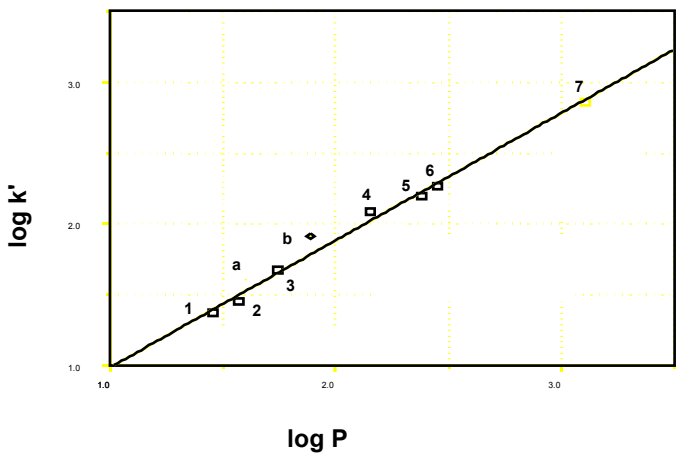
Reversed Phase HPLC

Dr. Shulamit Levin

Hydrophobicity

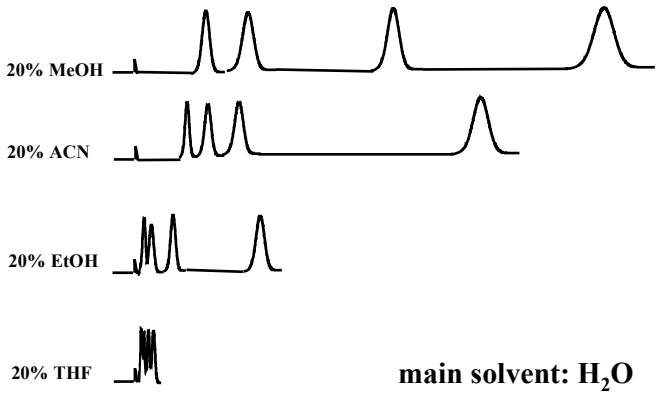


MOBILE PHASE

* 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



main solvent: H_2O

OPTIMIZATION: % SOLVENTS

IONIZATION and RETENTION

WEAK ACIDS

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

pKa~ 4-5

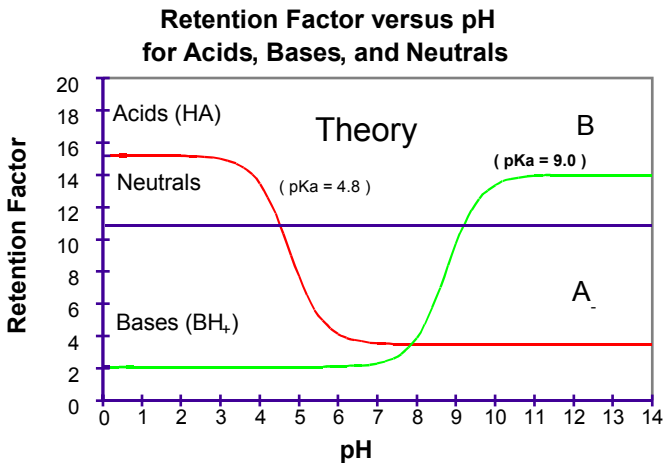
At pH >4-5 the main species is A^-

WEAK BASES

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

pKa ~ 7-8

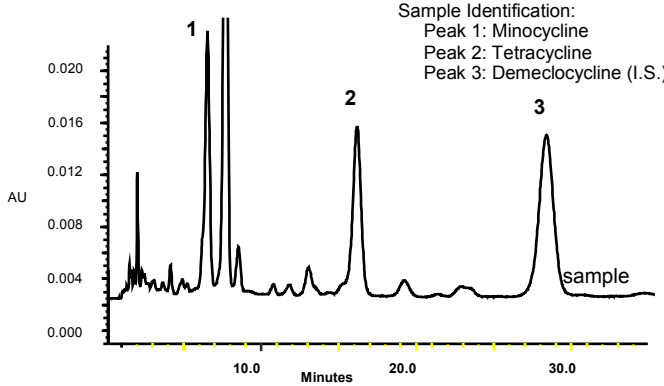
At pH < 7-8 the main species is BH^+



Antibiotics

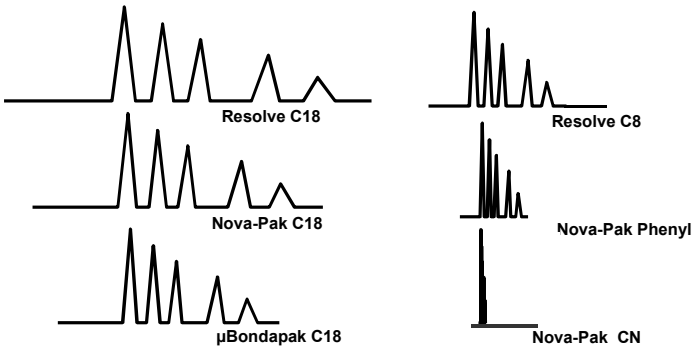
Column: SymmetryShield™ RP8, 5 µm, 3.0 x 150 mm
Mobile Phase: 0.1% TFA in Water : Acetonitrile: Methanol (91:7:2)
Detection: UV at 270 nm
