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The role of the dual nature of ionic liquids in the reversed-phase liquid chromatographic separation of basic drugs

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ABSTRACT

The cationic nature of basic drugs gives rise to broad asymmetrical chromatographic peaks with conventional C18 columns and hydro-organic mixtures, due to the ionic interaction of the positively charged solutes with the free silanol groups on the alkyl-bonded reversed-phase packing. Ionic liquids (ILs) have recently attracted some attention to reduce this undesirable silanol activity. ILs are dual modifiers (with a cationic and anionic character), which means that both cation and anion can be adsorbed on the stationary phase, giving rise to interesting interactions with the anionic free silanols and the cationic basic drugs. A comparative study of the performance of four imidazolium-based ILs as modifiers of the chromatographic behaviour of a group of β-blockers is shown. The ILs differed in the adsorption capability of the cation and anion on C18 columns. Mobile phases without additive and containing a cationic (triethylamine, TEA) or anionic (sodium dodecyl sulphate, SDS) additive were used as references for the interpretation of the behaviours. The changes in the nature of the chromatographic system, at increasing concentration of the additives, were followed based on the changes in retention and peak shape of the β-blockers. The silanol suppressing potency of the additives, and the association constants between the solutes and modified stationary phase or additive in the mobile phase, were estimated. The study revealed that SDS and the ionic liquid 1-hexyl-3-methylimidazolium tetrafluoroborate are the best enhancers of chromatographic peak shape among those studied.

1. Introduction

Separation in conventional reversed-phase liquid chromatography (RPLC) is based mainly on the hydrophobic interaction of solutes with the alkyl-bonded layer of the stationary phase, together with the solving power of the organic solvent. Additional ion-exchange interaction with free silanols on the packing may also take place with positively charged solutes, as is the case of basic drugs. Since this is a slow process, it results in poor peak shape [1–4]. The use of different types of additives in the hydro-organic mobile phases is an extended strategy to reduce or suppress the undesirable effects of residual silanols on the chromatographic performance of basic drugs [5,6]. This practice has the advantage of using conventional alkyl-bonded silica phase columns (octyl or octadecyl).

Additives traditionally used in RPLC as blocking agents have an ionic (cationic or anionic) nature, and can interact with the stationary phase through two main mechanisms, which can take place simultaneously: (i) direct electrostatic association with silanol sites (for cationic additives), which blocks ion-exchange processes with solutes, and (ii) hydrophobic interaction with the alkyl-bonded stationary phase creating a charged bilayer. These interactions reduce the pore size and affect the penetration depth of solutes into the bonded phase. Amines, such as triethylamine (TEA) [5,6], and anionic surfactants, such as sodium dodecyl sulphate (SDS) [7], are typical examples of mobile phase additives with cationic and anionic character, respectively, that modify the stationary phase through one or both described mechanisms, yielding changes in retention and enhancing the peak shape of basic drugs.

Ionic liquids (ILs) have recently been used as mobile phase modifiers in RPLC, with similar effects to those observed for amines and anionic surfactants [8–17]. ILs can be outlined as salts or solvents. By essence, they are made of cations and anions, and have the special feature of a low melting temperature (compounds arbitrarily classified as ILs melt at or below 100 °C). As solvents, ILs have a non-molecular nature with interesting features: mainly low volatility and flammability, and high thermal stability [18]. Due to these properties, in the 90s, ILs started to be considered as benign or green solvents that could candidate to replace pollutant organic solvents.

In the analytical chemistry field, ILs have been mainly used as stationary phases in gas chromatography [19], and as commented, as silanol blocking agents in RPLC. As mobile phase additives, they behave just as dissociated salts. ILs have the particular feature that

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both cation and anion are able to interact with the stationary phase, which confers them a dual character [13,14,17]. This increases the complexity of the chromatographic system. On the other hand, the wide variety of cation–anion combinations complicates the selection of the most convenient IL (more than two thousand ILs are known currently).

The potency of ILs as peak shape enhancers has been extensively reviewed, and mechanisms involving ion-pairing and solute interaction with the IL modified stationary phase have been discussed [17,20–22]. However, the narrow range of IL concentrations usually employed, and the attribution of a role mainly focused on silanol blocking by the cation in the IL, have resulted into a loss of information on the combined effect of the two opposite charged ions on the retention and peak shape of basic compounds. In this work, the performance of mobile phase ionic modifiers of different nature: cationic (TEA), anionic (SDS) and dual (ILs), added to acetonitrile–water mixtures, using a Kromasil C18 column, is examined for the separation of a set of basic drugs. Changes in the nature of the chromatographic system are interpreted through the changes in retention, peak broadening and tailing, the estimation of the binding capacity to silanols, and the association constants between solutes and the modified stationary phase, or the additive in the mobile phase. The goal of the study was to ultimately shed light into the effect of the cation/anion combination in ionic liquids and how these affect solute retention and peak shape.

2. Experimental

2.1. Reagents

The following seven basic drugs were used as probe compounds: acebutolol, atenolol, metoprolol, timolol (Sigma, St. Louis, MO, USA), celiprolol (Rhône-Poulenc Rorer, Alcorcón, Spain), esmolol (Du Pont-De Nemours, Le Grand Saconnex, Switzerland), and oxprenolol (Ciba-Geigy, Barcelona, Spain). The drugs were dissolved in a small amount of acetonitrile and diluted with water. The concentration of the injected solutions was 40 μg/mL.

The hydro-organic RPLC mobile phases contained acetonitrile (Scharlab) and one of the following ionic liquids (Table 1): 1-ethyl-3-methylimidazolium hexafluorophosphate ($EMIMPF_6$), 1-butyl-3-methylimidazolium hexafluorophosphate ($BMIMPF_6$), 1-butyl-3-methylimidazolium tetrafluoroborate ($BMIMBF_4$) (all from Sigma), and 1-hexyl-3-methylimidazolium tetrafluoroborate ($HMIMBF_4$) (Merck, Darmstadt, Germany). Occasionally, acetonitrile–water mixtures in the absence of additive were used. For comparison purposes, mobile phases containing triethylamine (Sigma) and acetonitrile, or sodium dodecyl sulphate (99% purity, Merck) and 1-propanol (Sharlab) were prepared. In all cases, mobile phases were buffered at pH 3 with 0.01 M citric acid monohydrate and sodium hydroxide (Panreac, Barcelona).

Nanopure water (Barnstead, Sybron, Boston, MA, USA) was used throughout. The drug solutions and mobile phases were filtered through 0.45 μm nylon membranes (Micron Separations, Westboro, MA, USA). The usual cautions required when working with salt or surfactant-containing mobile phases were followed [23].

