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Small Molecule Separations as Molecular Interaction Amplifiers

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Analytical Separations

- Analysis of complicated samples
  - Sample components with a range of interaction properties
  - More sample components
- Separation throughput
- Separation robustness
- MS interfacing

Chromatographic Separations

- "Witchcraft"
  - Disconnect from
    - Solute:stationary phase interactions
    - Solute:mobile phase interactions
    - Mobile phase:stationary phase interactions
- "Trivial"
  - Synthetic products
    - Starting materials
      - Alcohol from ester
    - By-products

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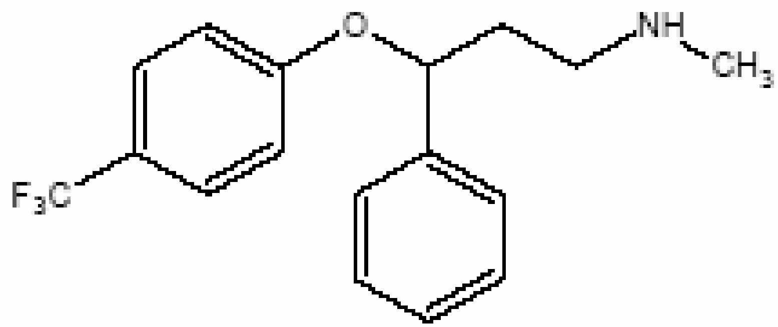
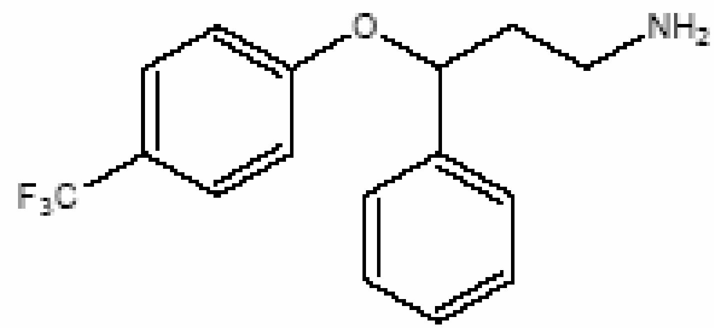
Fluoxetine



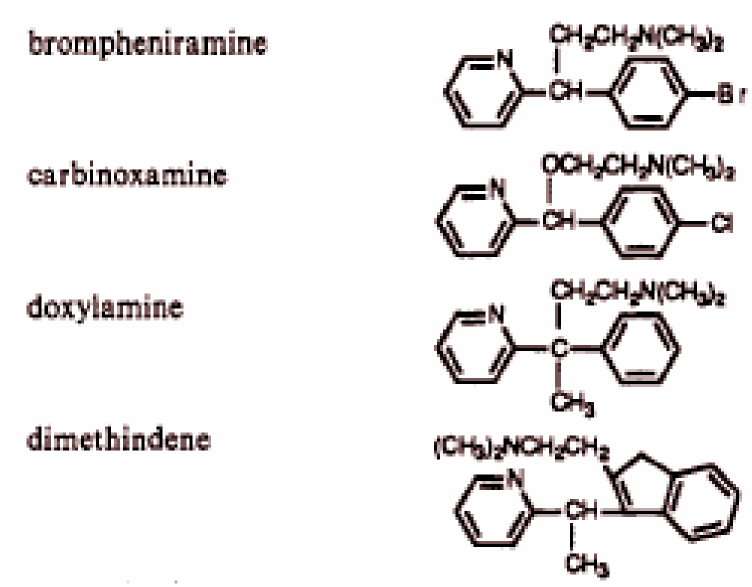


$t_{1/2}$ ~ 1-3 days

$t_{1/2}$ ~ 4-16 days

Sample prep
Chromatographic analysis

The ultimate in small molecule separations as molecular interaction amplifiers



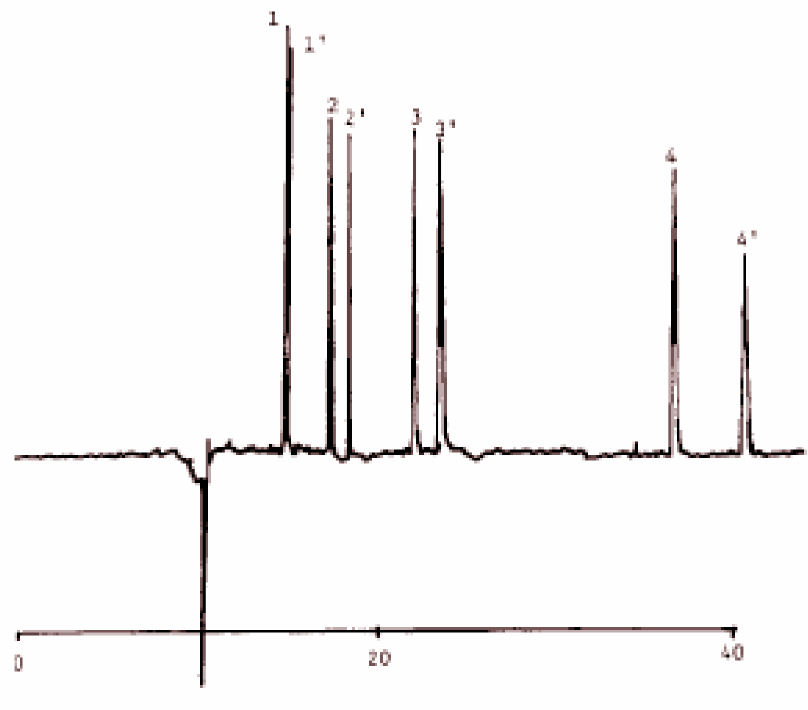


Figure 3. Electropherogram for the enantioseparation of a mixture of antihistamines: 1,1′: carbinoxamine; 2,2′: brompheniramine; 3,3′: doxylamine; 4,4′: dimethindene. Electrolyte: 2% heparin sodium, 10 mM phosphate buffer, pH 5.0.

Anal. Chem. **1994,** *66,* 3054–3059

Nutraceutical Analysis

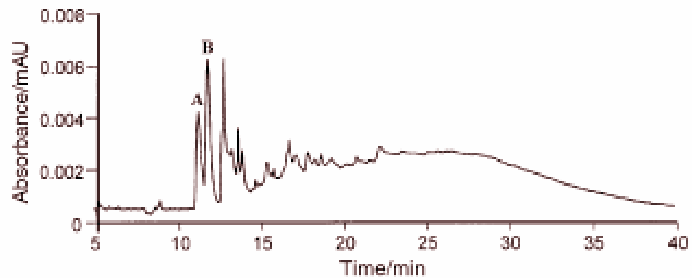
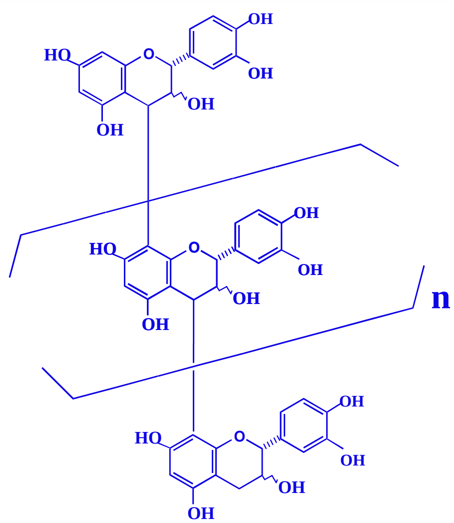


Fig. 1 Separation of polyphenols using sodium tetraborate (150 mM, pH 8.9). A: Epicatechin. B: Catechin.Voltage: 20 kV, cathodic detection at 200 nm.



