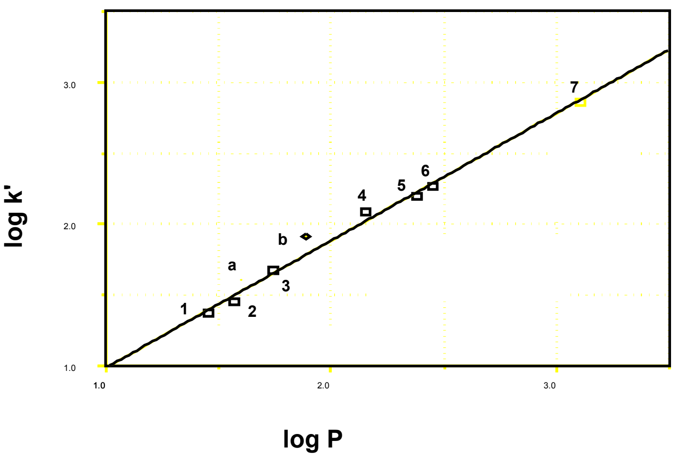
Reversed Phase HPLC

Dr. Shulamit Levin

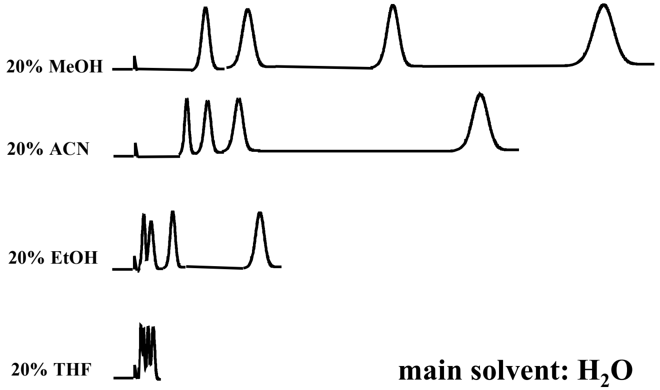
Hydrophobicity



MOBILE PHASE

- * TYPE OF MODIFIER (MeOH, ACN)
- * SOLVENT STRENGTH (% modifier)
- * pH
- * TYPE OF BUFFER (phosphate, acetate)
- * IONIC STRENGTH (Salts, buffer concentration)
- * ION-PAIRING REAGENTS (alkyl-amines, -sulfonates)

OPTIMIZATION: CHOICE OF SOLVENTS REVERSED PHASE



OPTIMIZATION: % SOLVENTS

IONIZATION and RETENTION

WEAK ACIDS

$HA \rightleftharpoons H^+ + A^-$

pKa~ 4-5

At pH >4-5 the main species is A^-

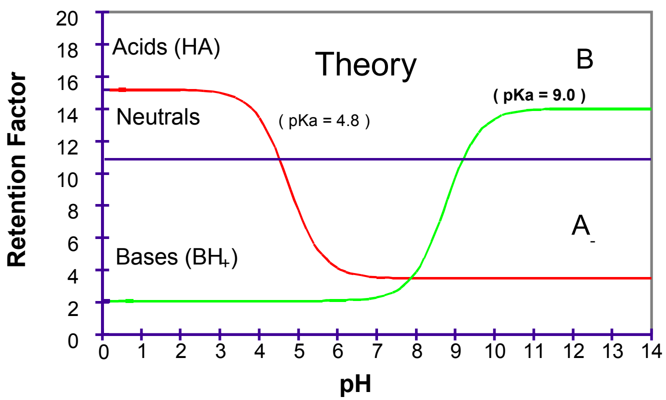
WEAK BASES

$HB^+ \rightleftharpoons H^+ + B$

pKa ~ 7-8

At pH < 7-8 the main species is BH^+

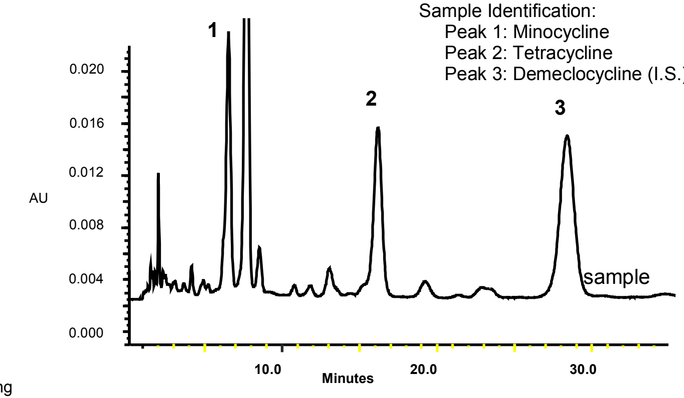
Retention Factor versus pH for Acids, Bases, and Neutrals



Antibiotics

Column: SymmetryShield™ RP8, 5 µm, 3.0 x 150 mm
Mobile Phase: 0.1% TFA in Water : Acetonitrile: Methanol (91:7:2)
Detection: UV at 270 nm
Flow Rate: 0.9 mL/min.
Injection Volume: 20 µL

Sample Identification:
Peak 1: Minocycline
Peak 2: Tetracycline
Peak 3: Demeclocycline (I.S.)



