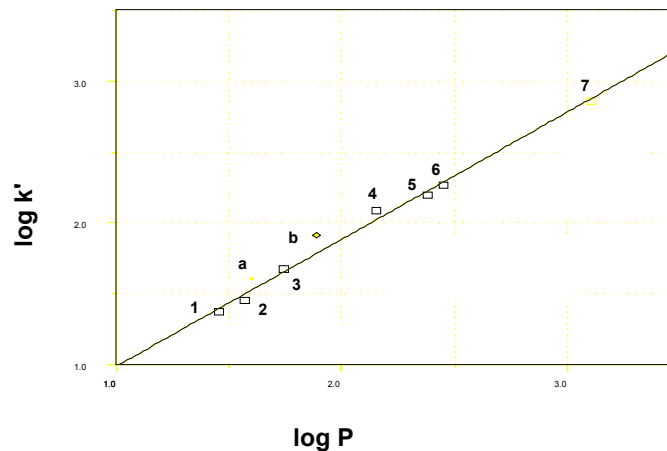


Reversed Phase HPLC

Dr. Shulamit Levin
Medtechnica

Hydrophobicity

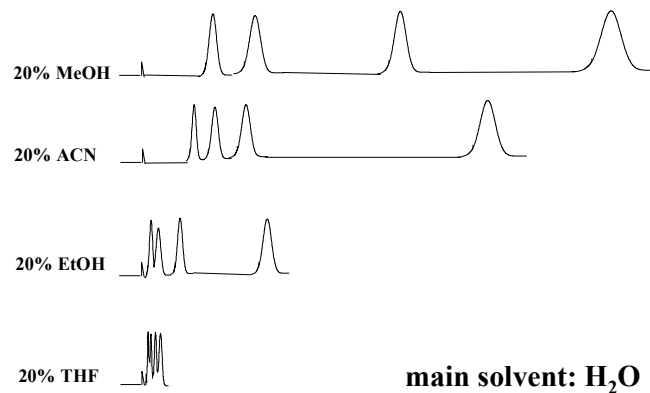


MOBILE PHASE

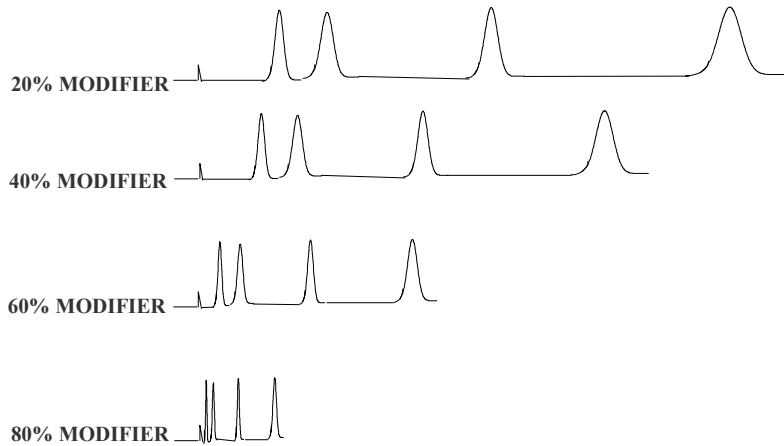
- * TYPE OF MODIFIER (MeOH, ACN)
- * SOLVENT STRENGTH (% modifier)
- * pH
- * TYPE OF BUFFER (phosphate, acetate)
- * IONIC STRENGTH (Salts, buffer concentration)
- * ION-PAIRING REAGENTS (alkyl-amines, -sulfonates)

