Retention and Selectivity in Aqueous Normal Phase/HILIC Separations

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Abstract

Interest in chromatography using aqueous-organic mobile phases high in organic content (aqueous normal-phase, ANP, HILIC) has continued to build in recent years.(1,2) In this mode of chromatography, analyte retention increases monotonically with an increase in the organic component of the mobile phase. In previous studies, significant contribution of stationary phase chemistry toward the manipulation of retention and selectivity in ANP has been demonstrated.(3) The aim of this continuing study was to further enhance the knowledge base of stationary phase chemistry contributions and, in turn, further the understanding of the governing retention mechanisms in this interesting mode of chromatography.

Abstract (contd.)

Retention and selectivity data for a wide variety of analytes, covering ranges of polarity, pKa values and functional groups, was obtained on bare silica and pentafluorophenylpropyl (PFP) bonded phase. Mobile phase variables known to be important in this mode, such as buffer type and concentration, have been contrasted and compared on each of the phases. Further evidence of operationally different retention mechanism is observed on the different polar phases. These differences in dominant retention mechanisms lead to important insights into method development strategies as well as operative parameter considerations that are often neglected in reversed-phase processes.

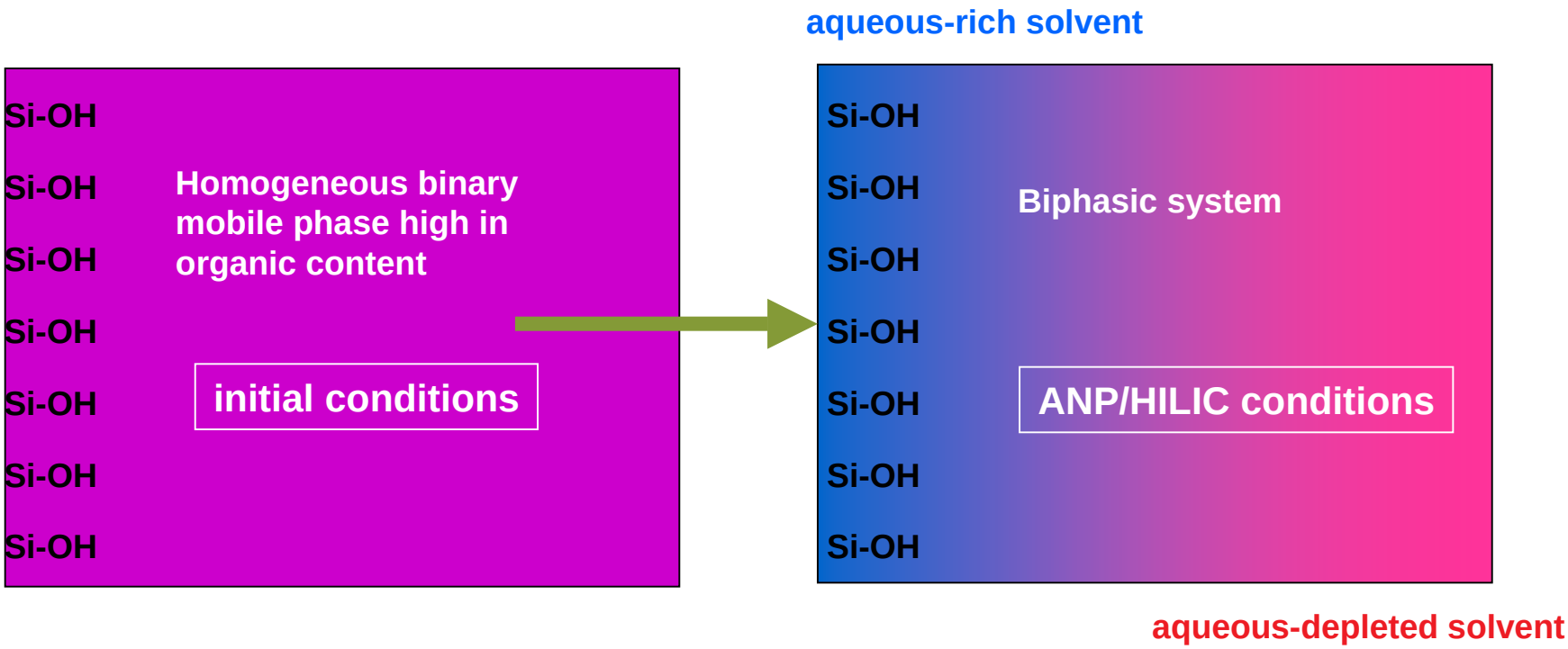
Introduction

Hydrophilic interaction chromatography utilizes a high organic mobile phase (typically acetonitrile) and a polar stationary phase to facilitate resolution of polar anlaytes. As shown in Figure 1, the aqueous portion of the mobile phase preferentially adsorbs onto the polar stationary phase, providing a semi-immobilized polar region of solvent. A polar analyte will partition into this polar region according to the polarity of the molecule, if this is the only mechanism present.

Polar molecules, more often than not, exhibit active functional groups (amines, hydroxyl groups, etc) that are capable of interacting with an active surface such as bare silica. For this reason, HILIC chromatography is often a combination of both true partitioning and other retention mechanisms such as ion-exchange and dipole interactions (hydrogen bonding, for example).

In this presentation, a model is developed for retention of basic polar and non-polar compounds on two ‘polar’ stationary phases:bare silica and pentafluorophenylpropyl (PFP).

