Utility of U-Shaped Retention Profiles Under Common Reversed Phase HPLC Conditions

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Introduction

• RP & NP retention profiles
• HILIC phases & U-shaped retention
• RP packings - prediction of silanol activity level
• Phases showing U-shaped retention
• Advantages
Reversed-Phase Chromatography

- Retention decreases with increasing organic
- Strong solvents: MeOH, ACN, etc.

Discovery ® C18
Flow = 1mL/min
MP: A = ACN w/ 5mM Ammonium acetate
   B = Water w/ 5mM Ammonium acetate (pH 6.8)
Normal Phase Chromatography

- Retention increases with increasing organic
- Strong solvent: Aqueous

SUPELCOSIL® Si (Silica)
Flow = 1mL/min
MP: A = ACN w/ 5mM Ammonium acetate
   B = Water w/ 5mM Ammonium acetate (pH 6.8)

Propranolol
U-Shaped Retention

- Retention increases at high organic and high aqueous MP
- Strong solvent: Mixture of Aqueous:Organic

Discovery Cyano
Flow = 1mL/min
MP:  A = ACN w/ 5mM Ammonium acetate
     B = Water w/ 5mM Ammonium acetate (pH 6.8)

Amitriptyline

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\text{CH}_3 \\
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\end{array}}}
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HILIC Phases

• “HILIC” first proposed by Andrew Alpert (1990)
• Polar phases can show U-shaped retention profiles
• Normal phase region explained by polar interactions
HILIC Chromatography

- Polar stationary phase - polar analyte interactions
- Example - Amino acids on PolySulfoethyl Aspartamide (left) and PolyHydroxyethyl Aspartamide (right) phases

HILIC On Reversed-Phase Columns

• Bij, et. al., found U-shaped curves on C8 & C18 phases for dibenzo-crown ethers, some peptides, and free amino acids (Ref.: J. Chromatogr., 203, (1981) 65).
• Bare silica columns also showed U-shaped retention.
• Hypothesis: The level of base deactivation can be approximated by the NP (HILIC) region of RP packings.
U- Shaped Retention Profile of C18 Phases

• NP (HILIC) region attributed to polar silanol interactions with polar analyte

• More active, Type A C18 phases (e.g., Spherisorb ODS 2) show more NP character

• Base deactivated, Type B C18 phases (e.g., Discovery C18, Discovery HS C18) show little NP character

Flow = 1mL/min
Propranolol, 0.33mg/mL
MP:  A = ACN w/ 5mM Ammonium acetate
     B = Water w/ 5mM Ammonium acetate (pH 6.8)
Pyridine/Phenol Test

VWD1 A, Wavelength=230 nm (URET\SPHER021.D)

Spherisorb ODS 2

Pyridine

Phenol

Uracil

MP = 90:10 - H2O:ACN, each w/ 5mM Ammonium Acetate; 35oC; Flow = 1mL/min; UV @ 230nm
Prediction of Level of Base Deactivation In RP Packings

- HILIC region relates well to traditional methods of measuring level of base deactivation for RP packings (e.g., Pyridine/Phenol Test)
- Pyridine asymmetry may be used to predict amount of tailing of basic compounds in RP region

Amitriptyline $k'$ (99% ACN) VS. Pyridine Asymmetry (10% ACN)
Surface Silanol Equilibrium

- Surface silanol equilibrium
- Range of silica pKa ~4-5

HILIC - pH 4

- HILIC increases when pH is lowered below pKa of basic compounds
- HILIC interaction can still occur even when surface silanols are protonated

Flow = 1mL/min
MP:  A = ACN w/ 5mM Ammonium acetate
     B = Water w/ 5mM Ammonium acetate (pH 4.0)
C18 Packing vs. Capacity Factor

- More HILIC retention with lower pH
- Dominant mechanism not electrostatic

Amitriptyline
MP = 99:1 - ACN/ H₂O both w/ 5mM NH₄OAc
B = ACN w/ 5mM NH₄OAC
Other Phases Exhibiting HILIC Character

Amitriptyline; flow = 1mL/min; M.P. = 5mM NH₄OAc, pH 6.8; 35ºC; 230nm
Advantages of Using Phases Exhibiting U-Shaped Retention

- Provide unique selectivity vs. C18 phases
- Presents twice the opportunity for retention/resolution (high and low % organic)
- Can use simple water:organic MP’s
- Often faster analysis
- Retain aqueous-soluble analytes
- Retain organic-soluble analytes
- Increase LC/MS sensitivity at high organic MP’s
- Reduce sample pre-treatment requirements
- Do not collapse
Summary

• HPLC phases can contain both RP and NP modes of retention
• For basic analytes on C18 phases, the mechanism in the HILIC region is not readily explained by siloxide anion electrostatic interactions as the major contributing factor
• Level of base deactivation of C18 phases can be approximated by their amount of HILIC character
• Several advantages exist for developing methods on phases that give U-shaped retention curves
• A wide variety of phases exist that do show U-shaped curves, including fluorinated, C18, silica, NH₂, and CN
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