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Aqueous liquid chromatography with mobile phases of sodium dodecyl sulphate and ionic liquid

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ABSTRACT

In conventional reversed-phase liquid chromatography (RPLC) with hydro-organic solvents, basic cationic solutes yield retained, broad, asymmetric peaks, owing to their interaction with free anionic silanols in the stationary phase. RPLC mobile phases to which the anionic surfactant sodium dodecyl sulphate (SDS), or an ionic liquid (IL) are added, have been proposed as solutions, since these additives are able to block the silanol effect thus improving the chromatographic performance. With these additives, it is however necessary to increase the elution strength by adding an organic solvent, such as an alcohol or acetonitrile. A novel aqueous liquid chromatographic mode (in the absence of organic solvent) is here proposed, where the mobile phases contain only a mixture of aqueous solutions of SDS and an IL derived from 1-alkyl-3-methylimidazolium associated to chloride, both environmentally friendly. When these reagents are added, the anionic surfactant adsorbed on the stationary phase is able to attract the cationic solutes, whereas the adsorbed IL cation repels them. The combination of both effects (attraction and repulsion) allows the modulation of retention, by varying the IL/SDS ratio. Given the character of the additives, a type of green liquid chromatography is achieved. In this work, the chromatographic behavior of six basic compounds of pharmaceutical interest, the β-adrenoceptor antagonists acebutolol, atenolol, carteolol, metroprolol, oxprenolol and propranolol, is examined. In order to assess the chromatographic behavior of the mixed mobile phases containing SDS and IL, changes in retention, peak profile and resolution of mixtures of the analytes were explored at varying concentration of the additives.

1. Introduction

The removal of toxic and volatile organic solvents, traditionally used in many chemical analytical procedures and different chemical processes, has attracted great attention in the last decade. The ideal situation would be not to use any organic solvent, taking into account the risks to health, and the generation and treatment of wastes, to which their cost must be added [1]. However, the removal of an organic solvent is not always possible. Instead, some less harmful alternatives to the environment and health have been proposed.

Among these alternatives are the use of aqueous solutions of surfactants and ionic liquids (ILs). Typical surfactants consist of a hydrocarbon chain attached to a hydrophilic ionic or strongly polar group, which can be adsorbed on surfaces or interphases of materials, markedly altering their interfacial free energy [2]. ILs are neutral salts with a low melting temperature, formed by a bulky organic cation associated to a smaller organic or inorganic anion [3]. In aqueous solution, surfactant monomers can associate to form clusters called micelles, which are organised structures able to interact with diverse chemical compounds [4]. These behaviors are also found in ILs with long alkyl chains, such as 1-alkyl-3-methylimidazoliums, which are known as surface active ILs (SAILs) [5]. However, it is also possible to form a SAIL by mixing an imidazolium IL, containing a short or relatively short alkyl side chain, with a conventional charged surfactant. Examples of SAILs are formed with 1-butyl–3-methylimidazolium cation ($[C_4C_1IM]^+$) combined with the anionic surfactants sodium dodecyl sulphate (SDS, with a single chain), dioctyl sulphosuccinate sodium (AOT, double chain), and tris(hexyl) sulphosuccinate (triple chain) [6].

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During the last decades, the interest in the use of surfactants and ILs to modify the mobile phase in reversed-phase liquid chromatography (RPLC) has been extended. The RPLC mode that has attracted more attention (with hundreds of references) is the so called micellar liquid chromatography (MLC), where the surfactants are added above their critical micellar concentration (CMC) [7–9]. This technique gives rise to hydrophobic, ionic and specific interactions between the solutes and the mobile and stationary phases, which modulate the retention and selectivity [10]. Meanwhile, ILs act just as dissociated salts in the mobile phase, and depending on their character, both the cation and the anion may be adsorbed on the stationary phase, creating a bilayer [11,12]. It is interesting to note that some MLC procedures have been developed with SAILs forming micelles [13].

Both the RPLC modes with mobile phases of surfactant micelles and ILs provide a solution to the broad and asymmetric peaks obtained for basic compounds in RPLC with hydro-organic solvents, as a result of ionic interactions with the residual free anionic silanols on silica-based stationary phases [14,15]. Surfactant monomers, or the IL cation and anion adsorbed on the stationary phase, provide a protection that masks the residual silanols, which improve the peak profiles [10,16]. However, the MLC mode with only surfactant gives rise to excessive retention for low polar compounds and, in general, poor peak efficiency. To avoid this, an organic solvent (acetonitrile or the short chain alcohols propanol, butanol and pentanol) is frequently added to the mobile phase to reduce the retention and enhance the efficiency, although this can also change the CMC, and eventually, produce micelle breakdown [7].