2.2. Apparatus and column

An Agilent chromatograph (Waldbronn, Germany), equipped with an isocratic pump (Series 1200), an autosampler, a UV–Visible detector (Series 1100) set at 254 nm (except for timolol, which was detected at 300 nm), and an HPChemStation (Agilent, B.02.01) for data acquisition, was used.

The analytical column (a Kromasil C18 column, Análisis Vínicos, Ciudad Real, Spain) had the following characteristics: 150 mm × 4.6 mm i.d., 5 μm particle size, 19% carbon load, 320 m^2/g surface area, and 110 Å pore diameter. The column was connected to a similar 30 mm guard column. The flow-rate was 1 mL/min. Duplicate injections were made using an injection volume of 20 μL.

2.3. Experimental designs

An experimental design consisting of four mobile phases with acetonitrile contents in the range 15–30% (v/v), without additive, was first explored to obtain a reference. A fixed concentration of 15% acetonitrile was finally selected. An ionic liquid or TEA was then added at concentrations 0.01, 0.02, 0.04 and 0.06 M. $EMIMPF_6$ was also used at 0.001 M, and $BMIMPF_6$ at 0.001 and 0.005 M. The minimal and maximal concentrations of the additives in the mobile phase were selected to achieve enough retention for the most polar β-blockers, and not excessive retention for the most apolar ones. When SDS was used, 1-propanol (15%) was selected as organic solvent, and the concentrations of SDS in the mobile phases were 0.02, 0.04, 0.075 and 0.15 M.

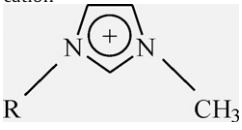
3. Results and discussion

3.1. Effect of ionic additives on RPLC retention of basic drugs

3.1.1. Mobile phases without additive

In order to get a reference for exploring the effect of several additives (cationic, anionic and dual) on the chromatographic behaviour of the basic drugs, acetonitrile–water mobile phases in the absence of additive were first prepared. The polarity range of β-blockers limited the content of acetonitrile between 15 and 30%. A concentration of 15% acetonitrile was selected to explore the effects of the additives on the RPLC system. This percentage of organic solvent prevented extremely low retention times upon the addition of some of the selected additives.

Table 1
Structure and properties of the ionic liquids.

Ionic liquid	1-R-3-methylimidazolium cation	Anion	m.p. (°C)[a]	*d* (g/ml)[a]	Water solubility[a]	Physical state at room temperature
						
$EMIMPF_6$	1-Ethyl–	PF_6^-	59	1.48	Partially soluble	Solid
$BMIMBF_4$	1-Butyl–	BF_4^-	−71	1.21	Soluble	Liquid
$BMIMPF_6$	1-Butyl–	PF_6^-	11	1.38	Non soluble	Liquid
$HMIMBF_4$	1-Hexyl–	BF_4^-	−81	1.15	Immiscible	Liquid

[a] From Refs. [18] and [21].

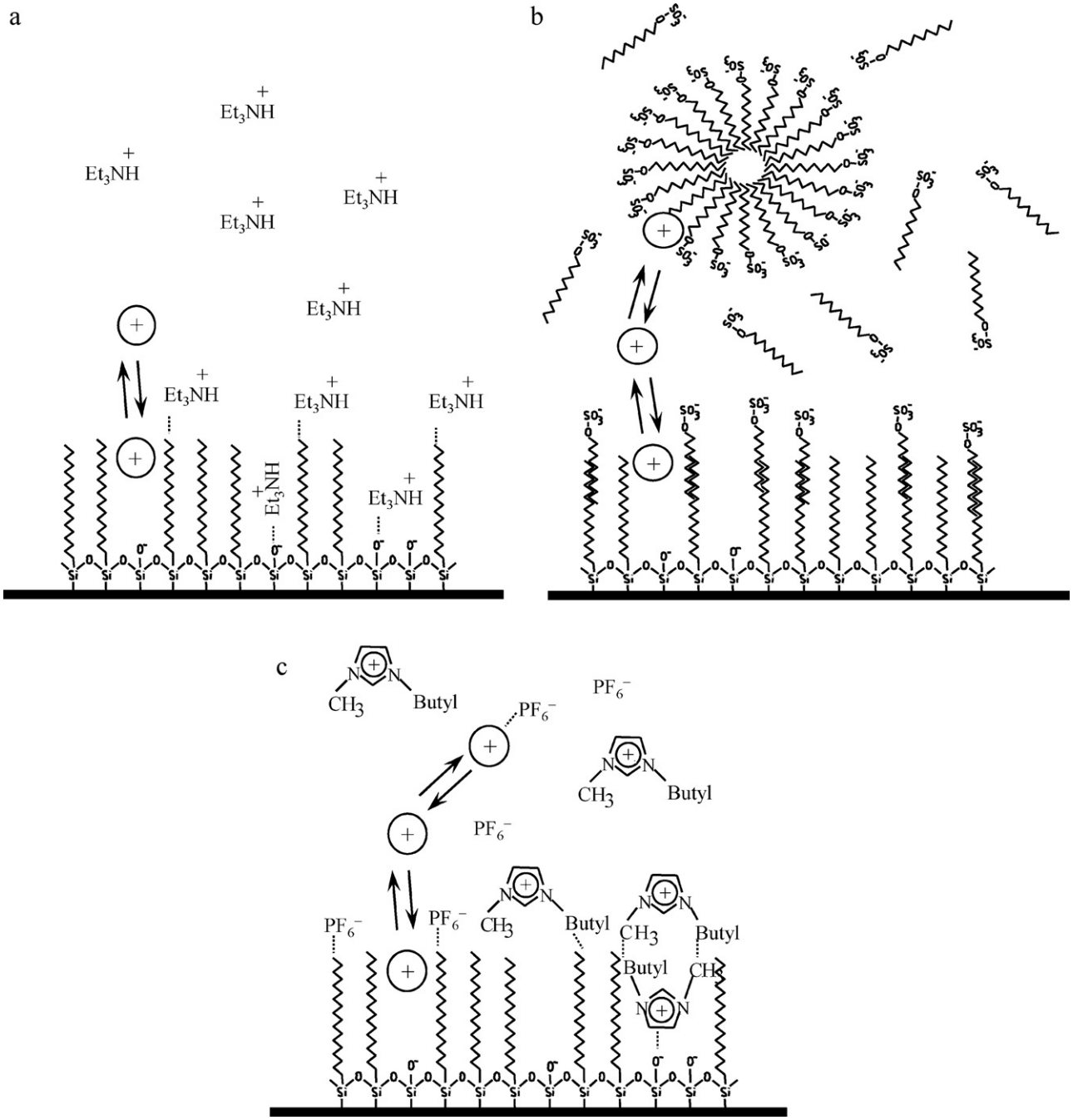


Fig. 1. Solute environment in a C18 stationary phase in the presence of different mobile phase additives: (a) TEA, (b) SDS, and (c) $BMIMPF_6$.

