Influence of the additives on the retention of analytes on the polar stationary phases under conditions of supercritical fluid chromatography

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ABSTRACT

Supercritical fluid chromatography (SFC) is a versatile analytical method, having a number of advantages compared with high-performance liquid chromatography. One of the common ways for achieve acceptable separation is the use of additives. Despite the numerous studies, their role in separation is not always clear as it may combine several different mechanisms.

For the first time the effect of the type and concentration of the additive on the retention of analytes on polar stationary phases in the SFC is estimated by the method of linear solvation energy relationship. It's shown that additives greatly effect only on the coefficients d^+ and d^-, reflecting the intensity of ionic interactions. The use of water as an additive increases the role of interactions with the formation of hydrogen bonds, and for a number of stationary phases - enhances dipole-dipole interactions.

INTRODUCTION

Supercritical fluid chromatography (SFC) is a modern analytical method, acting as an alternative to ultra-high-performance liquid chromatography, and having a number of advantages due to the specific properties of the fluid [1].

Additive is a component of mobile phase, which is introduced at much lower concentrations than the co-solvent (usually less than 1.0%). Nevertheless, they can cause significant improvements in peak shapes and changes in retention.

The additives can be organic acids (formic, acetic, trifluoroacetic) [2], bases (diethylamine, ammonium hydroxide) [3] or salts like ammonium acetate [4]. In addition, water is also used like an additive, often in combination with other additives [5].

Additives have been the topic of many works but still, their role in separation is not always clear as it may combine several different mechanisms, for example, changing the acidity of the mobile phase, adsorption on the active sites of stationary phase or forming ion pairs with ionic analytes.

One of the methods for determining the relative intensity of specific molecular interactions in a chromatographic system is Linear Solvation Energy Relationship (LSER) [6] methodology, which relies on an equation, relating free-energy interaction terms to retention factors (k):

$$\log k = c + eE + sS + aA + bB + vV + d^-D^- + d^+D^+, \tag{1}$$

In this equation, capital letters represent the solute descriptors, related to particular interaction properties, while lower case letters represent the system constants, related to the complementary effect of the phases on these interactions. c is the model intercept term and is

dominated by the phase ratio. E is the excess molar refraction (calculated from the refractive index of the molecule) and models polarizability contributions from n and π electrons; S is the solute dipolarity/polarizability; A and B are the solute overall hydrogen-bond acidity and basicity; V is the McGowan characteristic volume in units of $cm^3 \cdot mol^{-1}/100$; D^- represents the negative charge carried by anionic and zwitterionic species, and D^+ represents the positive charge carried by cationic and zwitterionic species. The system constants (e, s, a, b, v, d^-, d^+) obtained through a multilinear regression of the retention data for a certain number of solutes with known descriptors, reflect the magnitude of difference for that particular property between the mobile and stationary phases. Thus, if a particular coefficient is numerically large, then any solute having the complementary property will interact very strongly with either the mobile phase (if the coefficient is negative) or the stationary phase (if the coefficient is positive) [6].

When the mobile phase is constant, stationary phases can be compared, and, on the contrary, when the stationary phase is constant, the effects of mobile phases and operating conditions can be compared.

Thus, the aim of this study is to investigate the effect of additives on the characteristics of «stationary phase CO_2-MeOH» system using linear solvation energy relationship methodology.

MATERIALS AND METHODS

The experiments were performed using an Acquity UPC^2 SFC system (Waters, USA), including a pump for feeding carbon dioxide and co-solvent, autosampler, column thermostat, back pressure regulator, and diode matrix spectrophotometric detector. The instrument was controlled and the chromatographic data collected and processed using the Empower 3.0 program package (Waters, USA).

The following chromatographic columns were used:
– UPC^2 BEH (Waters, USA), 150 x 3.0 mm, particle size 1.7 um;
– HSS Cyano (Waters, США), 150 x 3.0 mm, particle size 1.8 um;
– UPC^2 BEH 2-EP (Waters, США), 150 x 3.0 mm, particle size 1.7 um;
– Nucleodur HILIC (Macherey-Nagel, Germany), 150 x 2.0 mm, particle size 1.8 um;

The mobile phase was carbon dioxide (extra-pure grade) and methanol (LiChrosolv HPLC grade, Merck, Germany). The additives were trifluoroacetic acid (HPLC grade, Sigma-Aldrich), diethylamine (≥99.5%, Sigma-Aldrich), ammonium acetate (HPLC grade, J.T. Baker) and ultra-pure water, obtained immediately before the experiment using the system Simplicity UV (Millipore, France).

89 test compounds of an aromatic nature were used during the work. All compounds with more than 98% main substance were purchased from Sigma-Aldrich (Germany) and Fluka (Switzerland). The choice of these compounds was dictated by the fact that this set of compounds were used for LSER characterization of polar sorbents in SFC [6], which allows justified comparison of the results with the literature data.

