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Reversed-phase high-performance liquid chromatography behavior of chaotropic counteranions

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Abstract

The retention behavior of inorganic liophilic anions in reversed-phase HPLC columns was studied. Usually, the addition of these ions to the mobile phase influences the retention of protonated basic analytes similar to the effect of amphiphilic ions (ion-pairing agents). The nature of this influence is the subject of this paper. HPLC retention of perchlorate (ClO_4^-), tetrafluoroborate (BF_4^-), and hexafluorophosphate (PF_6^-) ions was studied on six columns with different bonded phases including alkyl, phenyl and perfluorophenyl phases. The effect of the mobile phase ionic strength on the retention of liophilic ions was investigated. The influence of the type of organic modifier, acetonitrile and methanol, on the retention of inorganic ions was also studied and interpreted on the basis of adsorption from solutions. Semi-empirical expression is suggested for the description of the retention profile of studied liophilic ions versus the eluent composition. Significant retention of these ions is observed in acetonitrile–water eluents. Multilayer-type adsorption of the acetonitrile on the reversed-phase surface and its strong dispersive (or π–π) interactions with liophilic ions are responsible for significant retention of these ions. This accumulation of liophilic ions in the adsorbed layer on the surface of reversed-phase material introduces an electrostatic component in the retention of protonated basic analytes.

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1. Introduction

HPLC retention behavior of ionizable analytes and the influence of the type of buffer and other mobile phase additives have attracted increasing attention due to its importance for effective development of separation methods in pharmaceutical industry, where a majority of analytes are ionizable [1–4].

It was shown recently that type and concentration of the added salt could play a significant role in altering the retention of basic analytes. The effect of the counteranions on the HPLC behavior of basic compounds in reversed-phase was previously explained as an effect of analyte desolvation [5,6]

and described as a chaotropic effect when ion association of positively charged analyte with corresponding counteranion results in the disruption of the solvation shell, thus increasing apparent analyte hydrophobicity and retention. Mathematical description of this theory employs a Langmuir-type model of the retention dependence on the counteranion concentration. This model allows prediction of the change of the analyte retention with the change of the salt concentration. It was also observed that different type of counteranions have an inherently different effect on the retention of charged analytes. Further studies had shown the dependence of this increase in analyte retention at an equimolar concentration of counteranion is also dependent on the type of counteranion employed [7].

The disruption of the analyte solvation shell (chaotropic model) with the addition of the counteranions in the mobile phase does not explain the dramatic differences in the retention of basic analytes when different salts of similar size and charge were employed.

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Three distinct processes could be envisioned in the effect of chaotropic ions on the retention of basic analytes:

1. *Classic ion-pairing*: formation of essentially neutral ion-pairs and their retention according to the reversed-phase mechanism.
2. *Chaotropic*: counteranions disrupt the analyte solvation shell, thus increasing its apparent hydrophobicity and retention.
3. *Liophilic*: counteranions are adsorbed on the surface of the stationary phase, thus introducing an electrostatic component into the general hydrophobic analyte retention mechanism.

Gritti and Guiochon in their recent papers are essentially advocating for the domination of the first process [8–10]. They are explaining the counterion effect on the basis of the formation of a neutral ionic complex followed by its adsorption on the hydrophobic stationary phase. Similarity in adsorption behavior of anionic and cationic species is interpreted as a confirmation of their adsorption in the form of neutral complexes.

The retention of ionic components on reversed-phase columns is essentially regarded as ion-pair chromatography, which has been extensively developed by Horvath et al. [11] and Sokolowski [12,13] in the form of stochiometric adsorption of ionic species and by Hagglund and Stahlberg in the form of adsorption of ions and formation of an electrical double layer [14].

Most probably, all three mechanisms exist while one of them is dominating depending upon the eluent type, composition and adsorbent surface properties.

Recently, the effect of the type of counteranion on basic analyte retention was associated with the position of this ion in a Hoffmeister series [15]. Authors discuss only the effect of counteranion on the analyte solvation and briefly mention a possible ion-pairing mechanism with the formation of the ion-pairs in the solution.

All studies mentioned in this paper were performed using either methanol or acetonitrile as an organic modifier in the mobile phase. In the studies of the adsorption of the organic eluent modifier on the surface of the reversed-phase adsorbents, the formation of the thick adsorbed layer for acetonitrile was shown, while methanol adsorption from water formed a classical monomolecular adsorbed layer [16]. The thick adsorbed layer of acetonitrile provides a suitable media for the adsorption of liophilic ions on the stationary phase adding an electrostatic component to the retention mechanism, while monomolecular adsorption of methanol should not significantly affect adsorption of ions.

In this paper, we compare the chromatographic retention of inorganic anions themselves on different reversed-phase columns in various acetonitrile–water and methanol–water mixtures.

2. Experimental

2.1. Chemicals

The solvents used in this work are acetonitrile (Fisher Scientific, Fair Lawn, NJ, USA; HPLC grade), methanol (Fisher Scientific; HPLC grade), and HPLC grade water purified through Milli-Q system (Millipore). KPF_6, NH_4PF_6, KBF_4, and $KClO_4$ were $\geq 99.9\%$ purity (purchased from Aldrich, Milwaukee, WI, USA).

2.2. Columns

Columns used in this study are shown in Table 1.

Column void volume was measured by the injection of deuterated acetonitrile in 100% acetonitrile eluent and deuterated water in 100% water eluent with RI detection. The relative percent difference in both measurements for each column was less than 0.3%. Detailed description and justification of this method for void volume measurements is described in a previous paper [17].

2.3. Apparatus

Retention of anions was measured using Alliance-EZ-Q LC/MS (Waters, Millford, MA, USA) system equipped with an electrospray interface operated in negative ion mode with Mass Lynx data acquisition system. A 1 μL injection of a 100 ppb solution of corresponding salt in aqueous portion of the eluent was used. Flow rate of 1 mL/min was used and approximately 1/9 split of the column effluent (Upchurch Scientific splitter, WA, USA) was used to transfer 10% of the flow into the electrospray interface and 90% into the parallel diode array detector.

