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HIGH TEMPERATURE CHROMATOGRAPHY ON
HYBRID STATIONARY PHASES

Nelu Grinberg

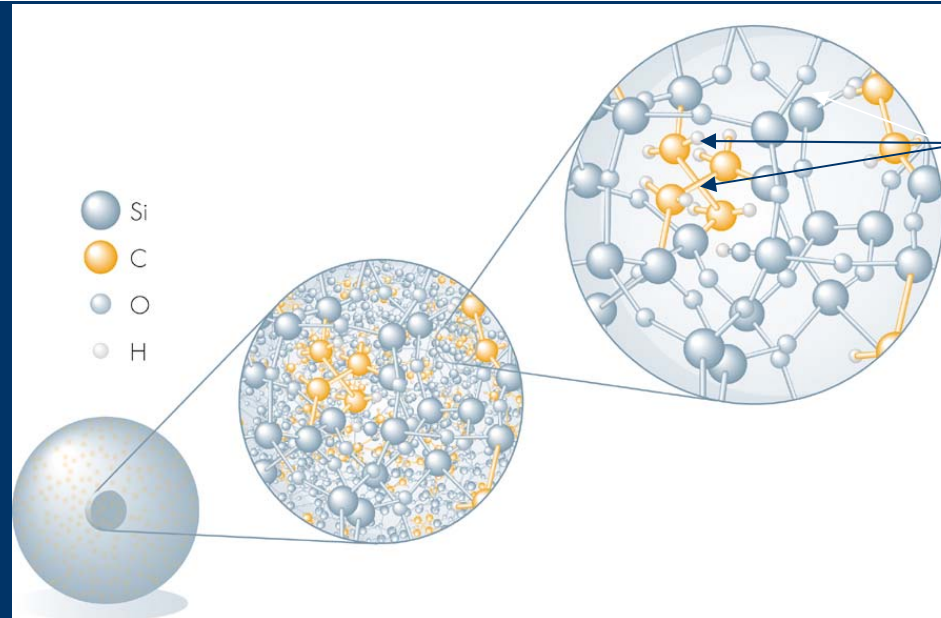
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- Liquid chromatography at elevated temperatures has traditionally not been widely used, with the exception of:
- Polymers of low solubility - polyolefins –
140 °C -150 °C
- Ion exchange chromatography - improve column efficiency.
- Development of more efficient packing materials decreased the use of higher temperature.

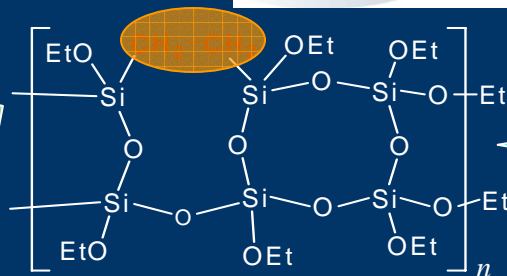
- Few silica based stationary phases are able to sustain high temperatures.
- Few HPLC systems had means for column thermostating.

BRIDGED ETHYL HYBRID PARTICLES

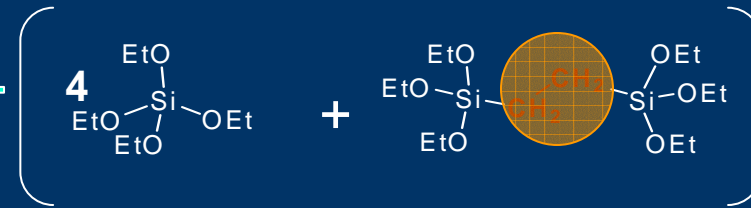
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Bridged Ethyl
In Silica Matrix



Polyethoxysilane
(BPEOS)

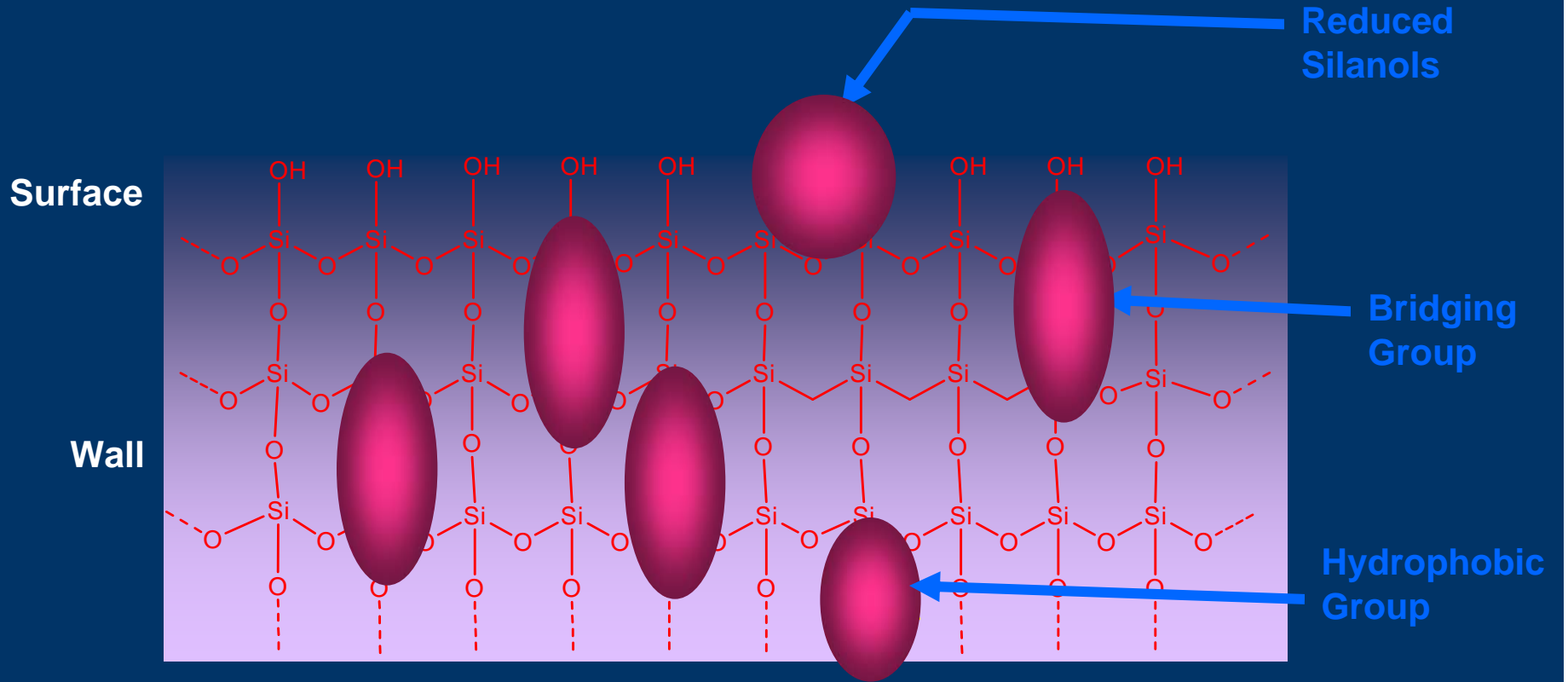


Tetraethoxysilane
(TEOS)

Bis(triethoxysilyl)ethane
(BTEE)