Figure 1. HILIC - Biphasic Solvent Distribution at Silica Surface



Polar analytes can partition into aqueous-enriched phase

Potential for polar and ionic interactions with surface

Introduction (contd.)

Figure 2 shows a retention profile for synephrine (log D(7.4) = -1.9 (ACD calc)) using both a PFP and a bare silica column. Here initial buffer concentration of 13 mM ammonium acetate, pH unadjusted (6.7), followed by addition of acetonitrile up to 90% (also diluting buffer concentration). The plots exhibit similar profiles that could be interpreted as similar retention mechanisms between the two phases…but is it true (?)

Figure 3 shows the retention profile for synephrine on the same two columns, however in this case the buffer concentration is held constant while increasing the acetonitrile content. Note that synephrine still exhibits increased retention on the bare silica phase, but not on the PFP, something is obviously different in terms of the retention mechanisms.

Figure 2. Retention Profile of Synephrine on Bare Silica and Pentafluorophenyl Phases – varying Organic and Buffer Concentration

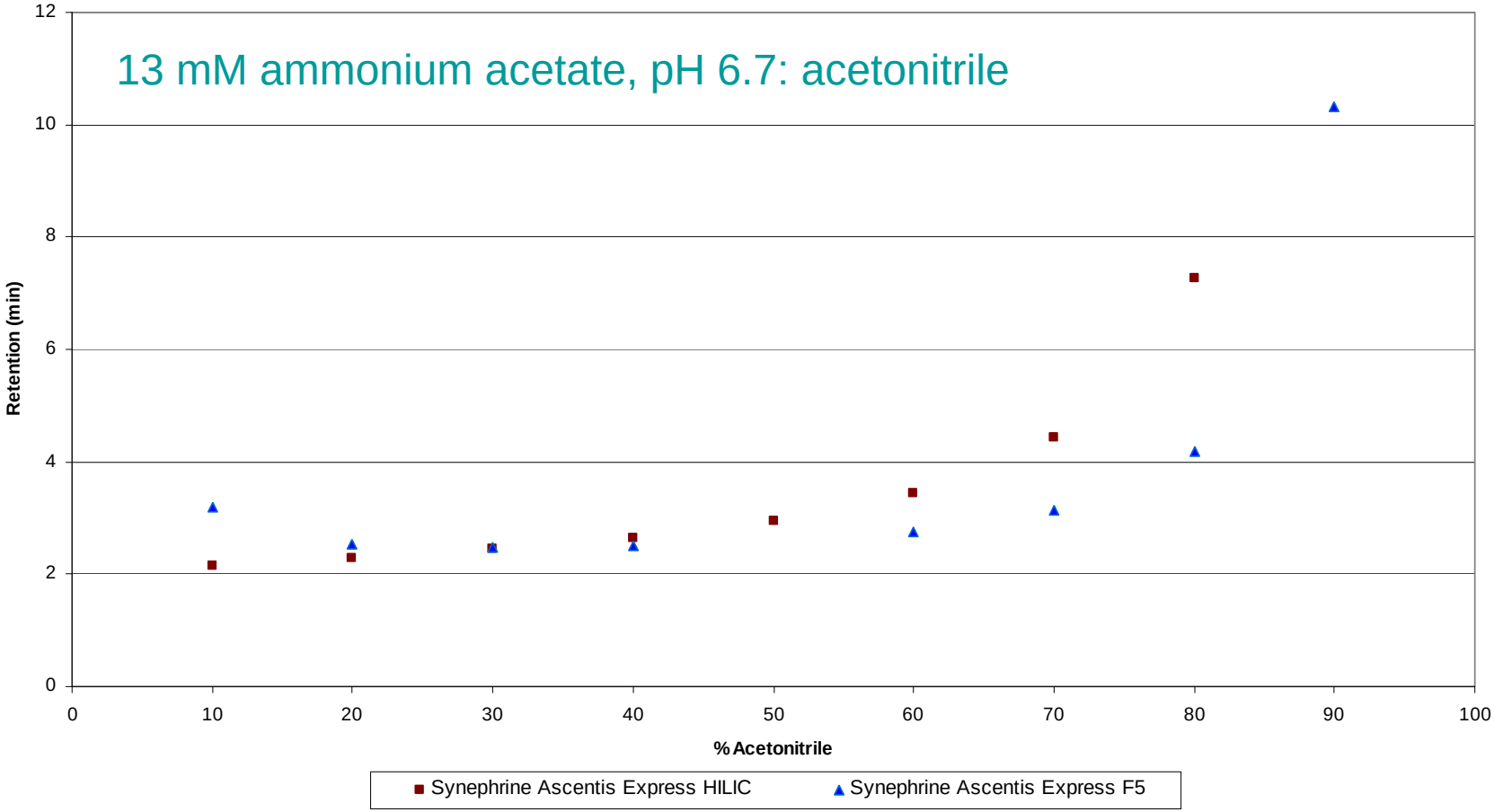


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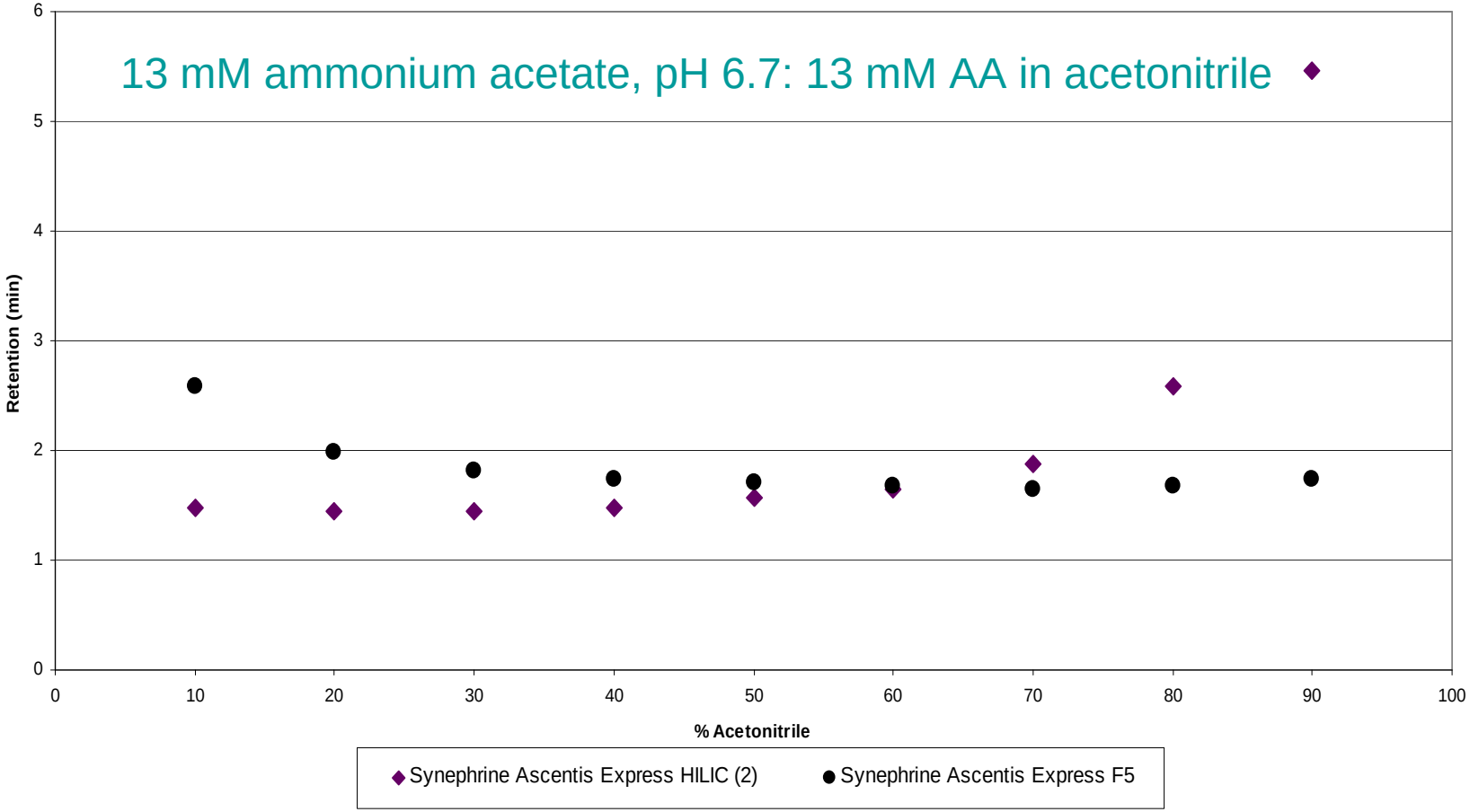
Figure 3. Retention Profile of Synephrine on Bare Silica and Pentafluorophenyl Phases – Constant Buffer Concentration



Retention Mechanism Model

To explain the chromatographic observations, the following two models are introduced.

Figure 4 depicts the semi-immobilized aqueous enriched phase near the surface of a polar stationary support and the mobile, organic enriched phase.

Enter a polar compound (P) and one can imagine a partitioning between the organic enriched and aqueous enriched phase.

In Figure 5 the added possibility (even probability) of additional interactions of the polar analyte with the active surface are introduced – hydrogen bonding, ion-exchange (depicted here) as well as others are highly probable.

Note that the partitioning into the aqueous layer draws polar molecules close to the surface, thereby enhancing polar interactions with it.