Fig. 1 shows mass spectra for all ions studied. MS parameters were optimized for maximum response of the characteristic ion. Single ion monitoring mode was used for measurements of all retention dependencies. Molecular ions were selected as characteristic ions for detection in SIM mode (shown in Table 2) together with control ions used for identification on the basis of abundance ratio.

2.4. Adsorbed layer volume

The amount of acetonitrile accumulated on the surface of studied reversed-phase column was calculated on the basis of the minor disturbance peak retention dependence on the acetonitrile composition measured for each column according to the principles discussed in previous paper [16]. Here, we briefly describe the calculation procedure and present necessary experimental data, since they may be useful for other studies performed on similar columns.

2.5. Determination of the mass of SiO_2 in the column

As we discussed in [17], the determination of specific surface area of the modified adsorbent possesses a significant

Table 1
Column parameters

Column	Column length (mm)	S_{SiO_2} (m²/g)	V_0 (mL)	V_{p,SiO_2} (mL/g)	$V_{p,mod}$ (mL/g)	V_{ip} (mL)	d_b (μmol/m²)	MW_{ligand} (g/mol)	f_{corr}	m_{total} (g)	m_{SiO_2} (g)
Luna-C18	100	392	1.088	0.96	0.412	0.636	3.1	311	0.73	1.097	0.796
Luna-PhHex	100	357	1.13	0.96	0.508	0.615	3.79	219	0.77	1.014	0.782
Allure-PFP	100	460	1.1	1.097	0.515	0.584	4.01	267	0.67	1.002	0.671
Allure-C18	150	460	1.44	1.097	0.508	0.86	3.5	311	0.67	1.142	0.761
Zorbax C8	75	180	0.725	0.6	0.35	0.4	3.4	311	0.84	0.929	0.780

Luna-C18 is Luna-C18, 100 mm × 4.6 mm (Phenomenex, Torrance, CA, USA); Luna-PhHex is Luna-Phenyl-Hexyl, 100 mm × 4.6 mm (Phenomenex, Torrance, CA, USA), Allure-PFP and Allure-C18 were both 4.6 mm i.d. and lengths were 100 and 150 mm, respectively (Restek Corp., Bellefonte, PA, USA); Zorbax C8 is Zorbax-Eclipse-XDB-C8, 75 mm × 4.6 mm (Agilent, Little Falls, DE, USA).
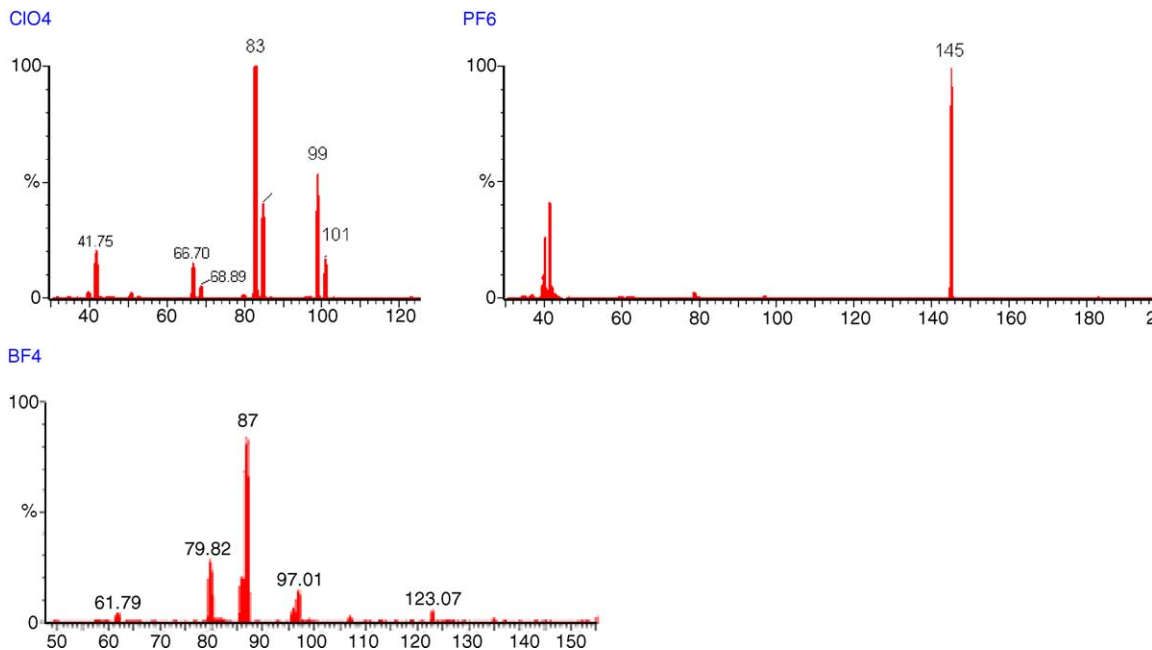

Fig. 1. Electrospray negative ionization spectra of ClO_4^-, PF_6^-, and BF_4^- ions.

uncertainty: (a) due to the surface roughness (variation in the conformation of bonded chains); and (b) due to the uncertainty of the nitrogen molecular area on the hydrophobic surface (variation from 13.6 or 16.4 Å² on silica to 21 Å² on alkyl surface). More convenient and universal approach is to refer all effects to the unit of the surface of the base silica used for a particular adsorbent. This allows the cross comparison between different columns with different bonded phases, and surface area values measured for bare silica are more reliable.

In previous papers [16,17], we had shown that the bonded layer is always in its densest conformation. This allows for the calculation of the exact mass of adsorbent in the column by measuring column void volume, V_0 (mL/column), interpar-

Table 2
Masses detected in ESI negative ion mode

Ion	Molecular ion	ID ion	Abundance ratio
PF_6^-	145	No fragments	–
BF_4^-	87	No fragments	–
ClO_4^-	99	83	0.59

ticle volume, V_{ip} (mL/column), and the specific pore volume of modified adsorbent, $V_{p,mod}$ (mL/g):

$$m_{mod} = \frac{V_0 - V_{ip}}{V_{p,mod}} \tag{1}$$

Mass of the base silica in the column is a fraction of the mass determined by Eq. (1). Corresponding correction coefficient could be calculated if the bonding density for used adsorbent is known:

$$f = \frac{1}{1 + S_{SiO_2} d_b MW_{ligand}} \tag{2}$$

where S_{SiO_2} is the specific surface area of base silica (m²/g), d_b is the bonding density (μmol/m²), and MW_{ligand} is the molecular weight of bonded ligands. Adsorbent and column specific parameters are given in Table 1.