Particle Attributes

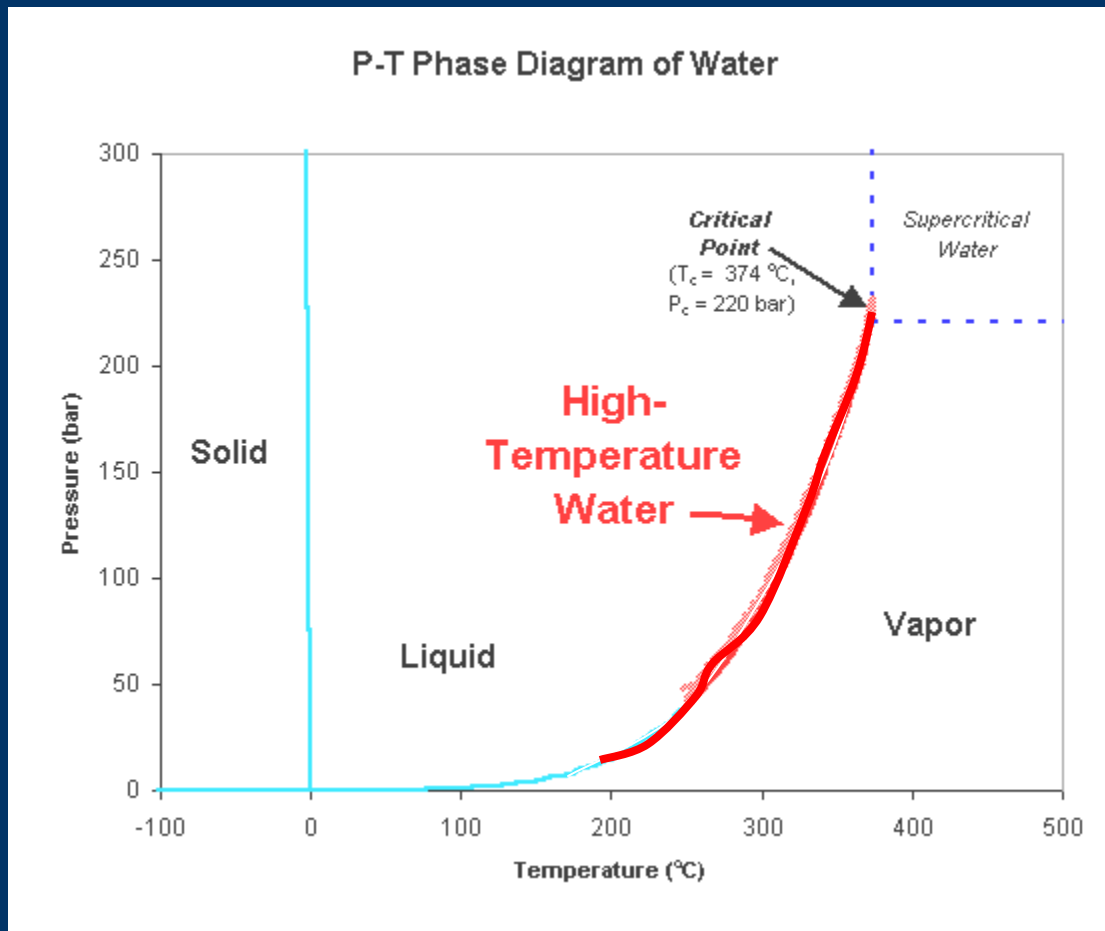
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- Recently a more general acceptance of the importance of temperature as a variable in HPLC.
- Reasons:
 - ❖ Decrease in viscosity of the mobile phase.
 - ❖ Lower back pressure.
 - ❖ Increased flow rates - shorter analysis time.
 - ❖ Increase analyte diffusivity.
 - ❖ Obtain more information on chromatographic system.

P-T PHASE DIAGRAM OF WATER

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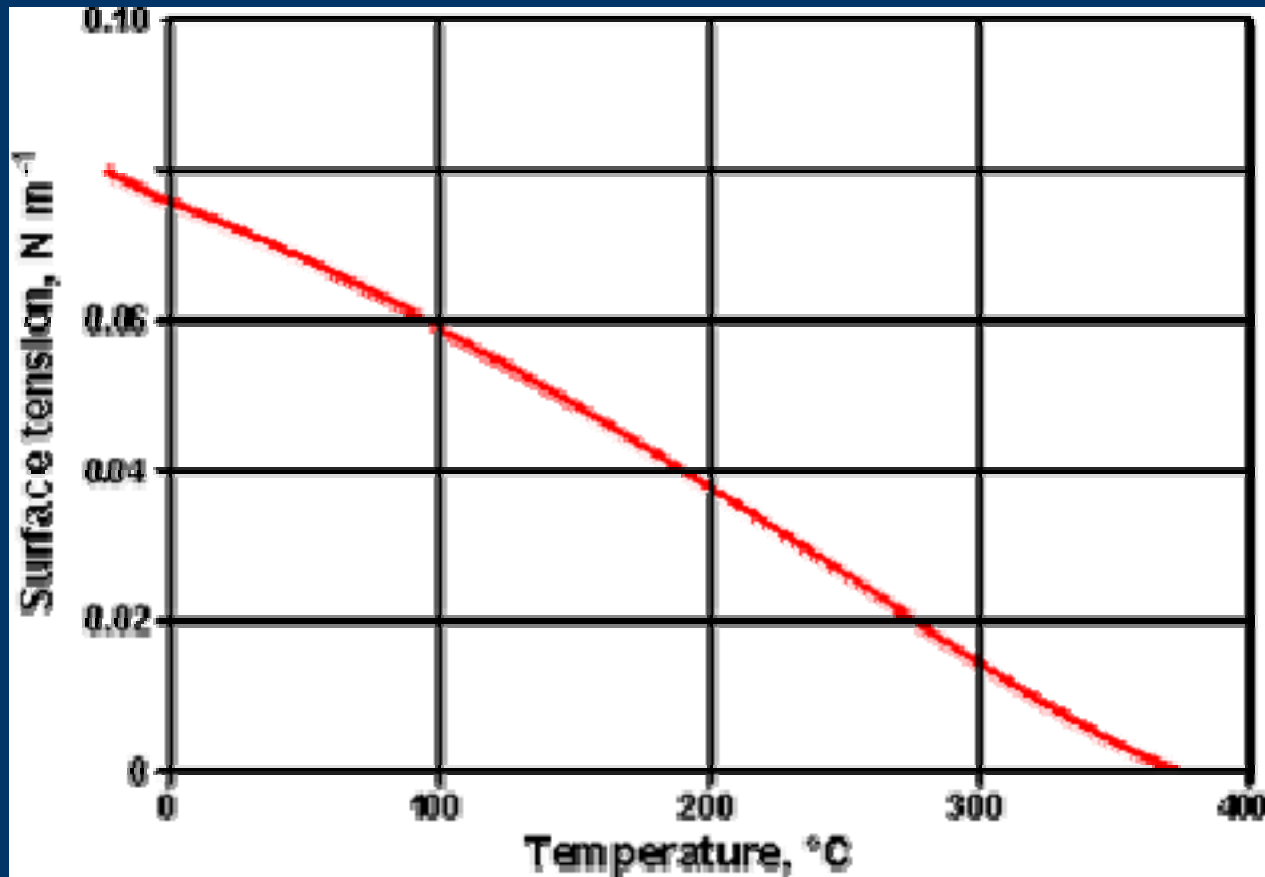
PROPERTIES OF HIGH TEMPERATURE WATER (HTW)

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- HTW is structurally different from ambient liquid water.
- Hydrogen bonding in water becomes weaker and less persistent with increasing temperature. At the same time the increase in temperature produces a decrease in density.
- Unlike the infinite percolating network of H-bonds found in ambient liquid water, the H-bond network in HTW exists in the form of small clusters of H-bonded water molecules. With increasing temperature and decreasing density, the average cluster size decreases.

VARIATION OF SURFACE TENSION OF WATER WITH TEMPERATURE

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PROPERTIES OF HIGH TEMPERATURE WATER (HTW)

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- The changes in the extent of hydrogen bonding are accompanied by corresponding changes in the dielectric constant.
- The Kirkwood equation states

$$\frac{(2D+1)(D-1)}{9D} = \frac{4\pi N_o d}{3M} \left(\alpha + \frac{\mu^2 g}{3kT} \right)$$

where:

D - the dielectric constant

N_o - Avogadro's number

k - Boltzmann constant

d - density

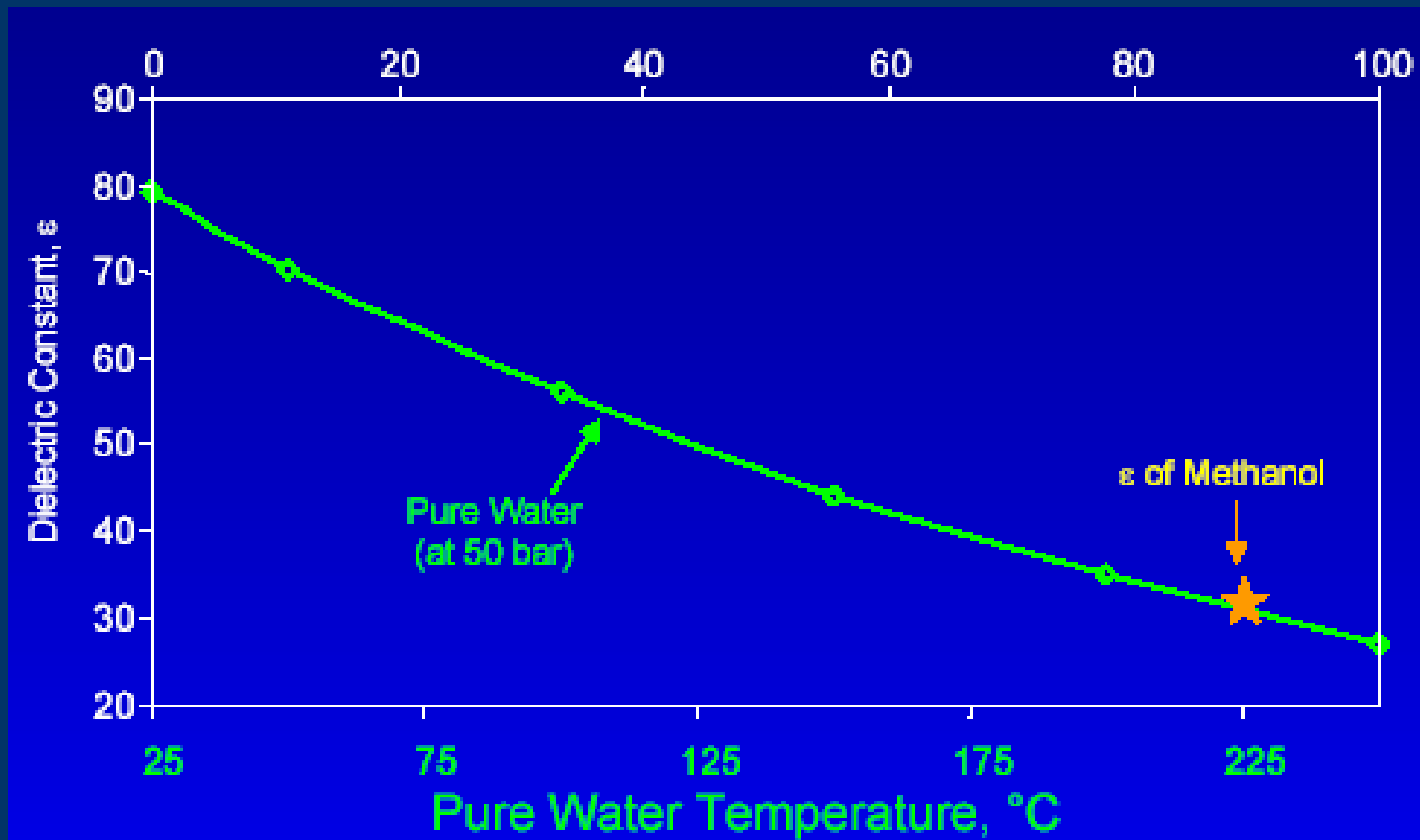
α - the molecular polarizability

μ - the molecular dipole moment

g - the Kirkwood correlation factor which accounts for nonrandom orientation of neighboring molecules

SOLVENT POLARITY AS A FUNCTION OF TEMPERATURE

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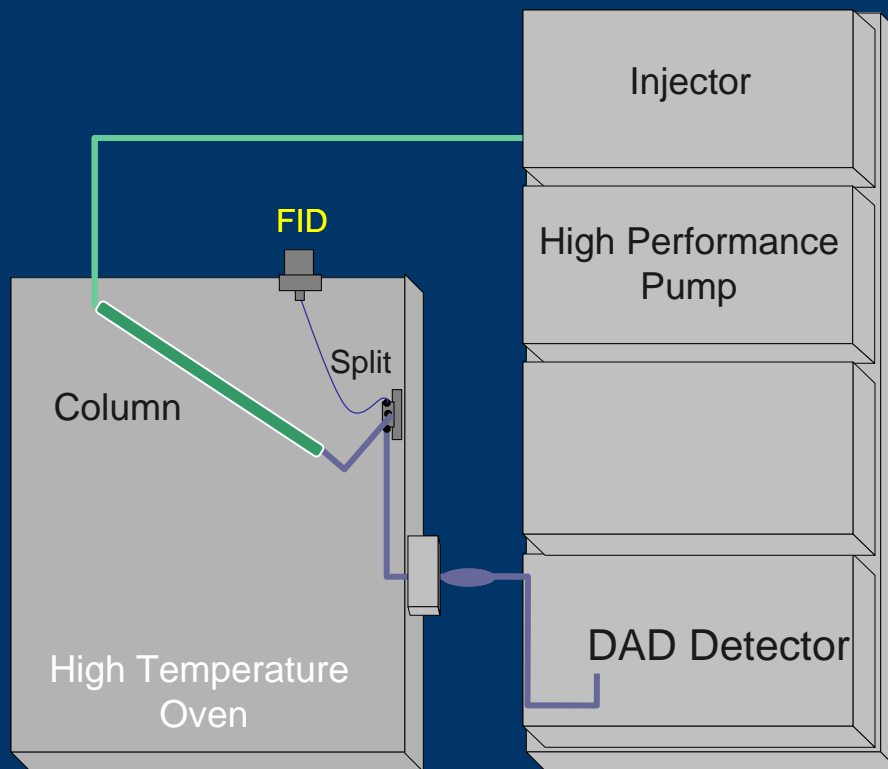


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- HTW behaves more like a polar organic solvent when temperature is increased causing a decrease in the density, dielectric constant and surface tension. **Consequently, small organic compounds are highly soluble in HTW.**
- The breaking of the H-bond network reduces the barrier for translational and rotational motion. This effect contributes to the increase in the self-diffusivity of water which occurs with increasing temperature and decreasing density.

