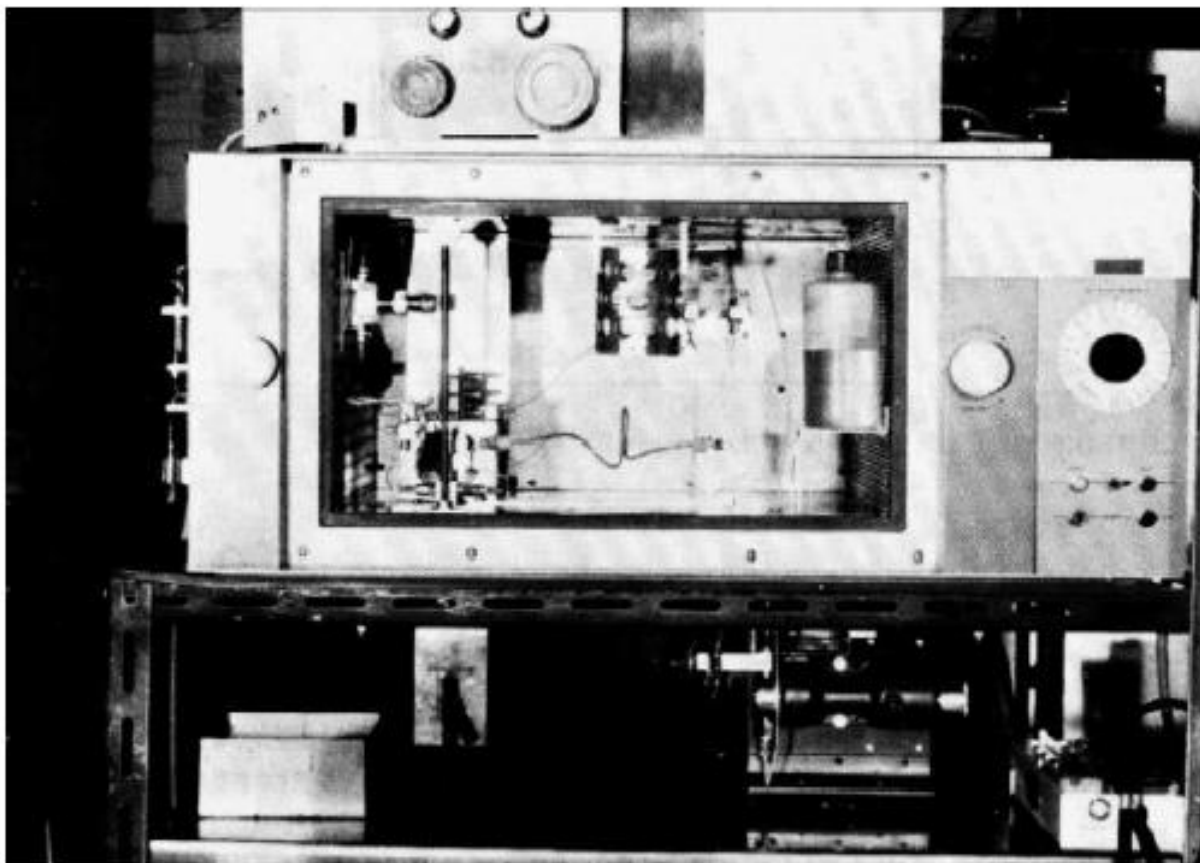


# **Fundamentals of Reversed Phase Chromatography: Solvophobic Theory Redux**

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Lecture at the “Csaba Horváth Memorial Session”  
at HPLC 2006 in San Francisco, organized by Imre Molnár

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**The first HPLC-Instrument (From the collection of Csaba Horváth)**

# Outline

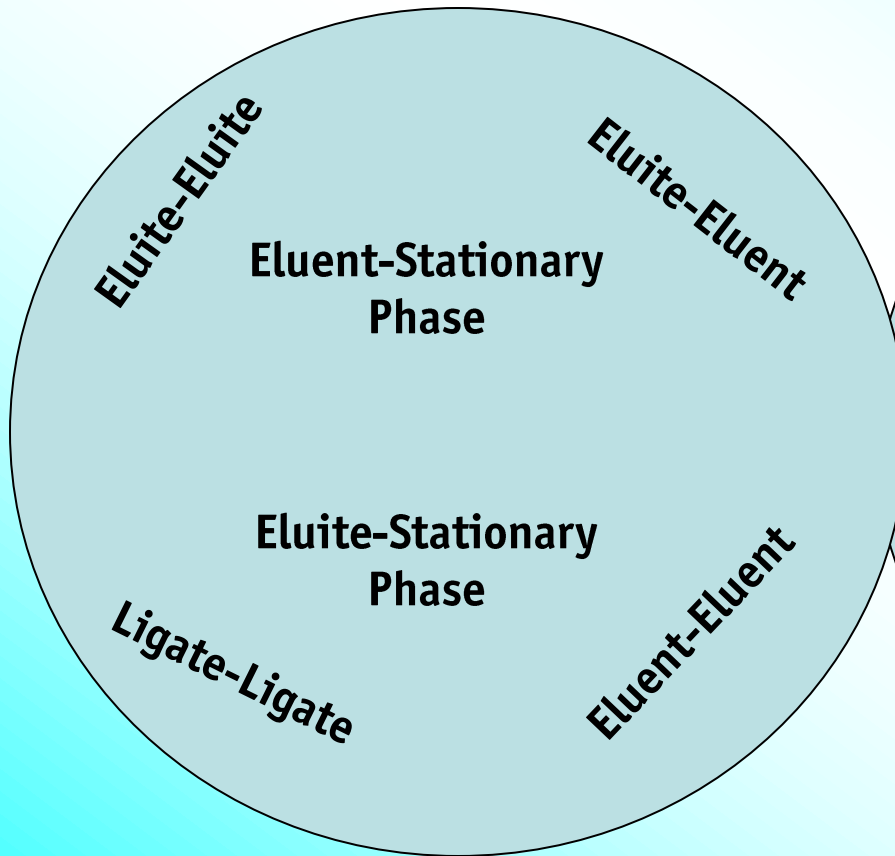
- Review of Various RPC Theories & Models
- Retention Mechanism Controversies
- Solvophobic Theory Redux
- Areas of Future Research

# Objective

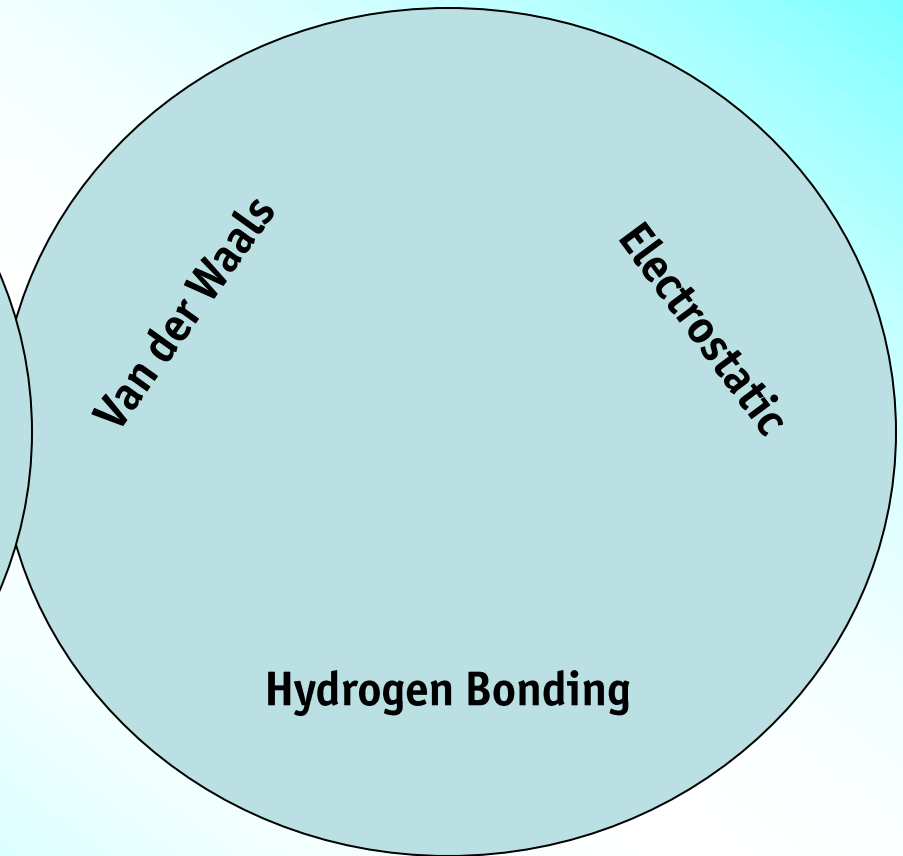
- Shed light on controversy enshrouding the fundamentals of RPC retention
  - Discuss merits of various points of contention
  - Address discordant views arising from semantics or misinterpretation
- To offer a unified and rigorous treatment of RPC retention of nonpolar compounds, acids, bases, proteins and peptides based on the Solvophobic Theory