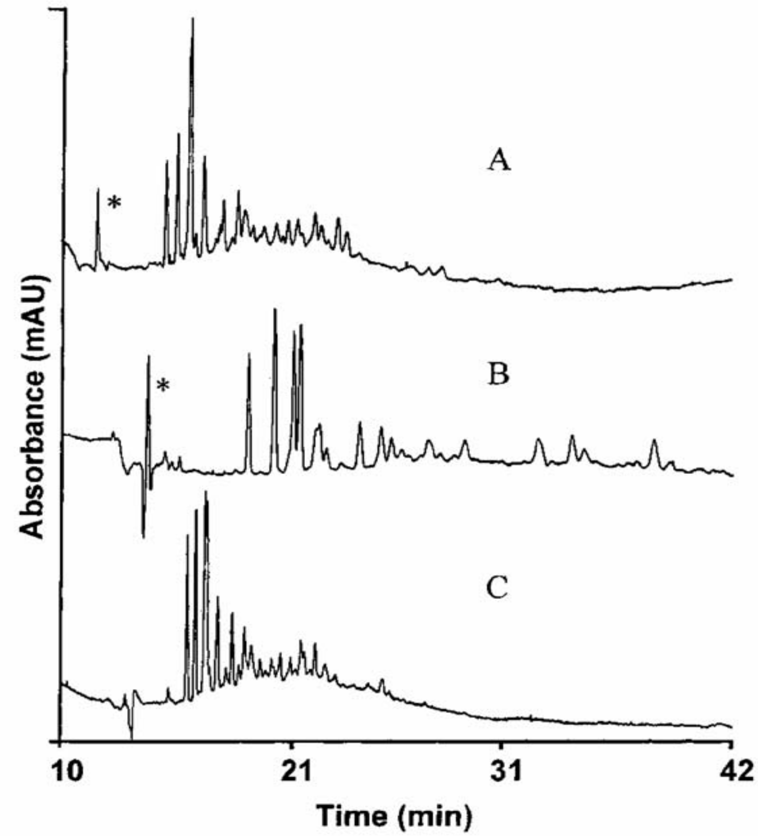


Figure 5. Separation of polyphenols using (A) 1-ethyl-3-methylimidazolium tetrafluoroborate, (B) 1-butyl-3-methylimidazolium tetrafluoroborate, and (C) 1-ethyl-3-methylimidazolium hexafluorophosphate [150 mM]; voltage, 16 kV, with anodic detection at 240 nm; asterisk (*), nitromethane.

Yanes, E.G.; Gratz, S.R.; Baldwin, M.J.; Robison, S.E.; Stalcup, A.M. Anal. Chem., 2001, *73*, 3838-3844.

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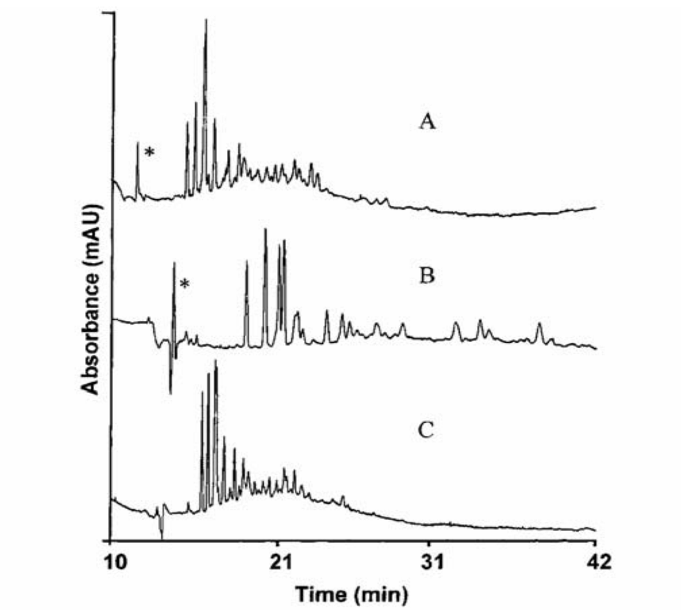
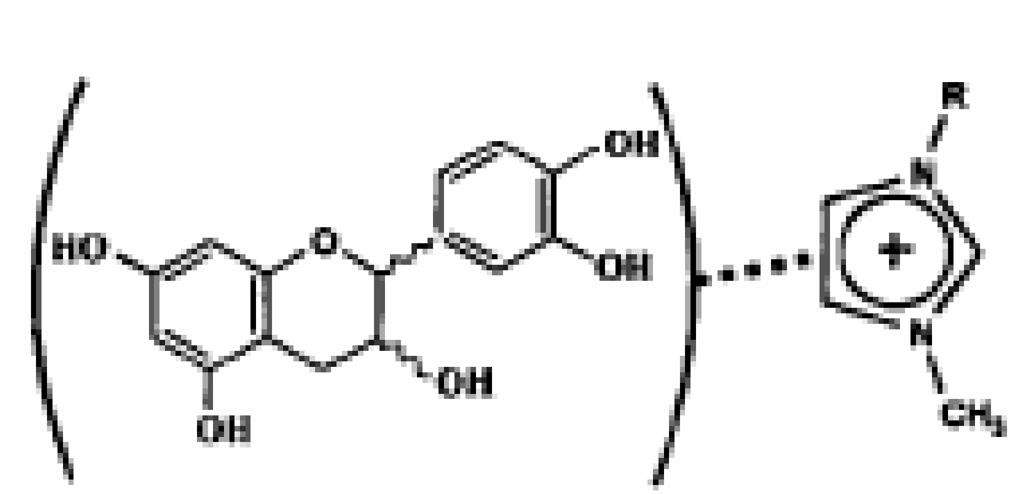


Figure 5. Separation of polyphenols using (A) 1-ethyl-3-methylimidazolium tetrafluoroborate, (B) 1-butyl-3-methylimidazolium tetrafluoroborate, and (C) 1-ethyl-3-methylimidazolium hexafluorophosphate [150 mM]; voltage, 16 kV, with anodic detection at 240 nm; asterisk (*), nitromethane.



$K = 5\ M^{-1}$

$\Delta G \sim 1$ kcal/mol

$kT \sim 0.6$ kcal/mol

$\Delta\Delta G << 1$ kcal/mol

Chromatography and Electrophoresis

- Analysis
- Kinetics
- Binding constants
- Competitive binding
- Columns as reactors

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Chromatographic Models

- **Witchcraft** → **Science**
- **Wanted**: model that allows prediction of likely conditions for optimum separations
- **Wanted**: model that allows quantitative comparison between separation systems
- **Wanted**: model that is based on chemical properties of analytes, mobile and stationary phases
- **Wanted**: model that allows reasonable peak assignment in the absence of standards

Characterizing Retention Properties

- 1st attempt to systematize differences in retention:
  - Kovatz Retention Index (1958)
    - Exploits relationship between retention and molecular weight for homologous series.