In the last decade, there has been great interest in another RPLC mode, where a microemulsion formed by an ionic surfactant (usually SDS), co-surfactant (a short chain alcohol), and an apolar solvent is used as mobile phase, which has been called microemulsion liquid chromatography (MELC) [17,18]. This mode has a special interest in the analysis of apolar compounds. In 2017, Peng et al. reported the replacement of the apolar solvent in the mobile phase by an IL, considering a microemulsion was still formed [19]. In Peng's work, several ILs, such as 1-butyl-, 1-hexyl, and 1-octyl-3-methyl imidazolium ($[C_4C_1IM]^+$, $[C_6C_1IM]^+$ and $[C_8C_1IM]^+$), associated to the anions BF_4^-, PF_6^- and bis(trifluoromethylsulfonyl)imide), adopted the role of oils in MELC with SDS as surfactant and 1-butanol as co-surfactant. It was found that $[C_6C_1IM][PF_6]$ was the most suitable for the analysis of a group of phenolic compounds, in terms of retention times and selectivity. Peng's suggestion is the starting point of a more recent study with the cations 1-methyl, 1-butyl, and 1-hexyl-3-methyl imidazolium ($[C_2C_1IM]^+$, $[C_4C_1IM]^+$ and $[C_6C_1IM]^+$), combined with the anions Cl^-, BF_4^- and PF_6^-, and 1-butanol as co-surfactant [20]. The anionic surfactant SDS was found to compete with the IL anions for column adsorption, with a behavior similar to that found in the absence of SDS, when an IL cation with strong affinity for the stationary phase is associated with a weakly adsorbed anion [11,21]. The peak profiles of the basic compounds were improved, especially in the presence of SDS. It was also found that the co-surfactant effect was negligible, and therefore, can be eliminated from the microemulsion.

The results above suggested that the use, in RPLC, of aqueous mobile phases composed by only SDS and 1-alkyl-3-methylimidazolium ILs could be useful in the analysis of basic compounds, without the need of adding an organic solvent. Basic compounds are attracted by the SDS anion and repelled by the IL cation, which can modulate the retention when the concentrations of SDS and IL in the mobile phase are varied. This behavior is explored in this work with a group of six β-adrenoceptor antagonists as probe compounds. The behavior is discussed in terms of retention, resolution and peak profile.

2. Experimental

2.1. Reagents

The probe compounds were the β-adrenoceptor antagonists acebutolol, atenolol, carteolol, metoprolol, oxprenolol, and propranolol (all from Sigma, St. Louis, MA, USA). The solutions were prepared in a small amount of ethanol from VWR International (Rosny-sous-Bois, France), with the aid of an Elmasonic S15 H ultrasonic bath from Elma (Singen, Germany), and dilution with water. The stock solutions, which were stable during at least two months at 4 °C, had a concentration of ca. 500 μg/mL, and were diluted with water to a final concentration of 40 µg/mL, prior to injection into the chromatograph.

The mobile phases contained sodium dodecyl sulphate from Merck (99% purity, Darmstadt, Germany) and one of the following ILs: 1-ethyl-, 1-butyl-, and 1-hexyl-3-methylimidazolium, associated to chloride ($[C_2C_1IM][Cl]$, $([C_4C_1IM][Cl]$, $[C_6C_1IM][Cl]$), all from Sigma. Mobile phases containing only SDS or $[C_6C_1IM][Cl]$ were also prepared to be used as references. In order to limit silanol ionization, RPLC mobile phases were buffered at pH ~ 3.0 with 0.01 M sodium dihydrogenphosphate (Sigma) and hydrochloric acid. The probe compounds had a strong basic character ($pK_a \geq 9$), which means that they were positively charged at the acidic pH of the mobile phases.

The β-adrenoceptor antagonists solutions and the mobile phases were filtered through 0.45 µm Nylon membranes from Micron Separations (Westboro, MA, USA) and degassed in the Elma ultrasonic bath. Nanopure water obtained with an Adrona system (Riga, Latvia) was used throughout.

2.2. Apparatus and column

An Agilent (Waldbronn, Germany) chromatograph equipped with an isocratic pump (Series 1200), an automatic injector (Series 1260 Infinity II), a thermostated column compartment (Series 1290 Infinity II), and a diode array detector (Series 1100) was used. The β-adrenoceptor antagonists were monitored at 254 nm. The retention data were obtained keeping the temperature at 25 °C and the flow rate at 1 ml/min. Duplicate injections of 20 µl were made. The dead time was obtained from the first baseline perturbation in the chromatograms.

The chromatographic system was controlled with an OpenLAB CDS LC Chemstation (Agilent C.02.01). The mathematical treatment was carried out with Excel (Microsoft Office 2010, Redmond, WA, USA). Chromatographic peaks were processed with the MICHROM software to obtain the peak parameters (retention times and peak half-widths) [22].

An XTerra-MS C18 analytical column from Waters (Milford, MA, USA), which replaces one out of every three silanols with a methyl group, was used. The characteristics of the column were: 150 mm × 4.6 mm i.d., 5 µm particle size, 120 Å mean pore diameter, 175 m^2/g surface area, and 12 wt% total carbon. This was preceded by a similar 30 mm guard column for protection from the mobile phase.

Mobile phases were recycled between runs and also during analysis to reduce reagent consumption and wastes. The absence of organic solvent in the mobile phases makes recycling possible, as long as the number of injections is sufficiently low. The chromatographic system was periodically rinsed with 10 vol of a methanol-water solution (5:95, *v*/*v*), followed by 10 vol of pure methanol (around 30 ml) to remove the surfactant and IL from the stationary phase [23]. Over the weekend, the column was maintained with methanol.