# Molecular Interactions in RPC

**Type**



**Nature**



# Stationary Phase Models in RPC

No.	Stationary phase model	Description	References
I	<b>Liquid hydrocarbon partition</b>	Bonded chains form a bulk liquid hydrocarbon layer over siliceous surface; Retention governed by partition mechanism.	Lochmüller and Wilder, Dorsey and Dill, Carr <i>et al.</i>
II	<b>Liquid-crystalline hydrocarbon partition</b>	Bonded chains form a liquid-crystalline hydrocarbon layer over siliceous surface; Retention governed by partition mechanism.	Martire and Boehm
III	<b>Amorphous-crystalline hydrocarbon partition</b>	Bonded chains form an amorphous-crystalline hydrocarbon layer over siliceous surface; Retention governed by partition mechanism.	Dill
IV	<b>Adsorptive hydrocarbon monolayer</b>	Bonded chains interact laterally with the tips forming an adsorptive monolayer surface; Retention governed by adsorption mechanism.	Dill
V	<b>Isolated solvated hydrocarbon chains</b>	Eluities associate with solvated bonded chains; Retention governed by the magnitude of contact area upon binding.	Horváth, Melander and Molnár
VI	<b>Collapsed hydrocarbon chains</b>	Eluities partition into adsorbed organic modifier layer, then adsorb on the collapsed ligate chains	Kazakevich <i>et al.</i>

# RPC Theories

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## CLASSICAL THERMODYNAMICS

Solvophobic Theory

Horváth, Melander and  
Molnár (1976)

Adsorption/Partition Theory

Jaroniec and Martire  
(1986)

Non-stoichiometric Ion-pair Theory

Ståhlberg (1986)

## STATISTICAL THERMODYNAMICS

Regular Solution Theory

Shoenmakers *et al.*  
(1976)

Liquid Crystalline Mean Field Lattice Theory

Martire and Boehm  
(1983)

Amorphous Crystalline Mean Field Lattice Theory

Dill (1980)

Self Consistent Field Theory

Tijssen *et al.* (1991)

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# Lacuna, Controversies & Misinterpretations

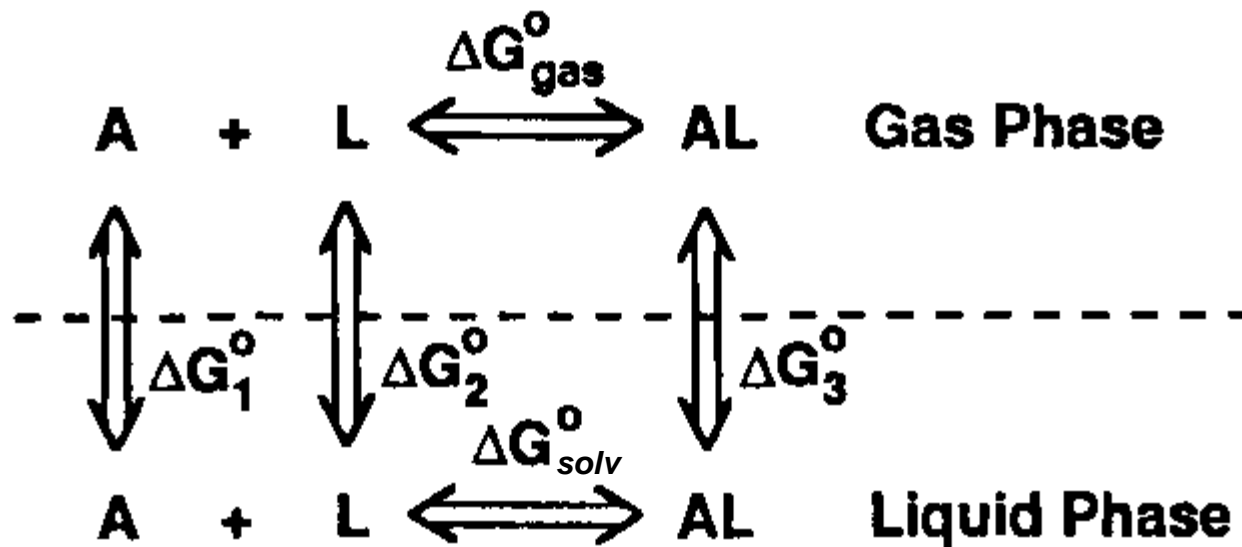
- Adsorption or Partition?
- Stationary vs. Mobile phase effects
- Entropic vs. Enthalpic effects



# Misinterpretations of the Solvophobic Theory

- Based on adsorption mechanism
- Neglects (or cannot describe) stationary phase effects in RPC
- Predicts, that retention in RPC is entropically driven

# Adaptation of the Solvophobic Theory to RPC Retention



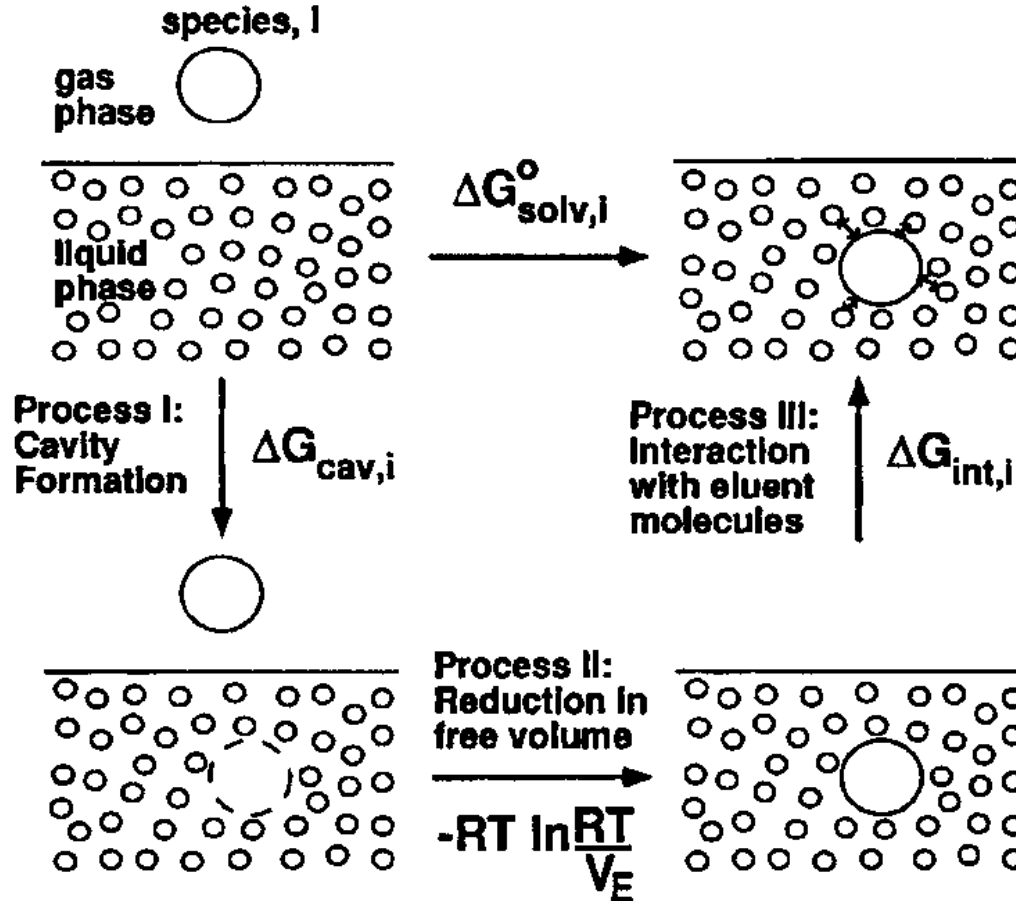
$$\Delta G^o = \Delta G_{\text{solv}}^o + \Delta G_{\text{gas}}^o$$

$$\Delta G_{\text{solv}}^o = \Delta G_3^o - \Delta G_1^o - \Delta G_2^o$$

Thermodynamic cycle illustrating the hypothetical gas phase binding of an eluite A with the ligate L, and the solvation of the individual species in RPC