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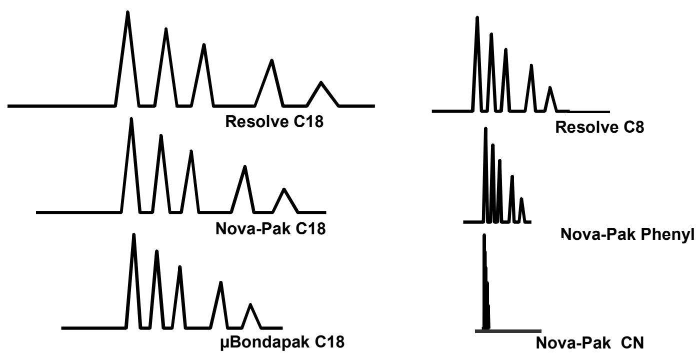
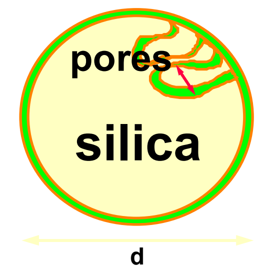
Types of Reversed Phase Columns



Stationary Phase Properties

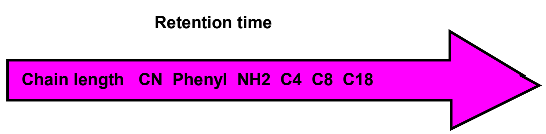
CHEMISTRY:
* BONDED HYDROCARBON: C-18, C-8, C-4, C-1, CN, phenyl
* % COVERAGE
* TYPE OF SILICA GEL

GEOMETRY
* SPHERE- IRREGULAR
* PARTICLE DIAMETER
* POROSITY



Stationary Phase Supports

Stationary phase	Functionality
C_{18}	$-Si(CH_3)_2C_{18}H_{37}$
C_8	$-Si(CH_3)_2C_8H_{17}$
tC_2	$-SiC_2H_5$
Aminopropyl	$-Si(CH_2)_3NH_2$
Cyanopropyl	$-Si(CH_3)_2(CH_2)_3CN$
Diol	$-Si(CH_2)_3OCH_2CH(OH)CH_2OH$



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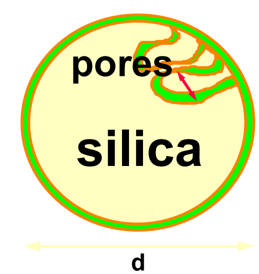
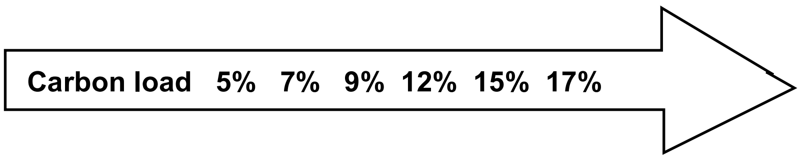


CARBON LOAD

Increasing carbon load on a similar geometrical shaped particles increases retention.

Retention time



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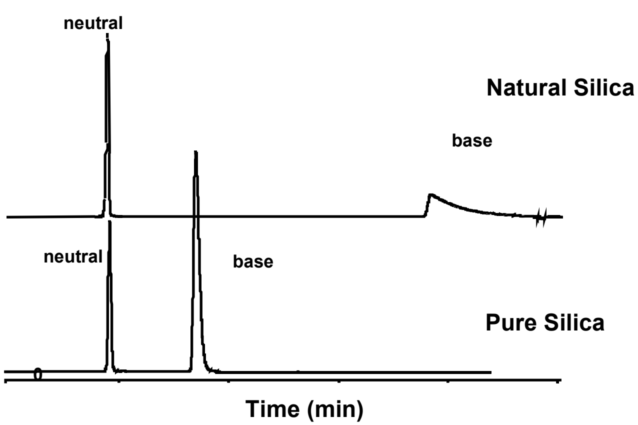
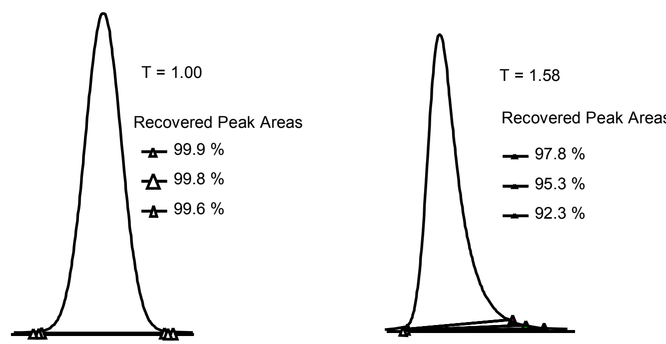
Quality of Columns Performance