Figure 4. HILIC Partitioning Model

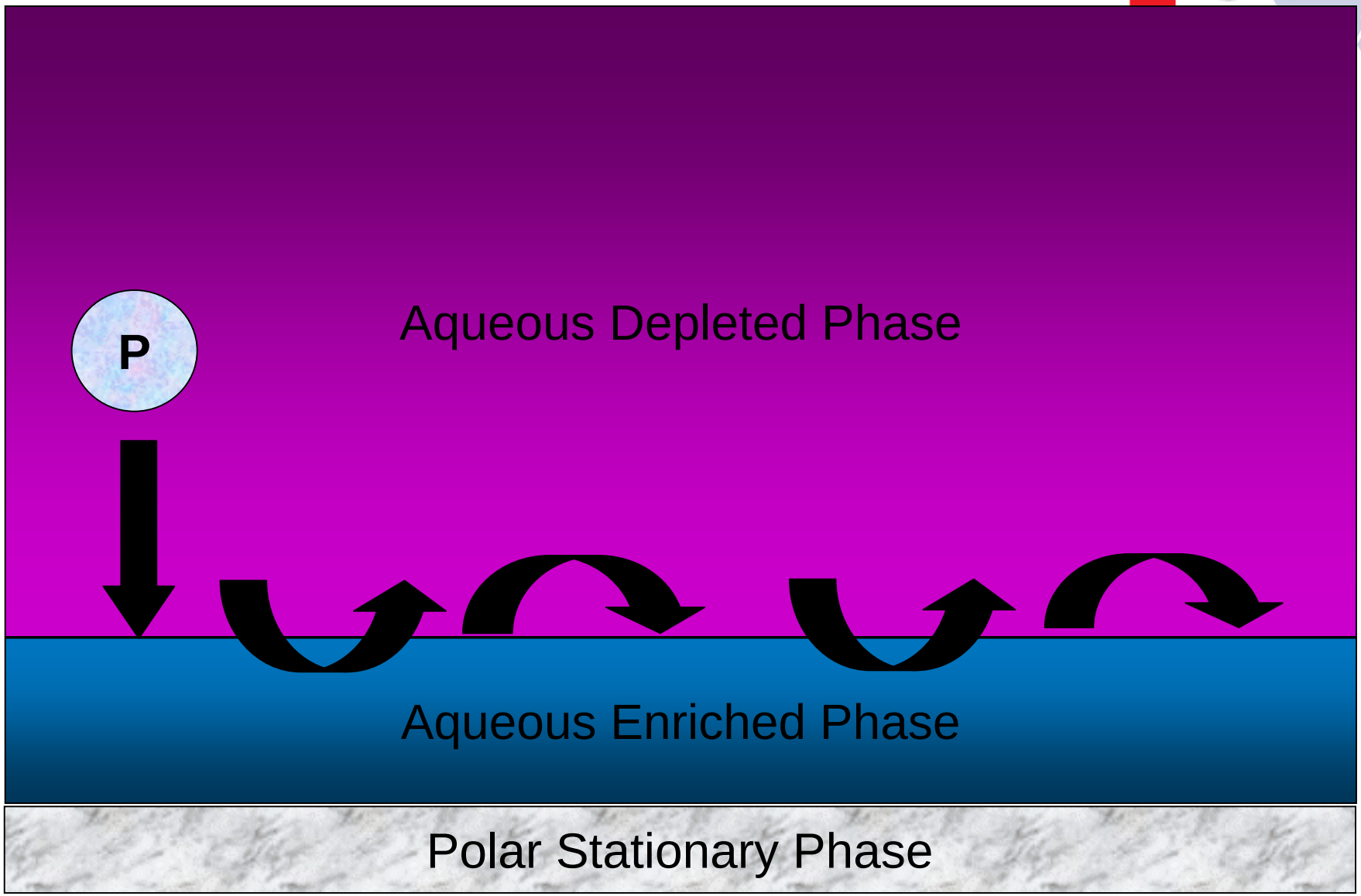
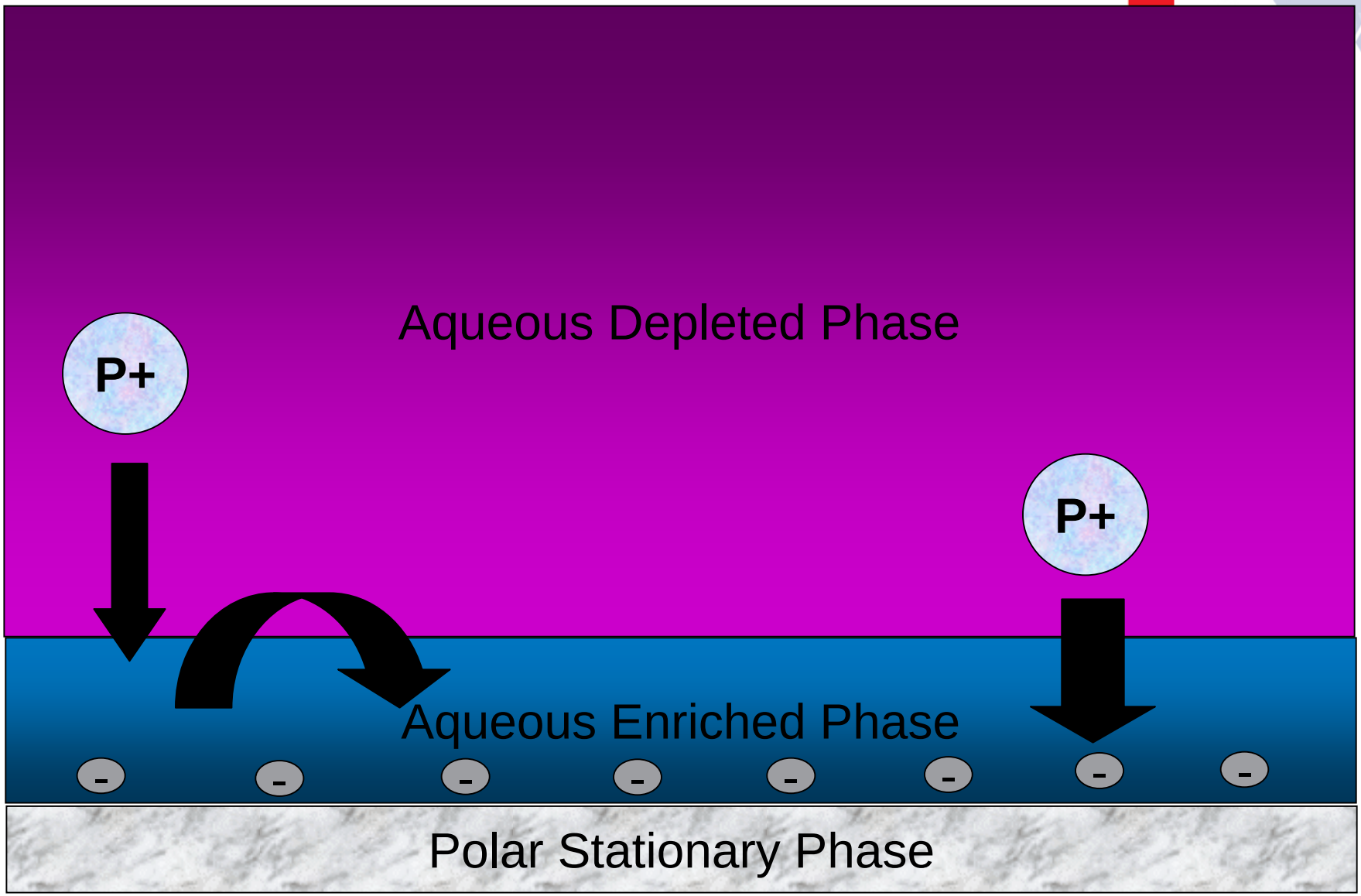


