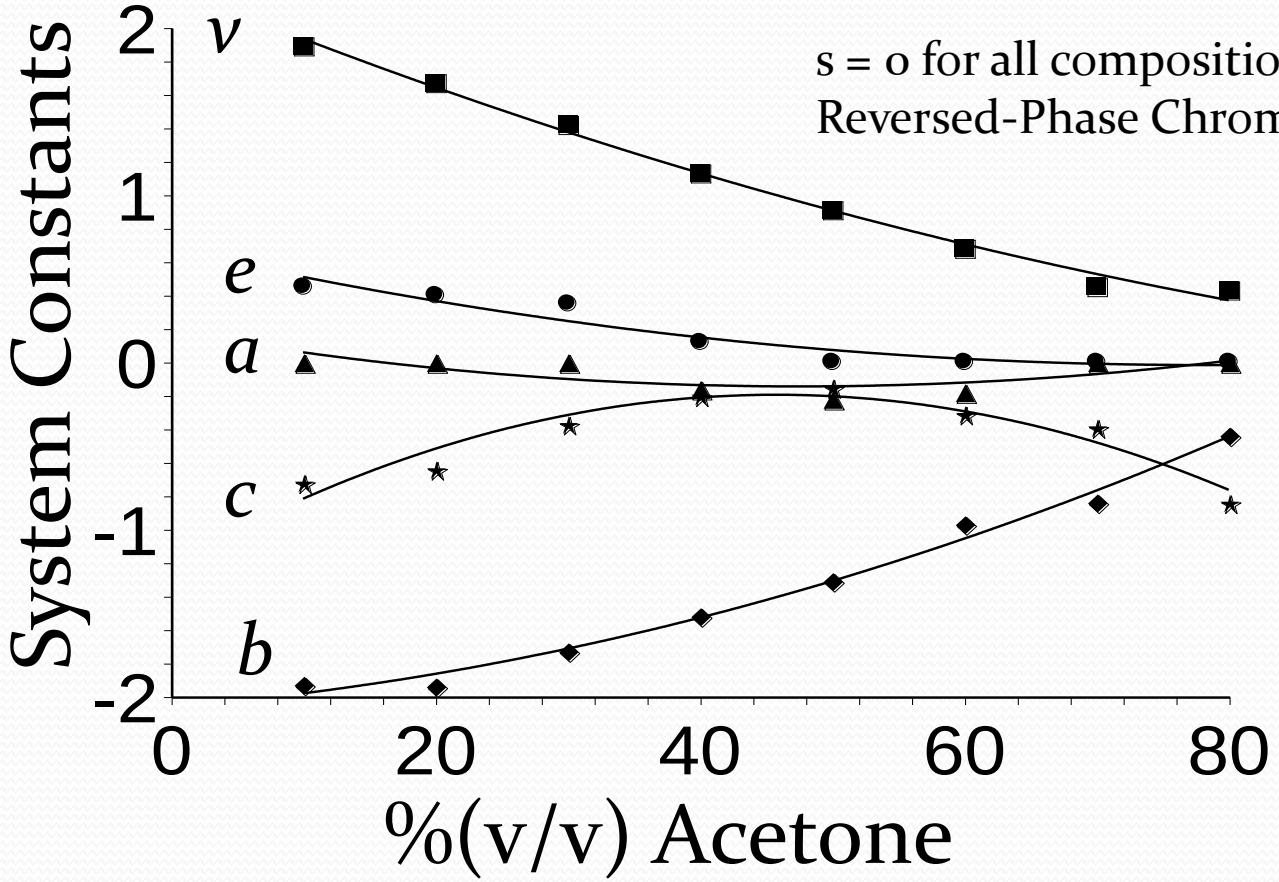




Octadecylsiloxane-bonded silica gel



Cyanopropylsiloxane-bonded silica



s = o for all compositions
Reversed-Phase Chromatography

AUTOMATED MULTIPLE DEVELOPMENT

For Mixtures of Wide Polarity

- Employs incremental multiple development with a fixed solvent entry position
- A different mobile phase is used for each development or for a large number of the developments
- The solvent gradient starts with the strongest solvent for shortest migration distance and ends with the weakest solvent over the longest development distance
- Final chromatogram is contained in a single lane

Automated Multiple Development

PROCESS VARIABLES

DRYING TIME	*Established by trail and error* avoid solvent of low volatility use vacuum monitor to indicate completion
LAYER CONDITIONING	*Optional* repeat sequentially to insure reproducibility
INITIAL DEVELOPMENT	Short (few mm) requires exact zone positions
SOLVENT ADVANCE DISTANCE	Depends on desired gradient shape monitored by a position sensing detector
NUMBER OF DEVELOPMENTS	Typically 10-30
RUN TIME	Minimize 25 developments ≈ 4 h 20 developments ≈ 2.5 h 10 developments ≈ 1.0 h