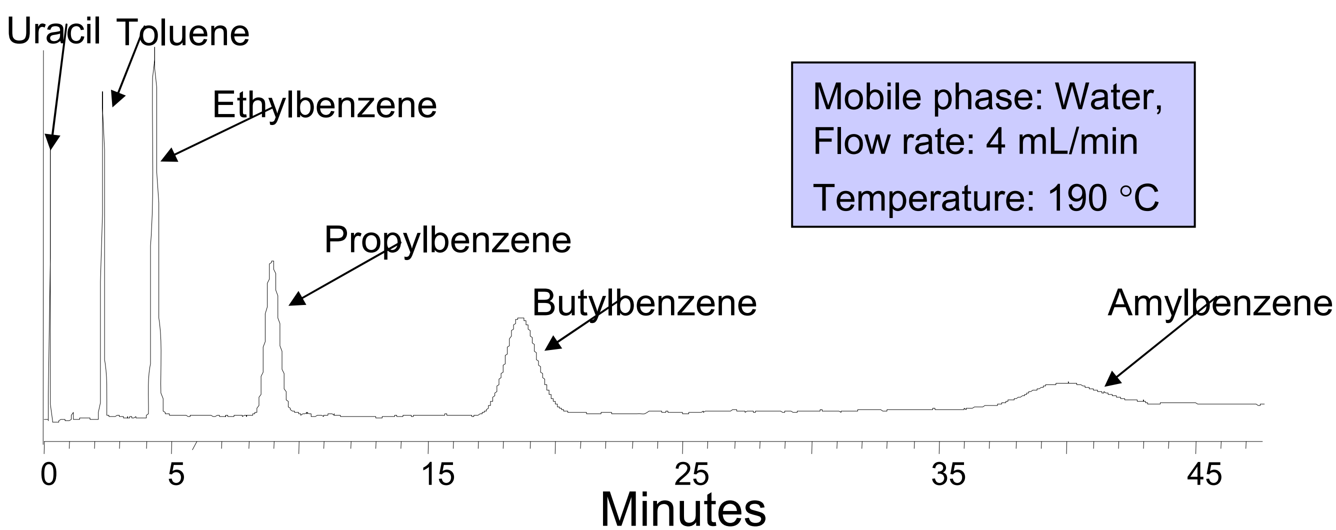


Figure courtesy of N. Grinberg

Plots of log k' vs #C's for homologous series

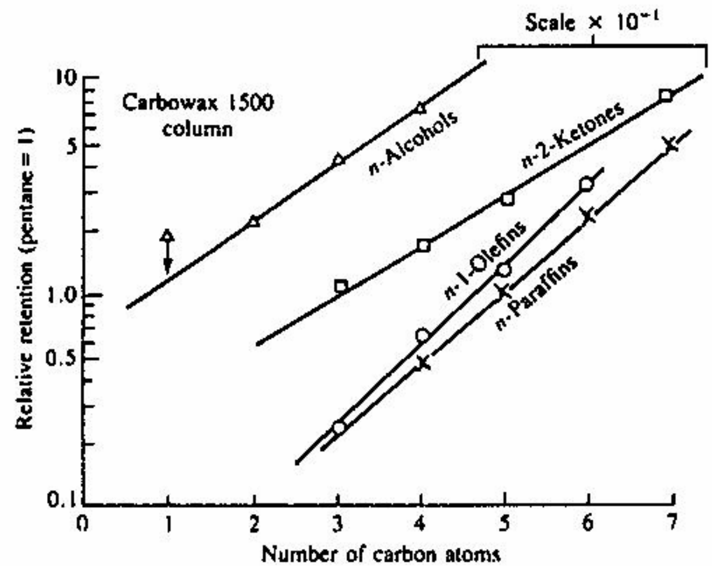


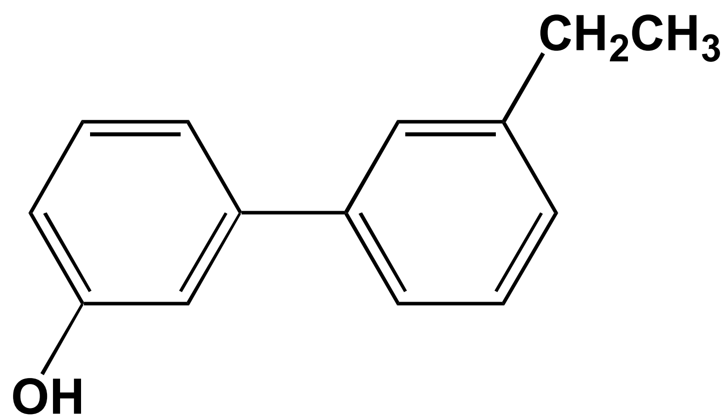
FIGURE 4.8 Plot of retention time (log scale) vs. number of carbon atoms for several homologous series of compounds.

$$\log k'_{tot} = n \log k'_{CH_2} + \log k'_{parent}$$

$$\log k'_{CH_2} \Rightarrow \Delta G^{o}_{CH_2}$$

$$\log k'_{parent} \Rightarrow \Delta G^{o}_{parent}$$

Characterizing Retention Properties



$$\log k'_{tot} \approx 2\log k'_{CH_2} + 2\log k'_{phenyl} + \log'_{OH}$$

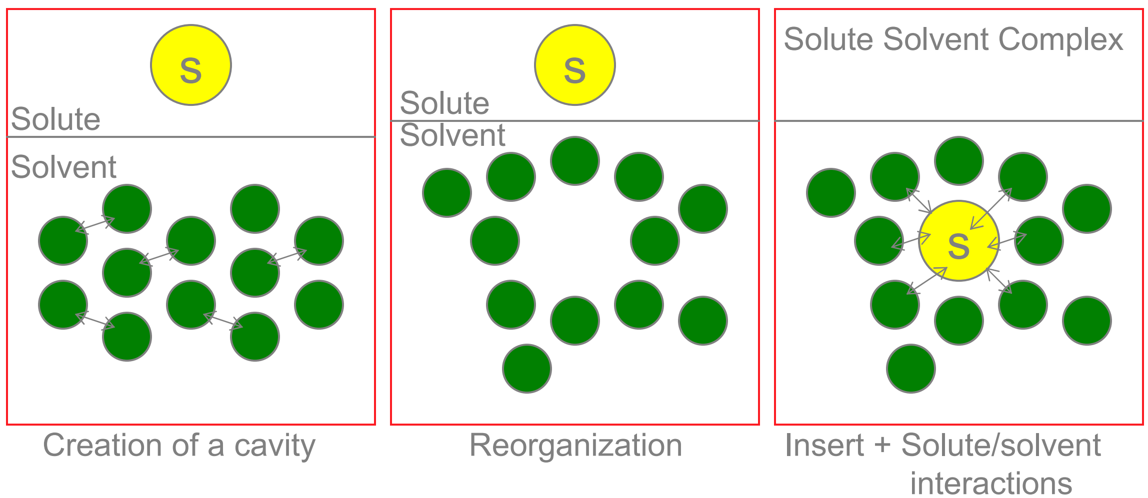
or

$$\log k'_{tot} \approx \log k'_{CH_3} + \log k'_{phenyl-CH_2} + \log'_{phenyl-OH}$$

Linear solvation free energy relationship (LSER)

$$\Delta G^0 = \Delta G^0_{dispersion} + \Delta G^0_{HB} + \Delta G^0_{\pi\text{-}\pi\ interaction} + \Delta G^0 .....$$

$$\log k' = \log k'_0 + rR_2 + s\pi_2^H + a\sum\alpha_2^H + b\sum\beta_2^H + vV_2$$



the cavity model of solvation

Adapted from: V. Pliska, B. Testa and H. van de Waterbeemd, *Lipophilicity in Drug Action and Toxicology*

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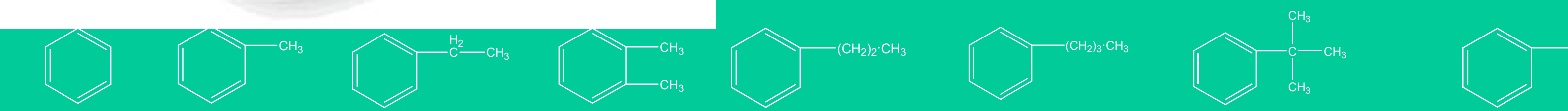
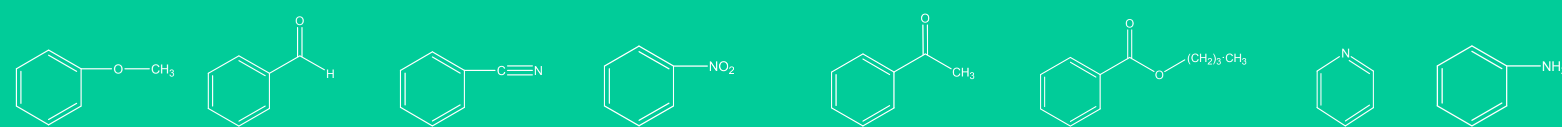
LSER training set

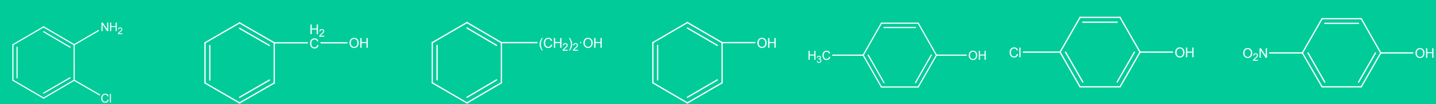


benzene toluene ethylbenzene *o*-xylene propylbenzene *n*-butylbenzene tert-butylbenzene iodobenzene

mesitylene biphenyl naphthalene anthracene fluorobenzene chlorobenzene 1,2-dichlorobenzene



anisole benzaldehyde benzonitrile nitrobenzene acetophenone butyl benzoate pyridine aniline



2-chloroaniline benzyl alcohol 2-phenylethanol phenol *p*-cresol *p*-chlorophenol *p*-nitrophenol

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LSER approach (cont.)

solute descriptors

$$\log k' = \log k'_0 + rR_2 + s\pi_2^H + a\sum \alpha_2^H + b\sum \beta_2^H + vV_2$$

Solute descriptors: values obtained from literature for each solute.