# The Solvation Process

Eluent-eluent  
Interactions



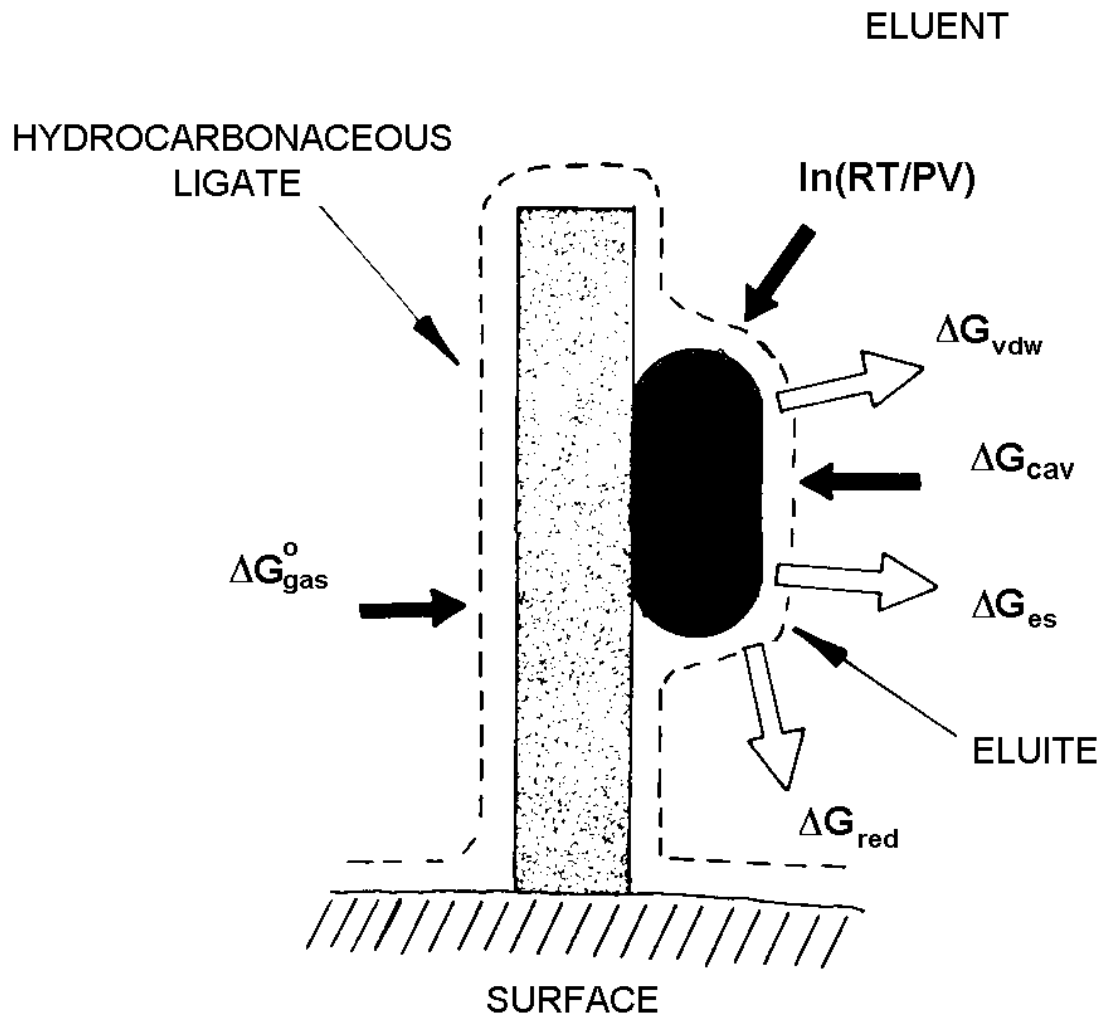
Eluent-eluite  
Interactions

$$\Delta G_{solv}^o = \Delta G_{cav} + \Delta G_{int} + \Delta \Delta G_{mix} + \Delta G_{red} - RT \ln \frac{RT}{PV}$$

$$\Delta G_{cav} = (\Delta G_{cav,AL} - \Delta G_{cav,A} - \Delta G_{cav,L})$$

$$\Delta G_{int} = (\Delta G_{int,AL} - \Delta G_{int,A} - \Delta G_{int,L})$$

# Forces Leading to the Association of an Elute Molecule with a Stationary Phase Ligate



The magnitude of the interactions between the elute and the ligate, which ultimately determines elute retention in RPC, is given by the difference between the two opposing effects, i.e., by the balance of all forces acting upon the two species.

# Quantifying RPC Retention

## Equation for Retention of Neutral Nonpolar Eluite

$$\ln k' = -\frac{N\Delta A_C \gamma_E}{RT} + \frac{4.836N^{1/3}(\kappa_E^e - 1)V^{2/3}\gamma_E}{RT} + \frac{0.60566I_f D_A D_E (Q' + Q'')}{RT} - \frac{(1 - I_f' M \frac{D_E}{1 + D_E})\Delta G_{gas}^o}{RT} + \ln \frac{RT}{PV} + \ln \phi$$

## Equation for Retention of Dipole in unionized form ( $k_o$ )

$$\ln k_o = -\frac{N\Delta A_C \gamma_E}{RT} + \frac{4.836N^{1/3}(\kappa_E^e - 1)V^{2/3}\gamma_E}{RT} + \frac{0.60566I_f D_A D_E (Q' + Q'')}{RT} - \frac{(\frac{N}{4\pi\epsilon_o})(\frac{1}{2} - \frac{V_A}{V_{AL}})(\frac{\mu_A^2}{v_A})(\frac{D}{1 - (\alpha_A/v_A)D})}{RT} - \frac{(1 - I_f' M \frac{D_E}{1 + D_E})\Delta G_{gas}^o}{RT} + \ln \frac{RT}{PV} + \ln \phi$$

## Equation for Retention of Dipole in Ionized Form ( $k_z$ ) at high ionic strength

$$\ln k_z = -\frac{N\Delta A_C \gamma_E}{RT} + \frac{4.836N^{1/3}(\kappa_E^e - 1)V^{2/3}\gamma_E}{RT} + \frac{0.60566I_f D_A D_E (Q' + Q'')}{RT} - \frac{\frac{Z^2 e^2 N}{4\pi\epsilon_o \epsilon} \left[ \frac{\epsilon - \epsilon^* (V_{AL}/V_L)^{1/3}}{\epsilon^* (V_{AL}/V_L)^{1/3}} \right]}{RT} - \left( \frac{\epsilon - \epsilon^*}{\epsilon^*} \right) (BI^{1/3} + CI) - \frac{(1 - I_f' M \frac{D_E}{1 + D_E})\Delta G_{gas}^o}{RT} - \frac{\Delta G_{gas,ez}^z}{\epsilon RT} + \ln \frac{RT}{PV} + \ln \phi$$

## Equation for Protein Retention

$$\ln k' = -\frac{N\Delta A_C \gamma_E}{RT} + \frac{4.836N^{1/3}(\kappa_E^e - 1)V^{2/3}\gamma_E}{RT} + \frac{0.60566I_f D_A D_E (Q' + Q'')}{RT} - \frac{\frac{Xm^{1/2}}{1 + Ym^{1/2}} + Z\mu_p m - A}{RT} - \frac{(1 - I_f' M \frac{D_E}{1 + D_E})\Delta G_{gas}^o}{RT} - \frac{\Delta G_{gas,ez}^z}{\epsilon RT} + \ln \frac{RT}{PV} + \ln \phi$$

A. Vailaya, J. Liq. Chromatogr., 28, (2005), 965-1054

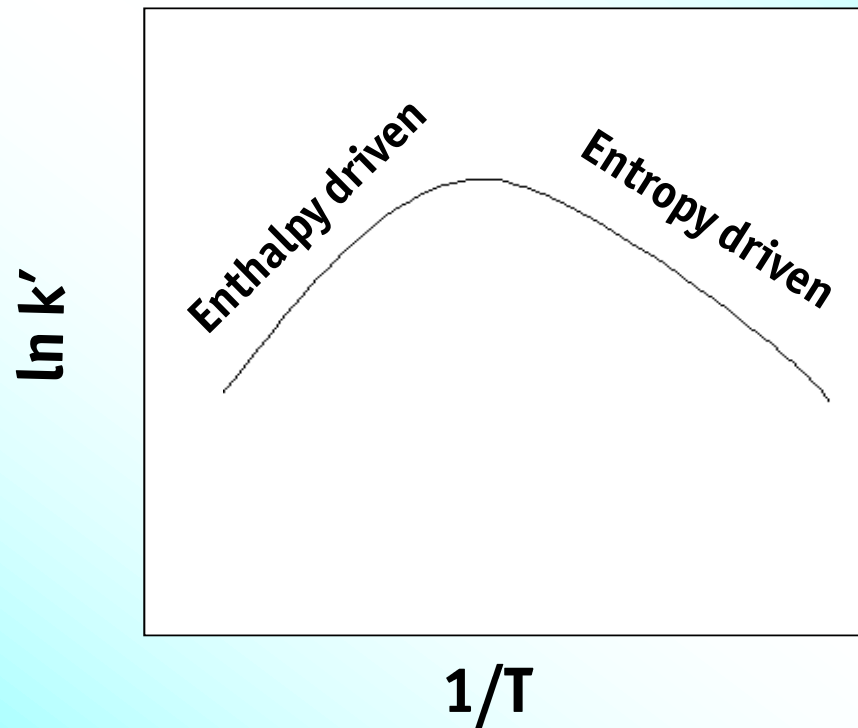
# Test of the Predictive Power of the Solvophobic Theory

