Aqueous Normal Phase ANP and how can it is useful for polar compounds – Tech Information

Aqueous Normal Phase or ANP chromatography is unique and extremely robust and useful.

***Definition: Aqueous Normal Phase* ANP** chromatography is an HPLC retention mechanism which encompasses the HPLC mobile phase region between reversed phase chromatography (RP) and organic normal phase chromatography (ONP) and is used mainly for polar compound separations.

Background Information: In normal phase chromatography, the stationary phase is more polar, and the mobile phase is less polar. In reversed phase it is the opposite; the stationary phase is more non-polar, and the mobile phase is more polar. Typical stationary phases for normal phase chromatography are silica or organic moieties with cyano and amino functional groups. For reversed phase, alkyl hydrocarbons are the preferred stationary phase; octadecyl (C18) is the most common stationary phase, but octyl (C8) and butyl (C4) are also used in some applications. The designations for the reversed phase materials refer to the length of the hydrocarbon chain.

In normal phase chromatography, the most nonpolar compounds elute first and the most polar compounds elute last. The mobile phase consists of a very nonpolar solvent like hexane or Heptane mixed with a slightly more polar solvent like isopropanol, ethyl acetate or chloroform. Retention increases as the amount of nonpolar solvent in the mobile phase increases. In reversed phase chromatography, the most polar compounds elute first with the most nonpolar compounds eluting last. The mobile phase is generally a binary mixture of water and a miscible polar organic solvent like methanol, acetonitrile or THF. Retention increases as the amount of the polar solvent (water) in the mobile phase increases. Normal phase chromatography, an adsorptive mechanism, is used for the analysis of solutes readily soluble in organic solvents, based on their polar differences such as amines, acids, metal complexes, etc. Reversed phase chromatography, a partition mechanism, is typically used for separations by non-polar differences.

"Silica Hydride" distinguishes as a support material from all other HPLC silica-based materials; most ordinary silica materials used for chromatography have a surface composed primarily of silanols (-Si-OH) as the terminal groups. On a "silica hydride surface" the terminal groups are primarily -Si-H (hydride) which makes the particle slightly hydrophobic instead of very hydrophilic. The silica hydride surface can also be functionalized with direct silicon to carbon bonds making the bond almost indestructible under any HPLC condition.

Mobile phases for ANP are based on an organic solvent (such as acetonitrile) with a small amount of water; thus, the mobile phase is both "**aqueous**" *(water is present)* and "**normal**" *(less polar than the stationary phase)*. **Thus, polar solutes *(such as acids and amines)* are most strongly retained, with retention decreasing as the amount of water in the mobile phase increases**.

The ANP retention mechanism is believed to be due primarily to adsorption of analytes directly onto the silica hydride surface, as investigations incorporating zeta potential measurements have demonstrated. In contrast, HILIC

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retention mechanism has been shown to be largely due to partitioning of the **analyte** in a thick water layer that naturally forms on the surface of silica based stationary phase materials, due to their hydrophilic character. Because the Si-H group is not polar like silica Si-OH moieties that predominate on traditional silica-based phases, a water layer does not form with silica hydride columns. This difference in retention mechanism can have notable advantages in terms of the **robustness** of a chromatographic separation, since the variable nature of the water shell can lead to differences in retention from run to run as well as unique Selectivity.

It is important to note that the most common mobile phase in reversed phase HPLC is DI water with formic acid or ammonium formate as the A solvent and acetonitrile with 2% water with formic acid or ammonium formate as the B solvent. This is the same mobile phase used in ANP with the different being the starting gradient concentration of B solvent is approximately 95%. The advantage is that HPLC systems and the column do not have to be purged or flushed when switching between polar and non-polar compounds.

Typically, the amount of the nonpolar component in the mobile phase must be 60% or greater with the exact point of increased retention depending on the solute and the organic component of the mobile phase. A silica hydride stationary phase is capable of ANP and will be able to function with both reversed phase and aqueous normal phase mobile phases with only the amount of water in the **eluent** varying. Thus, a continuum of solvents can be used from 100% aqueous to pure organic.

ANP retention has been demonstrated for a variety of polar compounds on the hydride based stationary phases in the article ***"Hydride-based stationary phases: A rapidly evolving technology for the development of new bio-analytical method", J.J. Pesek, R.I. Boysen, M.T.W. Hearn, M.T. Matyska, Anal. Methods, 6 (2014) 4496-4503.***

An interesting feature of HPLC columns based on silica hydride phases is that both polar and nonpolar compounds can be retained over some range of mobile phase composition *(organic/aqueous)* as a result of surface adsorption effects due to hydroxide ions in Aqueous Normal Phase ANP mode. This property distinguishes it from a pure HILIC (hydrophilic interaction chromatography) column where separation by polar differences is obtained, or a pure RP stationary phase on which separation by nonpolar differences in solutes is obtained with very limited secondary mechanisms operating.

Another important feature of the silica hydride-based phases is that for many analyses it is usually not necessary to use a high pH mobile phase to analyze polar compounds such as bases. The aqueous component of the mobile phase usually contains from 0.1 to 0.5% formic or acetic acid can also contain ammonium acetate or ammonium formate *(5-15mM)*, which is compatible with detector techniques that include mass spectral analysis.

Aqueous Normal Phase *HPLC is only possible on a hydrophobic particle such as the silica hydride stationary phases.*

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