2.6. Acetonitrile excess adsorption isotherms

Retention dependencies of acetonitrile–water minor disturbance peaks on the acetonitrile content in the eluent for the

Table 3
Retention of minor disturbance peaks (V_R) on studied columns

MeCN (%, v/v)	Retention volume of minor disturbance peak (mL)				
	Luna-C18	Luna-Phenyl-Hexyl	Allure-PFP	Allure-C18	Zorbax-Eclipse-XDB-C8
0	2.206	2.517	3.327	2.754	1.180
1	1.825	2.033	2.496	2.353	1.055
5	1.509	1.617	1.925	1.947	0.942
10	1.421	1.495	1.683	1.821	0.903
20	1.327	1.361	1.461	1.716	0.856
30	1.197	1.225	1.241	1.573	0.784
40	1.028	1.072	0.947	1.358	0.703
50	0.895	0.924	0.776	1.220	0.634
60	0.823	0.848	0.701	1.142	0.588
70	0.812	0.838	0.693	1.144	0.595
80	0.866	0.899	0.762	1.204	0.624
90	0.949	0.994	0.929	1.295	0.663
95	1.005	1.058	0.982	1.373	0.695
99	1.186	1.296	1.091	1.531	0.757
100	1.367	1.815	1.789	2.148	0.835

whole concentration range (0–100% (v/v) MeCN) are shown in Table 3.

Excess adsorption isotherm of acetonitrile from water was calculated using retention dependencies shown in Table 3 with the following equation:

$$\Gamma(c) = \frac{1}{S_{tot}} \int_0^c (V_R(c) - V_0)\, dc \qquad (3)$$

where S_{tot} is the total surface of the adsorbent in the column (silica surface). Fig. 2 shows an overlay of all five excess adsorption isotherms. As it was discussed in [16], by the nature of the excess isotherm, the linear decrease of the excess adsorption in the region of high concentration of acetonitrile represents the situation when surface adsorption forces are saturated with amount of the adsorbate already adsorbed and the increase in the equilibrium concentration of the bulk solution does not result in any additional adsorption. The amount of the adsorbate excessive to that in the bulk equilib-
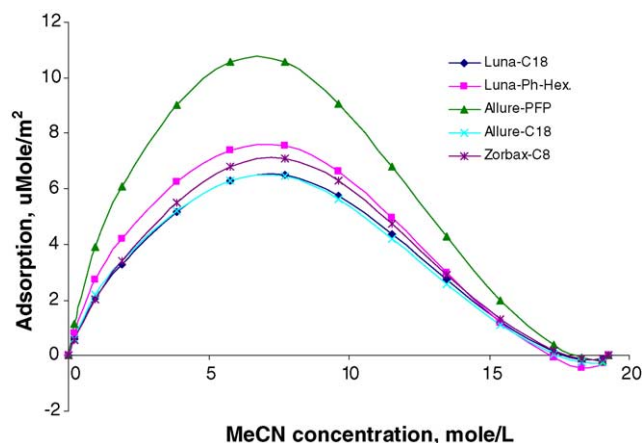
rium solution is decreasing in this region. This allows for the calculation of the maximum adsorption capacity:

$$a_{max} = \Gamma(c) - c \min\left[\frac{d\Gamma(c)}{dc}\right] \qquad (4)$$

where $\min[d\Gamma(c)/dc]$ is the minimum of the slope of excess adsorption isotherm. Eq. (4) is essentially the expression for the intercept of a linear function. As we discuss in [16], this value represents the maximum adsorption capacity of the adsorbed layer. These values are shown in Table 4 for all columns.

2.7. Adsorbed layer thickness

Phenyl modified columns show maximum adsorption capacity with the highest value for Allure-PFP column. Significant electron density on the fluorinated phenyl rings on the surface intensifies dispersive interactions with acetonitrile, which also has four π-electrons and could translate electrostatic fluctuations (basis for dispersive forces). This effect can explain the formation of the thick adsorbed layer of acetonitrile on the surface. The thickness of adsorbed layer shown in Table 4 is calculated for the flat surface, which is sufficient for the purposes of this paper. In reality, actual thickness is higher due to the curvature of the adsorbent inner pores and due to the presence of the boned layer. Thickness of the adsorbed layer shown in Table 4 essentially has a meaning of the volume of adsorbed MeCN per unit of surface. Total adsorption of acetonitrile on the surface could be calculated in this model as:

$$a_{tot}(c) = \Gamma(c) + \tau c \qquad (5)$$

Function of total adsorption of acetonitrile multiplied by the MeCN molecular weight and divided by the acetonitrile density essentially represents the function of the actual adsorbed layer thickness on the concentration of MeCN on the bulk

Fig. 2. Excess adsorption isotherms of acetonitrile from water on all studied columns shown in Table 1.

Table 4
Maximum adsorption capacity for acetonitrile and maximum adsorbed layer thickness

	Luna-C18	Luna-Phenyl-Hexyl	Allure-PFP	Allure-C18	Zorbax-Eclipse-XDB-C8
a_{max} (μmol/m^2)	14.42	16.96	22.47	14.061	15.96
τ (Å)	8.67	10.4	13.5	8.55	9.7

solution:

$$\tau(c) = a_{tot}(x) \frac{MW_{MeCN}}{d_{MeCN}} \qquad (6)$$

Acetonitrile adsorbed layer thicknesses calculated according to Eq. (6) are shown in Fig. 9 as a function of mole fraction of MeCN (MeCN mole fraction 0.2 corresponds to 42% (v/v) concentration).