2.3. Experimental designs

Experimental designs consisting of aqueous mobile phases with SDS contents in the range 0.10 to 0.25 M or $[C_6C_1IM][Cl]$ in the range 0.02 to 0.05 M were first explored to obtain a reference. SDS was then added at concentrations between 0.025 and 0.20 M to $[C_6C_1IM][Cl]$ solutions in the 0.005 to 0.03 M range to study the effect of both reagents in mixed mobile phases. To check the effect of the nature of the IL cation on mixed systems, mobile phases containing 0.05 M or 0.15 M SDS and $[C_2C_1IM][Cl]$ or $[C_4C_1IM][Cl]$, in the 0.005 to 0.03 M range, were prepared. All mobile phases were run from lower to higher concentrations. The minimal and maximal concentrations of SDS and the ILs were selected to achieve enough retention for the most polar β-adrenoceptor antagonists, and not excessive retention for the most apolar ones.

3. Results and discussion

As commented above, in RPLC with mobile phases containing SDS or IL, the stationary phase is covered by surfactant monomers, IL cation, and to a lesser extent, IL anion. SDS adsorption on C18 stationary phases has been demonstrated to be strong [7]. The adsorption of 1-alkyl-3-methylimidazolium ILs with long side chains (such as $[C_6C_1IM]^+$) and chaotropic anions (such as PF_6^-) is also substantial [11]. This work tries to gain more insight on the effects inside a chromatographic column when it is simultaneously modified with SDS and a 1-alkyl-3-methylimidazolium IL, whose adsorption capability differs from SDS. The stationary phase modified by the adsorbed ionic additives gives rise to a hydrophilic phase, where the charged sites act as ion exchangers with the cationic basic compounds. However, the numerous interactions existing in this environment make the elucidation of the retention mechanism rather complex. In this work, the research has been carried out with ILs associated to chloride (with negligible adsorption on alkyl bonded stationary phases), in order to reveal the real influence of the IL cations on the chromatographic behavior.

3.1. Retention behavior of the β-adrenoceptor antagonists with aqueous mobile phases containing SDS or $[C_6C_1IM][Cl]$

Fig. 1a shows the change in retention, for the set of β-adrenoceptor antagonists, in aqueous mobile phases to which the anionic surfactant SDS is added at increasing concentrations (0.10, 0.15, 0.20, and 0.25 M), without organic solvent. Retention factors are very high at 0.10 M SDS, especially for the most hydrophobic compounds (oxprenolol and propranolol), but successive additions of SDS reduce significantly the retention. The long retention times (especially at low surfactant concentration) can be explained by the strong electrostatic attraction of the basic compounds (positively charged) towards the anionic SDS monomers adsorbed on the stationary phase. It should be noted that the whole set of mobile phases was prepared at concentrations exceeding the CMC of SDS in water (0.008 M). Under these conditions, the number of surfactant monomers adsorbed on the stationary phase reaches saturation, and the remaining monomers in the mobile phase are organised as micelles with negative charge. These attract the cationic solutes towards the mobile phase. At increasing concentration of SDS, the number of micelles grows, and the mobile phase gains elution strength.

Fig. 1b shows the retention behavior of the β-adrenoceptor antagonists with mobile phases that contain only $[C_6C_1IM][Cl]$ at increasing concentration (0.02, 0.03, 0.04 and 0.05 M), in the absence of organic solvent and surfactant. The trend is similar to that observed with SDS (Fig. 1a): the retention factors are shortened at increasing concentration of the additive. However, the retention is

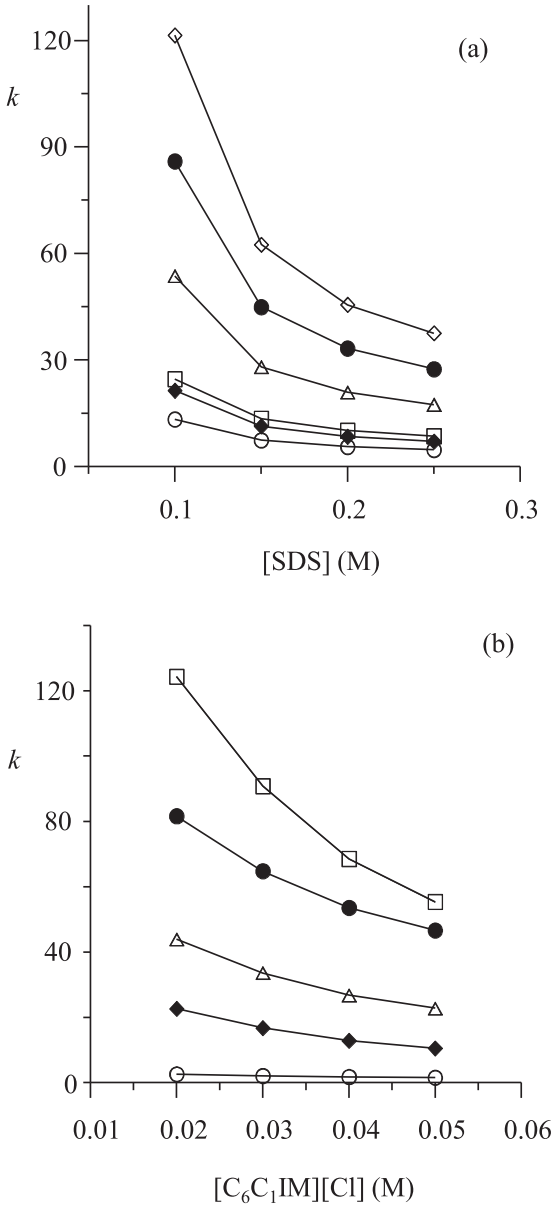

Fig. 1. Change in retention of several β-adrenoceptor antagonists, eluted with mobile phases containing only: (a) SDS, or (b) $[C_6C_1IM][Cl]$, at increasing concentrations. Solute identity: propranolol (◇), oxprenolol (●), metroprolol (△), acebutolol (□), carteolol (♦), and atenolol (○).

usually larger than in the case of adding the surfactant. In fact, the retention of propranolol was so high that it could not be measured.