3.1.2. Use of a cationic additive: triethylamine

TEA is the most popular amine used to block silanol groups in RPLC packings [5,6]. At the acidic working pH of the mobile phase, TEA is positively charged and interacts through electrostatic attraction with the negative silanols on the packing material. However, the hydrophobic part of the molecule can also associate with the alkyl chains bonded to the silica stationary phase, with the ammonium groups oriented away from the surface (Fig. 1a). This would give rise to a positively charged stationary phase repelling protonated basic drugs, which would elute at short retention times, or even with the void volume.

The chromatographic behaviour of several β-blockers under the presence of TEA, covering a domain of 0.01–0.06 M, is shown in Fig. 2a. A slight decrease in retention was observed with the first addition of TEA (0.01 M), which may be explained as a result of the electrostatic repulsion of the cationic β-blockers by the positively charged stationary phase due to adsorption of protonated TEA. Further addition of TEA yielded minimal changes on the retention factors. Kromasil column is a type-B silica-based material, which has been proven to be less affected in terms of retention upon addition of amines as silanol blockers [24].

3.1.3. Use of an anionic additive: sodium dodecyl sulphate

The anionic surfactant SDS has been also used as mobile phase additive in RPLC to modify the properties of alkyl-bonded stationary phases in the analysis of basic drugs [7,25–28]. The long hydrophobic chain of SDS monomers is inserted in the bonded organic layer with the sulphate group oriented outside [29]. This

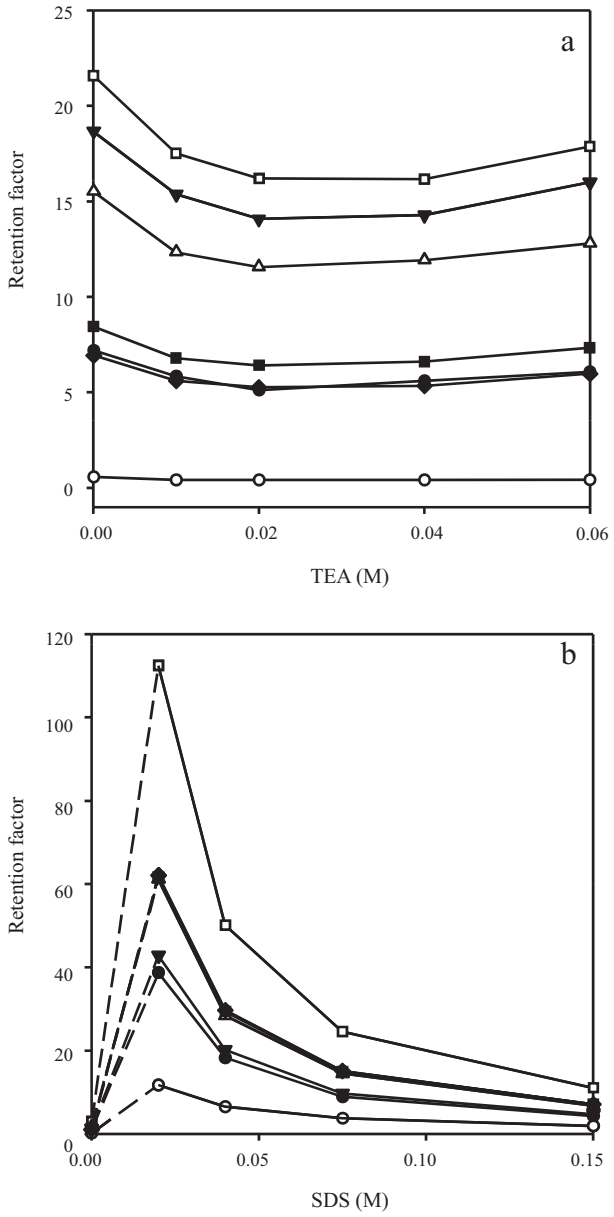


Fig. 2. Retention behaviour of β-blockers in different RPLC systems: (a) aqueous solutions containing 15% acetonitrile and different concentrations of TEA, and (b) aqueous solutions containing 15% 1-propanol and different concentrations of SDS. Solute identities: (○) atenolol, (♦) timolol (●) acebutolol, (■) metoprolol, (△) esmolol, (▼) celiprolol, and (□) oxprenolol.

results in a negatively charged stationary phase (Fig. 1b). The cationic solutes can interact hydrophobically with the alkyl-bonded layer, or through electrostatic attraction with the adsorbed anionic surfactant monomers.

The weak elution strength of acetonitrile when SDS was used as additive made 1-propanol instead of acetonitrile more convenient, in order to avoid extremely large retention times. However, the conclusions would be the same with both organic solvents. Mobile phases containing 15% 1-propanol, and covering a domain from 0.02 to 0.15 M SDS, were used to check the chromatographic behaviour of the β-blockers with the anionic additive. The concentration range of SDS and the selected percentage for 1-propanol allowed the existence of micelles [30].

A plot showing the change in retention with the concentration of SDS is shown in Fig. 2b. It should be noted that, below the critical micelle concentration (CMC, results not shown), a dramatic increase in the retention factors would be observed, owing to the strong electrostatic attraction of the positively charged basic drugs to the adsorbed surfactant monomers on the stationary phase. Addition of surfactant at concentrations exceeding the CMC yielded the expected decrease in retention, attributed to the progressive presence of micelles in the mobile phase, which attract the basic drugs, enhancing the solubilization capability. However, the retention factors were still larger than those obtained in the absence of SDS.

3.1.4. Use of dual additives: ionic liquids

Most ILs used as additives in RPLC are made of a large imidazolium cation associated with a relatively large anion [13]. For this study, we selected four ILs ($EMIMPF_6$, $BMIMPF_6$, $BMIMBF_4$ and $HMIMBF_4$), which are liquids at room temperature (except $EMIMPF_6$) and commercially available, and differ in their adsorption capability of the cation and anion [13].

Several simultaneous changes can take place in a chromatographic system upon addition of an IL to the mobile phase: the cation can associate to the anionic silanols or the alkyl-bonded phase, through electrostatic and hydrophobic interactions, respectively, and the anion can be adsorbed on the hydrophobic stationary phase. This creates an asymmetric bilayer, positively or negatively charged depending on the relative adsorption of the cation and anion. Therefore, the retention of a basic drug is the consequence of the combination of a mixed mechanism that involves ion-pairing, ion-exchange, and hydrophobic partitioning. Basic drugs can also interact with the IL anion in the mobile phase through ion-pair interactions. Fig. 1c shows a scheme of these interactions. The extension of the interactions of the cation and anion in the ILs with the stationary phase, and its importance on solute retention mechanisms, complicates the interpretation of the modification of the nature of the chromatographic system. We are making here an effort to elucidate this.