3. Results and discussion

3.1. Retention profile of anions in acetonitrile–water and methanol–water systems

The difference in the adsorption behavior of acetonitrile and methanol [16] on reversed-phase adsorbents described previously suggested that liophilic ions capable for dispersive type interactions would have significant differences in their retention from corresponding mobile phases. We indeed observed a marked difference in the retention of chaotropic anions themselves from acetonitrile–water and methanol–water mobile phases (Fig. 3)

In pure water, the slight retention of all ions is observed. An increase of the acetonitrile content led to the dramatic increase of the retention of all ions reaching the maximum at approximately 10% (v/v) of acetonitrile. PF_6^- retention increases the most. Further increase of the acetonitrile content led to the gradual decrease of the retention of all anions. Shape of the retention dependence in this concentration region (above 10% (v/v) of organic) can be attributed to the normal retention decay with increase of organic modifier in the eluent.

Retention of the same ions at similar chromatographic conditions and on the same column but from methanol–water eluent shows a dramatically different dependence (Fig. 3, right panel). Overall retention of these ions is lower and gradually decreases with increase of the organic content. Methanol forms only a monomolecular adsorbed layer on the surface of reversed-phase adsorbent and is mainly a proton donor, while acetonitrile forms a thick adsorbed layer [16] and with its four π-electrons can have strong dispersive interactions with studied ions.

Marked difference in the retention behavior of studied ions between acetonitrile–water and methanol–water eluents would indicate a principal difference in their retention mechanism. This may be associated with the differences in the possible interactions of ions containing a high degree of charge delocalization and significant polarizability with the organic adsorbed layer close to the adsorbent surface. For example, acetonitrile molecules with four π-electrons are prone to π–π type interactions, and thus could interact with PF_6^- ions. Methanol molecules on the other hand are primarily weak hydrogen donors and may not exhibit such interactions.

3.2. Effect of ionic strength

The retention of these ions is dependent on the ionic strength of the mobile phase. Figs. 4 and 5 show the dependencies of PF_6^- retention as a function of the mobile phase ionic strength at different concentrations of organic modifier.
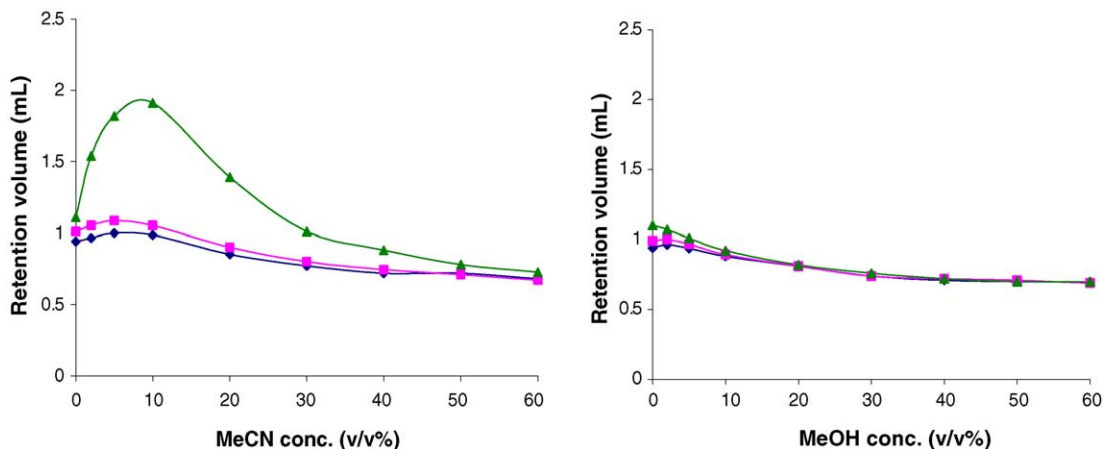

Fig. 3. Representative retention dependence of PF_6^- (triangles), ClO_4^- (squares), and BF_4^- (diamonds) anions on Zorbax-Eclipse-XDB-C8 column (row 5 in Table 1) from MeCN–water (left) and MeOH–water (right) eluents.

Either ammonium chloride or ammonium acetate was used for ionic strength adjustment. Employment of chloride or acetate ions shows no difference in the ionic strength effect on PF_6^- retention.

An increase of the PF_6^- retention with the increase of mobile phase ionic strength could possibly be explained on the basis of chaotropic theory. An increase of the counterion concentration (NH_4^+) [18] can lead to the disruption of the solvation shell of PF_6^- ions and to corresponding increase of its retention. This dependence follows the Langmuir-type isotherm (shown in Figs. 4 and 5) at all mobile phase compositions. Mathematical description of this isotherm is given in [6].

Retention behavior of studied ions in pure organic–water mixtures without ionic strength adjustment was essentially erratic and irreproducible. In some cases, all ions eluted with exclusion volume exhibiting an ionic exclusion effect. Increase of the ionic strength to just 1 mM led to a stabilization of the retention reproducibility of all studied ions.

All further studies were performed at 10 mM mobile phase ionic strength (calculated for aqueous portion of the mobile phase), since at this concentration the leveling of the ionic strength dependence was observed (Figs. 4 and 5).
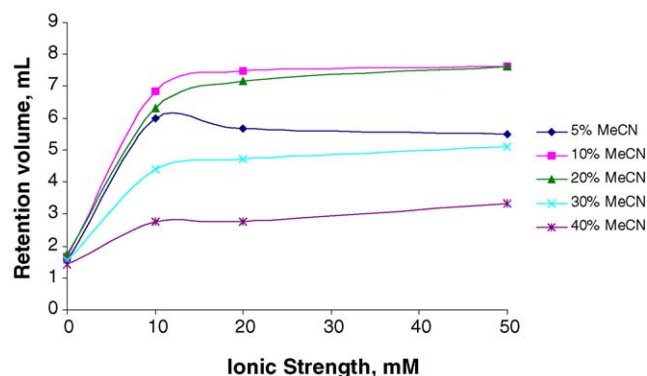

Fig. 4. Retention of PF_6^- vs. mobile phase ionic strength at different organic composition on Allure-C18 column.
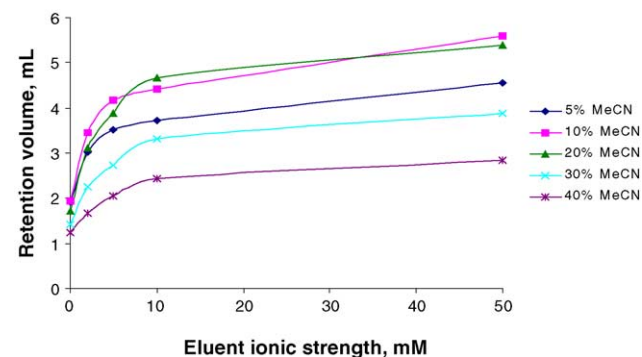

Fig. 5. Retention of PF_6^- vs. mobile phase ionic strength at different organic composition on Luna-Phenyl-Hexyl column.