Automated Multiple Development

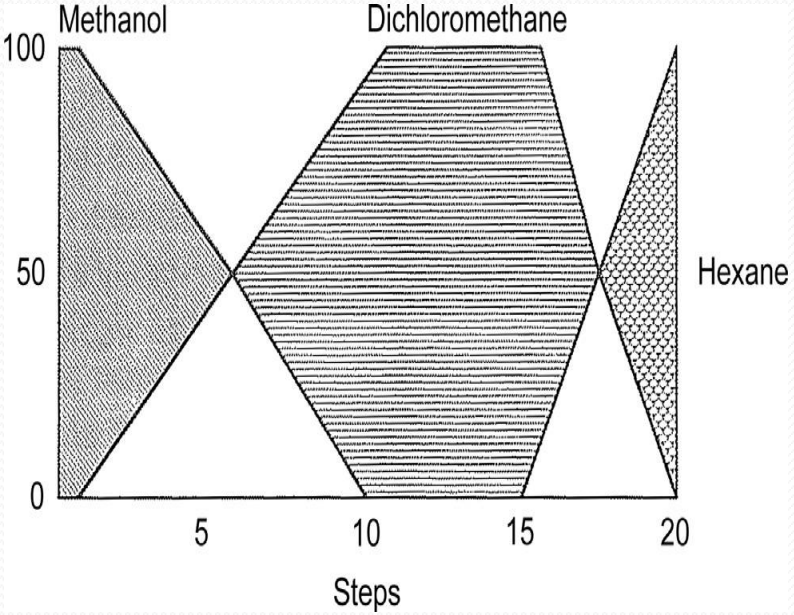
UNIVERSAL GRADIENT

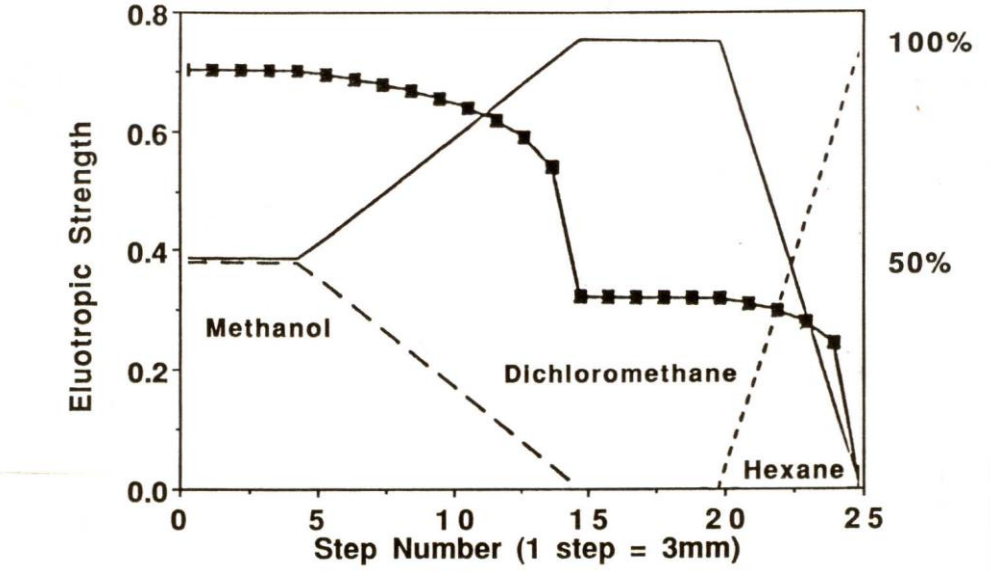
METHANOL ---> BASE SOLVENT --->HEXANE

Typical base solvents: dichloromethane or methyl t-butyl ether

Typical number of steps: 25 steps

Typical universal gradients do not correspond to linear solvent strength gradients





Automated Multiple Development

SUPERIMPOSE CHROMATOGRAM ABOVE THEORETICAL GRADIENT PROFILE

Facilitates Identification of:

- Initial strong solvent composition (first development)
- Terminal solvent strength (last development)

Gradient shape can be modified to enhance resolution

- Shallower gradient over those regions of the chromatogram where the peak separation is inadequate
- Steeper gradient over those regions where the zones are well separated
- If resolution remains inadequate after adjusting the gradient shape select a different base solvent(s)

AMD Separation

- Standard Lipid Mixture
- Gradient
  - 8 steps
  - Strong solvent methanol
  - Weak solvent hexane + toluene (95:5)
  - Base solvent chloroform