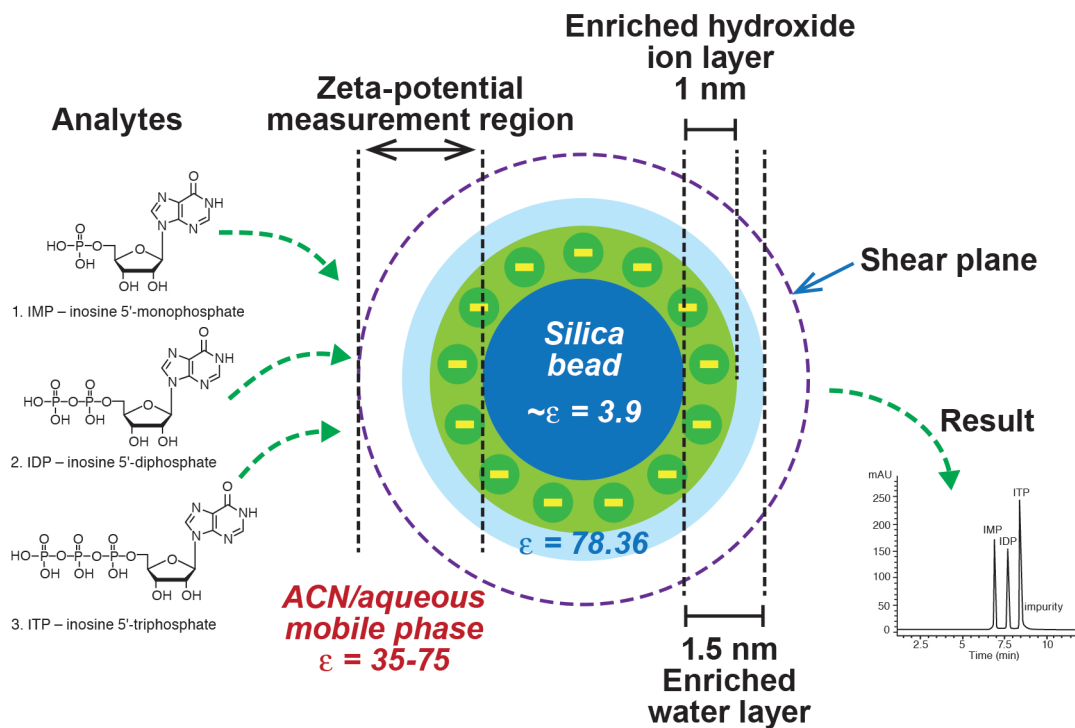


# ANP Mechanism<sup>1</sup>

The precise mechanism of ANP retention is an active area of investigation as of the publication of this catalog. However, a recent study involving zeta potential measurements to help characterize the surface has demonstrated that the water layer on a silica-hydride surface is, on average, only 0.5 of a monolayer, in contrast to 7-8 monolayers for ordinary Type B silica. This low amount of water on the surface precludes a partitioning process.

In addition, it has been determined that the TYPE-C silica surface possesses a negative charge. Instead of this charge being the result of surface silanols, as is the case for the ordinary unbonded silica used for HILIC methods, it has been ascribed to the presence of excess hydroxide ions adsorbed on the surface, derived from the aqueous component of the mobile phase (see Figure 5). Hydroxide ions from the surrounding liquid accumulate on the slightly hydrophobic silica-hydride surface. The mechanism of ANP is therefore thought to be a combination of ion attraction for positively charged species or ion displacement for negatively charged compounds. For polar neutral compounds a displacement/adsorption effect for retention is most likely.

Figure 5.



## ANP vs HILIC

Cogent TYPE-C silica columns perform similarly to HILIC (Hydrophilic Interaction Liquid Chromatography) columns in that they both show increased retention times for polar compounds (when using > 70% organic composition of the mobile phase) compared to reversed-phase HPLC. Both column types perform separations that are based on variations of normal-phase, but they each have different retention mechanisms and various other different properties.

HILIC stationary phases are typically more polar than TYPE-C silica bonded phases, which are relatively non-polar.

On HILIC columns, retention of polar compounds is achieved by partitioning in and out of the adsorbed water layer surrounding the stationary phase surface. As the acetonitrile concentration increases, the water layer decreases and the charged polar analytes are retained by a combination of cation-exchange with the silanols under the water layer and the partitioning effect.

*continued on next page*

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continued from previous page

With Cogent TYPE-C™ silica columns, charged polar compounds elute in a similar order as on HILIC columns. Since there are virtually no silanols on these columns, the polar compounds are retained more by the adsorptive character of the silica-hydrate surface, rather than by a partition mechanism. In addition, the non-polar ligand of the TYPE-C silica phases will also retain non-polar compounds.

As a result of the weak association of water with the TYPE-C silica-hydrate, there is a lack of a hydration shell at high organic content. This allows the column to equilibrate and change more rapidly than HILIC columns. This is a significant advantage for rapid gradients.

Another significant advantage of ANP over HILIC is reproducibility. Historically HILIC as a technique has suffered from a reputation for poor gradient method reproducibility. One of the main causes of this can be attributed to the variability in the thickness of the hydration shell surrounding the silica surface of Type B silicas. Conversely TYPE-C silica-hydrate phases used in ANP do not suffer from this because the enriched hydroxide ion water layer is much more stable, resulting in improved method reproducibility.

The Cogent TYPE-C silica columns are also more versatile, as they can be used in RP, ANP and ONP modes, without hysteresis or damage to the columns. HILIC columns can only retain polar compounds and are not suitable for RP analyses.

### Key Advantages of ANP over HILIC:

- Polar and non-polar compounds can be separated in the same isocratic run
- Precision run to run, day to day, batch to batch
- Equilibration time is much faster
- TYPE-C silica-hydrate columns can perform ANP, RP and ONP, whereas HILIC columns generally can only perform HILIC separations
- TYPE-C silica-hydrate columns offer low bleed for LC-MS



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