On the other hand, the reason for the drop in retention, when the concentration of the IL is increased, is different to the case of adding the anionic surfactant. It can be explained by the fact that the adsorbed IL cation ($[C_6C_1IM]^+$) on the stationary phase repels the positively charged solutes. We have not found information about the maximal amount of $[C_6C_1IM]^+$ that can be adsorbed on the C18 X-Terra column, but the observed behavior seems to indicate that at the assayed concentrations, saturation is not reached. It should be noted that $[C_6C_1IM]^+$ is not able to aggregate itself to form small clusters in the mobile phase. Anyway, the IL cation in the bulk aqueous phase does not seem to repel significantly the cationic solutes, which would shift them towards the stationary phase. Finally, the large retention of the β-adrenoceptor antagonists should be explained by their association with the C18 alkyl-bonded chains of the stationary phase and the electrostatic inter-

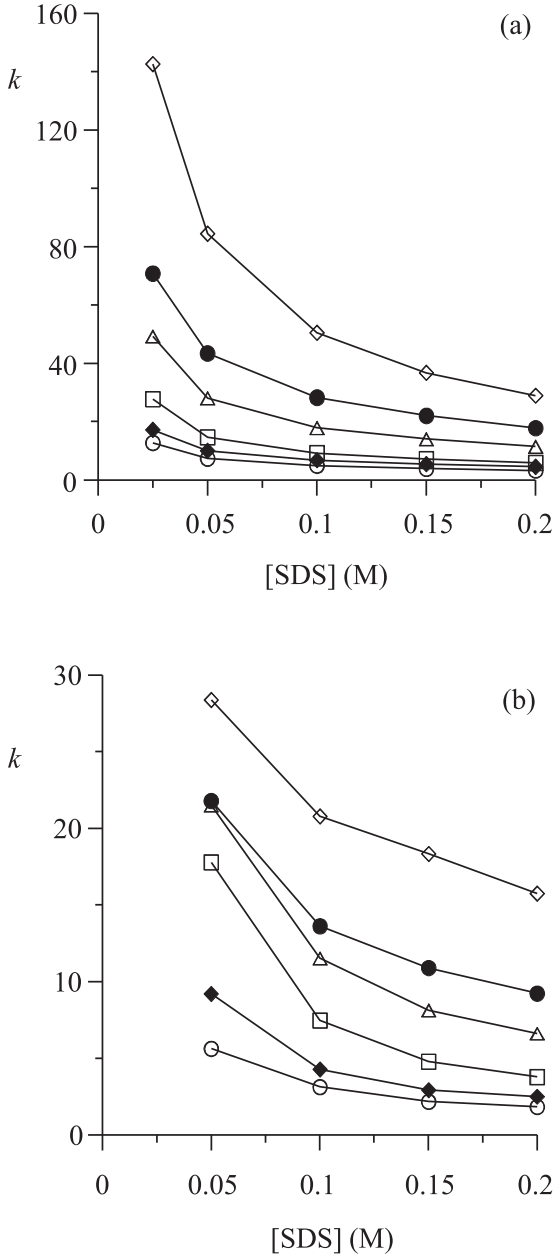

Fig. 2. Change in retention of several β-adrenoceptor antagonists, eluted with mixed mobile phases containing a fixed concentration of [C_6C_1IM][Cl] and increasing concentration of SDS: (a) 0.005 M [C_6C_1IM][Cl] and (b) 0.03 M [C_6C_1IM][Cl]. See Fig. 1 for solute identity.

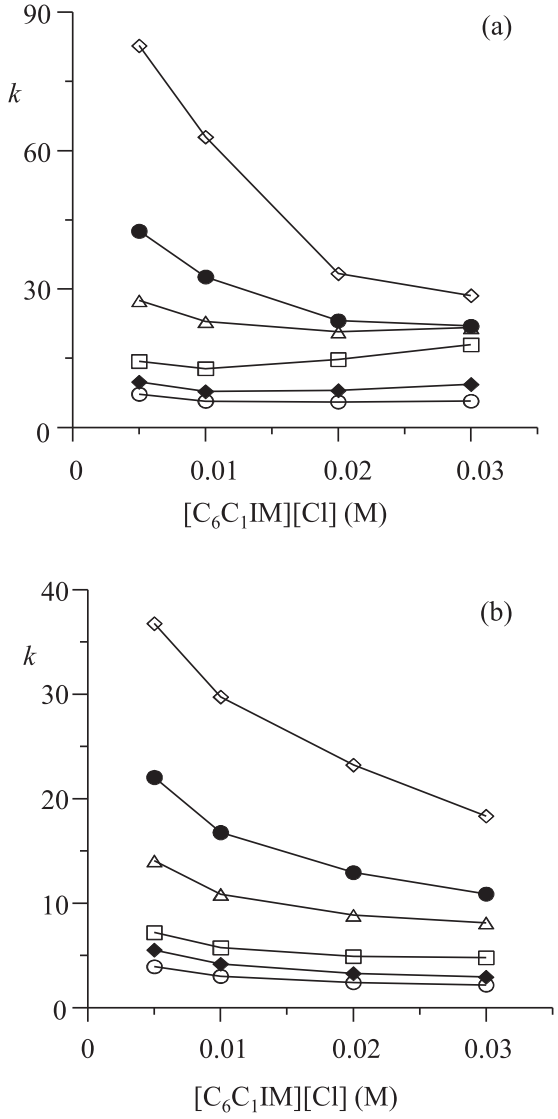

Fig. 3. Change in retention of several β-adrenoceptor antagonists, eluted with mixed mobile phases containing a fixed concentration of SDS and increasing concentration of [C_6C_1IM][Cl]: (a) 0.05 M SDS and (b) 0.15 M SDS. See Fig. 1 for solute identity.

action with the negatively charged residual silanols. This interpretation will be confirmed below.