The chromatographic behaviour of the mobile phases containing the four ILs selected in this work is shown in Figs. 3a–d. As observed, the retention factors of the β-blockers decreased upon addition of 0.01 M $BMIMBF_4$ and $HMIMBF_4$, being the change more intense for the latter (Figs. 3a and b). At higher concentrations, the changes in the retention factors were minimal. For this reason, the feasible experimental domain ranged from 0.01 to 0.06 M.

In a previous work [13], the anion BF_4^- was observed to show moderate adsorption on a Kromasil C18 stationary phase when compared with PF_6^- (according to the Hofsmeister series [31,32]): 15 versus 32 μmol, with a mobile phase containing 30% acetonitrile and 0.05 M $NaBF_4$ or $NaPF_6$. In the same report, the adsorbed amount of the IL imidazolium cations was shown to increase linearly with their concentration in the mobile phase, and with the hydrophobicity of the alkyl chain in the cation. Thus, for example, the adsorbed amount of the ionic liquid using mobile phases of 30% acetonitrile containing 0.05 M $EMIMPF_6$, $BMIMPF_6$ and $HMIMPF_6$ was ~50, 125 and 380 μmol, respectively.

In the presence of $BMIMBF_4$ or $HMIMBF_4$, the retention decreased with the concentration of the IL in the mobile phase (Figs. 3a and b). This means that the interaction of the imidazolium cation with the free silanols and/or the alkyl-bonded stationary phase prevails over the association of BF_4^- with the octadecyl layer. Therefore, the separation environment should be similar to that found in the presence of TEA: the electrostatic repulsion of the β-blockers with the adsorbed cations is dominant, especially in the presence of $HMIMBF_4$.

For $BMIMPF_6$ and $EMIMPF_6$, the retention times of the β-blockers were appreciably longer with respect to those in the absence of additive (Figs. 3c and d). This can be explained considering the strong adsorption of PF_6^- on C18 columns compared to

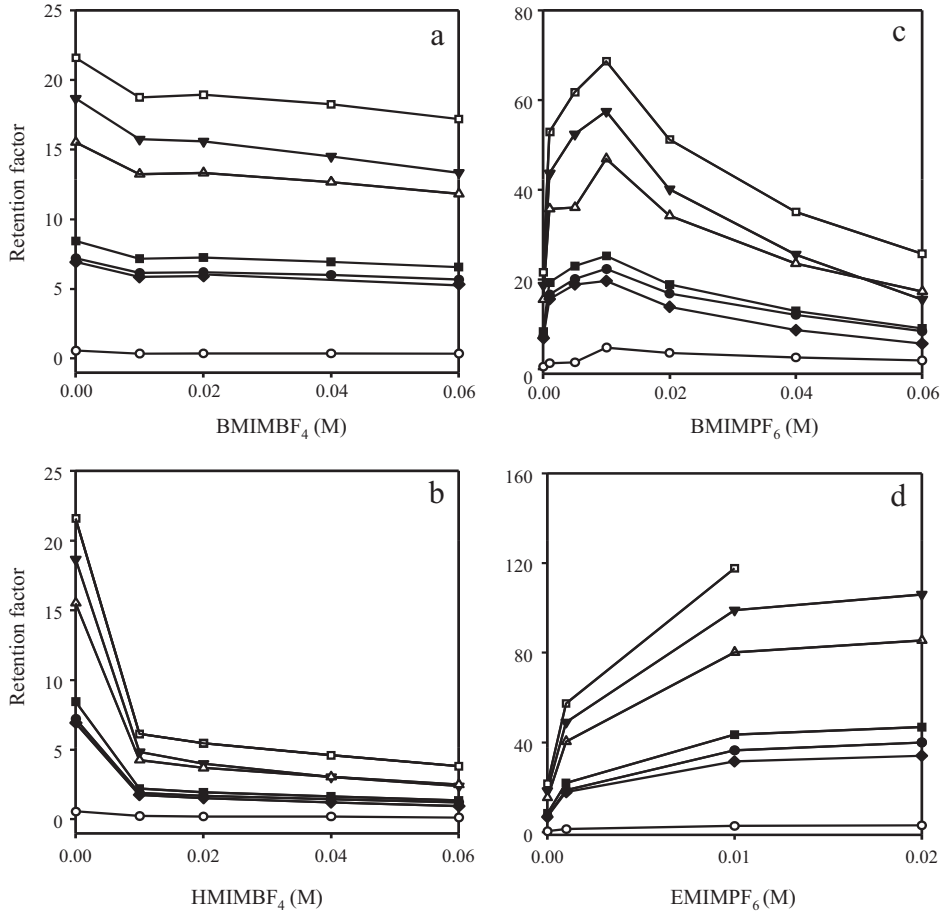


Fig. 3. Retention behaviour of β-blockers in different RPLC systems with aqueous solutions containing 15% acetonitrile and different concentrations of the four ILs. See Fig. 2 for solute identities.

BF_4^- [13]. For $BMIMPF_6$ (Fig. 3c), the retention factors increased in the range 0.001–0.01 M, but followed a gradual decrease between 0.01 and 0.06 M. This elution pattern suggests that the dominant retention mechanism in the presence of $BMIMPF_6$ depends on its concentration in the mobile phase. As long as the adsorbed amount of the IL does not reach the maximal capacity of the column, the interaction of the cationic solutes with the PF_6^- anion adsorbed on the stationary phase seems to be more important than that of the imidazolium cation, and consequently, ion-exchange will be the main responsible for the observed retention. Seemingly, once column saturation is reached, ion-pair interactions with PF_6^- present in the bulk mobile phase gain prominence, which reduces the retention. The elution behaviour is similar to that found upon addition of SDS (compare Figs. 1b and 3c).

Owing to the large retention observed in the presence of $EMIMPF_6$ (this IL is also costly), only a narrow range of concentrations (between 0.001 and 0.02 M) could be examined (Fig. 3d). In the studied range, again, the retention increased with the concentration of the IL, at the lowest concentrations assayed. The larger retention times (compared to $BMIMPF_6$) may be due to the smaller adsorption of $EMIM^+$ with respect to $BMIM^+$.