The stock methanol solutions of the individual components at a concentration of 1000 mg/L were prepared from exact samples and stored in a refrigerator at 4°C for no more than seven days. The working solutions were prepared from the stock solution immediately before the experiment at a concentration of each component of 100 mg/L.

The eluent flow rate was 1 mL/min, the temperature 25°C, and back pressure 150 atm. The detection was performed at wavelengths of 200–400 nm, spectral resolution of 4.8 nm, and data acquisition frequency of 40 Hz; the analytical wavelength was 210 nm. The amount of the introduced sample was 1.0 μL in all experiments, with two parallel injections made for each compound. The dead volume of the chromatographic system for calculation of the retention factors (k) was determined from the first negative peak on the baseline.

The multilinear regression analysis was performed using the Microsoft ®Excel program (Microsoft® Corporation, 2010).

RESULTS

According to the data obtained (Figure 1), trifluoroacetic acid primarily affects the retention of ionic analytes, i.e., acids and bases, which leads to a change in the coefficients d^+ and d^-, while the remaining coefficients do not change significantly. The greatest effect is observed for zwitter-ionic phase, while the lowest – for 2-ethylpyridinium phase.

Since the acid has an effect on unmodified silica gel, it can be concluded that silanol groups are primarily affected by the additives. On the other hand, according to the literature data [7], the introduction of acid into the system can lower the pH of the mobile phase up to 1, which can suppress the dissociation of acid-type analytes and convert the bases into a protonated form.
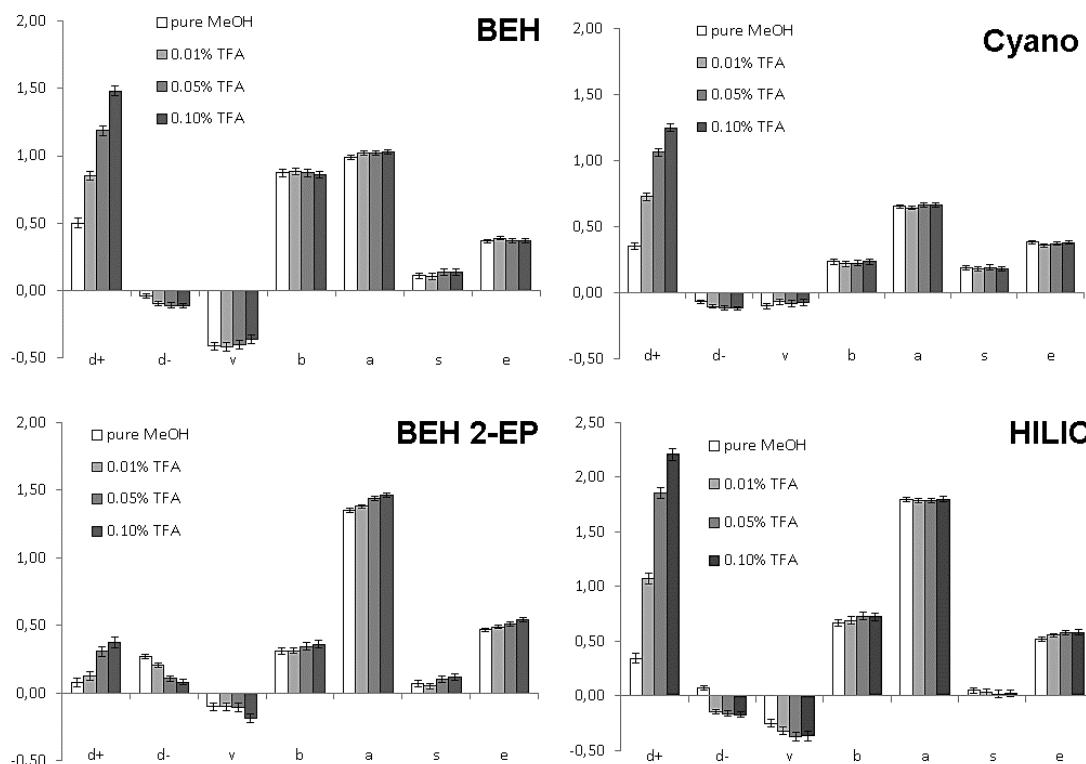

Figure 1. Effect of acid additive on the characteristics of the chromatographic system.

Base additive has a greater effect on the retention of analytes with acidic functional groups, which is characterized by a significant increase in the d^- coefficient (figure 2). For other descriptors, minor changes are observed, in particular, there is an increase in the ability to be a hydrogen bond donor (coefficient a increases) and a decrease in the ability to accept a hydrogen bond (coefficient b decreases).