OPTIMIZATION: CHOICE OF SOLVENTS

REVERSED PHASE

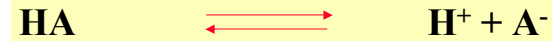


OPTIMIZATION: % SOLVENTS



IONIZATION and RETENTION

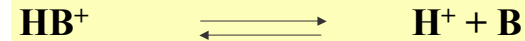
WEAK ACIDS



pKa ~ 4-5

At pH >4-5 the main species is A⁻

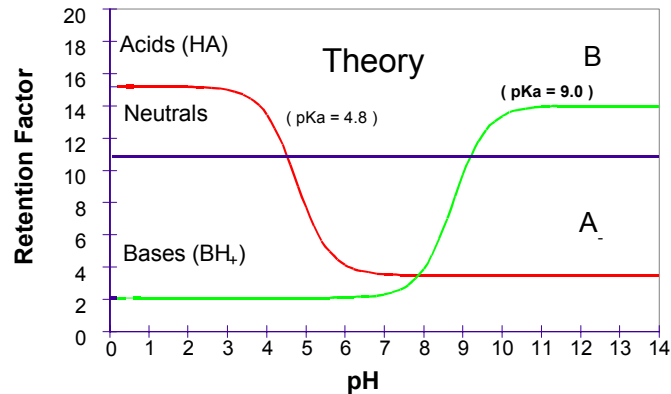
WEAK BASES



pKa ~ 7-8

At pH < 7-8 the main species is BH⁺

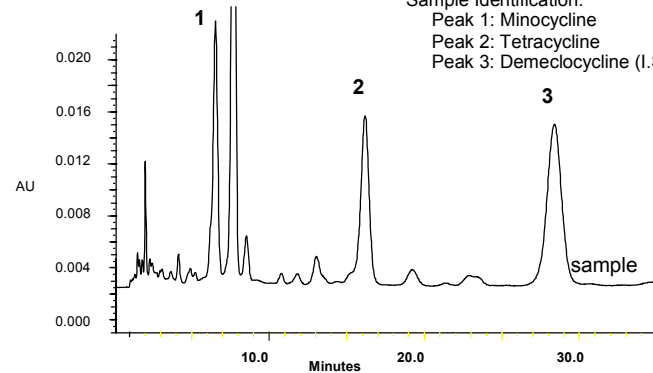
Retention Factor versus pH for Acids, Bases, and Neutrals



Antibiotics

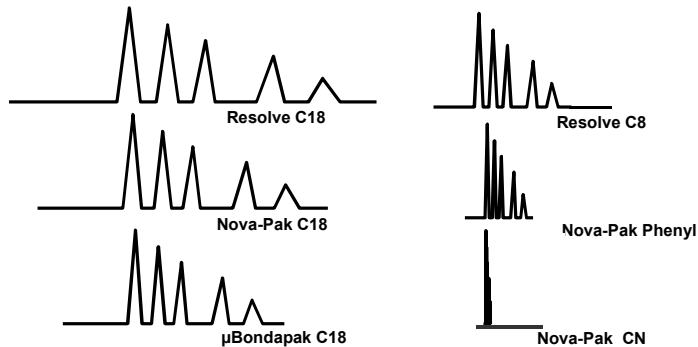
Column: SymmetryShield™ RP8, 5 μm, 3.0 x 150 mm
 Mobile Phase: 0.1% TFA in Water : Acetonitrile: Methanol (91:7:2)
 Detection: UV at 270 nm
 Flow Rate: 0.9 mL/min.
 Injection Volume: 20 μL

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



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) ₂ C ₁₈ H ₃₇
C ₈	-Si(CH ₃) ₂ C ₈ H ₁₇
tC ₂	-SiC ₂ H ₅
Aminopropyl	-Si(CH ₂) ₂ NH ₂
Cyanopropyl	-Si(CH ₂) ₂ (CH ₂) ₂ CN
Diol	-Si(CH ₂) ₂ OCH ₂ CH(OH)CH ₂ OH

Retention time

Chain length CN Phenyl NH₂ C₄ C₈ C₁₈

Stationary Phase Properties

CHEMISTRY:

* BONDED HYDROCARBON:
C-18, C-8, C-4, C-1, CN, phenyl

* % COVERAGE

* TYPE OF SILICA GEL

GEOMETRY

* SPHERE- IRREGULAR

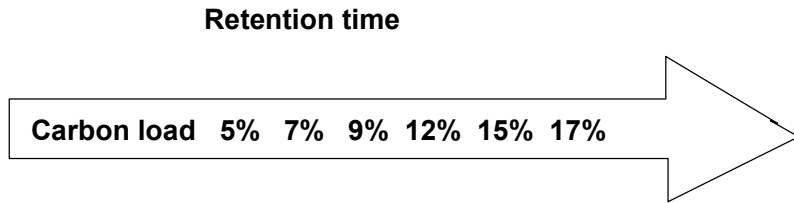
* PARTICLE DIAMETER

* POROSITY



CARBON LOAD

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



Stationary Phase Properties

CHEMISTRY:

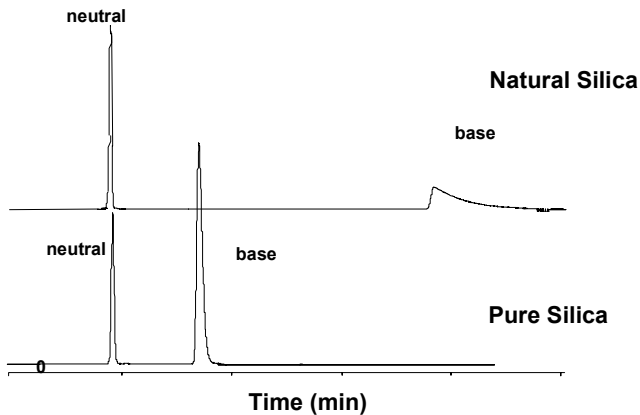
- * BONDED HYDROCARBON:
C-18, C-8, C-4, C-1, CN, phenyl
- * % COVERAGE
- * TYPE OF SILICA GEL