3.2. Solute-stationary phase and solute-mobile phase interactions

Early, in the development of RPLC with micellar mobile phases, there was great interest on the retention mechanism. A three phase (stationary phase, water/organic solvent, micelle) model was established, which gave rise to the proposal of equations that described the changes in solute retention [33,34]. According to Arunyanart and Cline-Love, two chemical equilibria are established in a micellar RPLC system [34]:

$$A + S \rightleftarrows AS \quad (1)$$

$$A + M \rightleftarrows AM \quad (2)$$

which describe the association of the solute in bulk water (A) with the stationary phase binding sites (S), or with a monomer of surfactant in the micelle dissolved in the mobile phase (M). The displacement of these equilibria is expressed by the constants K_{WS} and K_{AM} for Eqs. (1) and (2), respectively. Taking this into account, the retention factor will be given by:

$$k = \phi \frac{[AS]}{[A] + [AM]} = \frac{\phi K_{WS}[S]}{1 + K_{AM}[M]} \quad (3)$$

where ϕ is the phase ratio (the ratio between the volume of active surface of stationary phase and the column dead volume) and $[M]$ the molar concentration of surfactant monomers forming micelles. Since $[S]$ is constant (or practically constant), it can be included (together with ϕ) in the partition constant K_{WS}. Eq. (3) can thus be rewritten as:

$$\frac{1}{k} = \frac{1}{K_{AS}} + \frac{K_{AM}}{K_{AS}}[M] \quad (4)$$

K_{AS} ($\phi K_{WS}[S]$) and K_{AM} are the solute-stationary phase and solute-micelle association constants.

The linear plots of the inverse of the retention factor ($1/k$) versus the concentration of the surfactant, for mobile phases containing surfactant above the CMC, are an evidence of stationary phase saturation by the surfactant monomers. In fact, for SDS, it has been

Table 2
Association constants between solute and stationary phase (K_{AS}) and between solute and additive in the mobile phase (K_{AD}), and silanol-additive constant (K_A).

Probe compound	$BMIMBF_4$			$HMIMBF_4$			$BMIMPF_6$		TEA	
	K_{AS}	K_{AD}	K_A	K_{AS}	K_{AD}	K_A	K_{AS}	K_{AD}	K_{AS}	K_{AD}
Acebutolol	6.7	3.0	40.9	2.1	12.3	542	33.8	48.3	4.8	−3.6
Atenolol	0.4	0.9	–	0.3	23.4	–	6.7	39.9	0.4	−0.6
Celiprolol	17.5	5.2	29.0	6.0	25.4	295	154	141.4	13.1	−2.8
Esmolol	14.6	4.0	29.7	4.9	16.0	372	70.0	50.3	11.0	−2.3
Metoprolol	7. 9	3.3	39.0	2.5	13.8	458	40.3	55.8	6.0	−2.9
Oxprenolol	20.4	3.1	32.3	7.0	13.7	545	103	49.6	15.2	−2.2
Timolol	6.2	2.9	47.9	2.1	20.4	405	43.1	105	4.9	−2.8
Mean value			36.5 ± 7.4			436 ± 90				

checked that this saturation is reached close to the CMC, or the increase in the surfactant loading is small above the CMC [35]. On the other hand, the extrapolation of the linear segments gives a measurement of the strength of the interaction between the solute and stationary phase, expressed as the inverse of the intercept.

To our knowledge, Eq. (4) or similar have not been applied to measure the strength of the interaction of solutes with stationary phases modified by adsorption of ILs or TEA. ILs and TEA experience equilibria similar to those in Eqs. (1) and (2). Therefore, we fitted this equation to the data obtained in the presence of $BMIMBF_4$, $HMIMBF_4$, $BMIMPF_6$ and TEA. The estimated association constants are given in Table 2, where K_{AD} is the solute–additive association constant (analogous to K_{AM} in the presence of surfactant, see Eq. (4)).

We would like to indicate that the intercepts in Eq. (4) for all β-blockers eluted with SDS/1-propanol were null. This hindered the estimation of K_{AS}, but revealed a strong solute-stationary phase interaction (between the protonated β-blockers and the sulphate group in the surfactant).

Although we are centring our discussion on the solute-stationary phase interactions, the solutes interact also with the additive in the mobile phase. As observed in Table 2, the solute–additive association constants in the mobile phase (K_{AD}) for the ILs are positive, indicating a certain affinity of the basic drugs for the dissolved ILs. In contrast, K_{AD} values for TEA are slightly negative, which seem nonsense. However, in the micellar RPLC literature this behaviour (called "anti-binding") has been described, and explained as produced by electrostatic repulsion between the solutes and additive in the mobile phase, both bearing charge of the same sign [36]. We should also consider that the repulsion between charged solutes and stationary phase (without other kind of interaction) would make the solutes elute within the void volume region.

Turning back to the K_{AS} values for the ILs and TEA, the highest corresponded to $BMIMPF_6$ (suggesting a higher affinity of the β-blockers to the stationary phase modified with this IL), the lowest to $HMIMBF_4$, while the values for $BMIMBF_4$ and TEA were similar. These observations agree with those made in Section 3.1, and reveal a low or moderate adsorption for BF_4^-, and the greater interaction of $HMIM^+$ with the alkyl-bonded stationary phase (with respect to $BMIM^+$) that repels β-blockers and decreases the retention in a higher extent. In contrast, the adsorbed PF_6^- favours ion-pair interactions with the protonated solutes. Consequently, K_{AS} for $BMIMPF_6$ is appreciably higher with respect to $HMIMBF_4$. Finally, it should be indicated that we could not determine K_{AS} and K_{AD} for $EMIMPF_6$, for which we had no enough data.

3.3. Interaction of ILs with free silanols

3.3.1. Estimation of the suppressing potency based on retention

A combined retention mechanism for basic compounds was already postulated in the 80s for bonded-silica stationary phases with organic solvent–water eluents, as a result of hydrophobic and silanophilic interactions. Therefore, masking the silanol groups without any additional interaction will decrease the retention. In the pioneering reports by Horváth et al. [37,38] on the effect of several amine additives on the retention of a group of basic compounds, a mathematical model was proposed to evaluate the silanol

Table 3
Mean efficiencies (N) for the set of basic drugs, calculated according to Foley and Dorsey [39].

Additive	Additive concentration (M)							
	0.001	0.005	0.01	0.02	0.04	0.06	0.075	0.15
$BMIMBF_4$	–	–	2300 ± 600	2400 ± 800	2600 ± 1000	3000 ± 1100	–	–
$HMIMBF_4$	–	–	2400 ± 700	2400 ± 800	2600 ± 800	2600 ± 800	–	–
$EMIMPF_6$	1900 ± 500	–	3000 ± 600	3100 ± 100	–	–	–	–
$BMIMPF_6$	2500 ± 500	2800 ± 00	4000 ± 400	3800 ± 500	5000 ± 1000	7000 ± 2700	–	–
TEA	–	–	1900 ± 300	2300 ± 300	2700 ± 400	2000 ± 900	–	–
SDS	–	–	–	5000 ± 300	4300 ± 500	–	2800 ± 500	2100 ± 600

Table 4
Mean asymmetry factors (B/A) for the set of basic drugs.