3.3. Comparison of different ions

At all mobile phase conditions with acetonitrile–water, PF_6^- ion exhibits the greatest retention and this is the most liophilic ion in Hoffmeister series. This ion has the highest degree of charge delocalization and highest polarizability, which facilitates its possible dispersive (or van der Waals) interactions. These properties allow this ion to interact with acetonitrile molecules, which has significant π-electron density. Other studied ions have similar properties but their ability for dispersive interactions is lower. At acetonitrile concentrations up to 20% (v/v), all ions exhibit a maximum retention.

The retention of all ions decreases with the increase of the methanol concentration in the mobile phase from 0 to 50% (v/v) methanol. This contradicts the idea of the retention of these ions in the form of neutral ion-paired complexes. With an increase in the methanol concentration, there is consequent decrease of the bulk dielectric constant of the eluent, which essentially would facilitate the formation of ion-pairs [19] and thus should lead to the relative increase of their retention.

3.4. Comparison of different columns

Retention behavior of chaotropic ions was measured on columns with different bonded phases and different base material. Information on the column parameters is given in Table 1. General retention profile as a function of acetonitrile–water composition for all studied ions on all columns are similar and show maximum in the region between 10 and 20% (v/v) of acetonitrile in water (Fig. 6).

Further increase of the organic content (acetonitrile) in the mobile phase resulted in the exponential decrease of the retention of anions. The retention dependencies of the same anions on the same columns from methanol–water mixtures are completely different (Fig. 7). Overall retention is approximately on the order of magnitude lower and shows a monotonous decrease with increase of organic content.
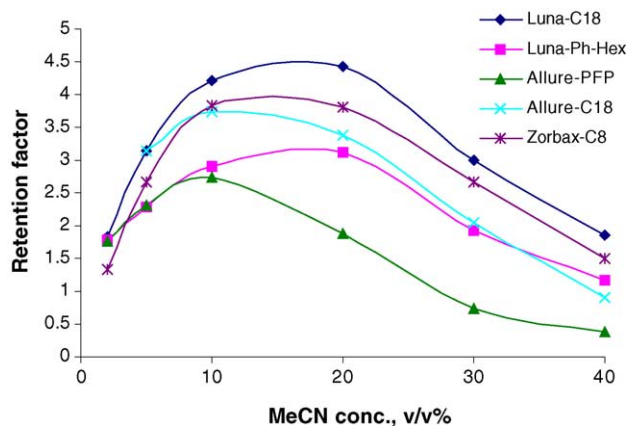

Fig. 6. Retention of PF_6^- ion on various columns from acetonitrile–water eluent. Ionic strength of the aqueous portion of the mobile phase was maintained at 10 mM.
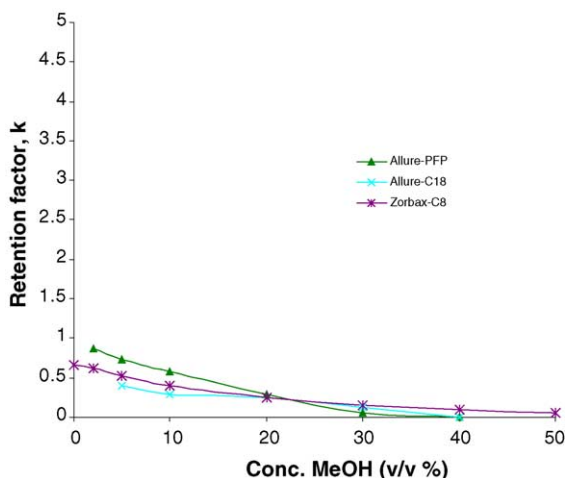
Fig. 7. Retention of PF_6^- ion on various columns from methanol–water eluent.

3.5. Retention model

Retention of studied ions in acetonitrile–water mixtures is non-monotonous versus the organic modifier content with a maximum around 20% (v/v) of MeCN in water. Retention dependence on the eluent composition in reversed-phase HPLC generally shows an exponential decay with the increase of the organic modifier concentration. This is usually described in the following form: $\ln(k') = a + xb$, where x is the eluent composition, and a and b are constants. This relationship has a thermodynamic background since in the partitioning retention model the retention factor is proportional to the distribution equilibrium constant, which in turn is an exponent of the excessive free Gibbs energy of the analyte in the chromatographic system. Excessive free Gibbs energy is the difference of the analyte potential in the stationary phase and its potential in the eluent. Assuming ideal behavior of the eluent and low analyte concentration, we can represent the analyte potential in the eluent as a linear function of the eluent composition.

It was shown that acetonitrile forms a thick adsorbed layer on the surface [16,20] of the stationary phase. The accumulation of acetonitrile on the surface resembles a Langmuir-type isotherm. Methanol, on the other hand [16], forms only a monomolecular adsorbed layer on the stationary phase surface. The thick acetonitrile layer adsorbed on the bonded phase surface acts as a pseudo-stationary phase. Recently, the theoretical model for the analyte retention based on dual adsorption–partitioning mechanism was suggested [16]. This model describes the retention as a superposition of two processes—partitioning into the acetonitrile layer and adsorption on the surface of the bonded phase.