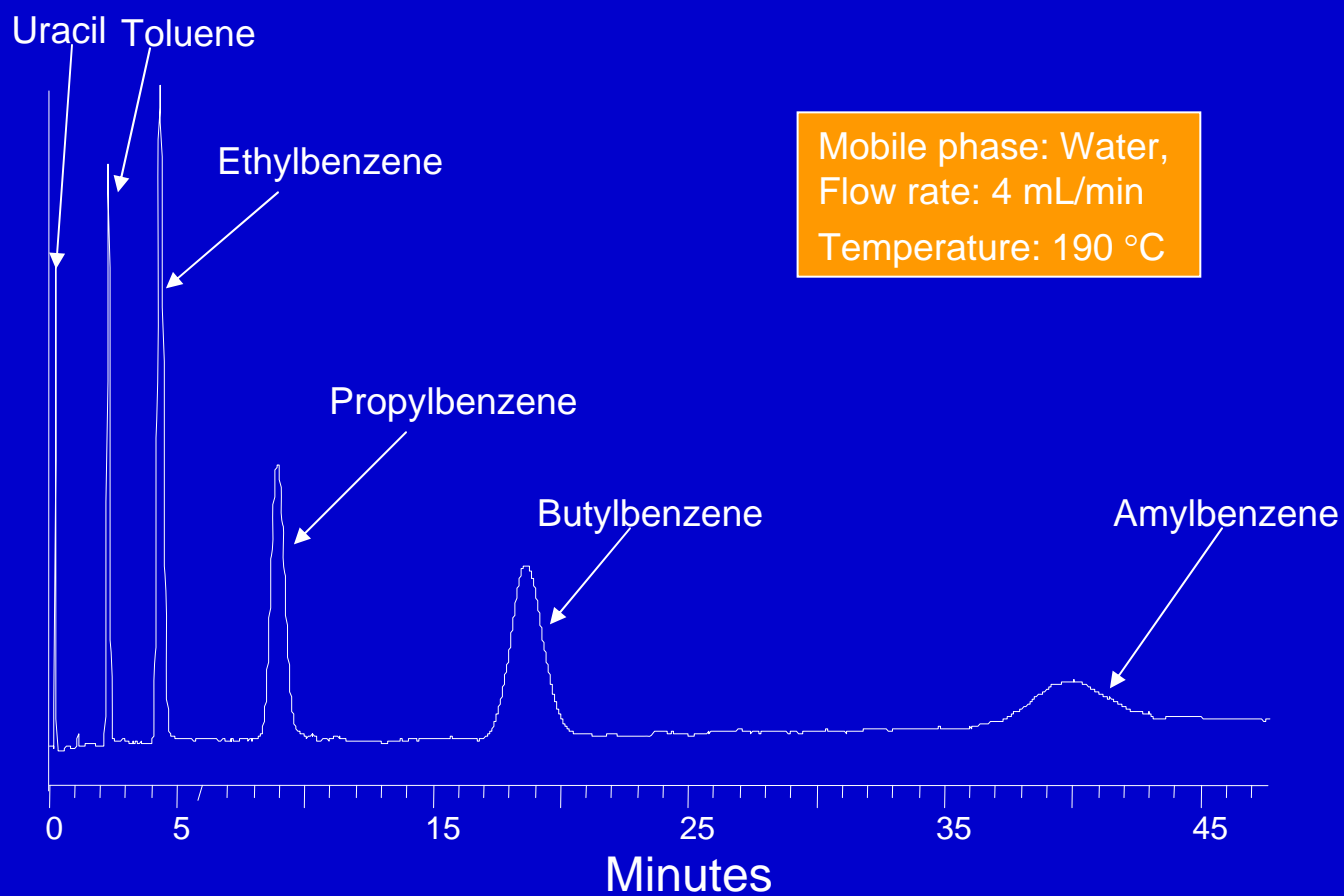
Instrument Diagram

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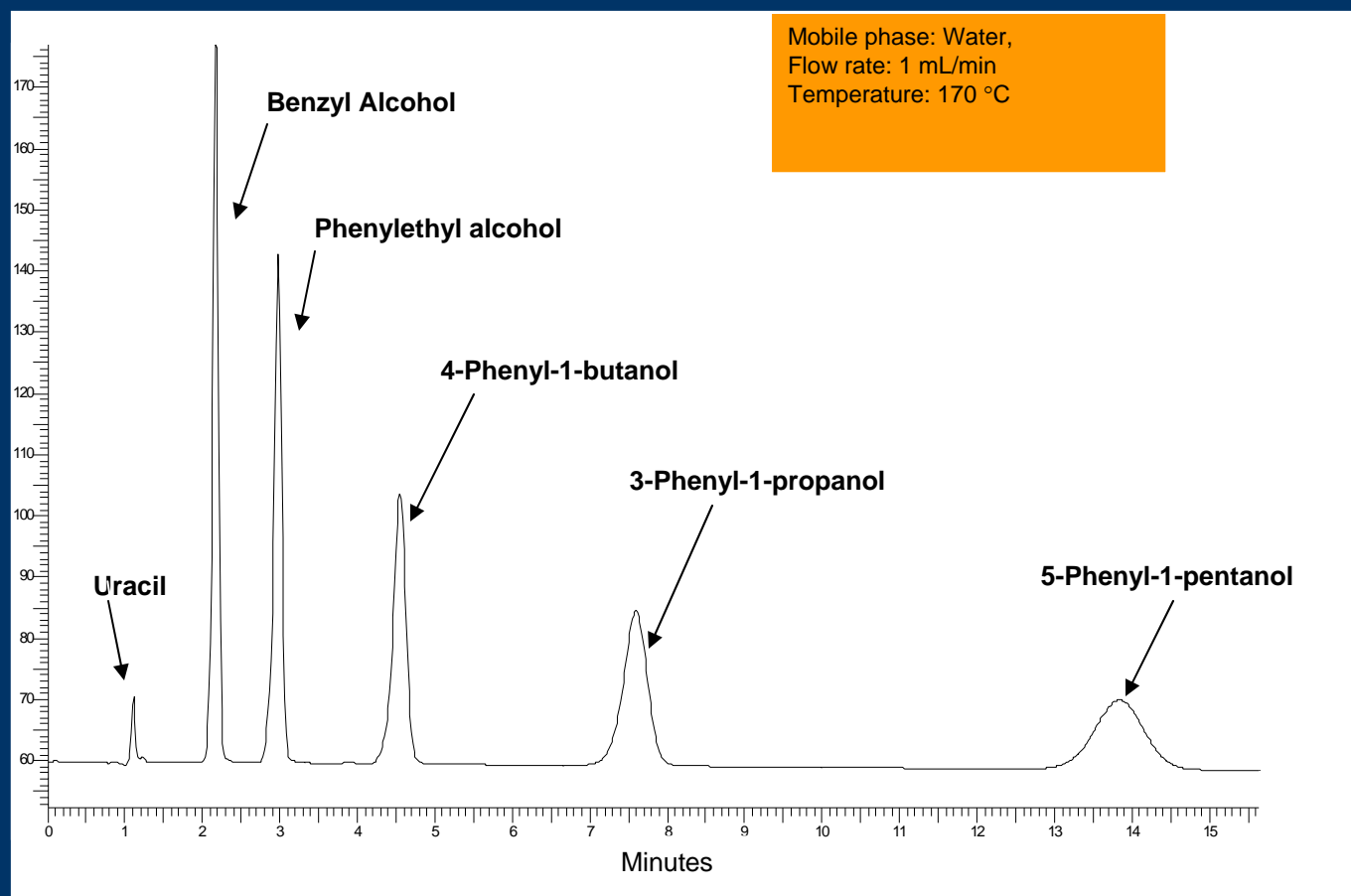
SEPARATION OF ALKYL BENZENES AT 190 °C USING WATER AS MOBILE PHASE

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CHROMATOGRAM OF AROMATIC ALCOHOLS

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TEMPERATURE EFFECT ON RETENTION IN CHROMATOGRAPHY

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$$RT \ln K = -\Delta G^\circ = -(\Delta H^\circ - T\Delta S^\circ) \quad (1)$$

$$K = k'/\Phi$$

$$RT \ln (k'/\Phi) = \Delta H^\circ - T\Delta S^\circ \quad (2)$$

$$\ln k' = -(\Delta H^\circ/RT) + (\Delta S^\circ/R) + \ln \Phi \quad (3)$$

where: ΔG° - difference in free energy of the solute in the two phases

Φ - phase ratio

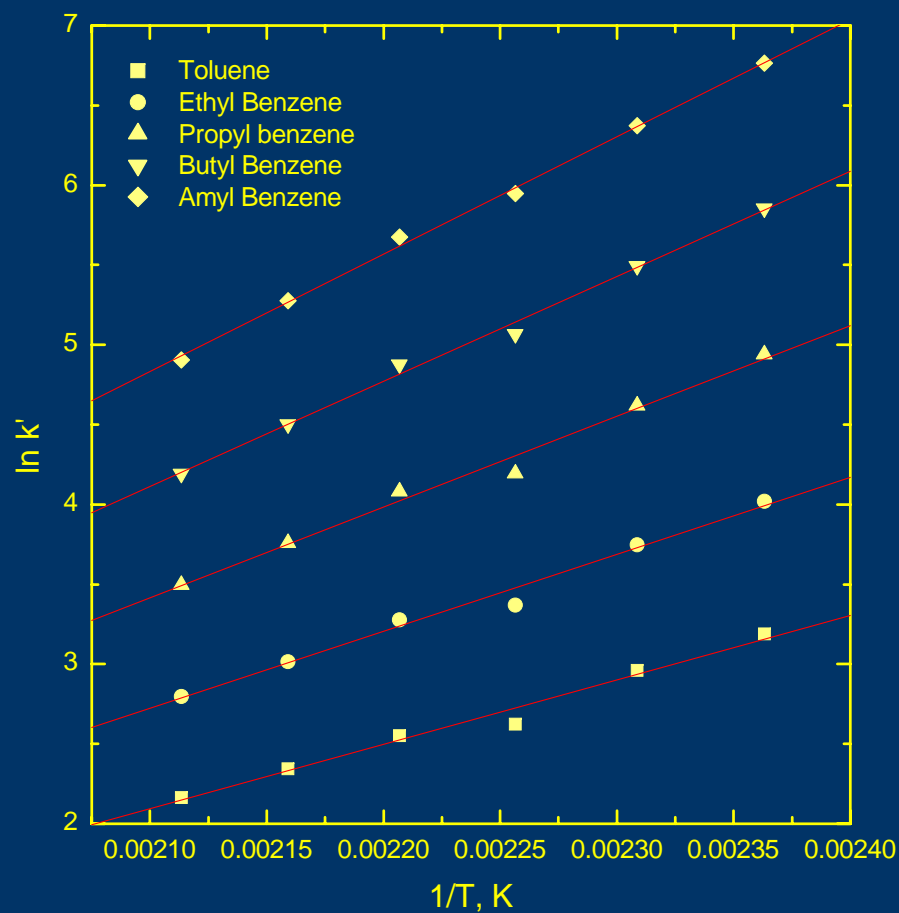
TEMPERATURE EFFECT ON RETENTION IN CHROMATOGRAPHY

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- Eq. (3) predicts that a plot of $\ln k'$ vs. $1/T$ will be a straight line with a slope of $-\Delta H^\circ/R$ and an intercept of $[(\Delta S^\circ/R) + \ln \Phi]$, provided that $\ln \Phi$ is independent of the temperature.