Additive	Additive concentration (M)							
	0.001	0.005	0.01	0.02	0.04	0.06	0.075	0.15
$BMIMBF_4$	–	–	1.8 ± 0.4	1.8 ± 0.2	1.8 ± 0.1	1.5 ± 0.2	–	–
$HMIMBF_4$	–	–	1.7 ± 0.1	1.7 ± 0.2	1.8 ± 0.8	1.8 ± 0.9	–	–
$EMIMPF_6$	2.3 ± 0.4	–	1.7 ± 0.2	1.6 ± 0.2	–	–	–	–
$BMIMPF_6$	1.8 ± 0.3	1.7 ± 0.1	1.5 ± 0.0	1.6 ± 0.3	1.3 ± 0.2	1.2 ± 0.1	–	–
TEA	–	–	2.5 ± 0.6	2.2 ± 0.5	2.1 ± 0.6	2.3 ± 0.9	–	–
SDS	–	–	–	1.0 ± 0.0	1.0 ± 0.0	–	1.1 ± 0.1	1.3 ± 0.2

suppressing potency based on solute retention. In the absence of additive, retention is given by:

$$k_0 = k_1 + k_2 \quad (5)$$

k_0 being the retention factor in the absence of additive, and k_1 and k_2 the hydrophobic and silanophilic contributions. When an amine is added to the mobile phase, a secondary equilibrium is established with the silanol groups:

$$\text{A} + \text{Silanol} \rightleftarrows \text{ASilanol} \quad (6)$$

Therefore, the retention factor in the presence of additive will be:

$$k = k_1 + \frac{k_2}{1 + K_A[A]} \quad (7)$$

where K_A is the binding constant between the silanol and additive, and $[A]$ its molar concentration. If Eqs. (5) and (7) are subtracted to isolate the silanophilic contribution to retention:

$$k_0 - k = k_2 - \frac{k_2}{1 + K_A[A]} = \frac{k_2 K_A[A]}{1 + K_A[A]} \quad (8)$$

This equation can be rearranged to obtain the classical Horváth equation:

$$\frac{[A]}{k_0 - k} = \frac{1}{k_2 K_A} + \frac{[A]}{k_2} \quad (9)$$

The binding constant K_A evaluates the ability of the additives to block the silanol sites, and can be obtained by regressing $[A]/(k_0 - k)$ versus $[A]$. More recently, Eq. (9) has been used to measure K_A for ILs added to the mobile phase, that is, their silanol suppressing potency [16].

Table 2 shows the K_A values estimated for the two tetrafluoroborates studied in this work. As observed, a particular value of K_A is obtained for each β-blocker. In the literature, K_A values for different basic compounds and ILs can be found [16]. A certain scattering is always observed among compounds. However, since K_A is the stability constant of the silanol complex with the cation of the additive, it should be a single value. From the values given in Table 2, we calculated $K_A = 36.5 \pm 7.4$ and 436 ± 90 for $BMIMBF_4$ and $HMIMBF_4$, respectively. The highest K_A obtained for $HMIMBF_4$ indicates, in principle, that this IL is a better silanol masking agent than $BMIMBF_4$.

On the other hand, the estimation of the ILs suppressing potency expressed as K_A assumes that Eq. (5) is valid. However, the dual nature of ILs should be considered, which may yield additional interactions. This is evident for the hexafluorophosphates, where owing to the high adsorption of the anion (PF_6^-), $k > k_0$ at all IL assayed concentrations. In previous section, we interpreted this behaviour as due to the attraction of the positively charged solutes to the adsorbed IL anion, with further ion-pair formation in the bulk mobile phase. Obviously, in this case, Eq. (9) cannot be applied. As we have commented, the adsorption of BF_4^- is significantly smaller with respect to PF_6^-. However, we can imagine that cationic solutes can also experience some interaction with BF_4^-, which will affect the estimation of K_A with this anion.

The anion associated to TEA (citrate($^{3-}$)) exhibits a negligible interaction with the alkyl-bonded phase [31,32]. Thus, Eqs. (5)–(9) should describe correctly the interaction of TEA with the silanol groups. However, with this additive, we observed practically null intercepts (slightly positive or negative) in Eq. (9) for all the β-blockers, which means that K_A should be large. This result does not agree with previous work, where K_A for TEA was found smaller compared to several ILs associated to anions weakly adsorbed on the stationary phase (e.g. BF_4^-, Cl^-, Br^- and OSO_4^-) [16]. TEA^+, which is a smaller cation than imidazolium with a better access to

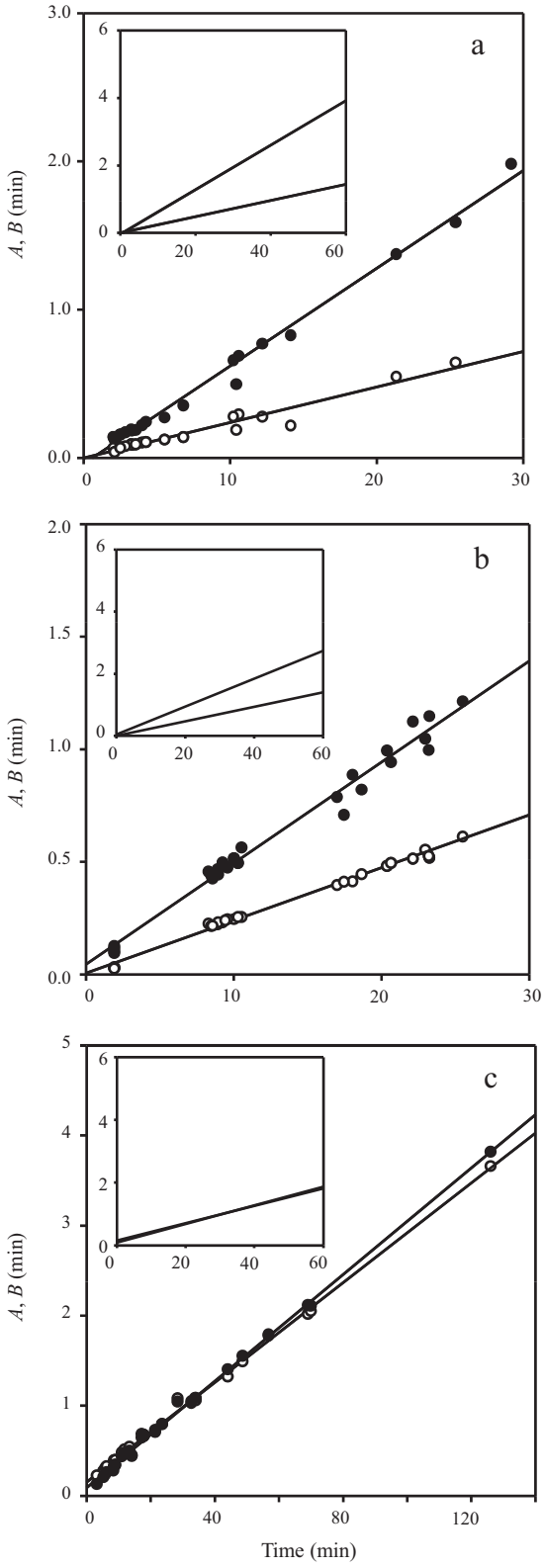


Fig. 4. Peak half-width plots ((○) A and (●) B), including the data for all probe solutes eluted with the whole set of mobile phases in the experimental designs for: (a) acetonitrile–water mobile phases in the absence of additive, (b) 15% acetonitrile and TEA, and (c) 15% 1-propanol and SDS.