3.2. Retention behavior of the β-adrenoceptor antagonists with aqueous mobile phases containing both SDS and [C_6C_1IM][Cl]

Although the retention of the β-adrenoceptor antagonists is also the result of the combination of hydrophobic and electrostatic interactions with the non-modified stationary phase, the charge of the mobile phase additives and solutes is mainly responsible for the changes in the retention. We considered interesting to study the effect on the retention of β-adrenoceptor antagonists of a mixture of the two additives with opposite charge (the anionic SDS and the cationic [C_6C_1IM][Cl]), which are significantly adsorbed on the stationary phase. The result should largely depend on the adsorption capability of the additives on the stationary phase and their concentration in the mobile phase. We have previously observed that pure mobile phases with only SDS or [C_6C_1IM][Cl] give rise to excessive retention. It was expected that by combining the two oppositely charged additives, at different concentrations, an adequate modulation of the retention of the cationic solutes should be possible.

3.2.1. Effect of SDS on retention

Mobile phases composed by mixtures of SDS and [C_6C_1IM][Cl] were prepared to study the effect of SDS on the retention. In the first set of experiments, the concentration of IL was fixed at two concentrations (0.005 M and 0.03 M), and SDS was increased (0.025, 0.05, 0.10, 0.15 and 0.20 M). In Fig. 2a and b, it can be appreciated that an increasing concentration of SDS in the mobile phase usually produces the reduction in the retention of the β-adrenoceptor antagonists, with a similar trend to that observed in the absence of IL (especially at the lowest concentration of IL, 0.005 M). The reduction in retention can be again attributed to the increased formation of SDS micelles in the mobile phase.