Figure 5. HILIC Model – Partition and Polar Interactions



Expectations According to the Model

According to this model

- Polar bases should retain very well in HILIC
  - Both partitioning AND ionic/polar interactions working toward retention
- Polar nonionic compounds should retain in HILIC, but perhaps not to the extent of polar bases
- Polar acids may be difficult to retain
  - due to charge repulsion unless the charge on the surface is minimized/or analytes neutralized
- Nonpolar bases may be retained
  - but not as much as polar (still have IEX mechanisms working in favor of retention)
- Nonpolar neutrals should not retain
- Nonpolar acids should not retain

But can the model explain differences in retention and selectivity between phases?

Experimental

Prepared composite sample at 10 µg/mL each base in methanol.

Compared retention profiles using Ascentis® Express HILIC (bare silica), Ascentis Express F5 (pentafluorophenylpropyl bonded).

Vary organic:aqueous ratio, 10:90 to 90:10 in 10% increments.

Methods:

1) 13 mM ammonium acetate in DI water:acetonitrile

2) 13 mM ammonium acetate in DI water:13 mM ammonium acetate in acetonitrile

Composite Test Probe - Basic Analytes

Compound Name	pKa(MB)	LogD(7.4)	LogP	Formula
synephrine	9.37	-1.90	0.13	C_{9}H_{13}NO_{2}
chloramphetamine	9.76	0.08	2.37	C_{9}H_{12}ClN
MDA	9.94	-0.80	1.64	C_{10}H_{13}NO_{2}
normetanephrine	8.31	-2.36	-0.86	C_{9}H_{13}NO_{3}
fenfluramine	10.23	0.91	3.55	C_{12}H_{16}F_{3}N
bupropion	7.16	2.13	2.32	C_{13}H_{18}ClNO
midodrine	7.75	-0.44	-0.27	C_{12}H_{18}N_{2}O_{4}
propranolol	9.5	0.79	2.9	C_{16}H_{21}NO_{2}
metoprolol	9.43	-0.47	1.63	C_{15}H_{25}NO_{3}
(±)-chlorpheniramine	9.33	1.04	2.97	C_{16}H_{19}ClN_{2}
pentazocine	8.94	2.61	4.15	C_{19}H_{27}NO
fluoxetine	10.05	1.41	3.93	C_{17}H_{18}F_{3}NO
verapamil	8.97	2.46	4.02	C_{27}H_{38}N_{2}O_{4}

Test probes varying in pK_a and hydrophobicity values

Results

Figures 6 and 7 show the retention profiles obtained for Ascentis Express HILIC (bare silica phase) using Method 1 (vary organic and buffer concentration) and Method 2 (vary organic, constant buffer concentration) for a subset of the probes.

- Figure 6 shows the expected increase in retention at higher concentrations of organic.
- Figure 7 shows that the increase in retention still exists, however retention is greatly reduced.
- The results indicate both a contribution from partitioning and IEX mechanisms.
- Similar profiles were acquired for each of the test probes – each depict increased retention in the ANP/HILIC region as expected.