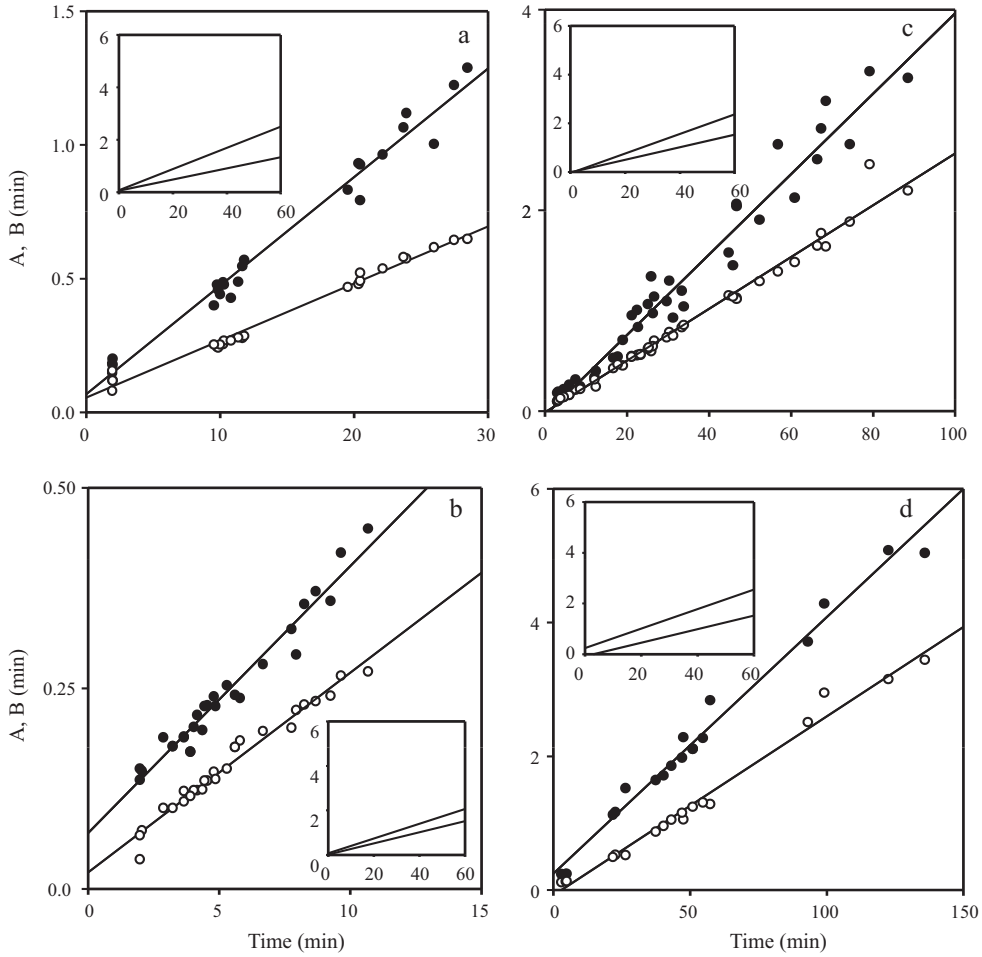


Fig. 5. Peak half-width plots ((○) A and (●) B), including the data for all probe solutes eluted with the whole set of mobile phases containing 15% acetonitrile and: (a) $BMIMBF_4$, (b) $HMIMBF_4$, (c) $BMIMPF_6$ and (d) $EMIMPF_6$.

silanol groups, should show a stronger interaction with the silanol groups, as we have measured.

It should be noted that the suppressing potency is measured by comparison with the stationary phase in the absence of additive (Eq. (9)). In a previous work, the authors measured larger K_A values (with respect to those obtained in this work) for several ILs in a LiChrospher RP-18 column, including $BMIMBF_4$ and $HMIMBF_4$ [16]. This column has a higher amount of unprotected silanol groups than the Kromasil column, as can be checked by the poor efficiency obtained for basic drugs eluted with hydro-organic mixtures without additive. Accordingly, the relative effect of ionic liquids in the LiChrospher column should be larger (larger K_A values). This constant is not an absolute value, it is relative to the stationary phase.

3.3.2. *Suppressing potency revealed by changes in peak shape*

Effective silanol blocking by ILs should be translated into a substantial enhancement in peak shape. In order to compare the performance of the studied additives (the four ILs, TEA and SDS), the peak efficiencies (expressed as theoretical plates, *N*, calculated according to Foley and Dorsey [39]), and asymmetries (*B*/*A*, *B* and *A* being the distance between the retention time and the tailing and leading edge of the peak, respectively) were evaluated at increasing concentration of the additives. Data concerning mobile phases in the absence of additive were included for comparison purposes.

In previous work, we reported enhanced peak shape for basic drugs eluted with hybrid mobile phases of SDS and acetonitrile or alcohol [7,40]. Elution with a solution containing only the surfactant gives rise to poor peak shape, which has been explained by the high carbon loading due to the adsorption of surfactant onto the packing [41]. This significantly increases the effective stationary phase thickness and diminishes solute diffusion in the stationary phase. The organic solvent added to the mobile phase decreases the stationary phase surfactant coating. A thinner surfactant layer permits a better diffusion of the protonated basic drugs and is effective in preventing their association with free silanols. The interaction of the charged solutes with the hydrophilic layer of SDS reduces also their penetration depth into the bonded phase. Thus, a significant improvement in peak shape is achieved using mobile phases containing both SDS and organic solvent. The kinetics of solute-sulphate electrostatic association seems to be more facile than ion-exchange processes involving silanols on the silica surface. However, the suppression of the silanol effect with SDS is not due to a direct electrostatic interaction with the free silanols, but due to a masking effect by the surfactant coating on the stationary phase.