Flow Rate: 0.9 mL/min.
Injection Volume: 20 µL

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



Cheng

Types of Reversed Phase Columns



Stationary Phase Properties

CHEMISTRY:

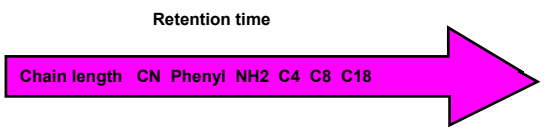
* **BONDED HYDROCARBON:** C-18, C-8, C-4, C-1, CN, phenyl
* **% COVERAGE**
* **TYPE OF SILICA GEL**

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	$-Si(CH_2)_3OCH_2CH(OH)CH_2OH$



Stationary Phase Properties

CHEMISTRY:

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

GEOMETRY

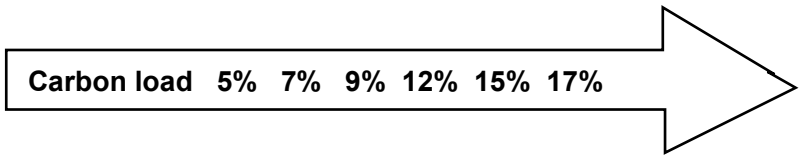
* **SPHERE- IRREGULAR**
* **PARTICLE DIAMETER**
* **POROSITY**

CARBON LOAD

Stationary Phase Properties

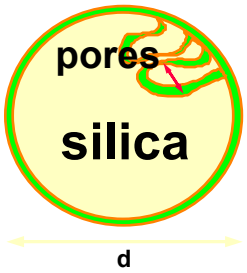
Increasing carbon load on a particles increases retention. **similar geometrical shaped**