R_2: excess molar refraction

$\Delta MR \equiv MR_{observed} - MR_{n\text{-}alkane\ of\ same\ V_2}$; $MR = (n^2 - 1)/(n^2 + 2)$

V_2: McGowan's characteristic volume

$V_2 = \Sigma$ (all atom contribution) - Σ $6.56 \times B$; $B = N - 1 + Rg$

π_2^H: dipolarity/polarizability

experimentally from GLC data and from water-solvent partition coefficients

$\Sigma\alpha_2^H$ and $\Sigma\beta_2^H$: effective hydrogen-bond acidity and basicity

from complexation constants and by GLC or partition measurements

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Range:

R_2: 0.477 to 2.290

π_2^H: 0.49 to 1.72

$\Sigma\alpha_2^H$: 0.00 to 0.82

$\Sigma\beta_2^H$: 0.04 to 0.56

V_2: 0.716 to 1.280

Table 1. Solvation solute descriptors for the training set

	Probe solute	Descriptors				
		R_2	π_2^H	$\Sigma\alpha_2^H$	$\Sigma\beta_2^H$	V_2
1	benzene	0.610	0.52	0.00	0.14	0.716
2	naphthalene	1.340	0.92	0.00	0.20	1.085
3	biphenyl	1.360	0.99	0.00	0.22	1.324
4	anthracene	2.290	1.34	0.00	0.26	1.454
5	toluene	0.601	0.52	0.00	0.14	0.857
6	*o*-xylene	0.663	0.56	0.00	0.16	0.998
7	mesitylene	0.649	0.52	0.00	0.19	1.139
8	ethylbenzene	0.613	0.51	0.00	0.15	0.998
9	propylbenzene	0.604	0.50	0.00	0.15	1.139
10	*n*-butylbenzene	0.600	0.51	0.00	0.15	1.280
11	tert-butylbenzene	0.619	0.49	0.00	0.16	1.280
12	fluo	[illegible]	[illegible]	[illegible]	[illegible]	.734
13	chlo	[illegible]	[illegible]	[illegible]	[illegible]	.839
14	iodo	[illegible]	[illegible]	[illegible]	[illegible]	.975
15	1,2-	[illegible]	[illegible]	[illegible]	[illegible]	.961
16	phe	[illegible]	[illegible]	[illegible]	[illegible]	.775
17	ben	[illegible]	[illegible]	[illegible]	[illegible]	.916
18	2-phenylethanol	0.811	0.91	0.30	0.64	1.057
19	*p*-cresol	0.820	0.87	0.57	0.31	0.916
20	*p*-chlorophenol	0.915	1.08	0.67	0.20	0.898
21	nitrobenzene	0.871	1.11	0.00	0.28	0.891
22	benzonitrile	0.742	1.11	0.00	0.33	0.871
23	benzaldehyde	0.820	1.00	0.00	0.39	0.873
24	aniline	0.955	0.96	0.26	0.50	0.816
25	acetophenone	0.818	1.01	0.00	0.48	1.014
26	2-chloroaniline	1.033	0.92	0.25	0.40	0.939
27	anisole	0.708	0.75	0.00	0.29	0.916
28	ethylphenyl ether	0.668	0.80	0.00	0.46	1.495
29	pyridine	0.631	0.84	0.00	0.47	0.675
30	*p*-nitrophenol	1.070	1.72	0.82	0.26	0.949
31	*o*-nitrophenol	1.015	1.05	0.05	0.37	0.949
32	*m*-nitrophenol	1.050	1.57	0.79	0.23	0.949

$$y = mx + b$$

$$y = m_1x_1 + m_2x_2 + m_3x_3 + \ldots + b$$

$$\log k' = rR_2 + s\pi_2^H + a\sum\alpha_2^H + \ldots + \log k_o'$$

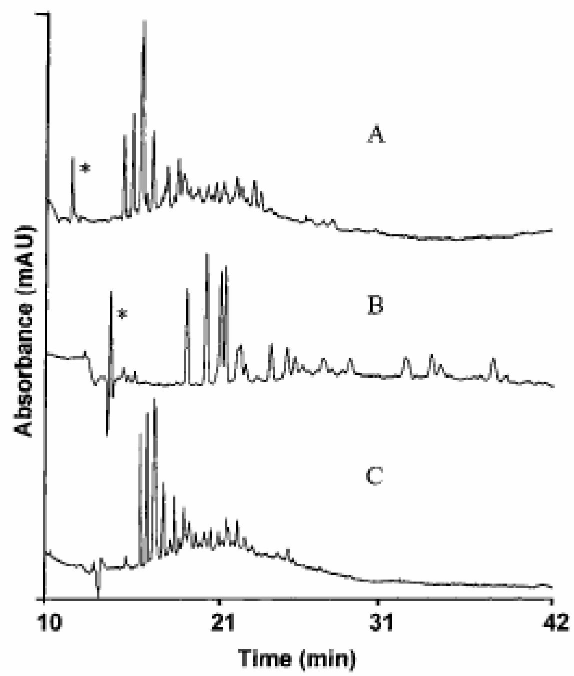
LSER approach (cont.)

- Extract coefficients from the data and use to calculate log k'_{calc}
  - Comparison of log k'_{calc} vs log k'_{exp}
    - Do selected molecular descriptors adequately describe solute/stationary phase interactions?
  - Comparison of coefficients on different phases
    - Quantitate differences between phases
  - Comparison of coefficients under different mobile phase conditions
  - Comparison of coefficients for specific analytes
    - Quantitate separation mechanism

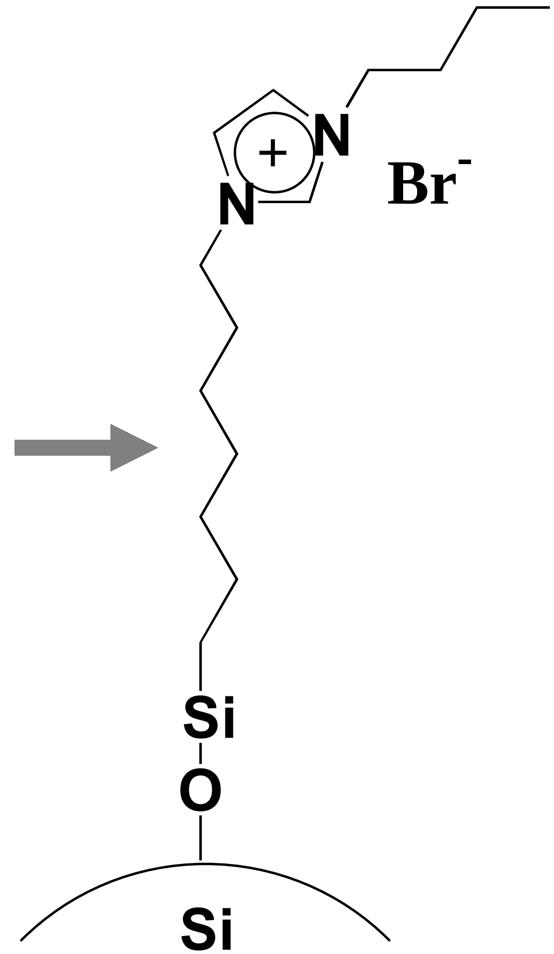
LSER: sure seems like a lot of trouble – why bother?

- **Witchcraft** ⟶ **Science**
- **Wanted**: model that allows prediction of likely conditions for optimum separations
- **Wanted**: model that allows quantitative comparison between separation systems
- **Wanted**: model that is based on chemical properties of analytes, mobile and stationary phases
- **Wanted**: model that allows reasonable peak assignment in the absence of standards

LSER Application: Butylimidazolium Phase for HPLC

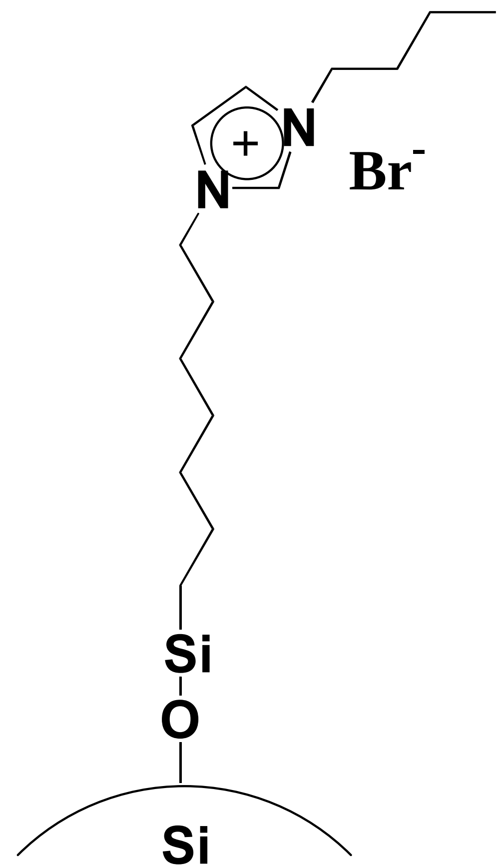


CE Method



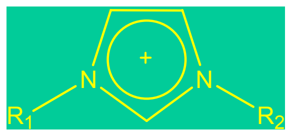
Goal

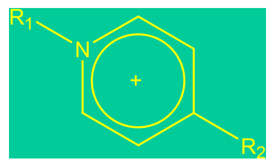
- Multimodal retention
  - Polar organics
    - Retains reversed phase selectivity
    - Retains reduced ion exchange
  - Reduced reliance on gradient elution?