Integration Errors Caused by Tailing



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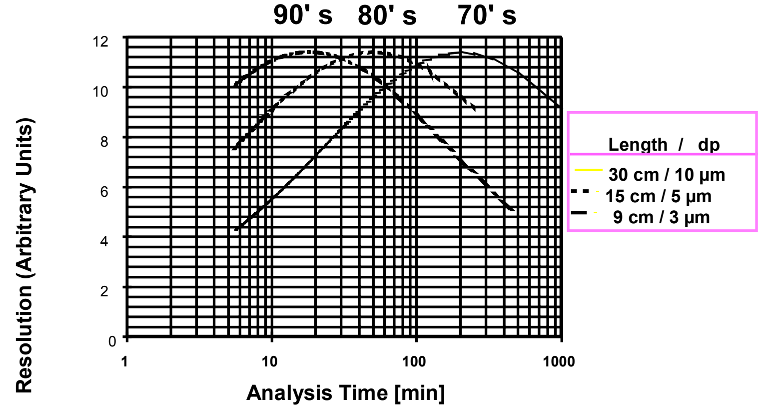
Resolution - Time Diagram



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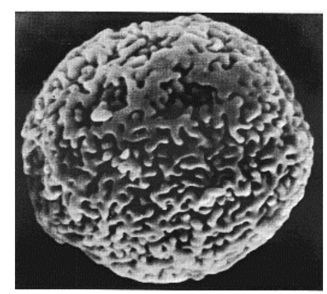
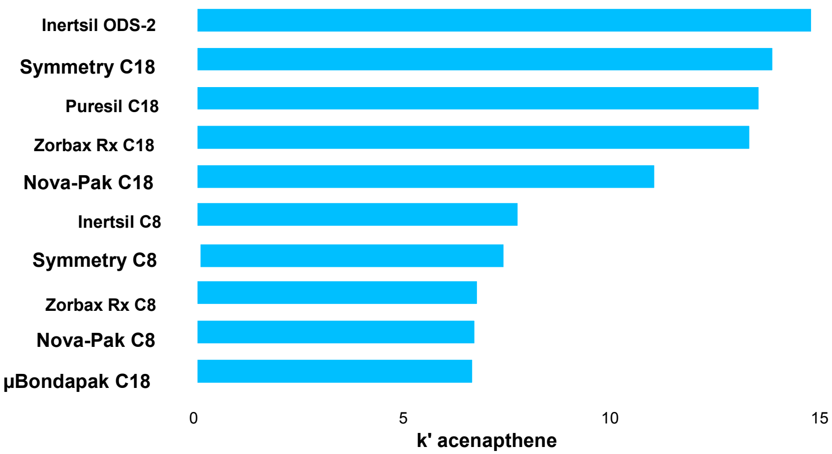
Pore size, shape and distribution



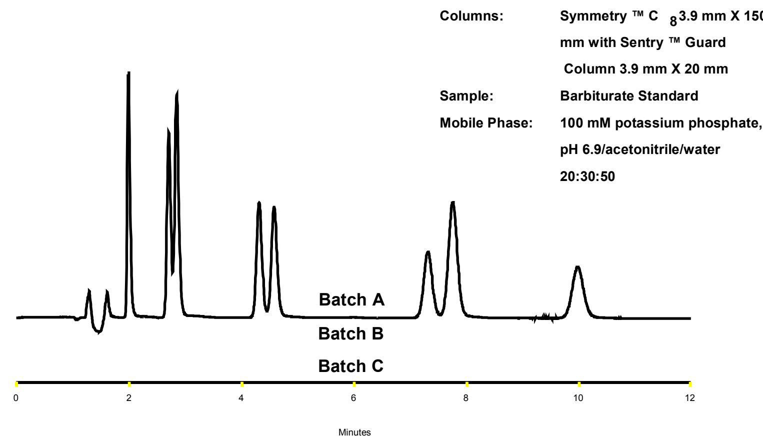
Macroporous spherical silica particle. [K.K.Unger, Porous silica, Elsevier, 1979]

Pore size defines an ability of the analyte molecules to penetrate inside the particle and interact with its inner surface. This is especially important because the ratio of the outer particle surface to its inner one is about 1:1000. The surface molecular interaction mainly occurs on the inner particle surface.

Relative Hydrophobicities of General Purpose Analytical Packings



Batch-to-Batch Reproducibility of Columns



Chromatogram of lifetime test