- Temperature Dependence
- Effect of Molecular Size of the Eluite
- Prediction of Hydrophobic Selectivity
- Effect of Organic Modifier in the Eluent
- Effect of Salt and pH
- Prediction of Retention Factor
- Effect of Stationary Phase
- Correlation between Octanol/Water Partitioning and RPC Retention

# Temperature Dependence in RPC

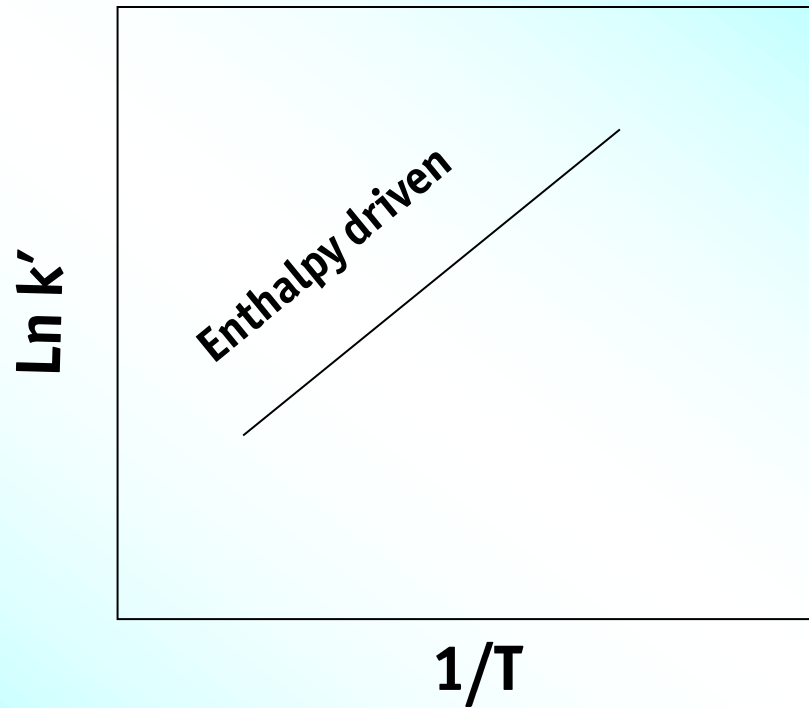
- Enthalpy change is due to a balance of large enthalpy changes associated with opposing forces of eluite-ligate, eluite-eluent and eluent-eluent interactions
- Solvophobic theory predicts curved van't Hoff plot in RPC, although deviation from linearity is relatively small (<5% over a 50°C temperature range)
- Curvature is expected to be more pronounced in water-rich mobile phases
- Temperature dependence of electrostatic interactions tends to counteract that of cavity reduction
- Enthalpy is largely negative due to dominating enthalpy contributions by eluite-eluent van der Waals interaction and cavity formation

# The Hydrophobic Effect



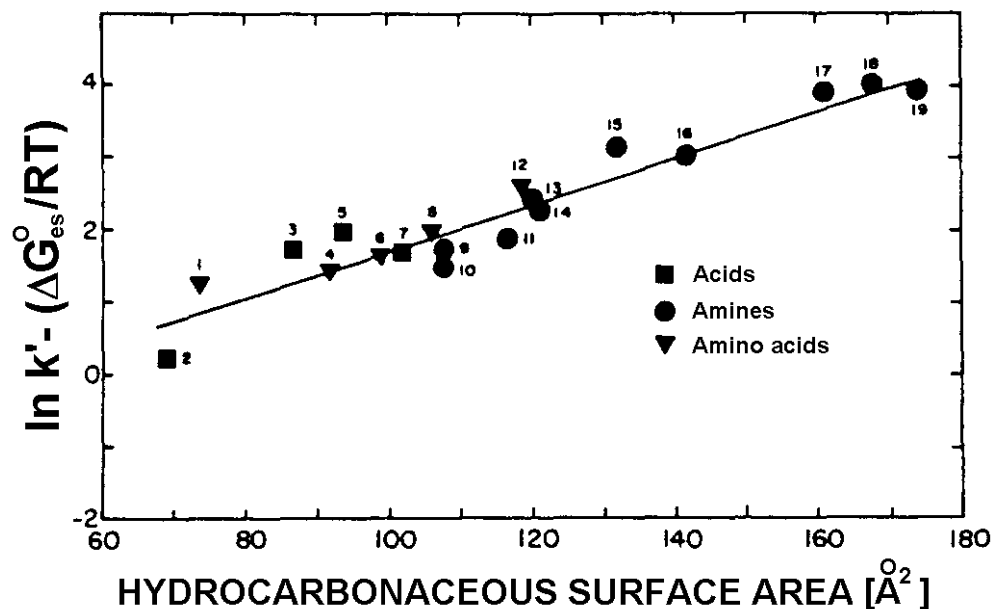
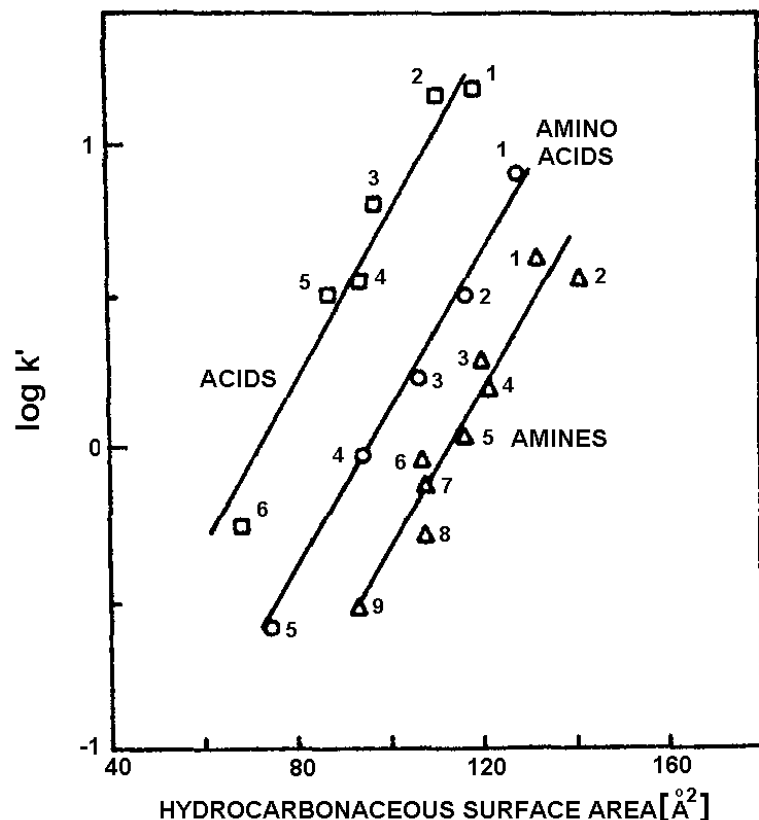