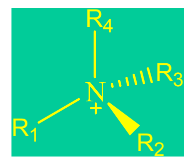


Ionic liquids

- Salts with melting points near room temperature
  - Bulky organic cations BF_4^-, PF_6^-, Br^-







- ~No vapor pressure
- Interesting solvent properties
- Wider range of properties than typical liquids

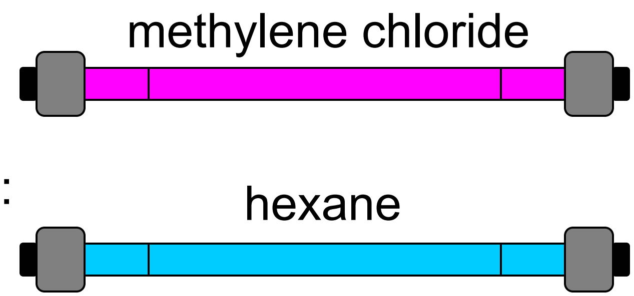
- Bonus: chromatography may tell us something about ionic liquids - Reciprocity

Strategy

- Explore reversed phase behavior
  - Models
    - LSER
    - Partitioning
- Explore ion exchange behavior

LSER approach (cont.)

Void volume determination:



Step 1:

Get solute descriptors and examine cross-correlation between the descriptors

Step 2:

Choose experimental conditions

Step 3:

Collect retention data and generate LSER system coefficients

Experimental Conditions:

250x4.6mm I.D. column, 20 μL loop
1 mL/min or less flow rate
254 nm or 310 nm wavelength
60 -100% methanol-water MP
triplicate injections

LSER approach (cont.)

- Extract coefficients from the data and use to calculate log k'_{calc}
  - Comparison of log k'_{calc} vs log k'_{exp}
    - Do selected molecular descriptors adequately describe solute/stationary phase interactions?
  - Comparison of coefficients on different phases
    - Quantitate differences between phases
  - Comparison of coefficients under different mobile phase conditions
  - Comparison of coefficients for specific analytes
    - Quantitate separation mechanism

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LSER MeOH / H_2O Results

$$\log k' = \log k'_0 + rR_2 + s\pi_2^H + a\sum\alpha_2^H + b\sum\beta_2^H + vV_2$$

Table 3. System coefficients as a function of mobile phase composition (volume fraction) for the solvent system methanol-water on the butylimidazolium bromide stationary phase

	System coefficients					
MeOH* -H_2O	$\log k_0'$	r	s	a	b	v
60 - 40	-0.97 ± 0.04	0.10 ± 0.03	-0.04 ± 0.05	-0.13 ± 0.03	-0.90 ± 0.05	0.89 ± 0.03
70 - 30	-1.00 ± 0.03	0.12 ± 0.02	-0.05 ± 0.04	-0.11 ± 0.02	-0.79 ± 0.04	0.66 ± 0.03
80 - 20	-1.28 ± 0.04	0.06 ± 0.03	0.11 ± 0.05	-0.10 ± 0.03	-0.85 ± 0.05	0.57 ± 0.04
90 - 10	-1.51 ± 0.07	0.06 ± 0.05	0.29 ± 0.08	-0.02 ± 0.06	-0.94 ± 0.09	0.41 ± 0.06
100 - 0	-1.66 ± 0.13	0.07 ± 0.10	0.62 ± 0.16	-0.02 ± 0.11	-1.06 ± 0.17	0.15 ± 0.12

*MeOH = methanol. number of the solutes=28.

LSER MeOH / H_2O Results

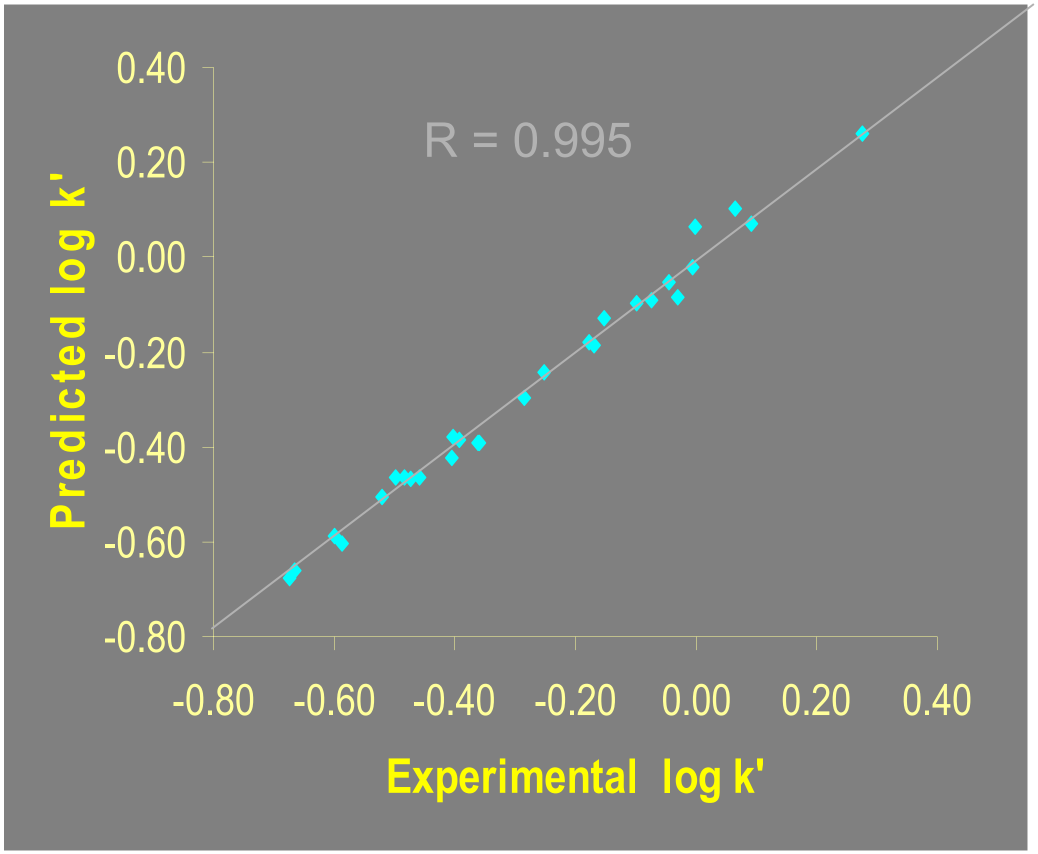
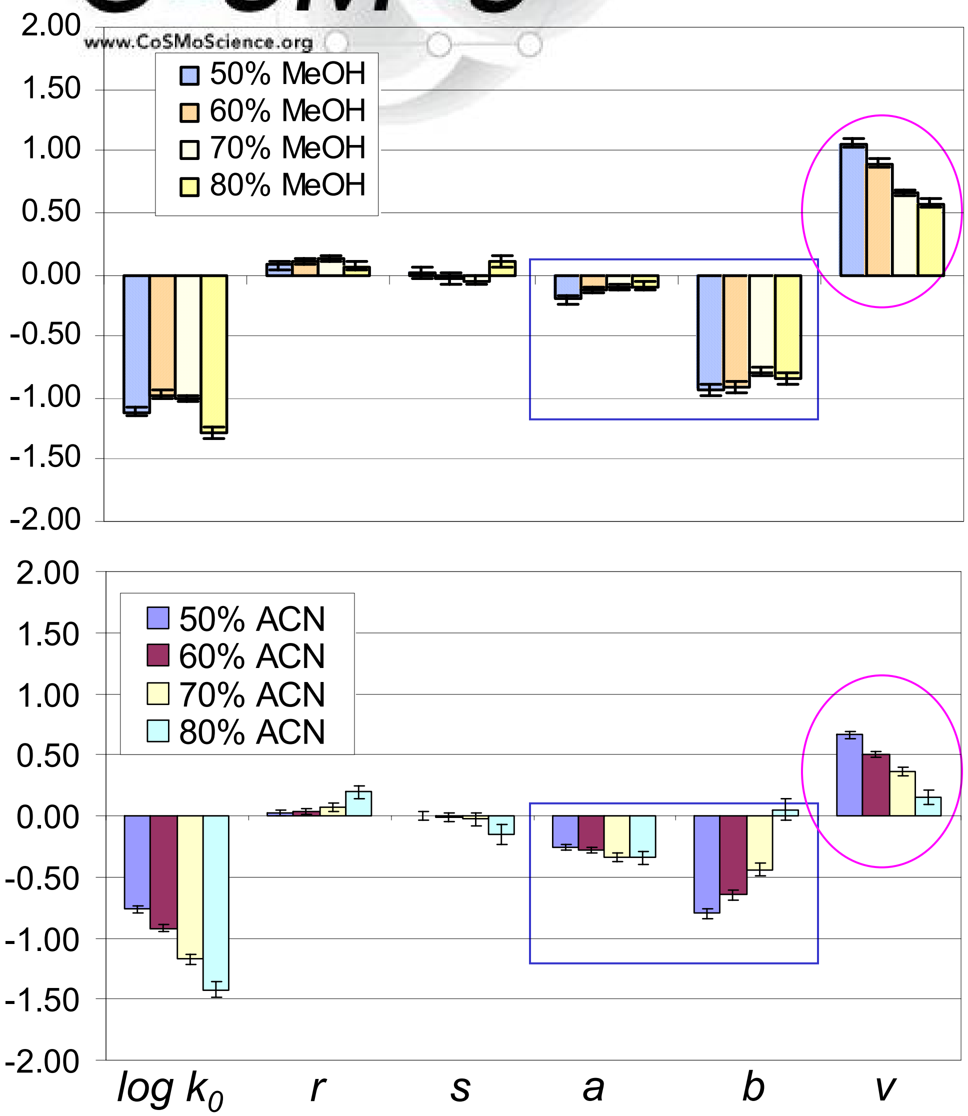