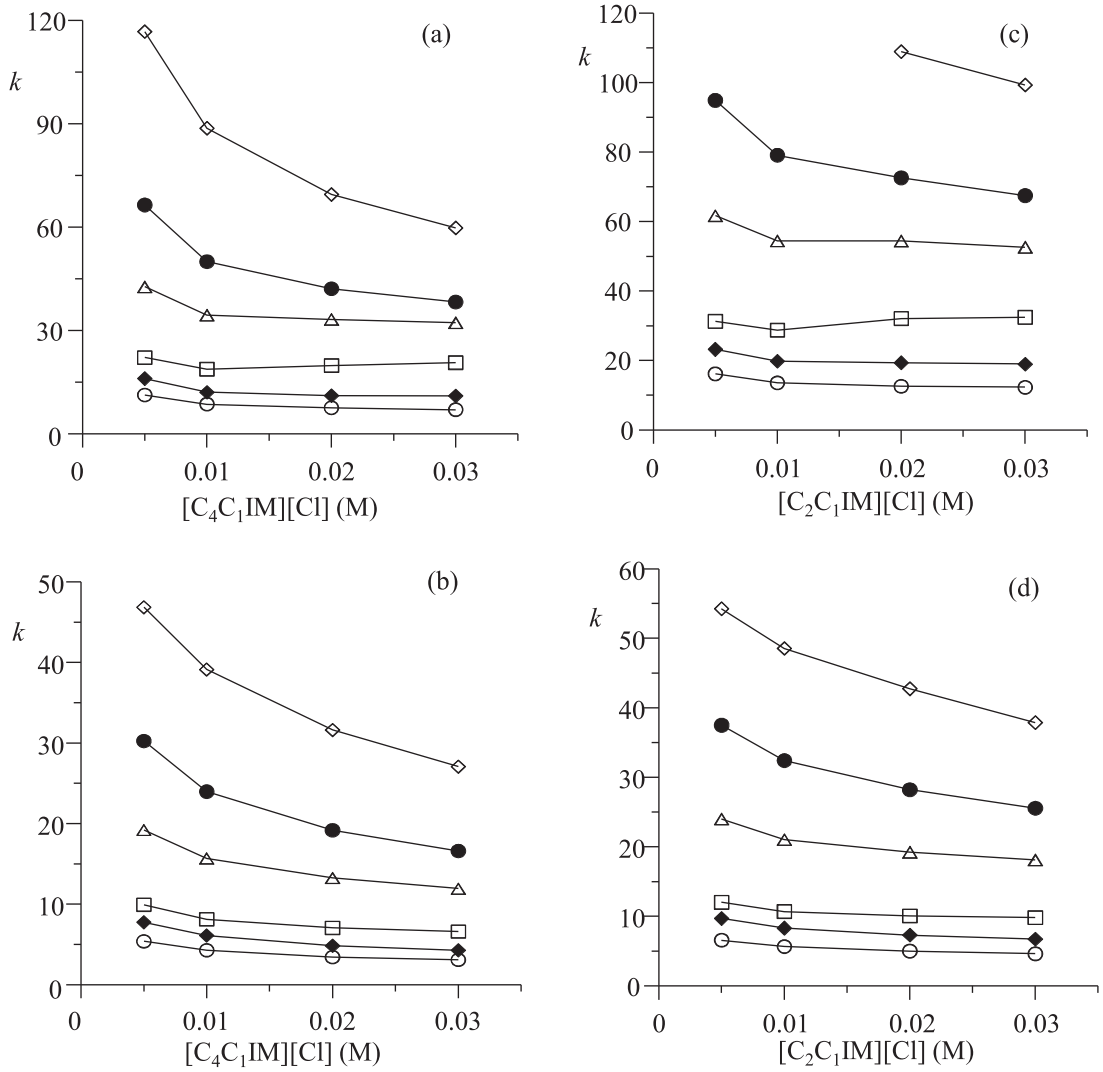

Fig. 4. Change in retention of several β-adrenoceptor antagonists, eluted with mixed mobile phases containing fixed concentrations of SDS: 0.05 M (a,c) and 0.15 M (b,d), and increasing concentration of: (a,b) $[C_4C_1IM][Cl]$ and (c,d) $[C_2C_1IM][Cl]$. See Fig. 1 for solute identity.

It should be noted that in the presence of IL, the reduction in the retention of β-adrenoceptor antagonists is substantial, especially at larger concentration of IL (0.03 M vs. 0.005 M). This indicates an appreciably lower elution strength, which should be interpreted by the higher IL/SDS ratio in the mobile phase, which makes the repulsion effect of IL cation on the cationic solutes more apparent. Thus, for example, for 0.10 M SDS in the absence of IL, the retention factor of propranolol was $k = 120$, and became $k = 60$ and 22 when 0.005 M and 0.03 M $[C_6C_1IM][Cl]$ was added, respectively. This reduction in retention can be again explained by the repulsion between the positively charged β-adrenoceptor antagonists and the IL cation. In this series, at both concentrations of $[C_6C_1IM][Cl]$, using increasing amounts of SDS, the retention of propranolol was low enough to be measured.

3.2.2. Effect of 1-hexyl-3-methylimidazolium on retention

Fig. 3a and b depict the changes in retention for mobile phases containing fixed concentration of SDS (0.05 M and 0.15 M, respectively) and increasing concentrations of $[C_6C_1IM][Cl]$ (0.005, 0.01, 0.02 and 0.03 M). In Fig. 3b (at the largest assayed concentration of SDS, 0.15 M), the retention followed the expected trend. In contrast, Fig. 3a (at 0.05 M SDS) shows that at the largest concentrations of IL (0.02 and 0.03 M) the retention slightly increased for some solutes (metoprolol, carteolol, atenolol and acebutolol). These results indicate that the anionic SDS and the IL cation compete for the adsorption sites on the stationary phase.

3.2.3. Effect of diverse imidazolium cations on retention

The results described in Sections 3.2.1 and 3.2.2 were obtained with $[C_6C_1IM][Cl]$. As indicated, the adsorption of the ILs on a C18 alkyl-bonded stationary phase depends on the length of the side hydrocarbon chain in position 1 in the imidazolium cation. Therefore, it was considered interesting to observe the behavior of the cationic solutes in the presence of ILs with shorter chains in the imidazolium group: 1-ethyl- and 1-butyl-3-methylimidazolium chlorides ($[C_2C_1IM][Cl]$ and $[C_4C_1IM][Cl]$), whose adsorption on C18 stationary phases is weaker [11].

The effect on retention was measured for mixed mobile phases composed of 0.05 or 0.15 M SDS and increasing concentrations of IL (0.005, 0.01, 0.02 and 0.03 M). Fig. 4 depicts the changes in retention in the presence of $[C_4C_1IM][Cl]$ and $[C_2C_1IM][Cl]$. These results must be compared with those in Fig. 3 for $[C_6C_1IM][Cl]$, using the same concentrations of SDS. As commented, the low adsorption of chloride ion on the stationary phase facilitates the observation of the effect produced by each cation.

The analysis of the results reveals that retention increases in the order $[C_6C_1IM]^+ < [C_4C_1IM]^+ < [C_2C_1IM]^+$. In all cases, the retention times decreased at larger concentration of the IL, the effect being more intense with a longer side chain in the imidazolium cation. Thus, for 0.005 M IL, the retention factor for propranolol was $k = 90$, 118 and above 120 in the presence of 0.05 M SDS, and $k = 28.5$, 47 and 54 in the presence of 0.15 M SDS, for $[C_6C_1IM]^+$, $[C_4C_1IM]^+$ and $[C_2C_1IM]^+$, respectively.