## Observed Behavior in RPC



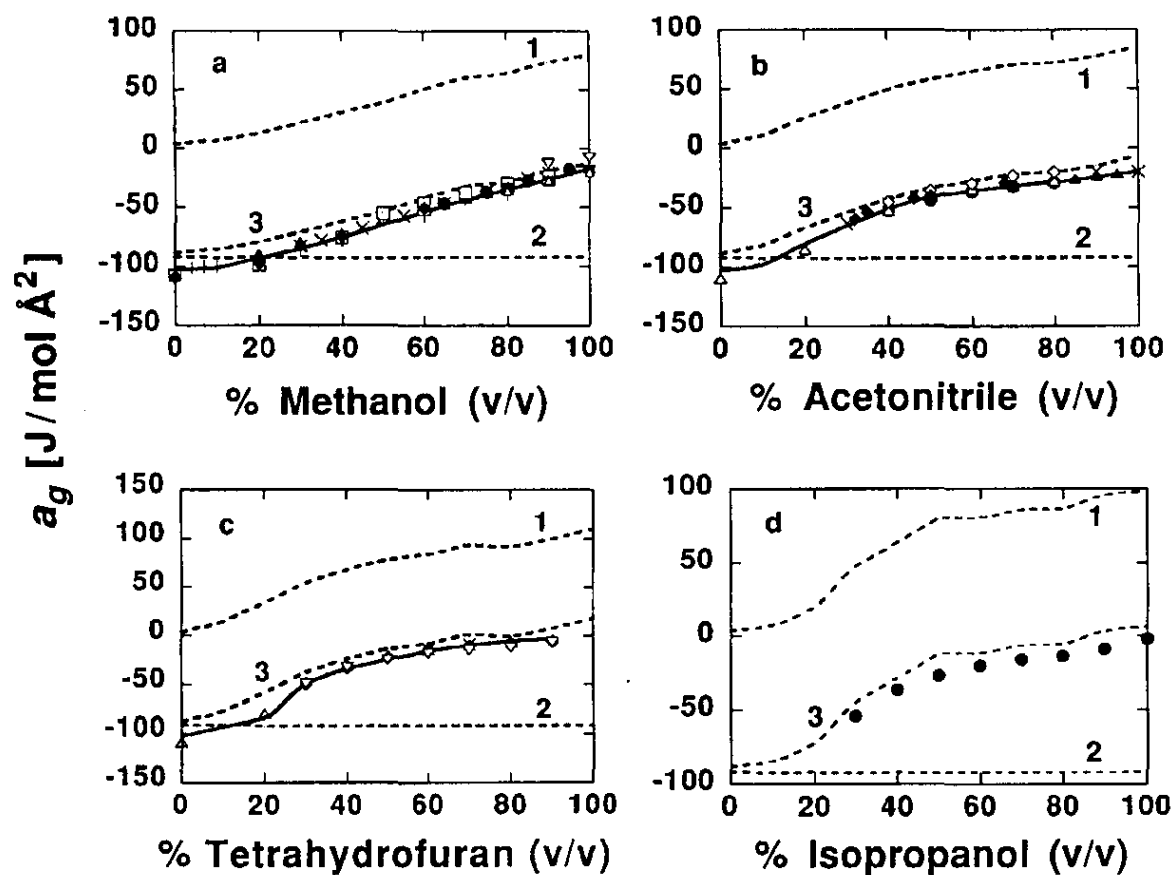
$$\Delta A_C = \alpha A_{np}$$
$$\ln k' = A' A_{np} + B'$$

# Effect of Molecular Size of the Eluite



Cs. Horváth, W. Melander, I. Molnár, J. Chromatogr., 125, 1976, 129-156

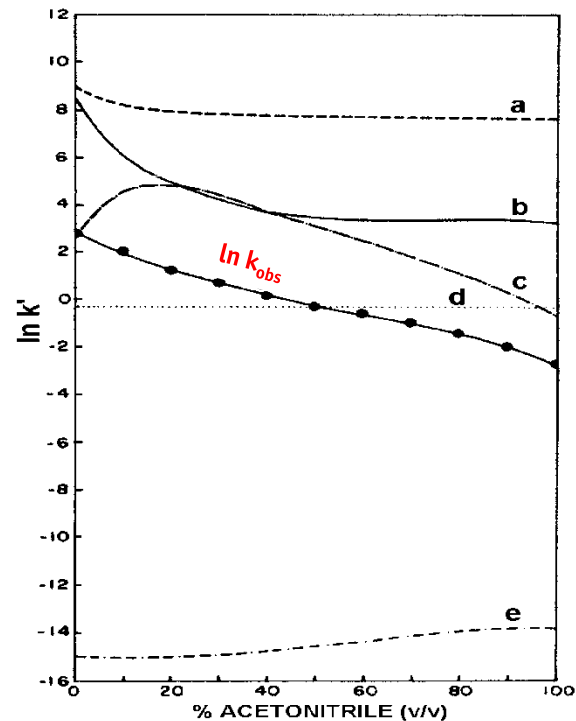
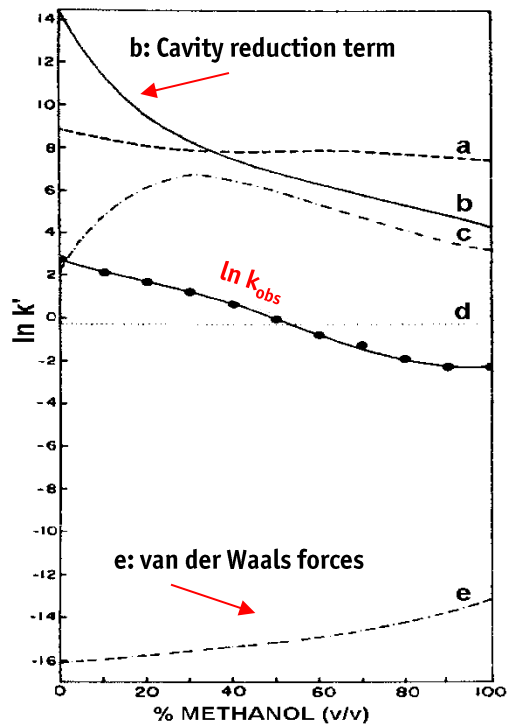
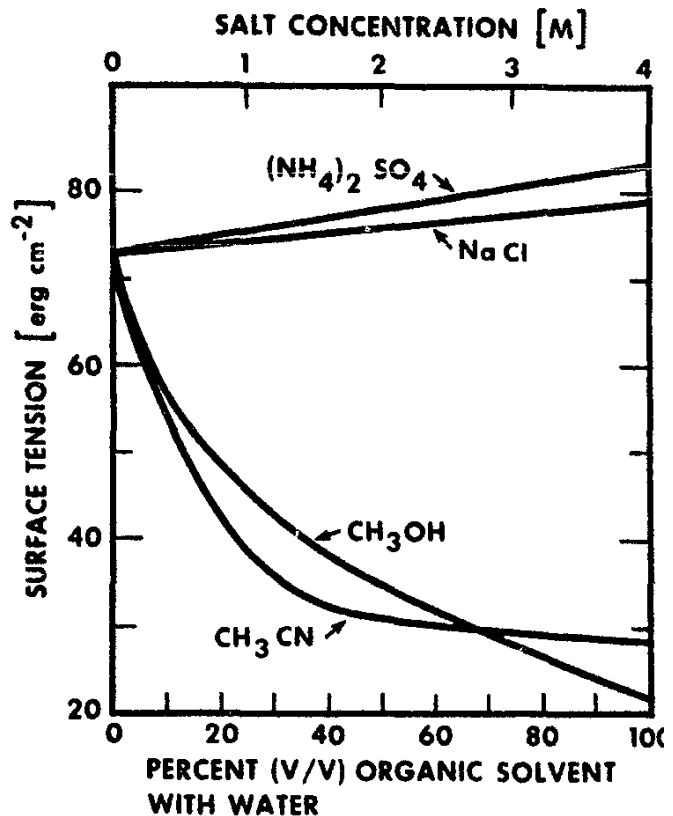
# Prediction of Hydrophobic Selectivity



A. Vailaya, Cs. Horváth, J. Phys. Chem. B, 101, 1997, 5875-5888

# Effect of Organic Modifier in the Eluent

$$\ln k' = A' + B'D + C'\gamma_E + D'(\kappa_E^e - 1)V^{2/3}\gamma_E + E' + \ln \frac{RT}{PV}$$