The estimated mean values for *N* and *B*/*A* in the absence of additives (considering the peaks for the seven β-blockers eluted in the range 15–30% acetonitrile) were rather poor ($N = 1400 \pm 300$, and $B/A = 2.2 \pm 0.4$ for 15% acetonitrile, and $N = 2000 \pm 300$, and $B/A = 1.6 \pm 0.5$ for 15% 1-propanol). The mean values for *N* and *B*/*A* in the presence of the additives are given in Tables 3 and 4, respectively. The peak shape enhancement was apparently moderate for the tetrafluoroborates and TEA, with mean efficiencies not exceeding 3000, and mean *B*/*A* values in the range 1.5–2.5. No clear trend was observed at varying additive concentration.

SDS and the hexafluorophosphates yielded, apparently, better peak shape. Observe that the efficiencies decreased and the peak asymmetry increased with SDS concentration, whereas it followed an opposite trend for the hexafluorophosphates (in the studied range). It should be considered, however, that the measured values for N and B/A are apparent values, since they depend strongly on solute retention. These values improve at increasing retention time, owing to the relative decrease in the extra-column contributions to peak width. This, together with the increase in the thickness of the surfactant layer at increasing SDS concentration in the mobile phase, can explain the trend for this additive. However, the peak shape was always enhanced at increasing concentration for the hexafluorophosphates, which is especially clear for $BMIMPF_6$, for which we could gather enough information.

3.3.3. Column behaviour related to peak shape

The plots of the left and right half-widths at 10% peak height versus the retention time are useful to assess the changes in peak broadening and symmetry inside a chromatographic column. In previous reports, we demonstrated the validity of these plots to compare the behaviour of several basic drugs eluted from microparticulate and monolithic columns [42,43], and to explore the effect of several organic solvents in conventional and micellar RPLC [44]. We apply here these plots to compare the effect of the silanol blocking agents on peak performance. The study involved the construction of individual plots (one for each mobile phase composition), and global plots (considering all studied mobile phases for each additive, where its concentration is varied).

Figs. 4 and 5 depict the global peak half-width plots obtained in the absence and presence of the different additives studied in this work. The inner plots show the region with maximal retention times of 60 min and maximal half-widths (for A or B) of 6. In the presence of SDS, the correlations were excellent ($r^2 > 0.99$ for A and B, Fig. 4c). The regression coefficients for the other situations were: without additive ($r_A^2 = 0.944$, $r_B^2 = 0.932$, Fig. 4a), with TEA ($r_A^2 = 0.992$, $r_B^2 = 0.982$, Fig. 4b), $BMIMBF_4$ ($r_A^2 = 0.987$, $r_B^2 = 0.978$, Fig. 5a), $HMIMBF_4$ ($r_A^2 = 0.972$, $r_B^2 = 0.960$, Fig. 5b), $BMIMPF_6$ ($r_A^2 = 0.982$, $r_B^2 = 0.956$, Fig. 5c), and $EMIMPF_6$ ($r_A^2 = 0.986$, $r_B^2 = 0.983$, Fig. 5d). The points on the global plots showed certain scattering for the ILs, mainly associated to the right half-width (B) (Fig. 5), but the correlations for the individual plots were highly satisfactory. This scattering suggests a continuous column modification at increasing additive concentration. In a previous work, half-width plots built for mobile phases containing SDS and acetonitrile, at varying concentration of both modifiers, also revealed a significant column modification (a change in the thickness of the surfactant layer on the stationary phase) [44].

The peak broadening rate (r_{pb}) inside the column is given by the sum of the slopes of the A and B half-width plots, whereas the extra-column contributions are associated to the intercepts [44]. The values obtained for the different modifiers are the following: without additive ($r_{pb} = 0.087$), and with TEA (0.068), SDS (0.057), $BMIMBF_4$ (0.062), $HMIMBF_4$ (0.058), $BMIMPF_6$ (0.066), and $EMIMPF_6$ (0.065). According to these values, the best column modifiers are SDS and $HMIMBF_4$, which yielded the smallest r_{pb} values. We should remind that the efficiency and asymmetry values given in Tables 3 and 4 are apparent values, affected by the retention times. The retention times with SDS and the hexafluorophosphates are appreciably longer than those with $HMIMBF_4$. This is partially the reason of the smaller apparent efficiencies. Finally, the half-width plots show clearly that only in the presence of the anionic surfactant, the peaks of the β-blockers are nearly symmetrical (the lines for A and B coincide, with a slope ratio of 1.07). Again, SDS seems to be the best peak enhancer, followed by $HMIMBF_4$ with a slope ratio of 1.34, against 1.92 for TEA.

4. Conclusions

In a recent work, we demonstrated that both changes in retention and peak shape of basic drugs β-blockers basic drugs provide information about the solute interactions in the mobile and stationary phases [7,40]. This research is here extended to increase the knowledge on the interactions of basic drugs in acetonitrile–water mobile phases containing ILs, which have a dual character. The behaviour in the presence of TEA (a cationic modifier) and SDS (an anionic modifier) assisted in the interpretation of the results.

The changes in retention of the basic drugs suggested that the estimation of the silanol suppressing potency of the ILs (K_A in Eq. (7)) should consider the influence of both the anion and the cation. The K_A values calculated from Eq. (9) are affected by the different nature of the interactions taking place simultaneously inside the column. Therefore, this equation can only be strictly applied to ILs where the anion is weakly adsorbed.

The high retention of β-blockers eluted with SDS-modified mobile phases is due to the strong association of the cationic solutes with the anionic surfactant adsorbed on the stationary phase. A similar behaviour is observed with $BMIMPF_6$, whose anion is also strongly adsorbed. On the other hand, the behaviour of $HMIMBF_4$ and TEA is similar, since the cations of these additives are significantly adsorbed on the stationary phase, whereas the corresponding anions are weakly or not adsorbed. Among the silanol blocking agents studied in this work, SDS appeared (followed by $HMIMBF_4$) as the best, and TEA as the poorest. It should be noted that SDS blocks silanol groups by coating the surface of the C18 stationary phase, forming a bilayer, whereas the blocking ability of TEA is related to the direct association with free silanols through electrostatic attraction.

The recommendation that the comparison of peak efficiencies and asymmetries achieved in different conditions (e.g. in the presence of different blocking agents) should be made for peaks eluting at similar retention times, is not always possible, due to the different ranges in retention times (compare Figs. 2 and 3). This can give rise to wrong conclusions about the best peak shape enhancer. In contrast, the half-width plots give information about the peak width and asymmetry at different retention times, and offer a measurement of peak broadening, independent of the extra-column contributions to the peak width. However, we should not forget that the retention time is an important parameter to take into account: some silanol blocking agents increase largely the retention, which forces to increase the concentration of organic solvent to achieve adequate retention. This is the case of SDS. Among the four ILs studied in this work, $HMIMBF_4$ seems to have the most interesting characteristics for the RPLC separation of basic drugs: a small peak broadening rate, combined with a low retention.

Acknowledgments

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