Figure 6. Bare Silica Phase – varying both Organic and Buffer Concentration

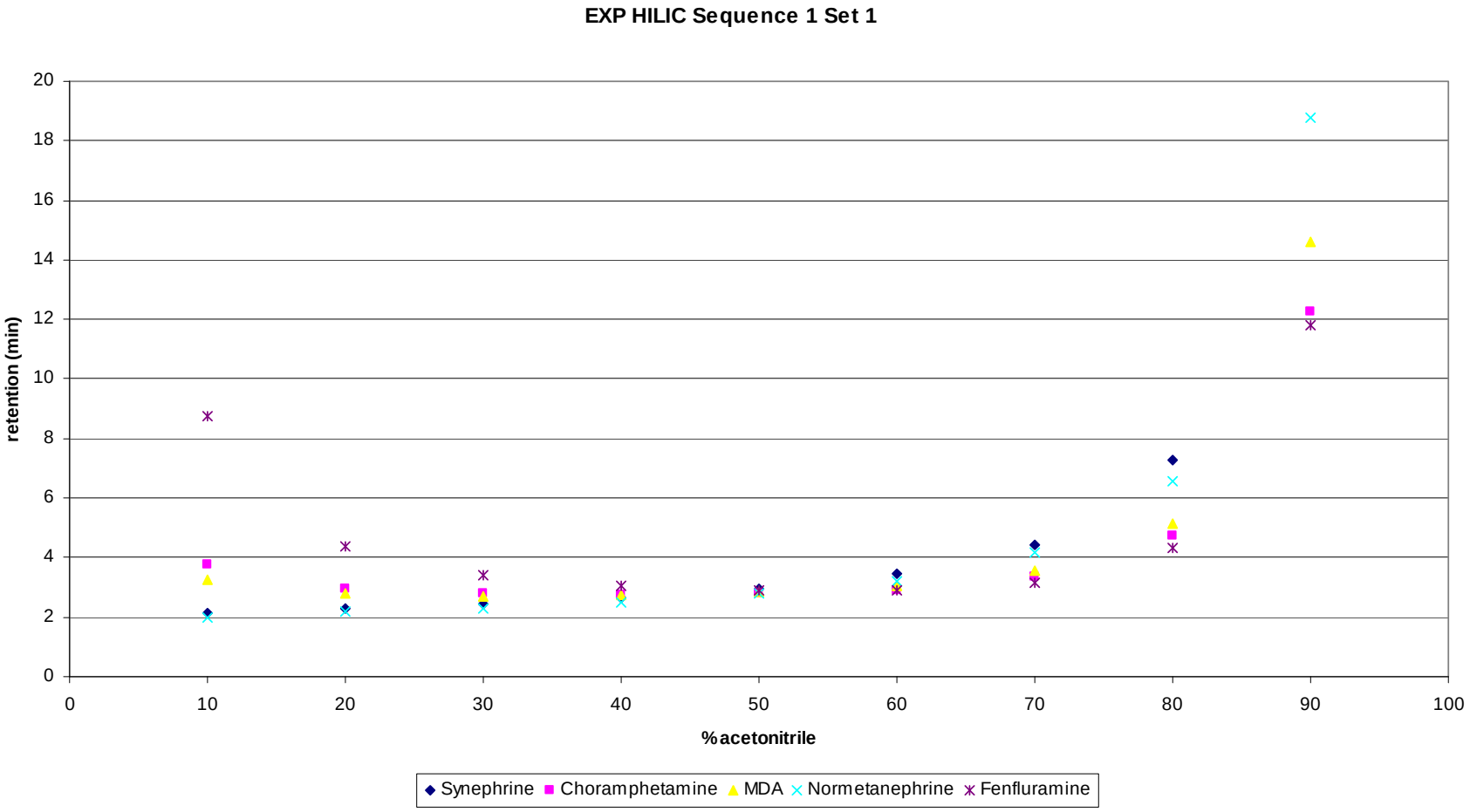
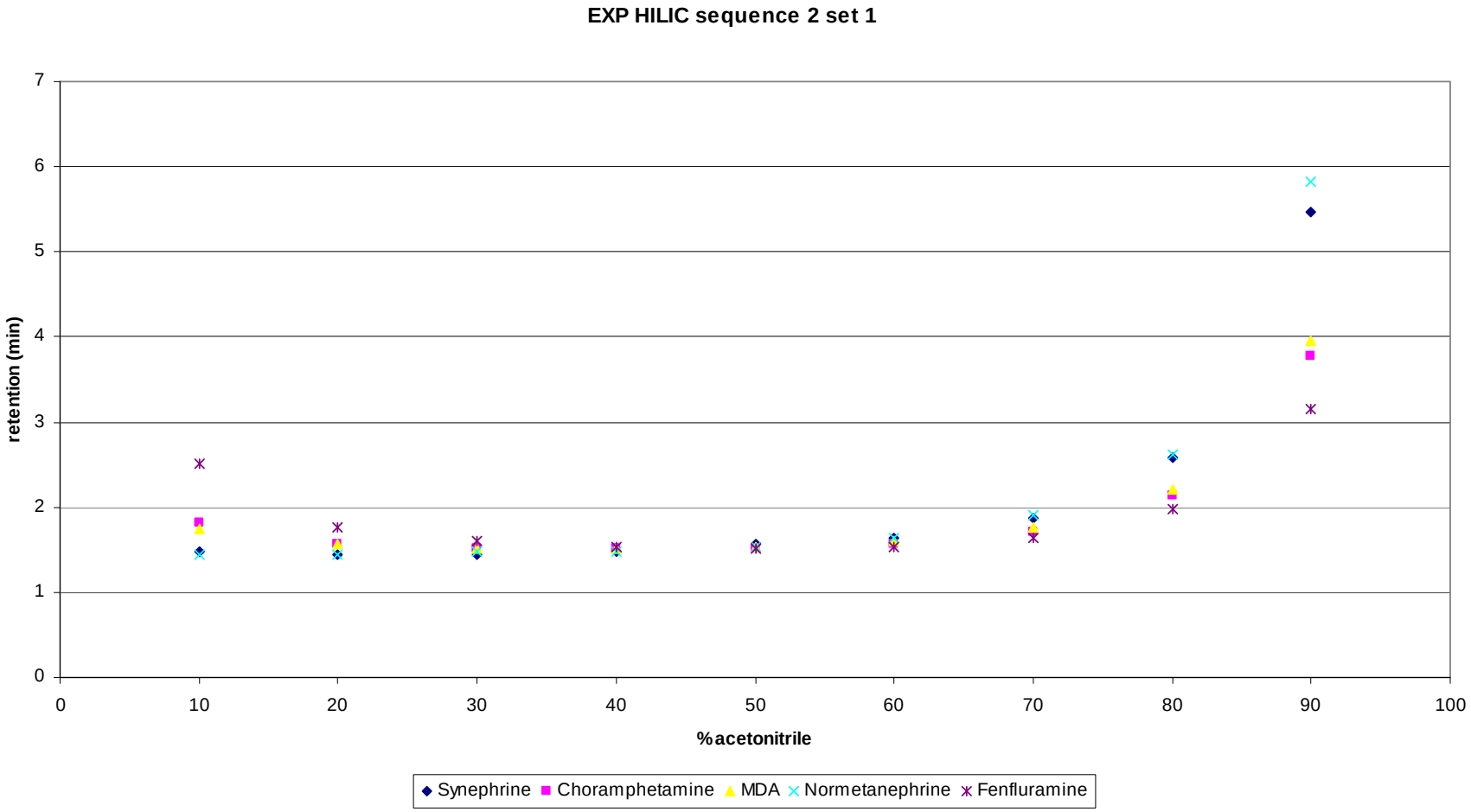