Figure 1 Plot of log $k'_{predicted}$ vs log $k'_{experimental}$ at 60% MeOH mobile phase composition

Comparison of CH_3CN / H_2O vs CH_3OH / H_2O systems



IN GENERAL:

Interaction capabilities: MeOH > ACN

$R_2, \Sigma\alpha_2^H$ and $\Sigma\beta_2^H$

Interaction capabilities: ACN > MeOH

π_2^H and V_2

OBSERVATIONS (*a*, *b* and *v* coefficients):

$v_{ACN} < v_{MeOH}$

---- ACN being a stronger/more nonpolar solvent than MeOH

b_{MeOH} is more negative than b_{ACN}

---- stronger HBing acidity interaction with MeOH/water than with ACN/water

b_{ACN} MP composition dependent

----consistent with the significant difference in HBing acidity between acetonitrile/water

a: independent of MP composition.

---- similar HBing acceptor ability of MeOH and water, or ACN and water.

Molecular descriptors

$$\log k' = \log k'_0 + rR_2 + s\pi_2^H + a\sum\alpha_2^H + b\sum\beta_2^H + vV_2$$

Range:

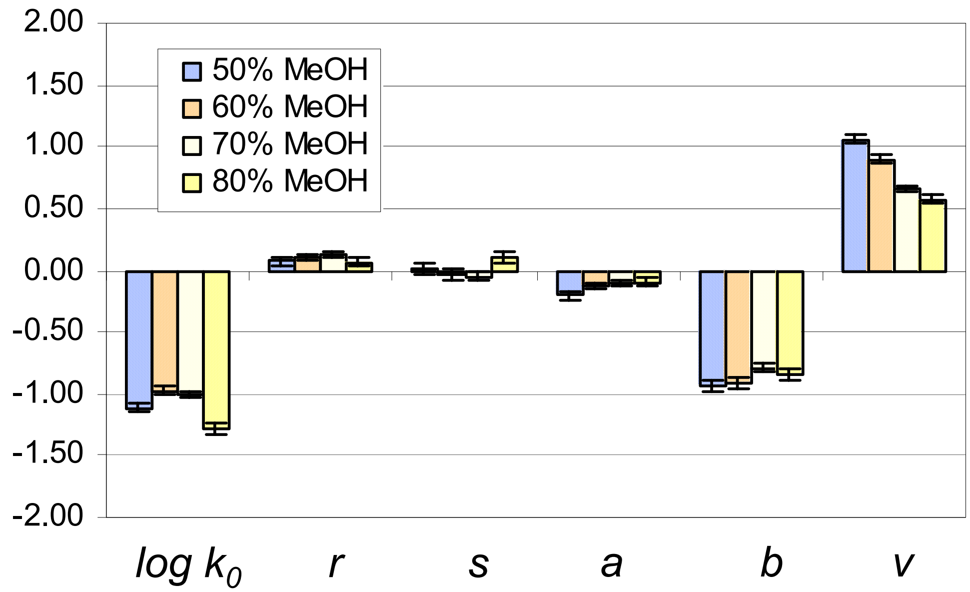
R_2: 0.477 to 2.290

π_2^H: 0.49 to 1.72

$\Sigma\alpha_2^H$: 0.00 to 0.82

$\Sigma\beta_2^H$: 0.04 to 0.56

V_2: 0.716 to 1.280



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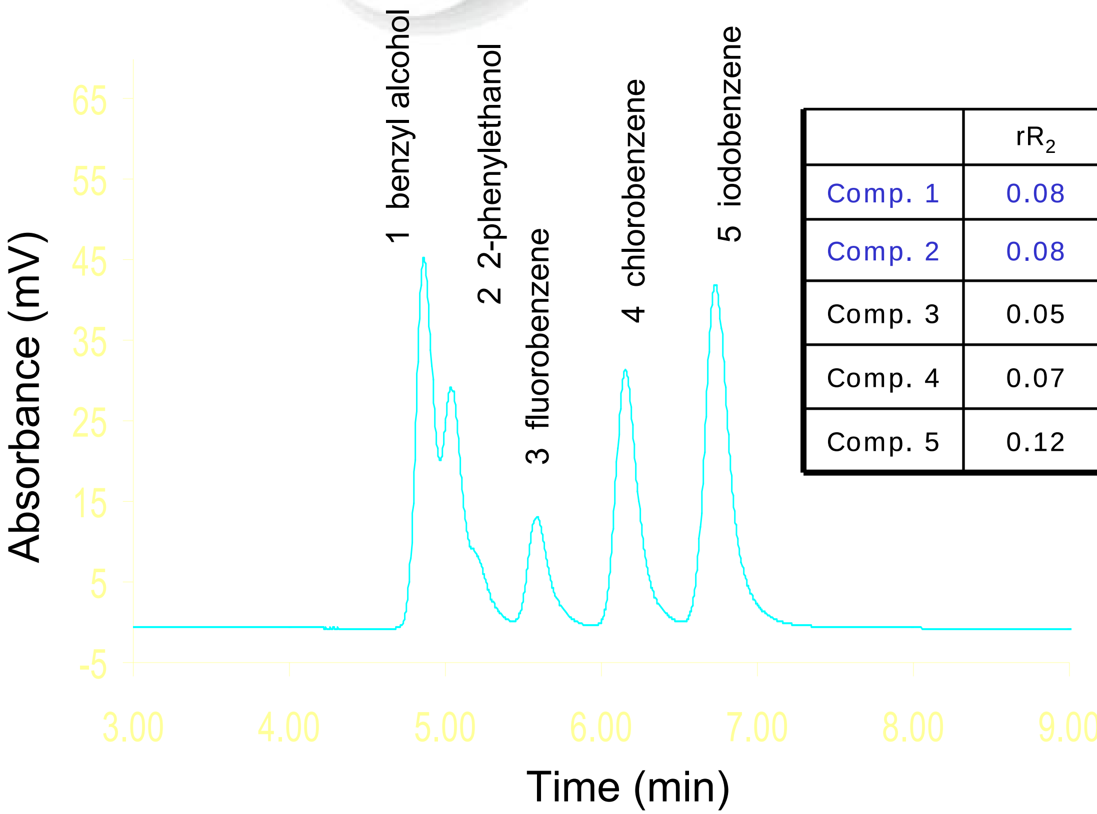
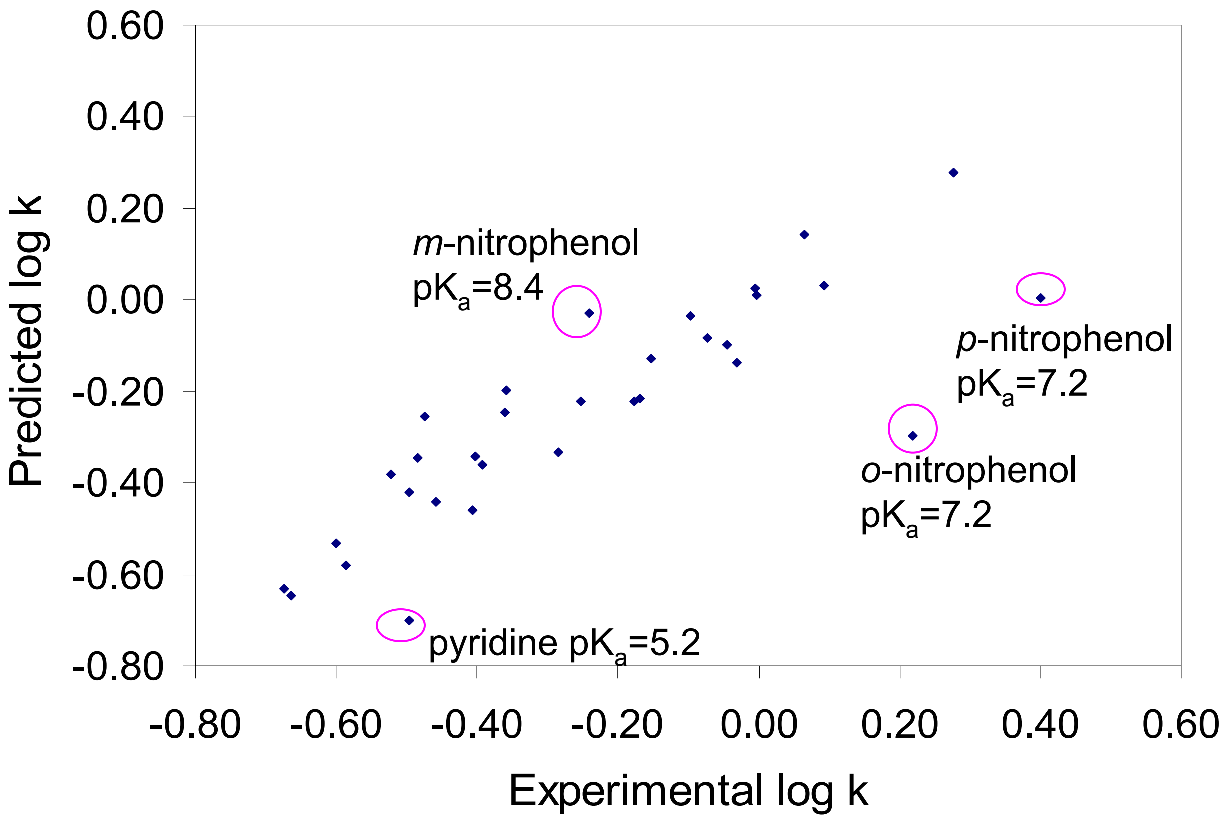
Understanding the retention behavior



	rR_2	$s\pi_2^H$	$a\Sigma\alpha_2^H$	$b\Sigma\beta_2^H$	vV_2	log k'
Comp. 1	0.08	-0.03	-0.04	-0.50	0.82	-0.67
Comp. 2	0.08	-0.04	-0.04	-0.58	0.94	-0.61
Comp. 3	0.05	-0.02	0	-0.09	0.65	-0.37
Comp. 4	0.07	-0.03	0	-0.06	0.75	-0.23
Comp. 5	0.12	-0.03	0	-0.11	0.87	-0.15

Figure 5. Separations at 60% MeOH mobile phase composition on the butylimidazolium-based stationary phase