The interpretation of the excess adsorption isotherms allows for an estimation of the total amount of the active component of a binary mixture (hydro-organic eluent) on the adsorbent surface, as well as allows for an estimation of the adsorbed layer thickness (organic), assuming a constant mo-
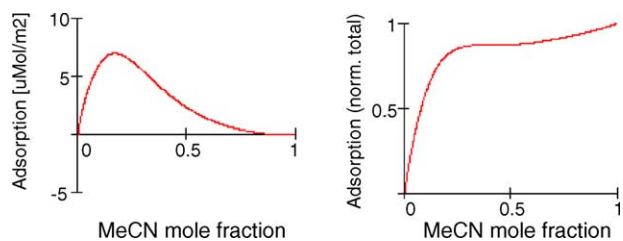

Fig. 8. Acetonitrile excess adsorption isotherm from water on Zorbax-Eclipse-XDB-C8 adsorbent (left); normalized filling of adsorbed layer (right).

lar volume of the organic molecules. Methodology for these calculations are discussed elsewhere [16].

Fig. 8 shows an excess adsorption isotherm of acetonitrile from water measured with minor disturbance method [21] method on a Zorbax-Eclipse-XDB-C8 (row 5 in Table 1). Right panel of same figure shows the normalized dependence of the adsorbed layer filling with acetonitrile molecules with increasing acetonitrile concentration.

Average maximum thickness of the acetonitrile adsorbed layer (estimated from the excess adsorption isotherm using method described in [16,20]) is approximately 9–11 Å (not corrected for the surface curvature). In the region where there is a low acetonitrile content in the mobile phase (below 10% (v/v)), the adsorbed layer has a relatively small amount of acetonitrile molecules and thus retention of chaotropic ions is weak. With the increase of the acetonitrile content in the mobile phase, there is a rapid increase in the amount of acetonitrile adsorbed (Fig. 9). This leads to a corresponding increase in the retention of the anions. This process essentially levels off at 30–42% (v/v) of acetonitrile in the mobile phase (0.15–0.2 in mole fractions). On the other hand, an increase of the acetonitrile content in the mobile phase also leads to a corresponding increase of π–π interactions of MeCN molecules with chaotropic ions in the mobile phase consequently decreasing their retention. Since at 30% (v/v) of MeCN in the eluent, the formation of adsorbed layer is complete there will
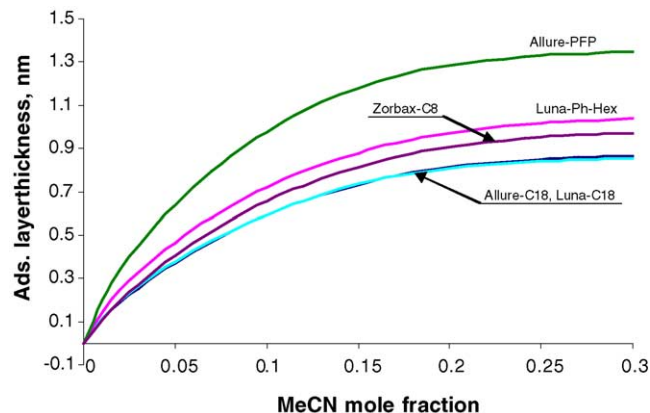

Fig. 9. Variation of the acetonitrile adsorbed layer thickness with its molar fraction in the mobile phase. Mole fraction region shown corresponds approximately from 0 to 53% (v/v) of MeCN in water.

be no further increase of the retention of the chaotropic anion and concurrently there will be a decrease of the retention due to the increase in interactions of the studied ions with MeCN in the mobile phase. This could be described mathematically as a superposition of two processes.

As a basis, we consider the retention model described in [16]. Basic retention equation is:

$$V_R(c_{el}) = V_0 + (K_p(c_{el}) - 1)V_{ads} + SK_H K_p(c_{el}) \qquad (7)$$

where $V_R(c_{el})$ is the retention of analyte ions as a function of the eluent composition, V_0 is the void volume, $K_p(c_{el})$ is the equilibrium constant for the distribution of the analyte ions between the eluent and adsorbed layer, V_{ads} is the volume of adsorbed layer, S is the adsorbent surface area, and K_H is the adsorption equilibrium constant for analyte ions adsorption from neat acetonitrile on the corresponding stationary phase.

The distribution constant, $K_p(x)$, by definition of thermodynamic equilibrium could be expressed as:

$$K_p(x) = A \exp\left(\frac{\Delta G}{RT}\right) \qquad (8)$$

where A is a proportionality coefficient, and ΔG is the free Gibbs energy of the analyte distribution between the mobile and stationary phases. This distribution is eluent composition dependant and the simplest description of this dependence will be a linear dependence of the free Gibbs energy versus the molar fraction of the acetonitrile in the mobile phase. Function (8) could be then written in the form:

$$K_p(x) = A \exp\left(\frac{B - xC}{RT}\right) \qquad (9)$$

where B is essentially the difference between the free Gibbs energy of analyte ions in acetonitrile phase and water phase, which we further denote as ΔG_{MeCN}, and C is essentially the span of the analyte ions free Gibbs energy through the whole eluent concentration range, which we denote as ΔG_{el}.

Volume of adsorbed layer (V_{ads}) is also an eluent composition dependent parameter, which could be calculated from the excess adsorption isotherm of acetonitrile (or methanol) from water. Fig. 9 shows the dependencies of the acetonitrile adsorbed layer thickness for all studied columns. Detailed description of these calculations is given in Section 2.

