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Newsletter

Effect of Buffer Concentration on Retention of Charged Analytes

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When charged molecules are analyzed by HPLC, the pH of the buffer plays an important role in obtaining efficient separation. In the reverse phase (RP) separation the buffer usually provides stable ionization form for analytes and eliminates or significantly reduces electrostatic interaction of the analytes with residual charge of the stationary phase (SP) surface. Usually the concentration of the buffer does not affect retention of the analytes significantly.

When a mixed-mode SP, such as Primesep (Figure 1) is used in separation, the buffer provides an additional function. Each Primesep column contains an embedded ion-exchange functional group, either positive or negative. The charged molecules strongly interact with the embedded charge on the SP, and by changing the concentration of the buffer you can substantially alter the retention of analytes. If the charge of analyte and the charge of SP surface are opposite, you will observe a strong interaction that in its turn will produce strong retention.

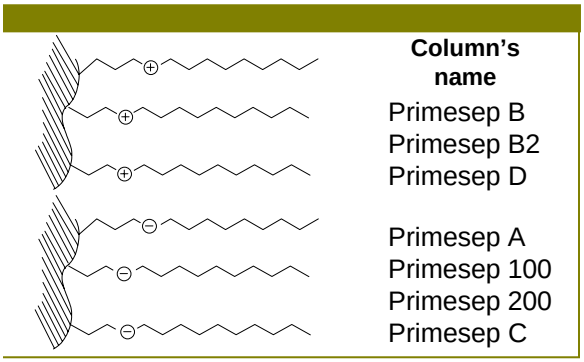
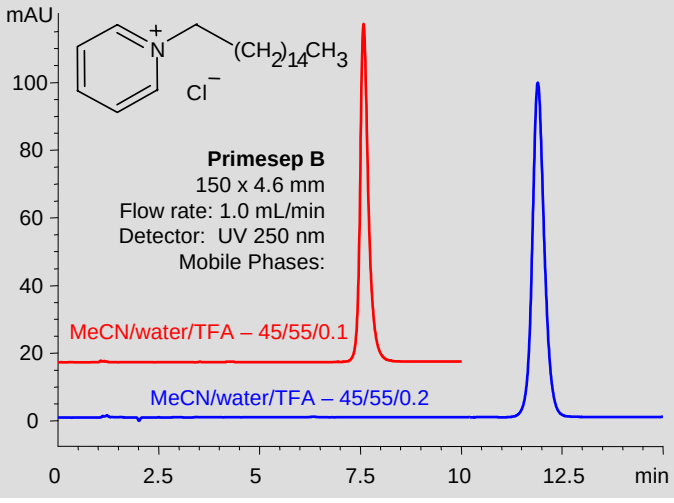


Figure 1: Simplified structure of stationary phase ligand attached to silica support

Figure 2: Effect of buffer concentration on retention of cetylpyridine on positively charged RP column



If the charge of analyte and the charge of SP surface are the same, you will observe a repulsion that will result in a reduced retention compared to the one received in ordinary RP chromatography.

The example on Fig. 2 demonstrates the effect of the buffer concentration on retention of the analyte on SP with the same charge. The increase in the buffer concentration (TFA) increases the retention of the positively charged analyte.

The example on Fig. 3 demonstrates the effect of buffer concentration on retention of positively charged amines on a negatively charged SP column. In this example, the increase in buffer concentration (TFA) decreases the retention of amines and amino acids and has no effect on the retention of neutral molecules such as benzonitrile and benzoic acid. The last is neutral at the shown concentration of TFA.

The example on Fig. 4 illustrates the sample consisting of both positively and negatively charged molecules. The increase in the concentration of the TFA moves the oppositely charged components in the opposite direction which changes their elution order.

To sum it all up, the buffer concentration is a powerful tool in the control of selectivity and retention of the charged compounds in the mixed-mode chromatography and should be monitored with the same accuracy as the concentration of the organic modifier.

Figure 3: Effect of buffer concentration on retention of positively charged compounds on negatively charged RP column

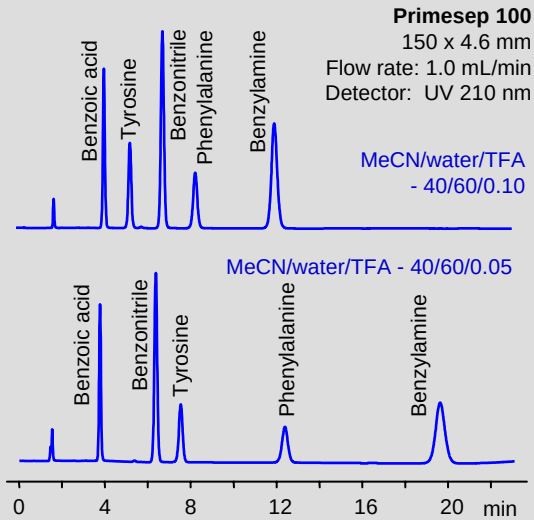


Figure 4: Effect of buffer concentration on retention of bromide ion and dextromethorphan on positively charged RP column