Inadequacy of the LSER model for ionizable compounds on a charged stationary phase



Plots log $k_{predicted}$ vs log $k_{experimental}$
at 60% MeOH mobile phase composition

LSER model for ionizable compounds on a charged stationary phase

$$\log k = \log k_0 + rR_2 + s\pi_2^H + a\Sigma\alpha_2^H + b\Sigma\beta_2^H + vV_2 + dD$$

$$D = [X^-]/([HX]+[X^-]) = 10^{(pH-pK_a)}/[1+10^{(pH-pK_a)}]$$

S. Espinosa, E. Bosch and M. Roses, *J Chromatogr A* 945 (2002) 83-96.

Extended LSER model for ionizable compounds

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$$\log k = \log k_0 + rR_2 + s\pi_2^H + a\sum\alpha_2^H + b\sum\beta_2^H + vV_2$$

$$\log k = \log k_0 + rR_2 + s\pi_2^H + a\Sigma\alpha_2^H + b\Sigma\beta_2^H + vV_2 + dD$$

Predicted regression coefficients and statistics for the LSER or extended LSER approaches*

	Without correction (in 60%MeOH)		With *D* descriptor (in 60%MeOH)
log k_0	-1.13 ± 0.18		-0.92 ± 0.07
r	-0.12 ± 0.14		0.08 ± 0.05
s	0.49 ± 0.18		0.01 ± 0.07
a	-0.18 ± 0.16		-0.18 ± 0.06
b	-1.08 ± 0.21		-0.84 ± 0.08
v	0.90 ± 0.17		0.82 ± 0.06
d			3.79 ± 0.29
R	0.846		0.982
SE	0.163		0.059
F	13		113

* Number of solutes = 32 ;
R = overall correlation coefficient, SE = standard error in the estimate, F = f statistic.

Two significant improvements:

Reduced uncertainties

Increased correlation

Two groups of system coefficients:

Larger group: *a*, *b* and *v*.

Smaller group: *r* and *s*.

r: positive value consistent w/ original LSER for neutral compounds.

s: provides *s* consistent w/ original LSER for neutral compounds.

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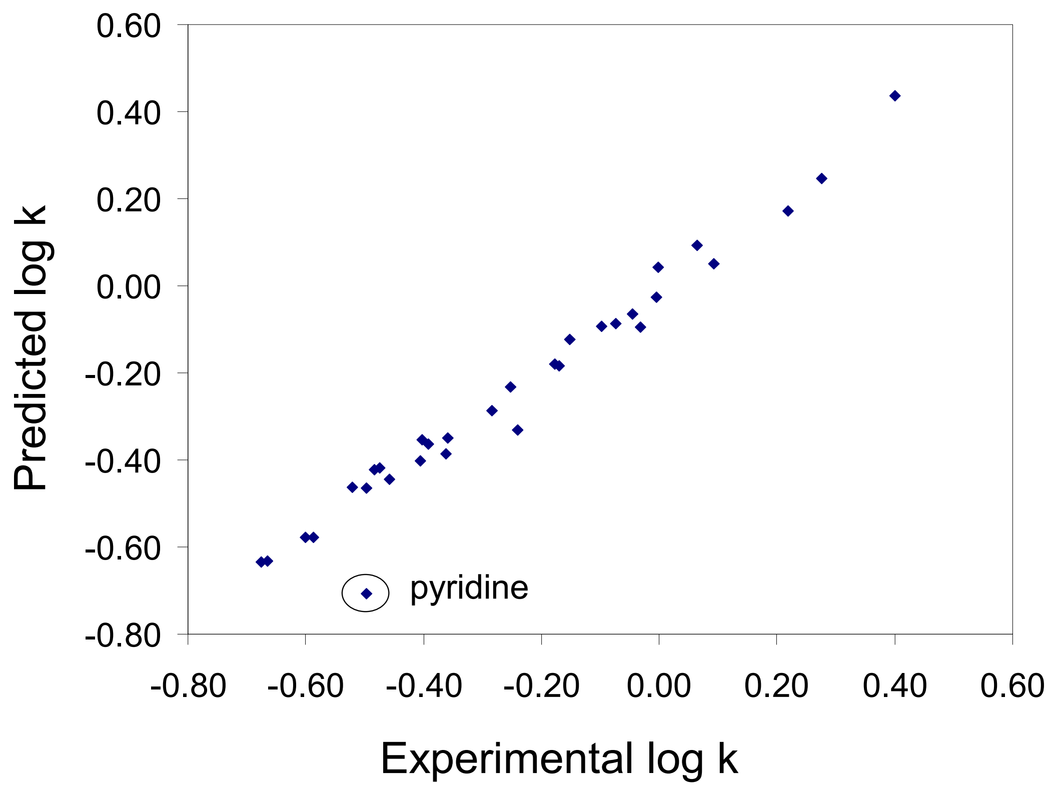
Extended LSER model for ionizable compounds