Figure 7. Bare Silica Phase – varying Organic, Buffer Concentration Constant at (13 mM)



Figures 8 and 9 show the same set of compounds run on an Ascentis Express F5 (pentafluorophenylpropyl) stationary phase using Methods 1 and 2, respectively.

When the buffer concentration is simultaneously reduced as organic is increased, retention increases. There is, however, a lack of this feature when the buffer concentration is held constant.

This is in contrast to what was observed on HILIC phase. Although increased buffer attenuated retention on the HILIC phase, the increase in retention as a function of increased organic was still evident in most cases.

The results provide evidence that the retention on the PFP phase is primarily due to IEX and not a true HILIC (partitioning) mechanism. This was true for each of the sets (data not shown).

Figure 8. PFP Phase – varying both Organic and Buffer Concentration

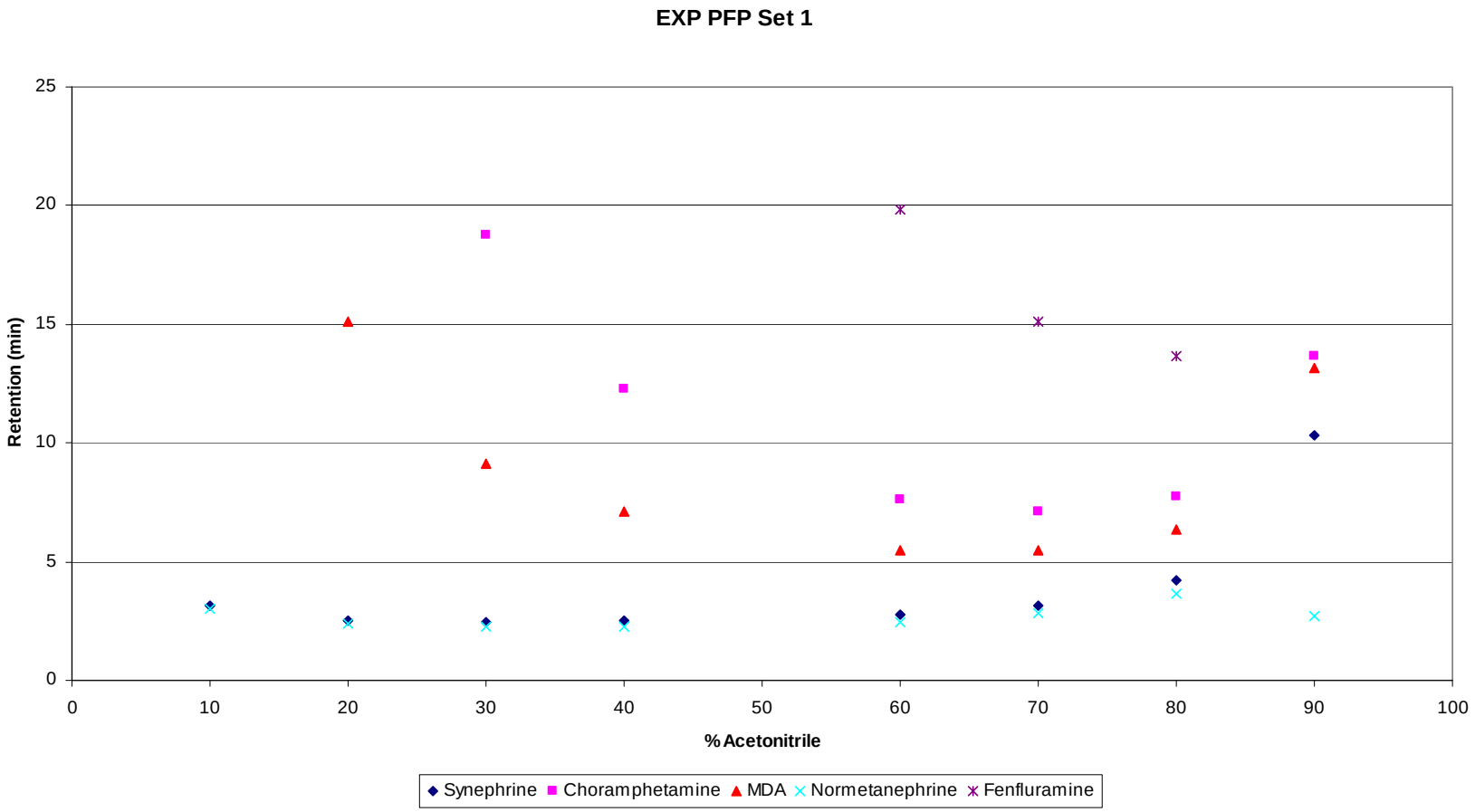


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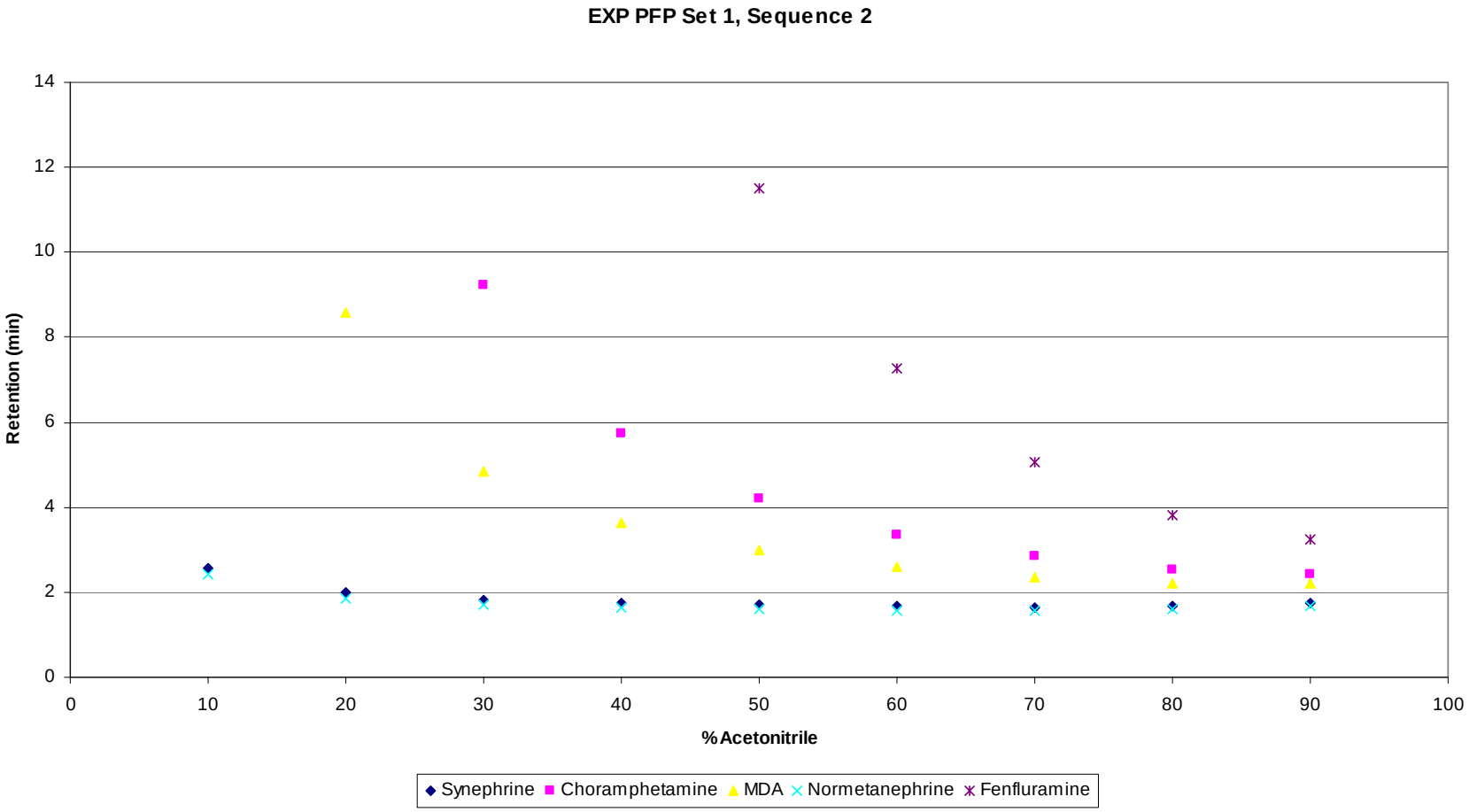
Figure 9. PFP Phase – varying Organic, Buffer Concentration Constant at 13 mM



Conclusions

Although the PFP and bare silica phases show a similar retention dependence on the percentage of organic (ie., show ANP/HILIC character), the mechanisms that dominate retention are shown to be different.

The data suggests that bare silica imparts both a partitioning mechanism (HILIC) as well as an IEX mechanism when polar basic analytes are present. Here the bases interact positively via both ion-exchange as well as by HILIC partitioning. A synergistic set of mechanisms.

The PFP bonded phase exhibited no evidence of HILIC (lack of retention for hydrophilic probes at higher buffer concentrations); however, IEX was shown to dominate retention where basic probes were present.

The presented model appears to fit the data obtained in the study.

The variables that influence IEX vs. partitioning mechanisms are very different. When developing robust methods in ANP/HILIC, it is essential to understand the dominant mechanisms that govern retention.

Acknowledgements

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4. A. Daniel Jones, MSU

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