3.3. Peak profile in the mixed surfactant-IL systems

In RPLC, chromatographic peaks are often somehow broad and asymmetric. Highly symmetric and narrow peaks favor chromatograms with high resolution. The main problem lies in the analysis of basic compounds, which tend to elute as tailed and asymmetric peaks. This section shows the changes in the peak profiles of the set of β-adrenoceptor antagonists in aqueous mobile phases containing different concentrations of SDS and IL. The study has been carried out using the so-called half-width plots. The representation of the values of the left (A) and right (B) half-widths, versus the retention time, allows visualizing the changes in the width and asymmetry of the chromatographic peaks in liquid chromatography, as the conditions inside the chromatographic column are modified. Half-widths were measured at 10% peak height to avoid the peak symmetry be affected by the baseline noise of chromatograms. In previous work [24,25], half-width plots have been shown useful to compare the chromatographic behavior of different compounds, different chromatographic columns, and mobile phases of diverse composition. The construction of the plots is simple and the depicted line for each half-width can be characterised by the following equations:

$$A = A_0 + m_A t_R \quad (1)$$

$$B = B_0 + m_B t_R \quad (2)$$

where m_A and m_B are the slopes of the linear correlations, and A_0 and B_0 the corresponding intercepts that represent the extra-column contribution to the peak broadening. Eqs. (1) and (2) are useful to predict the peak half-widths for compounds eluted at different retention times, and to characterise chromatographic columns. The sum of m_A and m_B represents the broadening rate of chromatographic peaks as the solutes elute inside the column, and the m_B/m_A ratio indicates the peak asymmetry at high retention times.

In this work, the half-width plots were built with the information obtained for the whole set of analytes. Fig. 5 shows the plots for mobile phases containing only SDS or $[C_6C_1IM][Cl]$ at increasing concentrations. In both cases, there is some scattering in the data points, but the slopes of the left (m_A) and right (m_B) half-widths almost agree, which indicates significantly symmetric peaks. However, the elution with mobile phases containing only SDS gave rise to more symmetric peaks than mobile phases prepared with only the IL.

Fig. 6 depicts the half-width plots for the set of β-adrenoceptor antagonists, eluted with mixed mobile phases of SDS and $[C_6C_1IM][Cl]$ (Fig. 6a and b), $[C_4C_1IM][Cl]$ (Fig. 6c and d), or $[C_2C_1IM][Cl]$ (Fig. 6e and f), where the concentration was fixed for SDS (0.05 and 0.15 M) and increased for the ILs. It should be noted that the half-width plots show two trends, which are delimited by elongated circles. The values at short retention times are aligned and show greater symmetry and smaller width, whereas the values at longer retention times are more dispersed, but also give rise to an approximated linear trend. The first group of values correspond to the most polar solutes (acebutolol, atenolol and carteolol), and the second group to those most hydrophobic in the set (metoprolol, oxprenolol and propranolol). On the other hand, the values are more scattered at the largest assayed SDS concentration

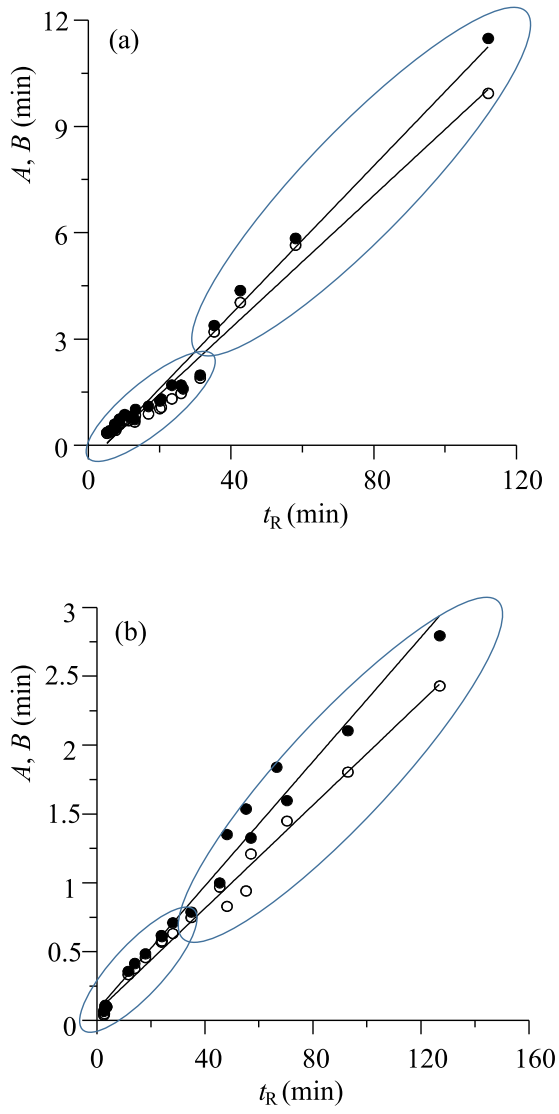

Fig. 5. Half-width plots (left, A (○) and right, B (●)), built with the data obtained for the set of β-adrenoceptor antagonists with mobile phases containing: (a) only SDS, and (b) only $[C_6C_1IM][Cl]$.

Table 1
Half-width plots parameters for the studied mobile phases: slopes for the left (m_A) and right (m_B) half-width plots, sum of slopes and slopes ratio.

Mobile phase	m_A	m_B	$m_A + m_B$	m_B/m_A
15% Acetonitrile-water[a]	0.0204	0.0482	0.0686	2.40
SDS	0.0935	0.1049	0.1984	1.12
$[C_6C_1IM]^+$	0.0187	0.0226	0.0413	1.21
SDS 0.05 M / $[C_6C_1IM]^+$	0.0745	0.0743	0.1488	0.997
SDS 0.15 M / $[C_6C_1IM]^+$	0.0788	0.0908	0.1696	1.15
SDS 0.05 M / $[C_4C_1IM]^+$	0.0639	0.0635	0.1274	0.994
SDS 0.15 M / $[C_4C_1IM]^+$	0.0819	0.0765	0.1584	0.93
SDS 0.05 M / $[C_2C_1IM]^+$	0.0470	0.0508	0.0978	1.08
SDS 0.15 M / $[C_2C_1IM]^+$	0.0689	0.0843	0.1532	1.22

[a] Ref. [26].