Plot of LSER-predicted log k vs log $k_{experimental}$ **accounting for organic modifier impact** at 60% MeOH

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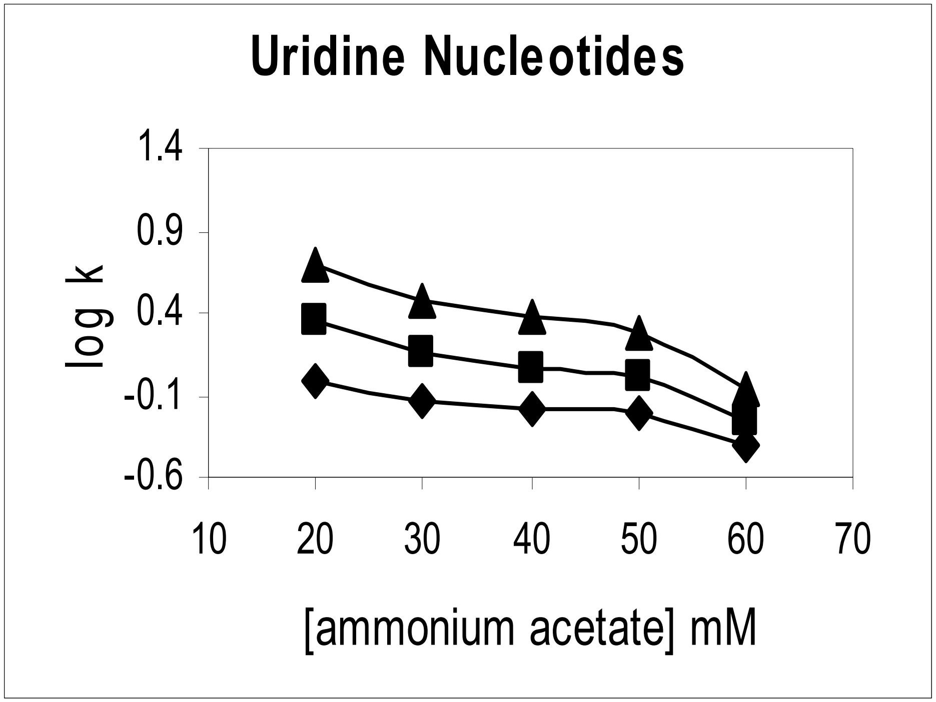
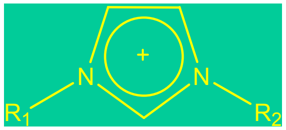
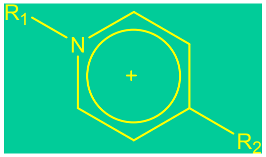
Ion exchange-based retention

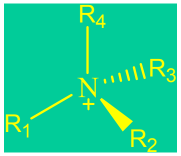


Ionic liquids

- Salts with melting points near room temperature
  - Bulky organic cations BF_4^-, PF_6^-, Br^-







- ~No vapor pressure
- Interesting solvent properties
- Wider range of properties than typical liquids

- **Bonus: chromatography may tell us something about ionic liquids - Reciprocity**

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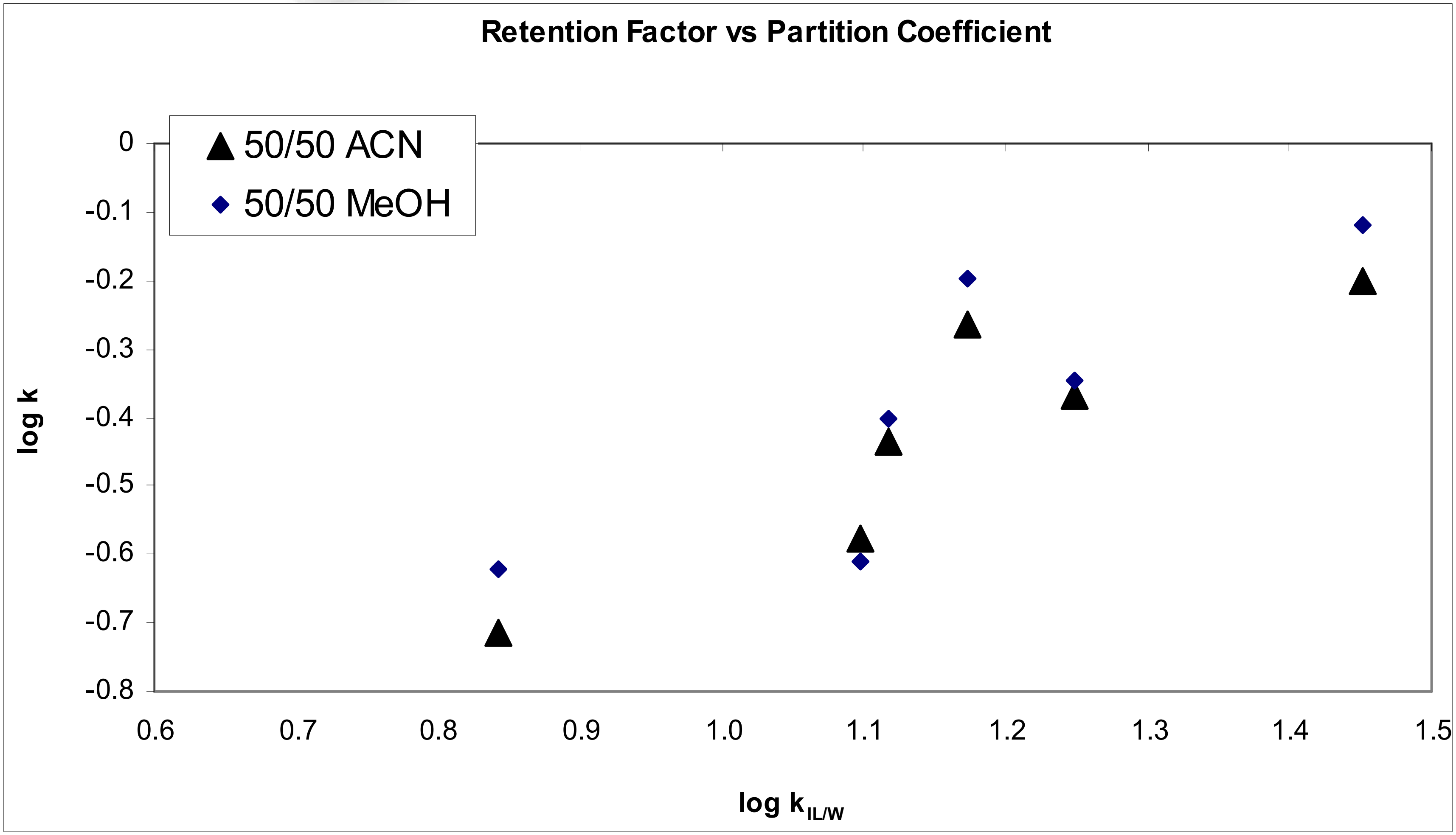

Impact of Counter-ion on Retention

k for 4-iodophenol and benzene using a 100% MeOH mobile phase*		
Counter ion	$k_{benzene}$	$k_{4\text{-iodophenol}}$
Br^-	0.18	0.49
CH_3COO^-	0.19	0.40
Cl^-	0.18	0.25
BF_4^-	0.17	0.22
I^-	0.21	0.19

Conclusions

- The butylimidazolium HPLC column exhibits multimodal retention
  - Phenyl-like reversed phase behavior in MeOH / H_2O or MeCN / H_2O mobile phases
  - Ion-exchange type behavior
    - Extended LSER model for nitrophenols
    - Comparison with commercial ion exchange column
- The butylimidazolium HPLC column exhibits retention which suggests ionic liquid-like character
  - Retention correlated to ionic liquid / H_2O partition coefficients
  - Retention of polar organics impacted by counter-ion

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Small Molecule Separations as Molecular Interaction Amplifiers

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