Retention time

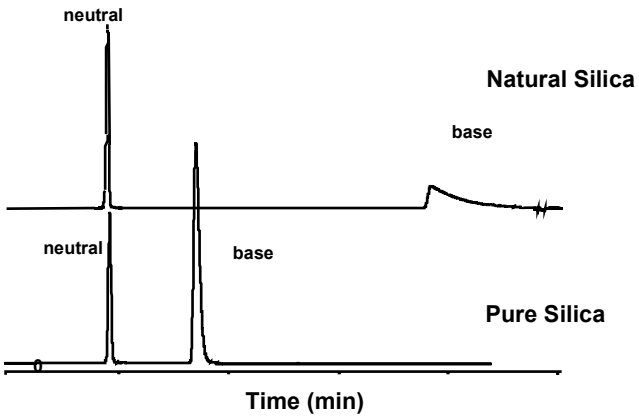


CHEMISTRY:
* **BONDED HYDROCARBON:** C-18, C-8, C-4, C-1, CN, phenyl
* **% COVERAGE**
* **TYPE OF SILICA GEL**

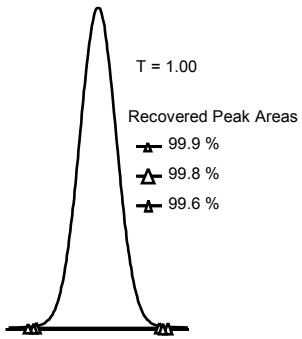
GEOMETRY
* **SPHERE- IRREGULAR**
* **PARTICLE DIAMETER**
* **POROSITY**

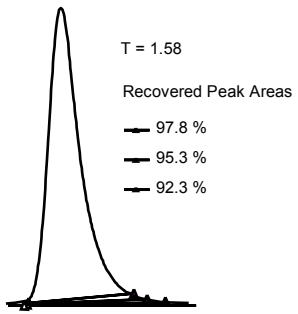


Quality of Columns Performance



Integration Errors Caused by Tailing



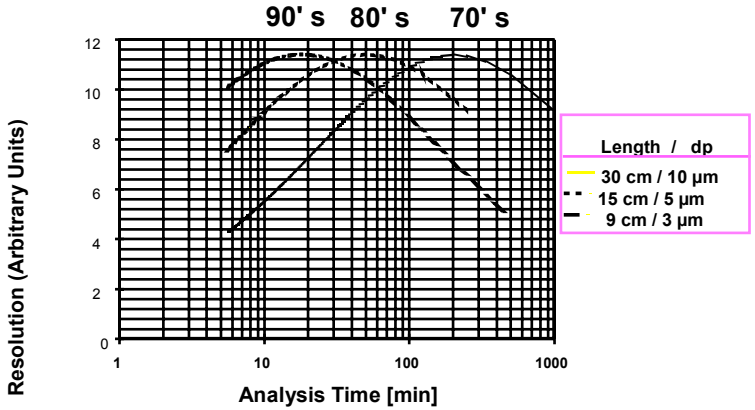


Stationary Phase Properties

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

GEOMETRY
* SPHERE- IRREGULAR
* PARTICLE DIAMETER
* POROSITY

Resolution - Time Diagram

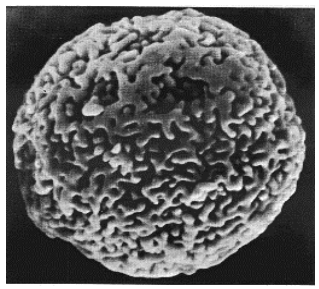


Stationary Phase Properties

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

GEOMETRY
* SPHERE- IRREGULAR
* PARTICLE DIAMETER
* POROSITY

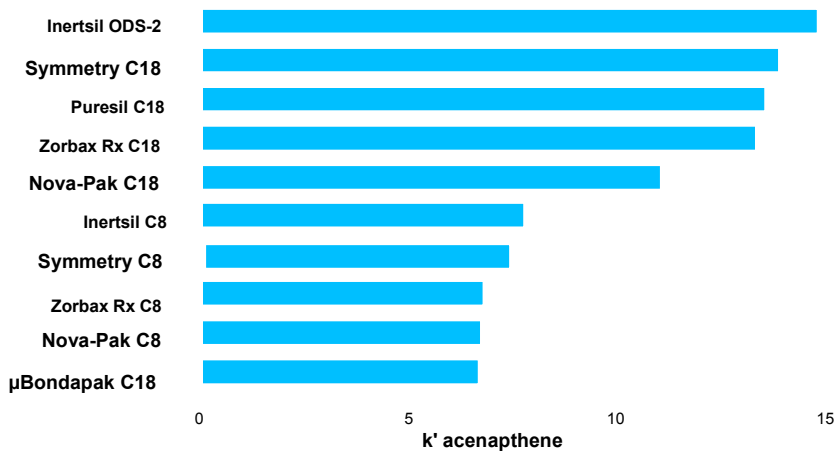
Pore size, shape and distribution



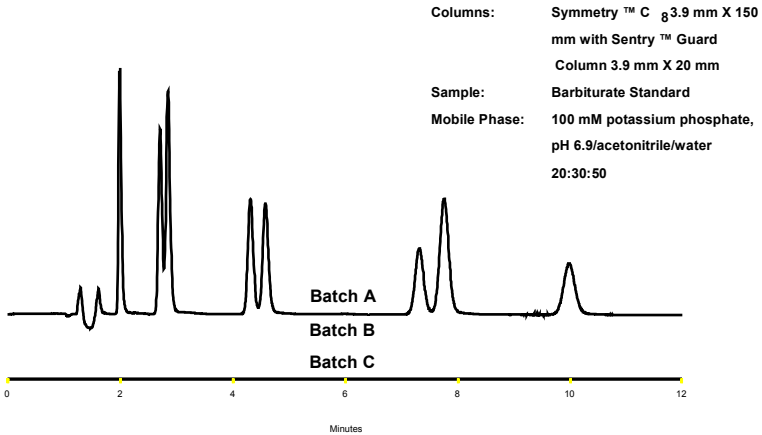
Macroporous spherical silica particle. [K.K.Unger, Porous silica, Elsevier, 1979]

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