Unlike other stationary phases, diethylamine does not have a significant effect on the sorbent with 2-ethylpyridinium groups. The possible reason of this effect is that 2-ethylpyridinium ligand is acting similarly to a basic additive to reduce basic interactions with silanols.
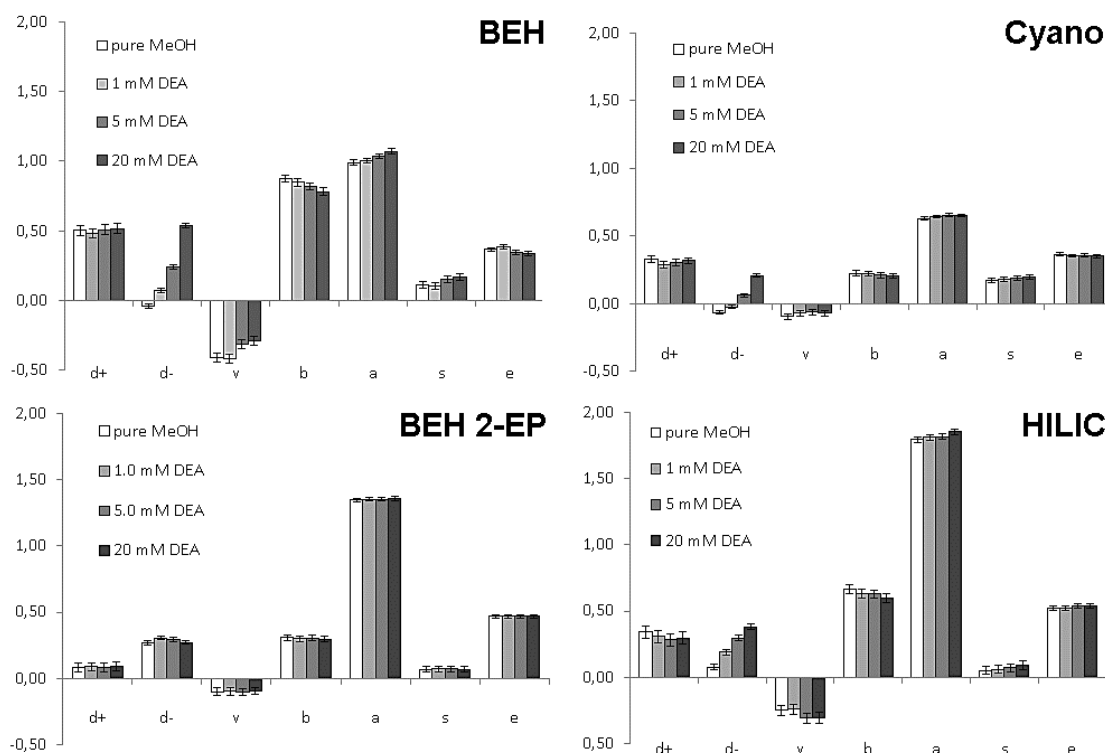
Figure 2. Effect of base additive on the characteristics of the chromatographic system.

Based on the obtained data (figure 3), it can be noted that for bare silica gel, as well as for the zwitterionic phase, ammonium acetate has the same effect as diethylamine, but somewhat more pronounced, consisting in increased retention of acid-type analytes, which corresponds to growth of the coefficient d^-. The possible reason is the shielding of silanol groups by ammonium and acetate ions. In the case of the zwitterionic phase ammonium ion can block the negatively charged sulfate ion of the functional groups of the sorbent. For the 2-ethylpyridine stationary phase ammonium acetate has practically no effect on the retention of the analytes, like diethylamine.
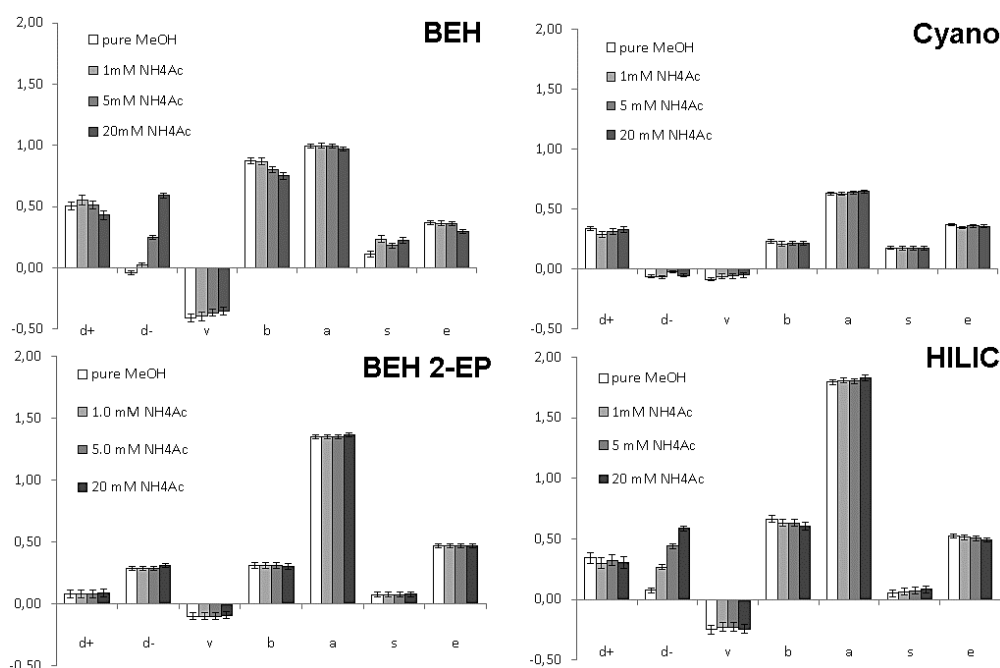