$$a = \ln(RT / PV)$$

$$d = B'D$$

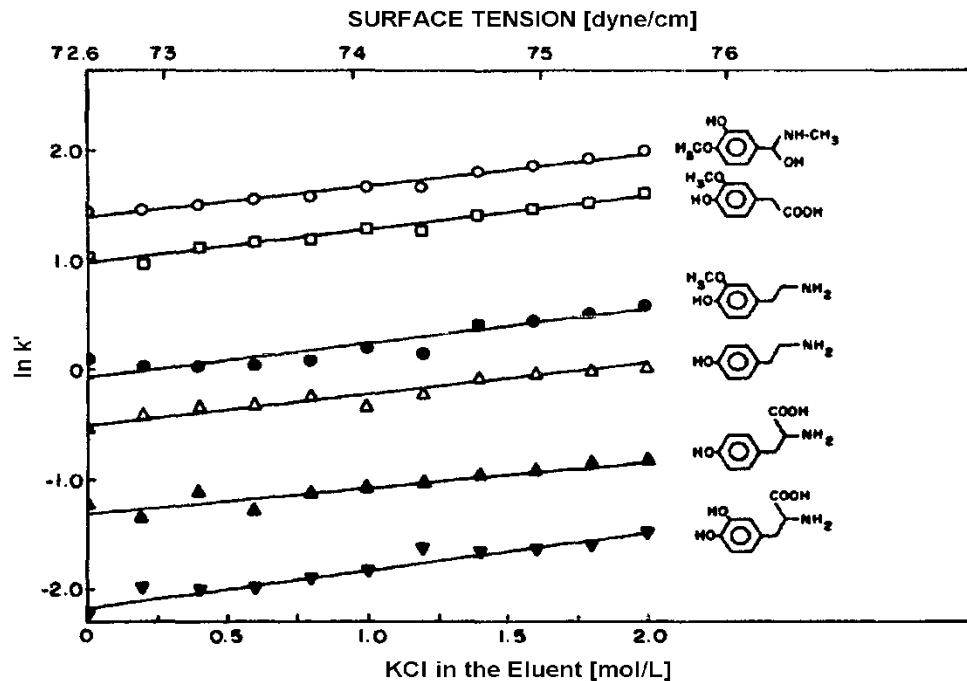
$$b = C'\gamma_E$$

$$e = E'$$

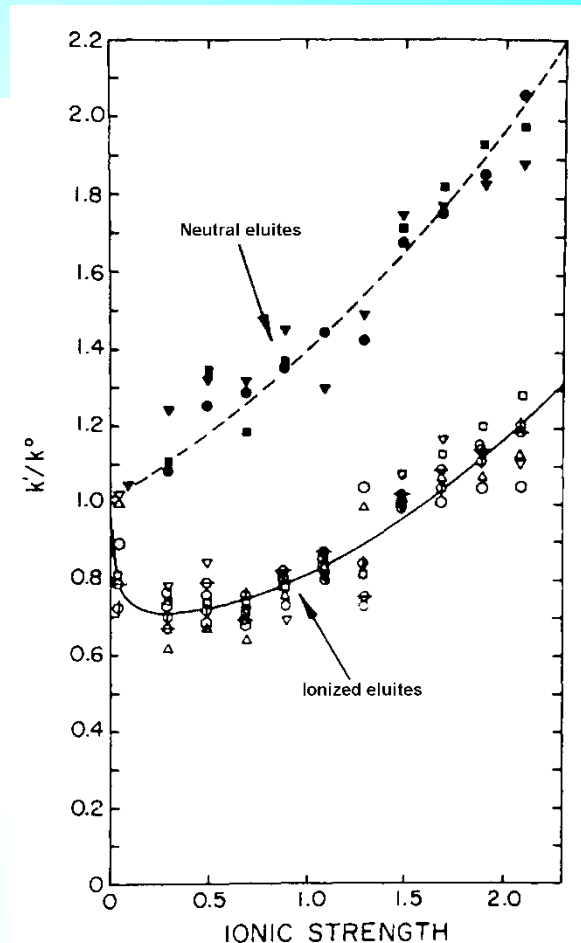
$$c = D'(\kappa_E^e - 1)V^{2/3}\gamma_E$$

The strongest and most similar energetic contribution term of (a-e) to  $\ln k'$  is the Cavity Reduction Term  $b = C'\gamma_E$

# Effect of Salt



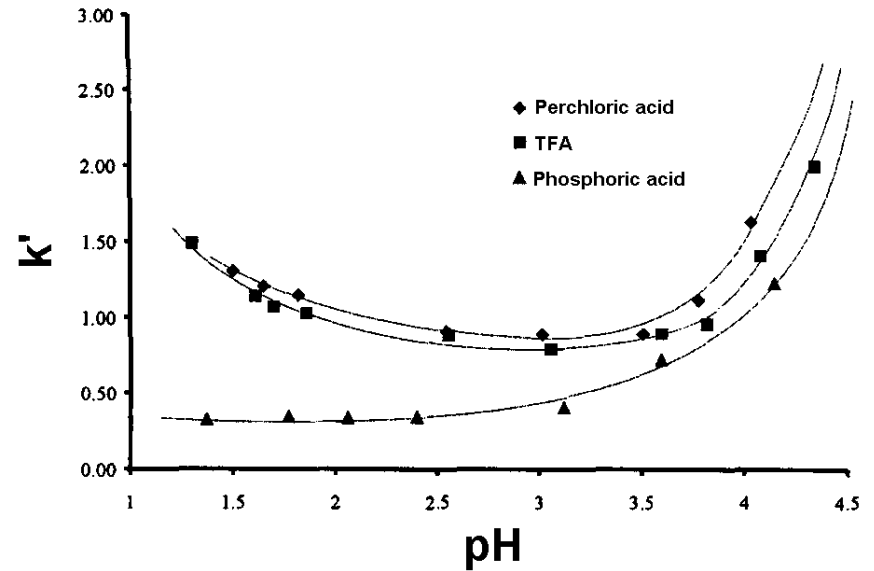
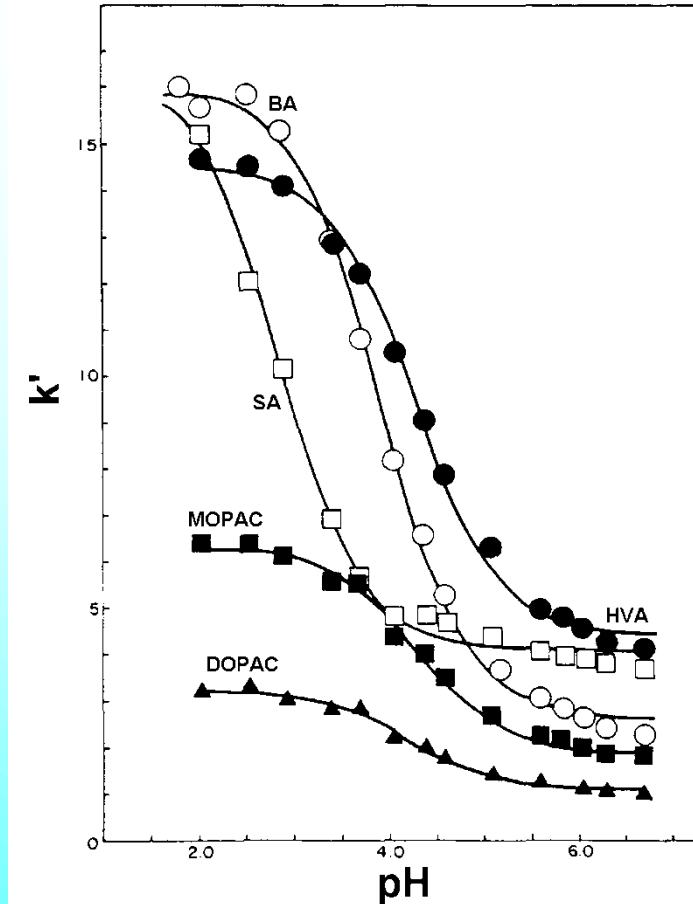
Effect of salt concentration on the retention factor in RPC is weak. However it is sufficient for the separation of proteins in HIC. Column: Partisil 1025 ODS; Flow Rate: 1 mL/min; Temperature: 25 °C.