GEOMETRY

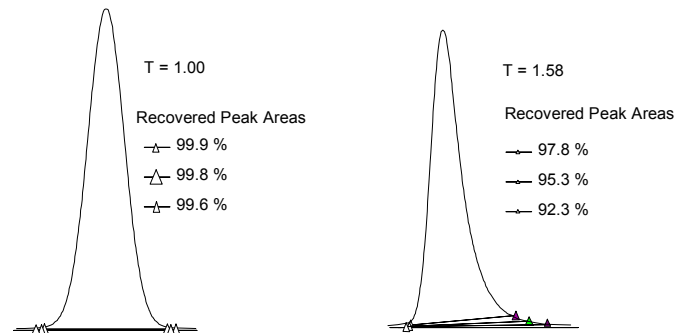
- * SPHERE- IRREGULAR
- * PARTICLE DIAMETER
- * POROSITY



Quality of Columns Performance



Integration Errors Caused by Tailing



Stationary Phase Properties

CHEMISTRY:

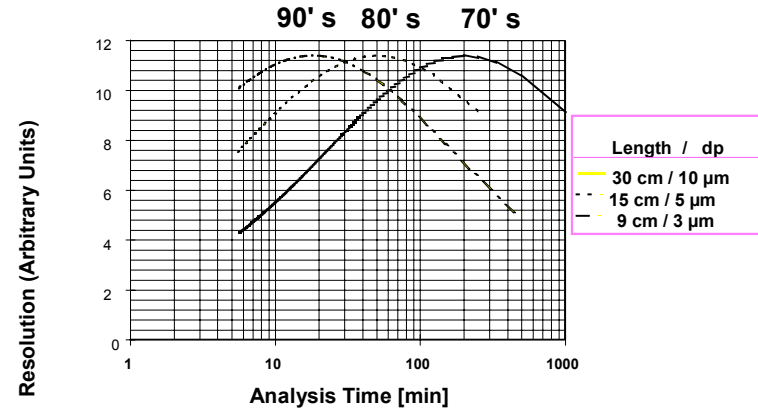
- * BONDED HYDROCARBON:
C-18, C-8, C-4, C-1, CN, phenyl
- * % COVERAGE
- * TYPE OF SILICA GEL

GEOMETRY

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



Resolution - Time Diagram



Stationary Phase Properties

CHEMISTRY:

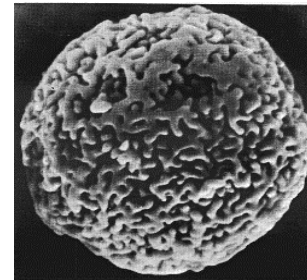
- * BONDED HYDROCARBON:
C-18, C-8, C-4, C-1, CN, phenyl
- * % COVERAGE
- * TYPE OF SILICA GEL

GEOMETRY

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



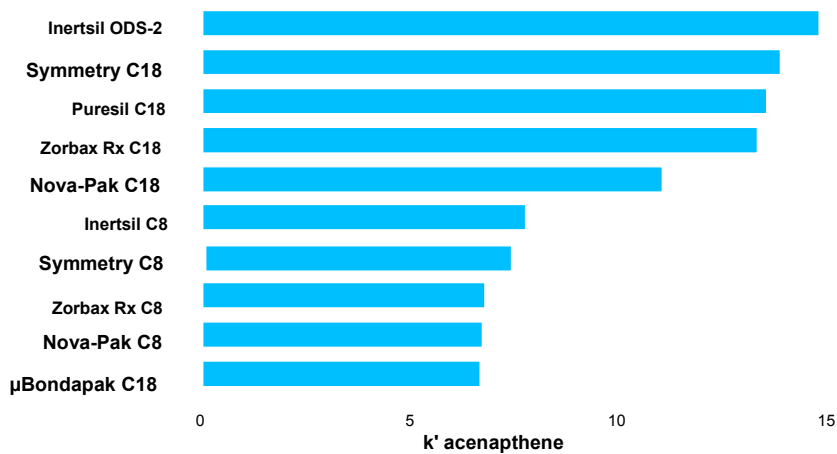
Pore size, shape and distribution



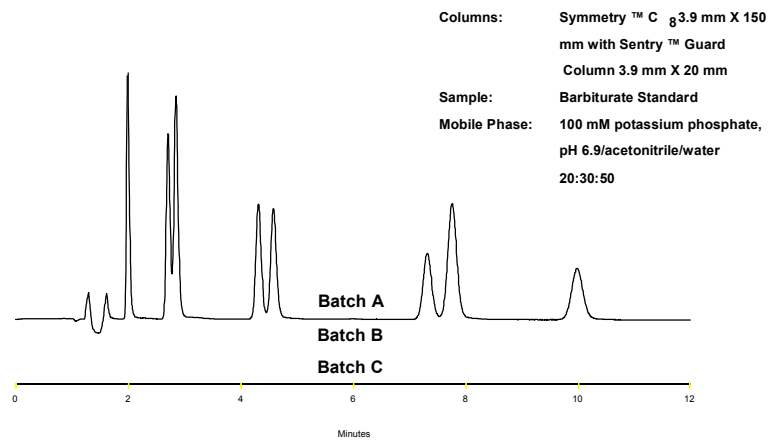
Macroporous spherical silica particle. [K.K.Unger, Porous silica, Elsevier, 1979]

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

