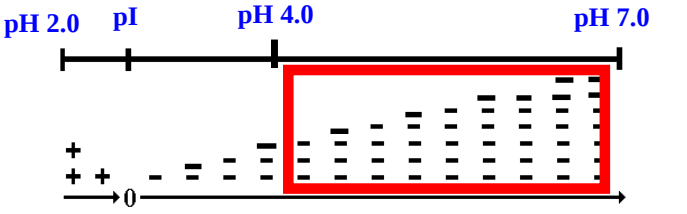
The secret behind CHIRAL-AGP and pH

With the **CHIRAL-AGP** column it is possible to separate enantiomers from many different compound classes; amines (primary, seconday, tertiary as well as quarternary ammonium compounds), acids (strong and weak) and nonprotolytes (esters, sulphoxides, amides, alcohols etc.). This broad applicability is a unique property for the **CHIRAL-AGP** column.

One reason for this broad applicability is the possibility to increase or induce enantioselectivity by changing the composition of the mobile phase. Especially important are changes in the **pH**.

The stationary phase on the **CHIRAL-AGP** column is a protein, α[1]-acid glycoprotein (AGP). The protein contains many different charged groups that are affected by changes in the pH of the mobile phase. AGP is a very acidic protein with a pKa-value of 2.7, i.e. at this pH the net charge of AGP is zero. At a pH below 2.7 the net charge of AGP is positive and at pH above 2.7 the net charge is negative. This is expressed in the figure below:



Increasing net negative charge of AGP at higher pH

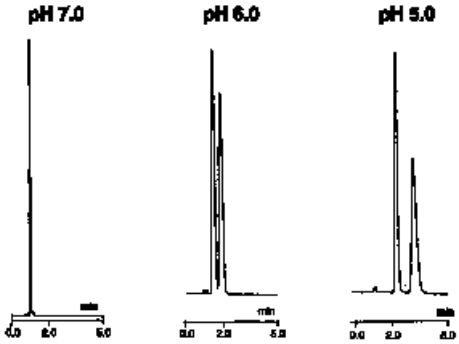
= pH range used in chromatography

The influence of pH on the AGP phase during chromatography of charged analytes will be shown in the examples below.

1. Acidic analytes

When a carboxylic acid (in this example 2-phenoxypropionic acid) is chromatographed at pH 7, both the acid and AGP has a net negative charge.

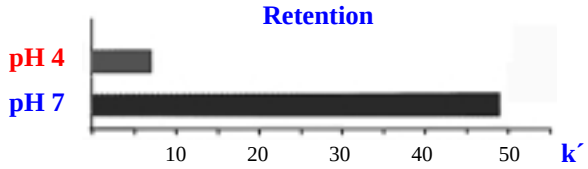
As a result there is a repulsion between the acid and the stationary phase, leading to no retention at all, ie. elution of the acid in the void volume.



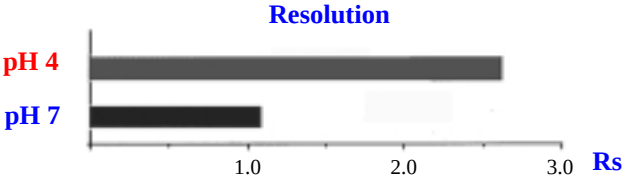
As pH is decreased, the negative charge is decreased on both the acid and AGP, giving higher retention and chiral recognition, leading to baseline separation.

2. Basic hydrophobic analytes

When a hydrophobic amine (in this example propranolol) is chromatographed at pH 7, the retention is very high (k´ almost 50). The reason is that AGP has a strong negative charge and the amine is positively charged, leading to strong attraction. If pH is decreased to 4, retention is much shorter, due to the decrease in negative charge of AGP.



Also the enantioselectivity is strongly affected when decreasing pH from 7 to 4. At pH 7, Rs is ca. 1, ie. no baseline resolution. However, at pH 4, Rs is increased to ca. 2.5.



The main reason is that at pH 4 the 2-propanol content of the mobile phase can be reduced (from 6 to 0.5%), giving higher α-value leading to higher resolution.

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