Cs. Horváth, W. Melander, I. Molnár, J. Chromatogr., 125, (1976) 129-156

Cs. Horváth, W. Melander, I. Molnár, Anal. Chem., 49, (1977) 142-154

# Effect of pH



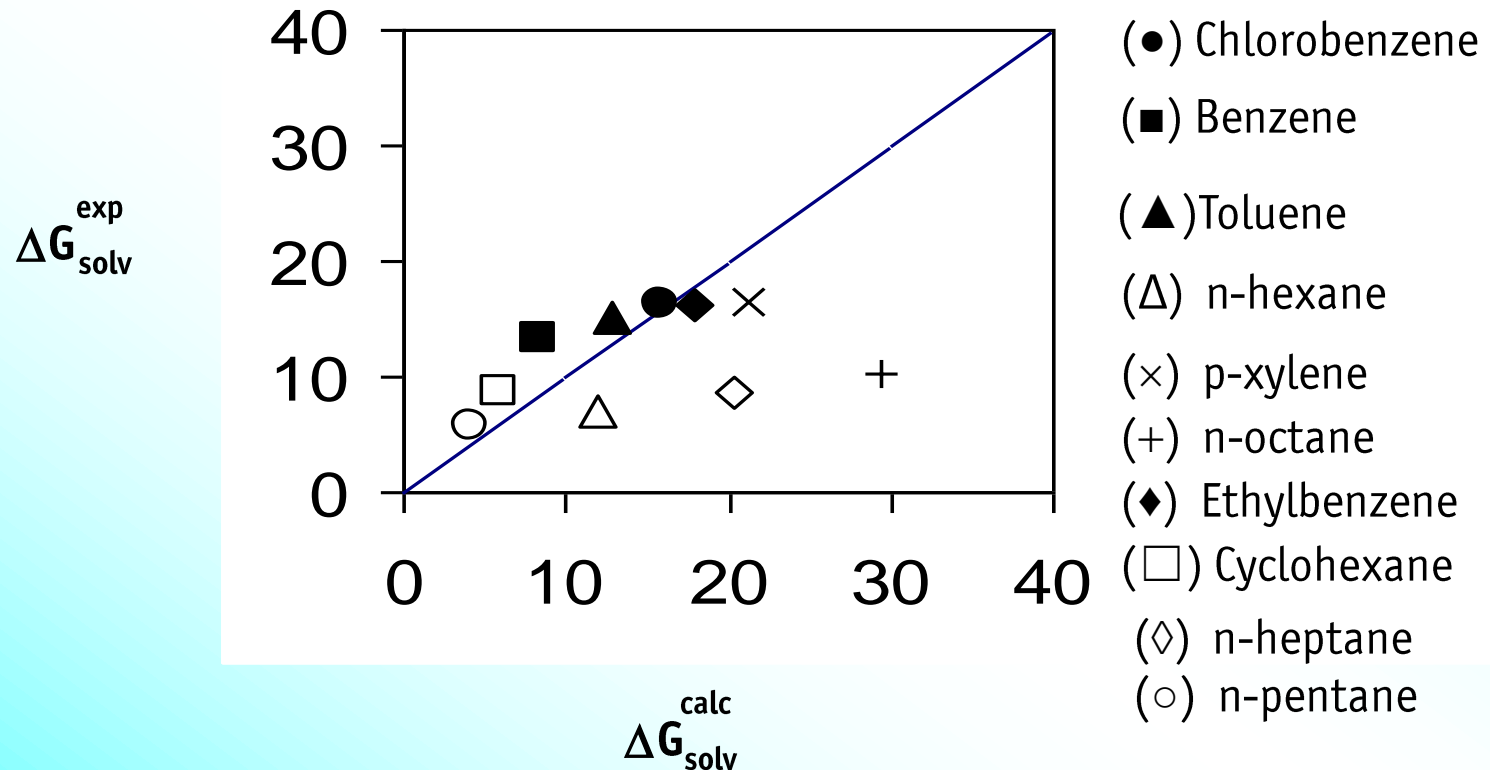
$$k' = \frac{k_o + k_z d}{1 + d}$$

Cs. Horváth, W. Melander, I. Molnár, Anal. Chem., 49, 1977, 142-154

R. LoBrutto, A. Jones, Y.V. Kazakevich, H.M. McNair, J. Chromatogr. A, 913, 2001, 173-187

# Prediction of Retention Factor

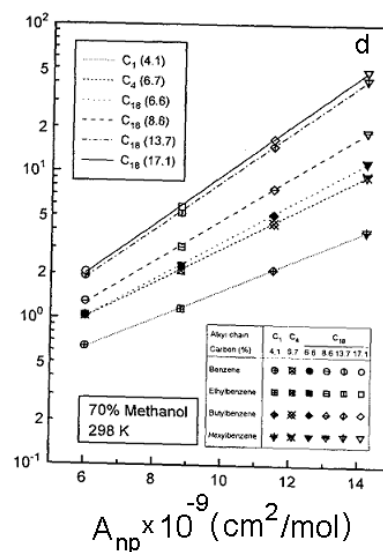
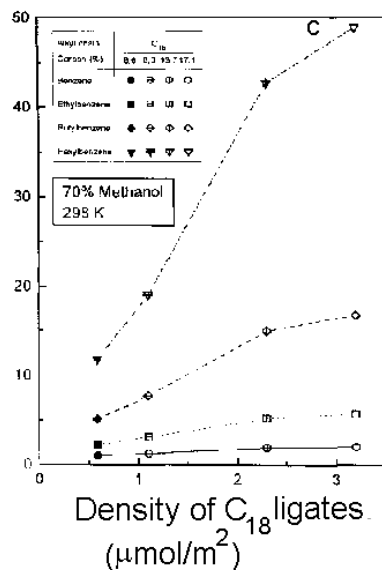
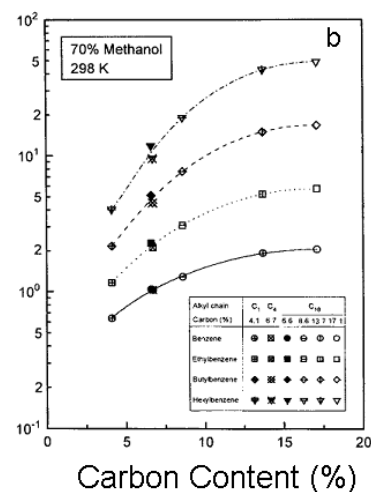
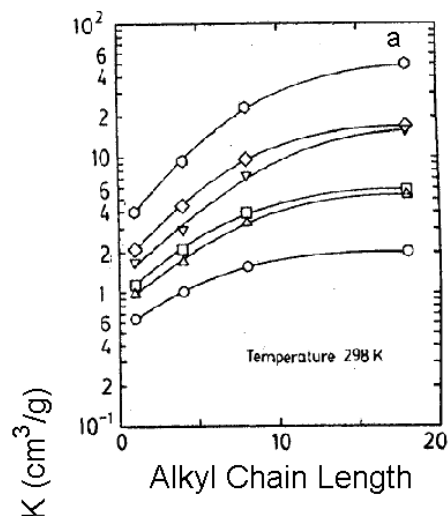
$$\Delta G^o = \Delta G_{solv}^o + \Delta G_{gas}^o$$



K. Miyabe, M. Suzuki, *AIChE J.*, 41, 1995, 536-547.

A. Vailaya, *J. Liq. Chromatogr.*, 28, 2005, 965-1054

# Effect of Stationary Phase



K. Miyabe, S. Takeuchi, *Anal. Chem.*, **69**, 1997, 2567-2574

H. Engelhardt, G. Ahr, *Chromatographia*, **14**, 1981, 227.

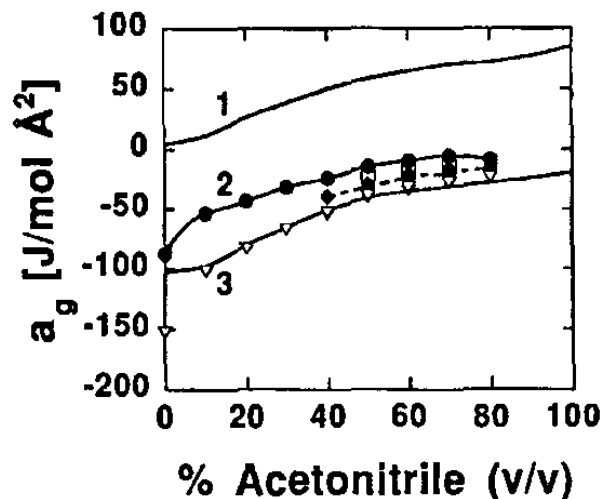
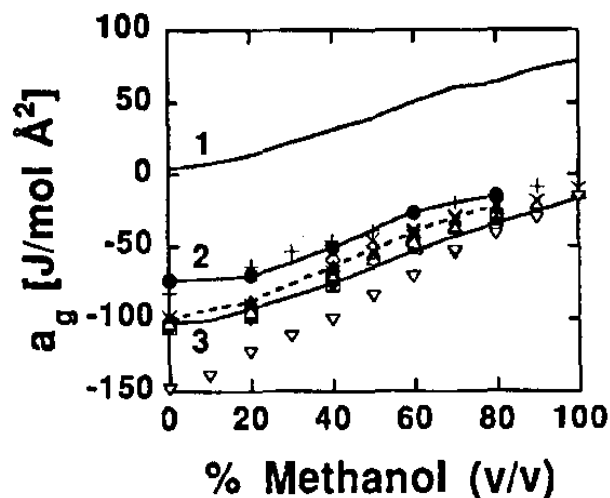