Overall expression for the description of the retention dependencies of analyte ions versus eluent composition will have only four unknowns and allow numerical approximation of experimental retention data (shown as a function of the mole fraction of organic eluent component):

$$V_R(x) = V_0 - V_{ads}(x) + A \exp\left(\frac{\Delta G_{MeCN} - x\,\Delta G_{el}}{RT}\right)$$
$$+ (V_{ads}(x) - SK_H) \qquad (10)$$

Essentially, Eq. (10) describes the retention volume of the analyte as a sum of the mobile phase volume ($V_0 - V_{ads}$, assuming that adsorbed acetonitrile layer is stagnant) and an energetic term which describes analyte partitioning into
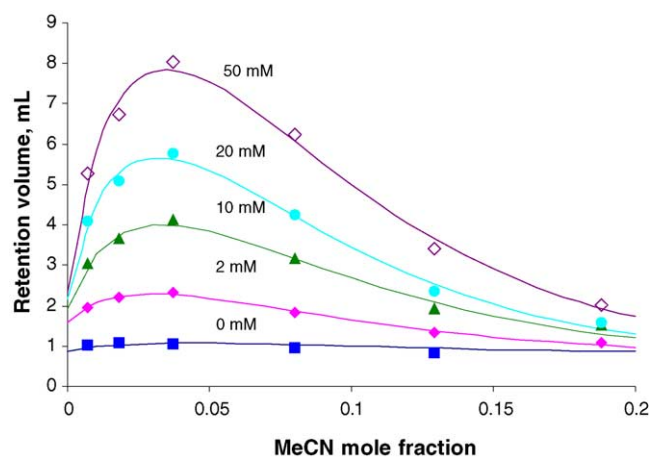

Fig. 10. Experimental (points) and mathematical model (lines) dependencies of PF_6^- retention on Allure-PFP (perfluorinated propyl-phenyl phase) column vs. the acetonitrile composition (shown in molar fractions) at different ionic strengths (0, 2, 10, 20, and 50 mM adjusted with NH_4Cl).

the adsorbed layer and its adsorption on the stationary phase surface. Volume of the adsorbed layer, as we discussed above (Fig. 9) is also a function of the acetonitrile concentration in the mobile phase.

In our experiments, we determine the retention dependencies of PF_6^- ions on the eluent composition and salt concentration at constant temperature. At these conditions, the influence of parameter A and ΔG_{MeCN} in Eq. (10) are indistinguishable. In the optimization, we were using the following form of Eq. (10):

$$V_R(x) = V_0 - V_{ads}(x) + \exp\left(\frac{\Delta G_{MeCN} - x\,\Delta G_{el}}{RT}\right)$$
$$\times (V_{ads}(x) - SK_H) \qquad (11)$$

Excess adsorption isotherm of acetonitrile from water, void volume values as well as the thickness of adsorbed layer were measured for each column and were determined according the procedure described in [16]. Non-linear optimization was made in MathCAD-2001 (MathSoft Inc., Cambridge, MA, USA) software using a generalized *genfit* function (essentially minimization of the gradients on a multidimensional surface, where number of dimensions corresponds to the number of parameters).

Function describes experimental values with 0.999 regression coefficient (non-linear) and between 0.1 and 3.5% R.S.D. range.

Figs. 10–13 show retention dependencies of PF_6^- ions versus acetonitrile molar fraction at different ionic strength of the mobile phase on columns with different types of bonded phases: Allure-PFP (perfluorinated propyl-phenyl) (Fig. 10), Luna-Phenyl-Hexyl (Fig. 11), Allure-C18 (Fig. 12), and Zorbax-Eclipse-XDB-C8 (Fig. 13) phases. All these phases had shown similar retention profile for PF_6^- over a wide range of acetonitrile concentrations. Solid lines in Figs. 10–13 are a functional description of experimental dependencies (points represent experimentally measured
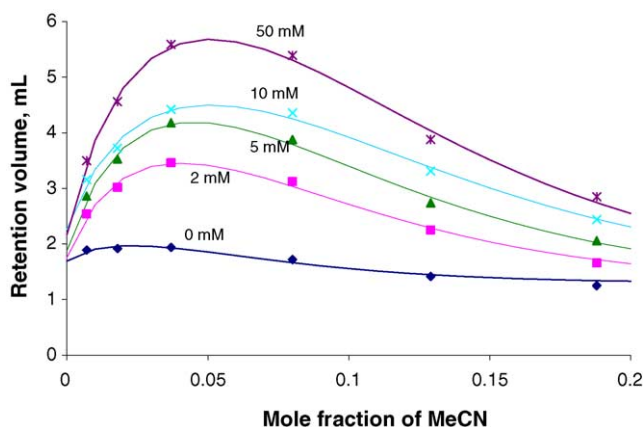
Fig. 11. Experimental (points) and mathematical model (lines) dependencies of PF_6^- retention on Luna-Phenyl-Hexyl (hexyl-phenyl phase) column vs. the acetonitrile composition (shown in molar fractions) at different ionic strengths (0, 2, 5, 10, and 50 mM adjusted with NH_4Cl).

values) obtained from non-linear regression optimization of Eq. (11). Calculated optimization parameters are shown in Tables 5–8 for corresponding columns.

K_H is the equilibrium constant describing the adsorption of studied ions on the surface of the bonded phase. This value is very low for all types of surfaces and at all ionic strengths studied, which indicates the very weak (if existent) interactions of PF_6^- ions with stationary phase surface.

Analyte partitioning into the acetonitrile adsorbed layer is described by the exponential term of Eq. (11). As it could be seen from Tables 5–8, parameters ΔG_{MeCN} and ΔG_{el} are essentially independent on the mobile phase ionic strength. Parameter ΔG_{MeCN} represents the interaction energy of studied ions with acetonitrile and for all column it is on the level of 7–12 kJ/mol, which is typical for dispersive interactions [22].
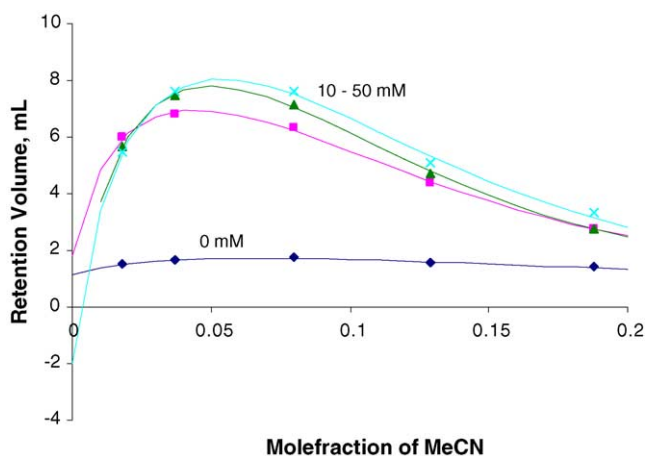