VAN'T HOFF PLOTS FOR ALKYL BENZENE

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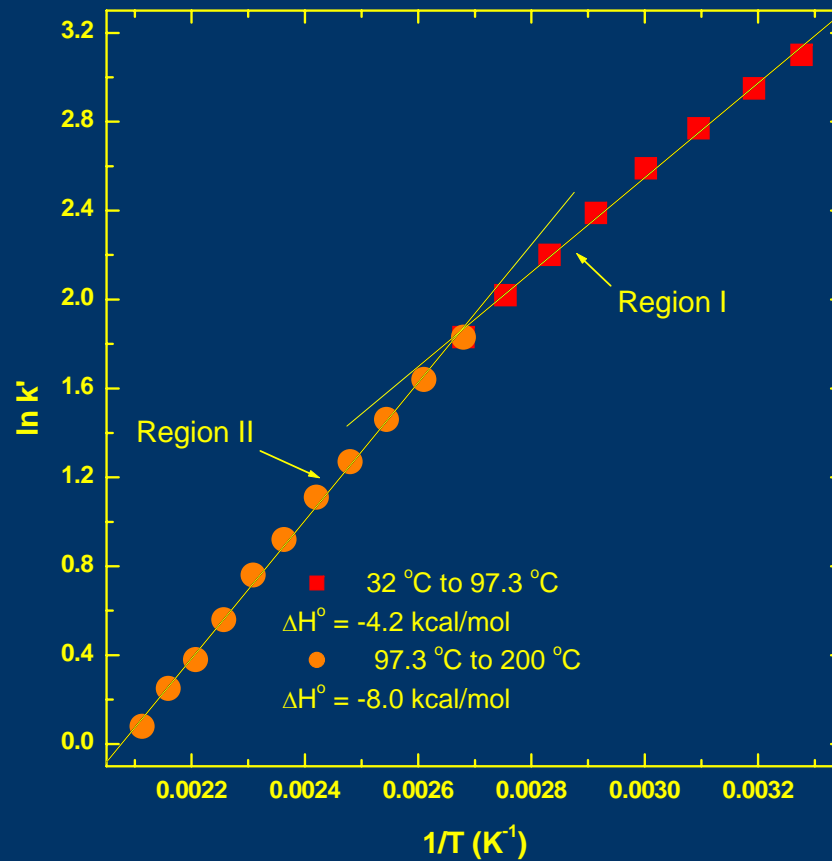


ΔH° Values for Alkyl Benzenes and Aromatic Alcohols

Analyte	ΔH° (kcal/mol)
Toluene	-8.00
Ethyl Benzenes	-9.55
Propyl Benzenes	-11.29
Butyl Benzene	-13.10
Amyl benzene	-14.53
Benzyl Alcohol	-7.88
Phenetyl Alcohol	-9.02
3-Phenyl-1-Propanol	-10.52
4-Phenyl-1-Butanol	-12.08
5-Phenyl-1-Pentanol	-13.73
Tert Butyl Benzene	-13.78
Isobutyl Benzene	-14.65

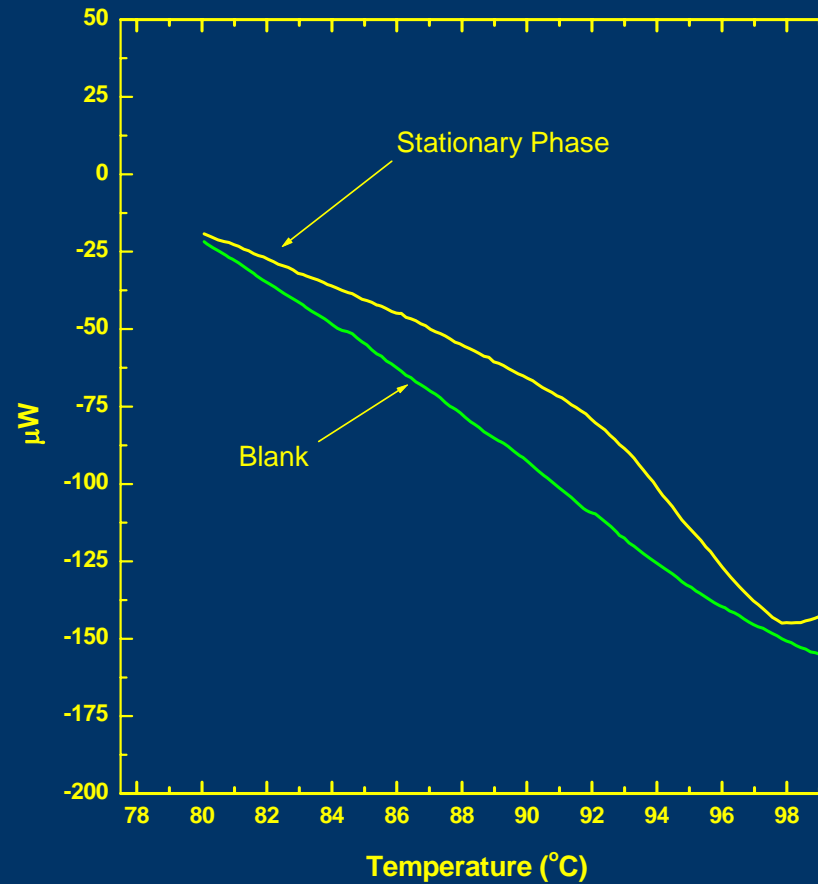
VAN'T HOFF PLOT FOR TOLUENE

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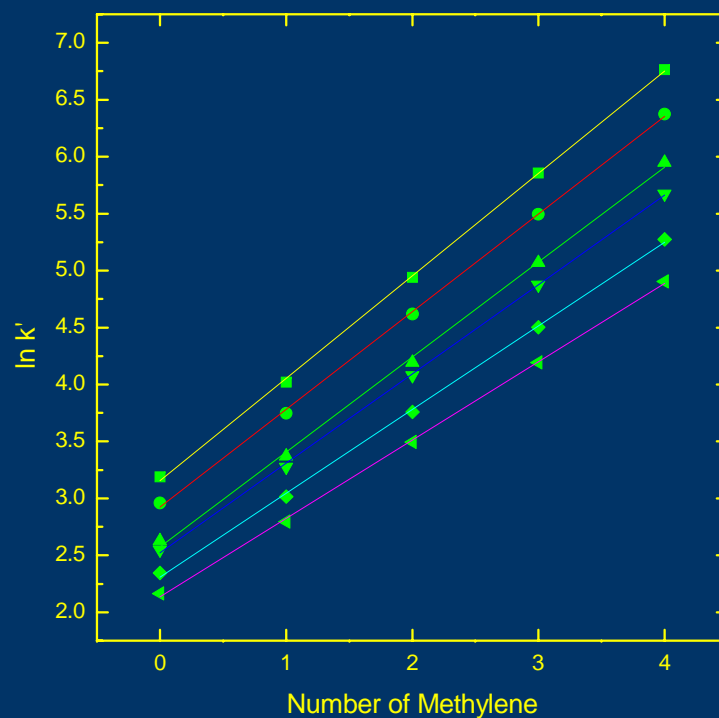
DSC OF HYBRID STATIONARY PHASE IN THE PRESENCE OF A MIXTURE OF ACETONITRILE-WATER (30:70, V/V)

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In k' VS. THE NUMBER OF METHYLENE GROUPS

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- 150 °C $Y = 3.1554 + 0.89881 \cdot X; r^2 = 0.999$
- 160 °C $Y = 2.9245 + 0.85734 \cdot X; r^2 = 0.999$
- ▲ 170 °C $Y = 2.5717 + 0.83429 \cdot X; r^2 = 0.999$
- ▼ 180 °C $Y = 2.5226 + 0.78462 \cdot X; r^2 = 0.999$
- ◆ 190 °C $Y = 2.3096 + 0.73485 \cdot X; r^2 = 0.999$
- ◄ 200 °C $Y = 2.1338 + 0.68822 \cdot X; r^2 = 0.999$

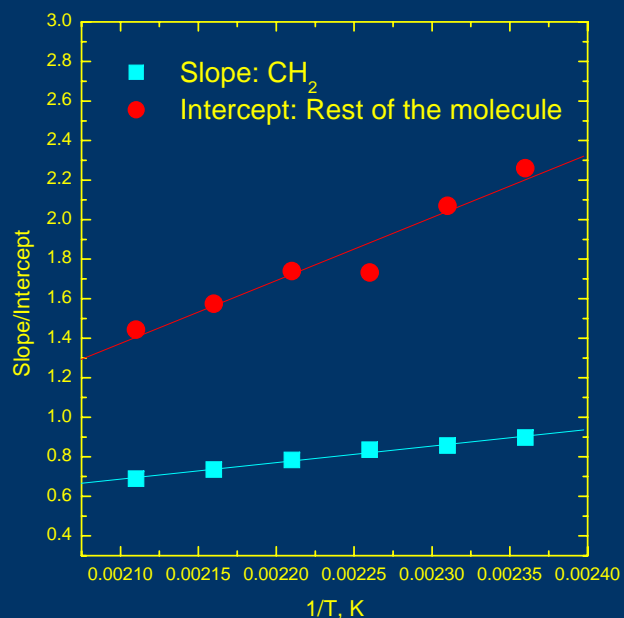
$$RT \ln k' = -n\Delta G^{\circ}_{(\text{Methylene Group})} - \Delta G^{\circ}_{(\text{Rest of the Molecule})}$$

The slope represents the portion of the standard free energy from each methylene group and intercept represents the contribution of the rest of the molecule e.g. the aromatic part and the end CH_3