# Hydrophobic Selectivity

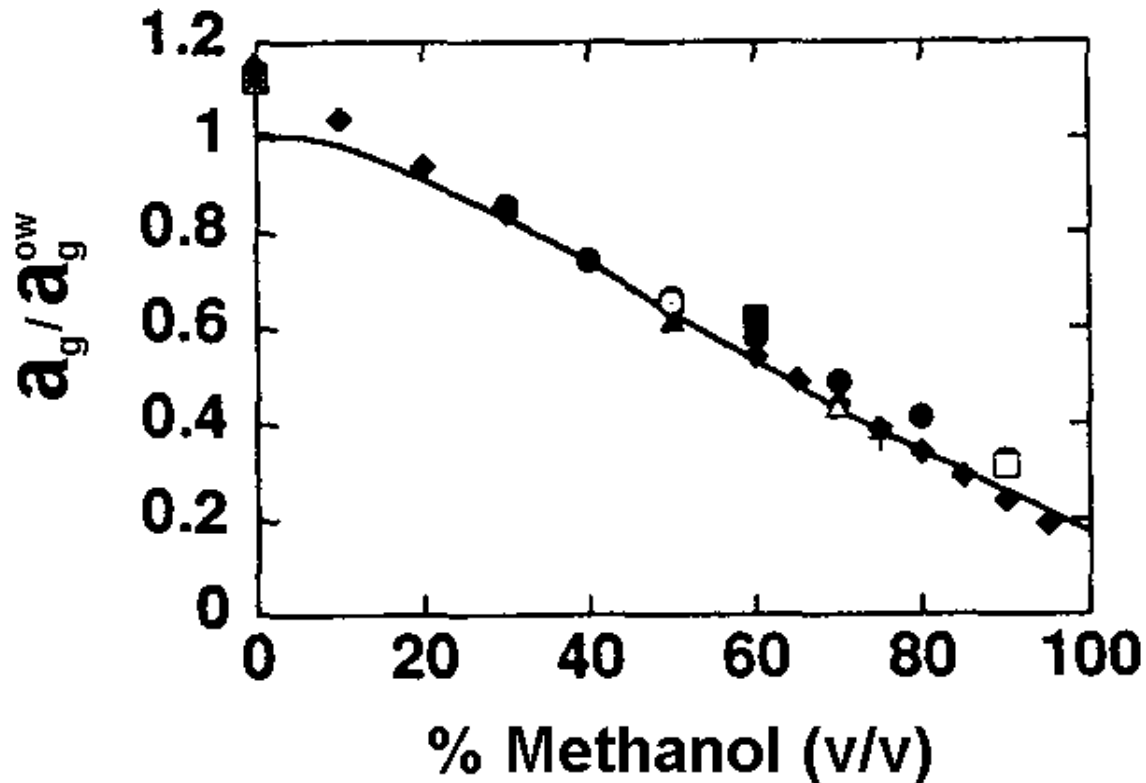
Hydrophobic selectivity is more fundamentally affected by changes in the mobile phase, than by changes in the chain length of the bonded stationary phase (insignificant change for C8, C10, C18, C22

-> **17 [J/mol Å<sup>2</sup>]** change from C18 to C1, **versus**

-> **86 [J/mol Å<sup>2</sup>]** change from **0 to 100% methanol**)



# Correlation between Octanol/Water Partitioning and RPC Retention



$$\ln k' - \ln K_{ow}$$

Solid line represents the ratio of hydrophobic selectivity values of RPC retention and octanol/water partitioning determined individually from experimental data. Symbols represent the slope values of linear  $\ln k' - \ln K_{ow}$ -plots of data from various sources.

A. Vailaya, Cs. Horváth, J. Chromatogr. A, **829**, (1998) 1-27

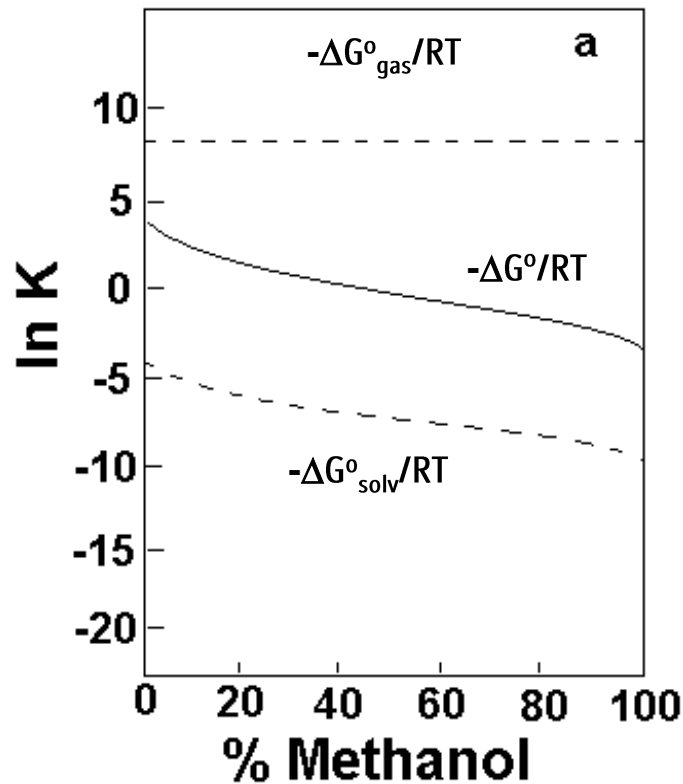
# Correlation between Octanol/Water Partitioning and RPC Retention

The free energy change for the partitioning of a solute between octanol and water phases can be expressed in terms of interfacial surface tension and molecular surface area  $A_A$  as

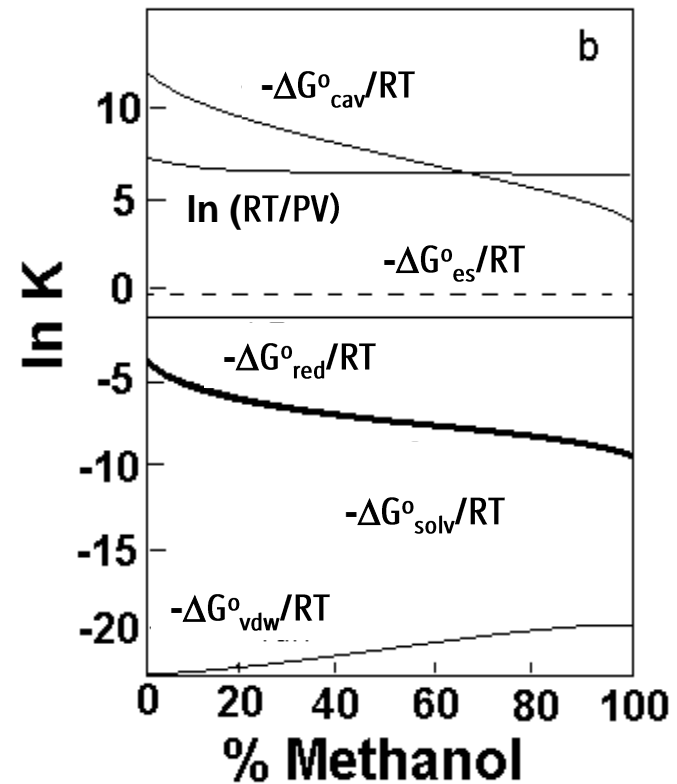
$$\Delta G_{ow}^o = (\kappa_{AO}^s \gamma_{AO} - \kappa_{AW}^s \gamma_{AW}) A_A + RT \ln \frac{V_w}{V_o}$$

assuming the electrostatic interactions in the two phases to be negligible. In the above equation  $\gamma_{AO}$  and  $\gamma_{AW}$  represent solute-octanol and solute-water interfacial tensions and  $V_o$  and  $V_w$  are molar volumes of octanol and water respectively. The  $\kappa^s$ 's convert the respective interfacial tensions to the microthermodynamic value applicable to molecular dimensions. Thus the equation predicts a linear relationship between  $\Delta G_{ow}^o$  and  $A_A$  of the solute for a set of hydrophobic compounds, with the slope of the linear plot determined by interfacial tensions.

# Interplay of Various Interactive Forces in RPC Retention



“Gedanken”-Experiment: showing the estimated equilibrium constant at room temperature for the retention of toluene on octadecylated silica gel column in **Gas Chromatography** with helium as carrier gas and in **RPC** with methanol/ water mixtures as the mobile phase. The net effect of the mobile phase,  $-\Delta G_{solv}^o / RT$  essentially reduces the magnitude of eluite-stationary-phase van der Waals interactions in the gas phase.



The individual contributions of various interactions **in RPC** contributing to the net solvent effects,  $\Delta G_{solv}^o$  for the RPC retention of toluene on an octadecylated silica column at room temperature.  $\Delta G_{vdw}$  = free energy change of eluite-eluent van der Waals interactions,  $\Delta G_{es}$  = free energy change of eluite-eluent electrostatic interactions,  $\Delta G_{cav}$  = free energy change associated with cavity reduction in the eluent (eluent-eluent van der Waals and hydrogen bond interactions),  $\ln(RT / PV)$  = reduction in free volume and  $\Delta G_{red}$  = free energy change associated with reduction of eluite-ligate van der Waals interactions in the gas phase.

# Future Challenges

- Quantifying Stationary Phase Effects
  - Shape selectivity
  - Topography of the stationary phase surface
  - Configuration and arrangement of ligate chains
  - Eluent adsorption
- Predicting Retention of Biologicals
  - Knowledge of 3-dimensional molecular structure of proteins at the chromatographic surface (conformational changes)
  - Influence of the nature of salts
  - Steric hindrance

# Conclusions

- The macroscopic view of the solvophobic theory predicts that the net free energy change associated with the stationary phase effect **is much greater**, than that associated with the mobile phase effect.
- At the molecular level, however, the solvophobic theory identifies the **dominant role played by the favorable energetics of cavity reduction in overcoming the unfavorable energetics of eluente-eluent van der Waals interactions.**

**Criticisms of the Solvophobic Theory are based on a straw man argument**

# Acknowledgement

***“In memory of Csaba Horváth!”***