Figure 3. Effect of salt additive on the characteristics of the chromatographic system.

Water has a significant effect on the retention of analytes, causing a complex effect on various types of interactions (figure 4). In particular, for all stationary phases, a significant increase in the coefficients a and b is observed, which indicates an increase in the intensity of interactions with the formation of a hydrogen bond. For 2-ethylpyridine and cyanopropyl phases, an increase in the parameter s, associated with the participation of dipole-dipole interactions, is also observed. In addition, for all the stationary phases studied, there are similar trends in the parameters related to ionic interactions: an increase in the coefficient d^+ and a decrease in the coefficient d^- are observed.
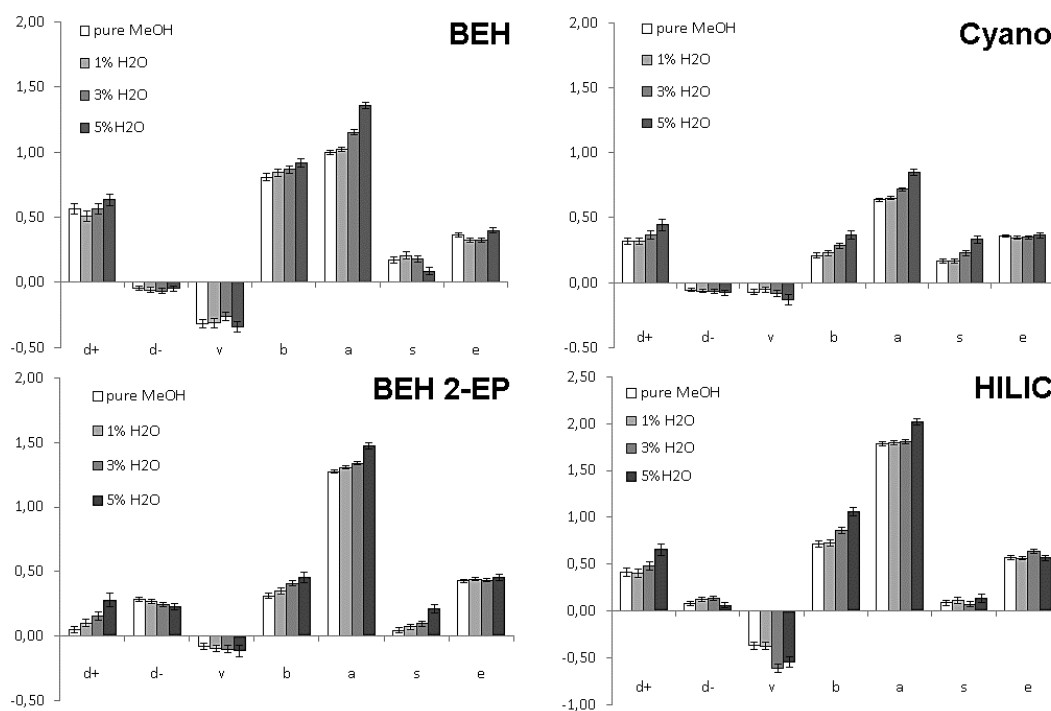

Figure 4. Effect of water additive on the characteristics of the chromatographic system.

CONCLUSION

The use of acids, bases and salts as additives has a significant effect on the retention of compounds possessing acidic or basic functional groups, with the greatest changes observed for the coefficients d^+ and d^-, reflecting the intensity of ionic interactions. The use of water as an additive has a complex effect on the retention of analytes, primarily increasing the role of interactions with the formation of hydrogen bonds, and for a number of stationary phases - enhancing dipole-dipole interactions.

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