GRAPH OF SLOPE/INTERCEPT VS. 1/T FOR ALKYL BENZENES (a) AND AROMATIC ALCOHOLS (b)

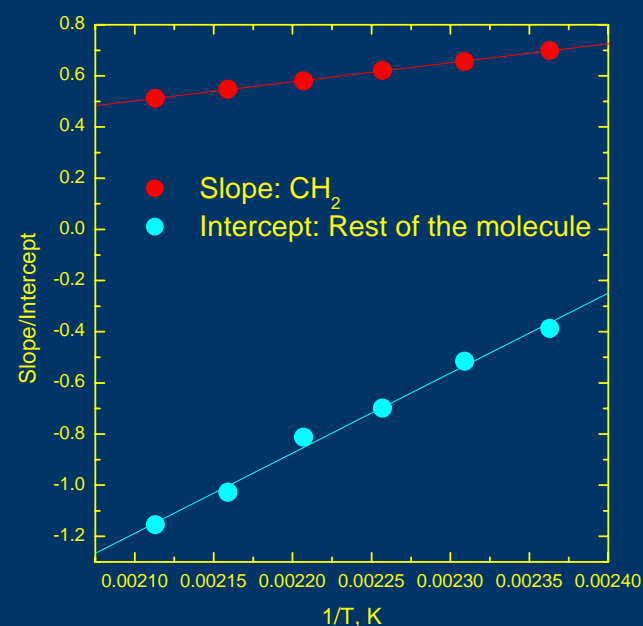
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a



$$\begin{aligned} \Delta H_{\text{CH}_2}^\circ &= -1.66 \text{ kcal/mol} \\ \Delta S_{\text{CH}_2}^\circ &= -2.13 \text{ cal/mol.K} \\ \Delta H_{\text{rest of the molecule}}^\circ &= -6.33 \text{ kcal/mol} \\ \Delta S_{\text{rest of the molecule}}^\circ &= -10.57 \text{ cal/mol.K} \end{aligned}$$

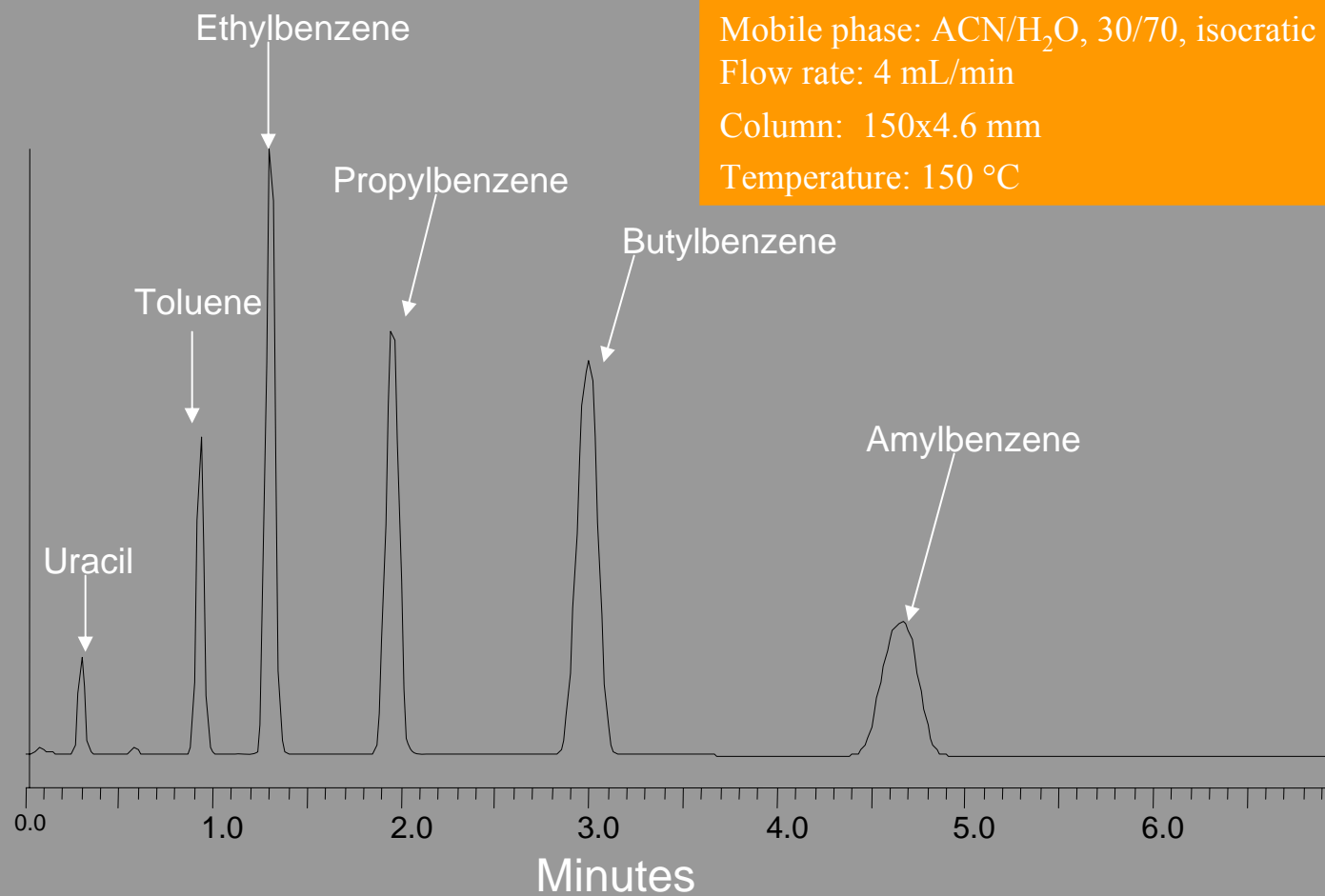
b



$$\begin{aligned} \Delta H_{\text{CH}_2}^\circ &= -1.48 \text{ kcal/mol} \\ \Delta S_{\text{CH}_2}^\circ &= -2.11 \text{ cal/mol.K} \\ \Delta H_{\text{rest of the molecule}}^\circ &= -6.22 \text{ kcal/mol} \\ \Delta S_{\text{rest of the molecule}}^\circ &= -15.42 \text{ cal/mol.K} \end{aligned}$$

SEPARATION OF ALKYL BENZENES USING WATER/ACETONITRILE AS MOBILE PHASE

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The relationship between the retention factor and mobile phase composition in HPLC is described by:

$$\ln k' = \ln k_w - S\phi$$

where: k' is the solute capacity factor

ϕ is the mobile phase composition in volume fraction

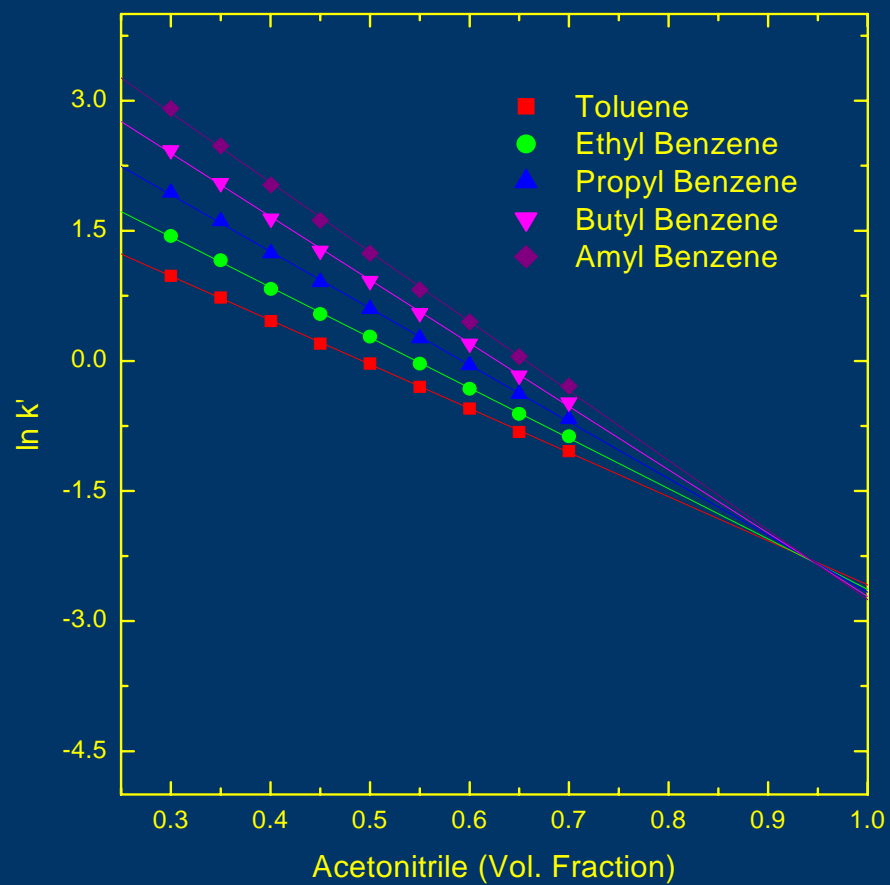
k_w is the extrapolated k' for pure water

S is the slope of the plot

- S is proportional to the free energy of solute transfer from pure water to pure organic mobile phase and correlates with the molecular size.