Fig. 12. Experimental (points) and mathematical model (lines) dependencies of PF_6^- retention on Allure-C18 column vs. the acetonitrile composition (shown in molar fractions) at different ionic strengths (0, 10, 20, and 50 mM adjusted with NH_4Cl).
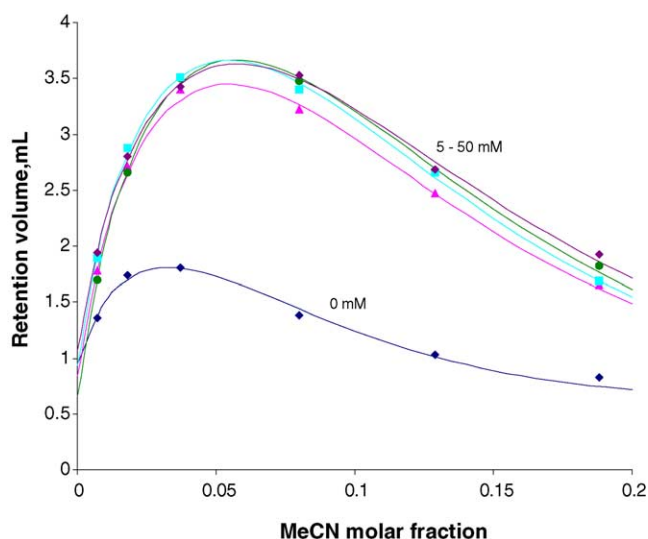

Fig. 13. Experimental (points) and mathematical model (lines) dependencies of PF_6^- retention on Zorbax-Eclipse-XDB-C8 column vs. the acetonitrile composition (shown in molar fractions) at different ionic strengths (0, 5, 10, 20, and 50 mM adjusted with NH_4Cl).

Coefficient ΔG_{el} has a meaning of energetic span of partitioning constant in the whole concentration region and it reflects the excessive interactions of studied ions with water and acetonitrile as well as structural organization of molecules.

Suggested phenomenological model describes the retention of PF_6^- ions on different reversed-phase columns very well. Average deviation of calculated values from experimentally measured is on the level of 1%, which confirms that indeed a superposition of several processes govern the retention of liophilic ions in acetonitrile–water systems. The interaction of these ions with the bonded phase surface is negligible as it could be seen from the coefficients K_H for all four columns (Tables 5–8) and thus adsorbed layer of acetonitrile is the major factor in the retention of studied ions.

Table 5
Calculated coefficients for PF_6^- retention on Allure-PFP column

Coefficient	Ionic strength				
	0 mM	2 mM	10 mM	20 mM	50 mM
ΔG_{MeCN} (kJ/mol)	0.2	6.5	8.6	10.1	11
ΔG_{el} (kJ/mol)	2.7	39.7	39.7	45.4	42.7
K_H	0	0.0001	0.00009	0.00006	0.00005
R.S.D. (%)	3.4	1.9	3.0	2.4	2.9

Table 6
Calculated coefficients for PF_6^- retention on Luna-Phenyl-Hexyl column

Coefficient	Ionic strength				
	0 mM	2 mM	5 mM	10 mM	50 mM
ΔG_{MeCN} (kJ/mol)	4.8	9	9.6	9.6	10.5
ΔG_{el} (kJ/mol)	26.1	33.4	31.4	26.8	28.9
K_H	0.0003	0.00005	0.00005	0.00006	0.00004
R.S.D. (%)	1.1	0.6	0.09	0.5	0.6

Table 7
Calculated coefficients for PF_6^- retention on Allure-C18 column

Coefficient	Ionic strength			
	0 mM	10 mM	20 mM	50 mM
ΔG_{MeCN} (kJ/mol)	3.7	11.2	11.8	12.2
ΔG_{el} (kJ/mol)	20.2	34.2	34.1	34.3
K_H	0.00004	0	0	0
R.S.D. (%)	1.0	0.8	0.9	1.2

Table 8
Calculated coefficients for PF_6^- retention on Zorbax-Eclipse-XDB-C8 column

Coefficient	Ionic strength				
	0 mM	5 mM	10 mM	20 mM	50 mM
ΔG_{MeCN} (kJ/mol)	10.7	11.7	11.9	11.4	11.4
ΔG_{el} (kJ/mol)	51.2	32.5	31.4	28.6	28.6
K_H	0.00003	0.000008	0.000004	0.00003	0.00003
R.S.D. (%)	2.1	1.7	0.9	0.9	1.1

Monomolecular character of methanol adsorption suggests simple competitive adsorption mechanism for the retention of any analyte in reversed-phase columns. Multilayered character of acetonitrile adsorption creates a pseudo-stationary phase of significant volume on the surface, which acts as a suitable phase for the ion accumulation. In the low organic concentration region (from 0 to 30% (v/v) of acetonitrile), studied ions show significant deviation from the ideal retention behavior (decrease in ion retention with increase in acetonitrile composition) due to the formation of the acetonitrile layer and significant adsorption of the chaotropic anions was observed. This creates an electrostatic potential on the surface, which provides an additional retentive force for protonated basic analytes.

4. Conclusion

In previous papers [7,23], we show that different chaotropic additives have different effect on the retention of basic analytes. This difference could not be explained on the basis of chaotropic theory as it was described in [6]. Chaotropic effect as a simple disruption of the analyte solvation shell should lead to the identical retention plateau when different chaotropic agents are employed. Accumulation of

the PF_6^- ions in the layer of acetonitrile adsorbed on the surface of the stationary phase explains its specific effect on the significant retention increase of protonated basic analytes compared to weaker chaotropic anions such as perchlorate which are not as strongly adsorbed in the acetonitrile layer. Moreover, there should be a significant difference in the effect of the same chaotropic additives on interaction with protonated basic analytes employing acetonitrile- and methanol-based mobile phases. Existence of the multilayered adsorption of acetonitrile leads to the significant increase of a protonated analyte's retention, as opposed to methanol which has only monolayer-type adsorption behavior and should lead to small differences in the analyte retention.

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