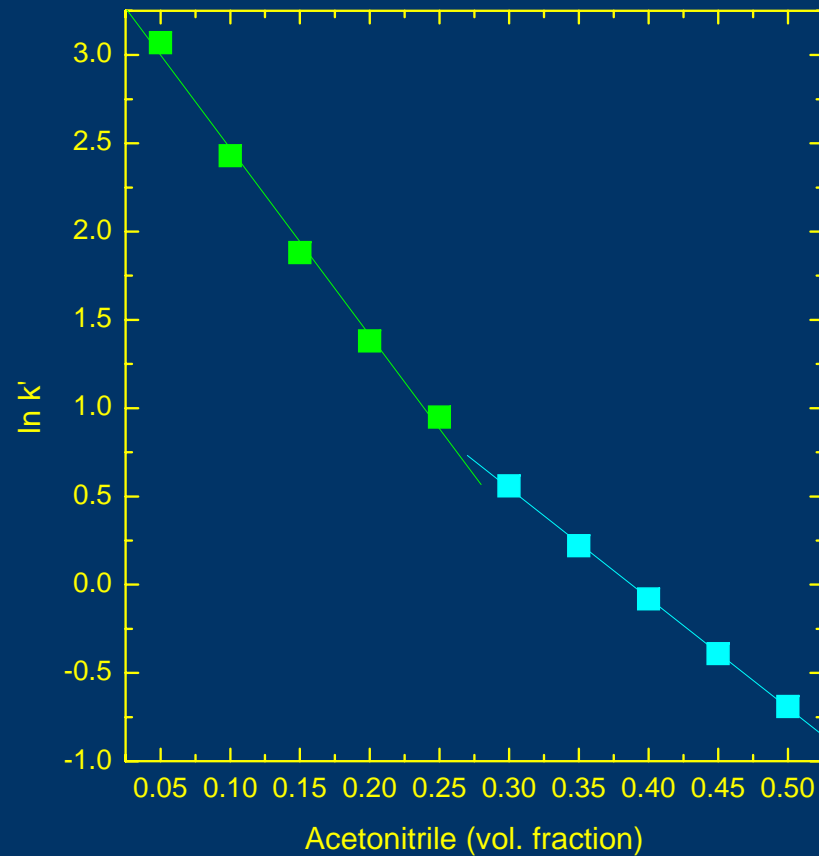
INFLUENCE OF CONCENTRATION OF ACETONITRILE ON THE k' OF ALKYL BENZENES AT 150 °C

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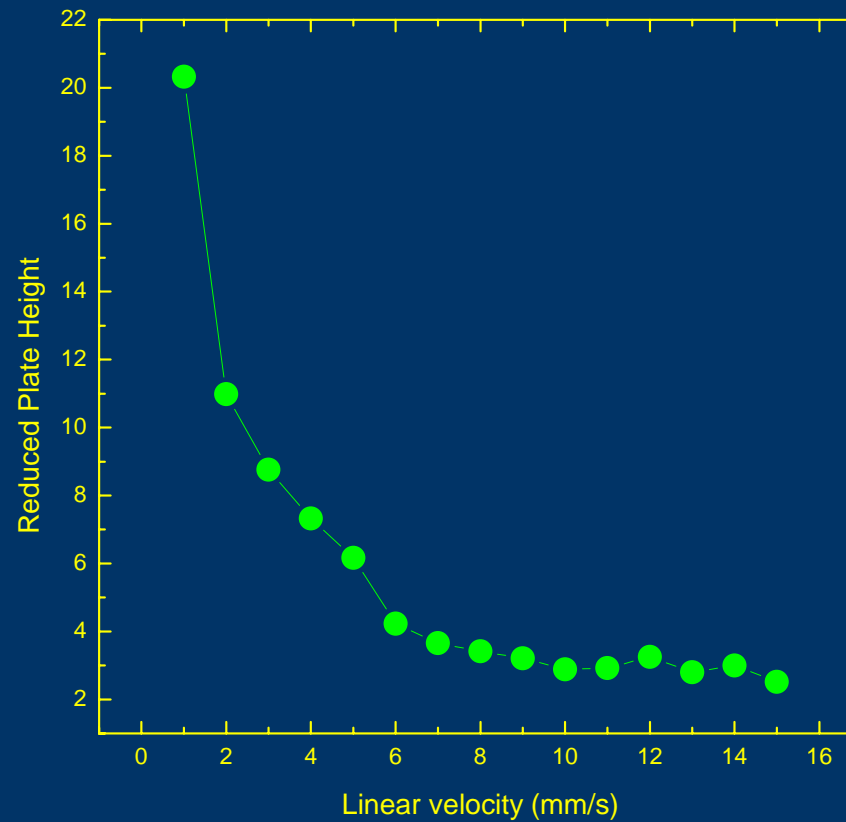
INFLUENCE OF CONCENTRATION OF ACETONITRILE ON THE k' OF 5-PENTY-1-PENTANOL 150 °C

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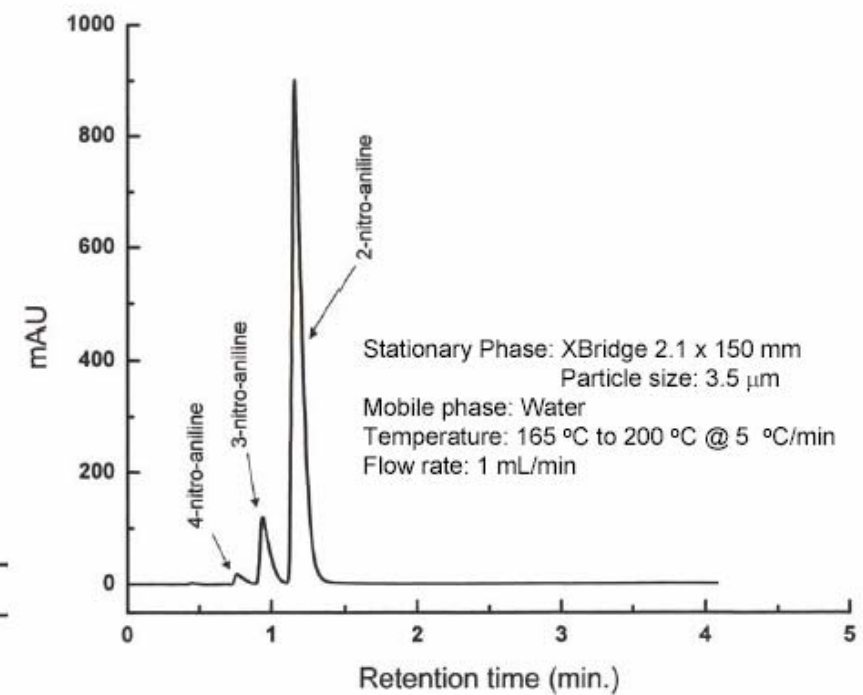
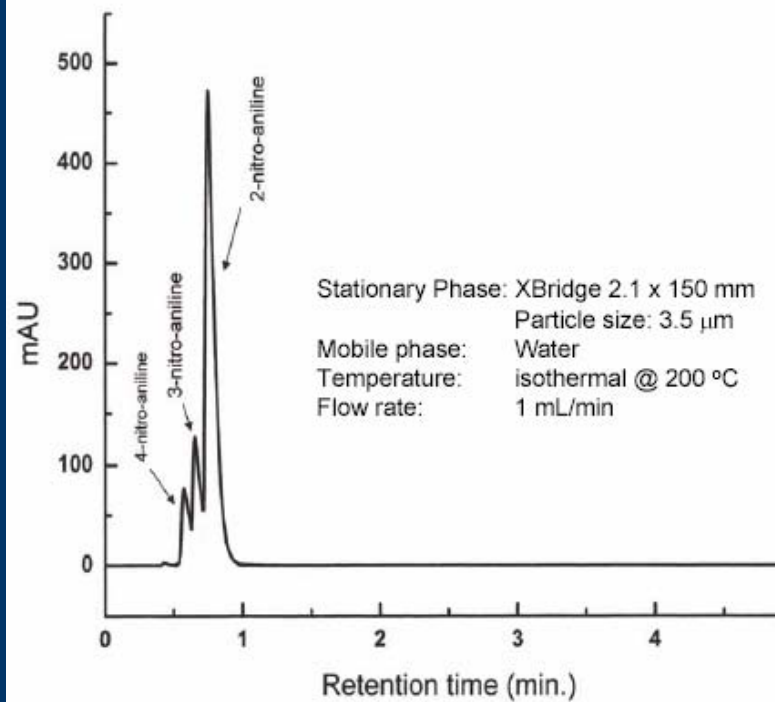
INFLUENCE OF TEMPERATURE ON REDUCED PLATE HEIGHT

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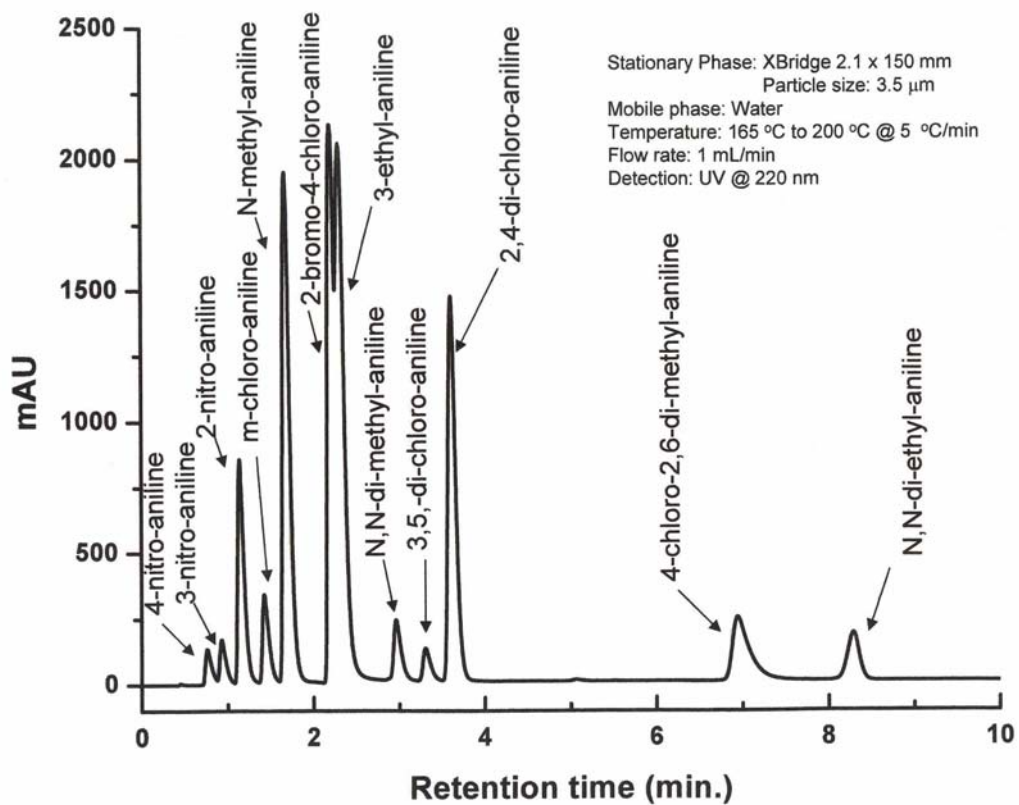
CHROMATOGRAPHY OF NITRO ANILINES

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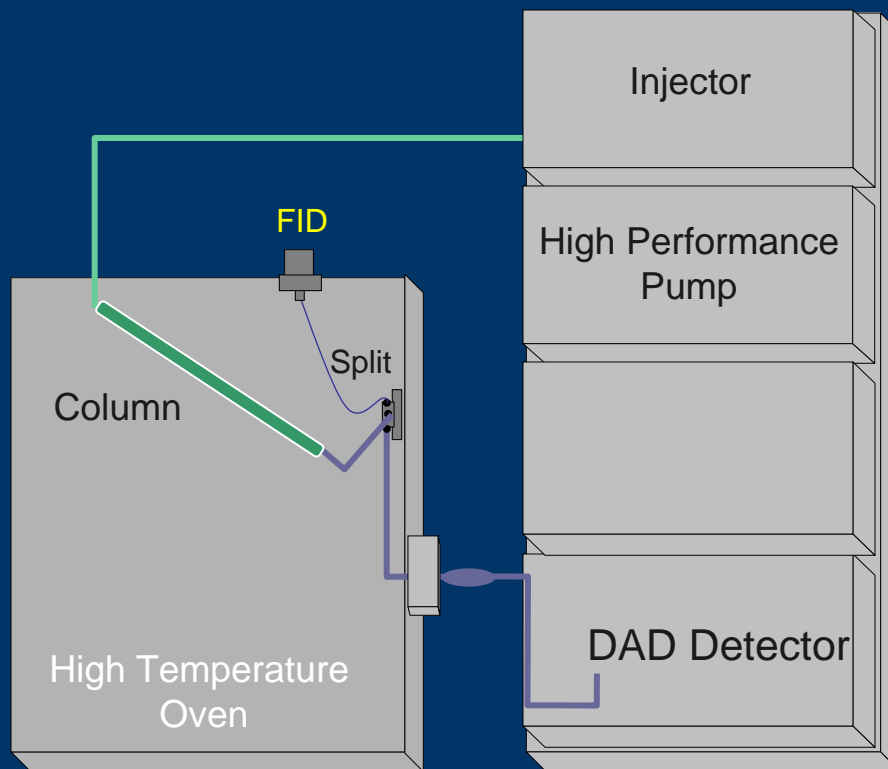
SEPARATION OF ANILINE DERIVATIVES

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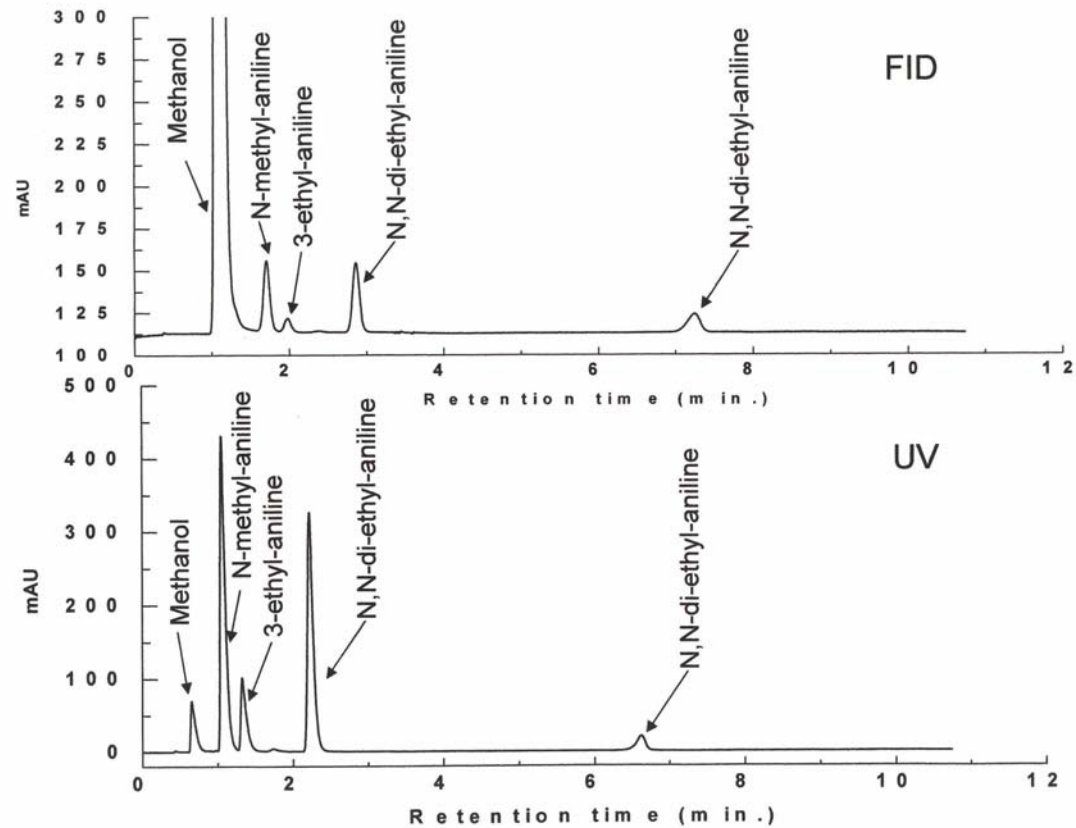
Instrument Diagram

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SEPARATION OF ALKYL ANILINE USING UV AND FID DETECTION

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