(0.15 M). The greater scattering of the plotted values indicates a larger difference between the interactions that the different compounds experience with the stationary phase modified with the additives.

Table 1 shows the parameters of the fitted straight-lines for the half-width plots (Figs. 5 and 6): the slopes of the left (m_A)

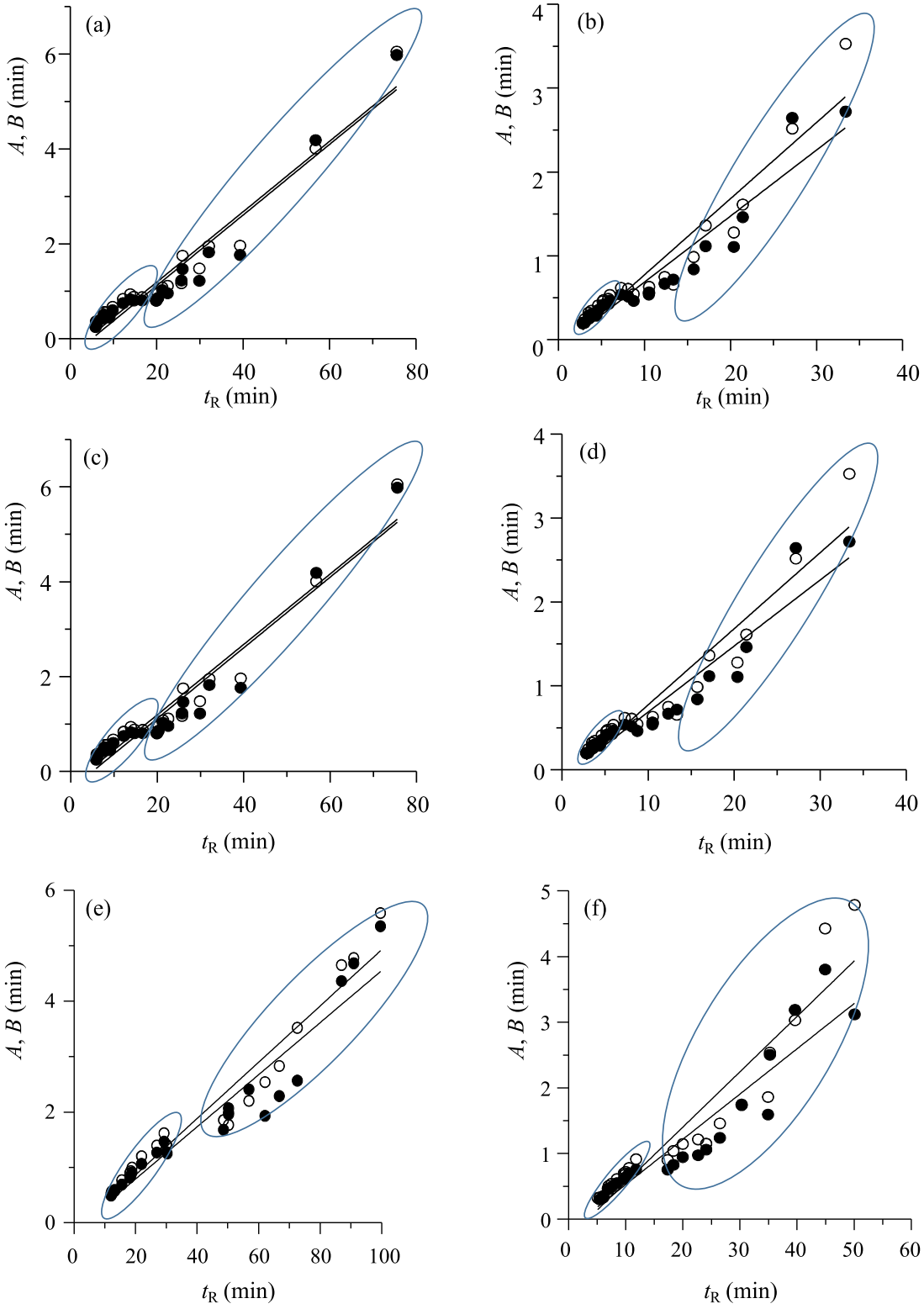

Fig. 6. Half-width plots (left, A (○) and right, B (●)), built with the data obtained for the set of β-adrenoceptor antagonists with mobile phases containing fixed concentrations of SDS: 0.05 M (a,c,e) and 0.15 M (b,d,f), and increasing concentration of: $[C_6C_1IM][Cl]$ (a,b), $[C_4C_1IM][Cl]$ (c,d), and $[C_2C_1IM][Cl]$ (e,f).

and right (m_B) half widths, and the sum and ratio of the slopes. All m_B/m_A ratios for the mobile phases containing additive are close to unity. This indicates low peak tailing, and therefore, more effective silanol masking when both additives are added to the mobile phase. Peak symmetry depended on the IL used, and can be ordered from greater to lesser as: $[C_6C_1IM]^+ \sim [C_4C_1IM]^+ > [C_2C_1IM]^+$. The peaks obtained with mobile phases formed by only SDS were associated to a larger $m_A + m_B$ value, which means a larger peak broadening rate at increasing retention time.

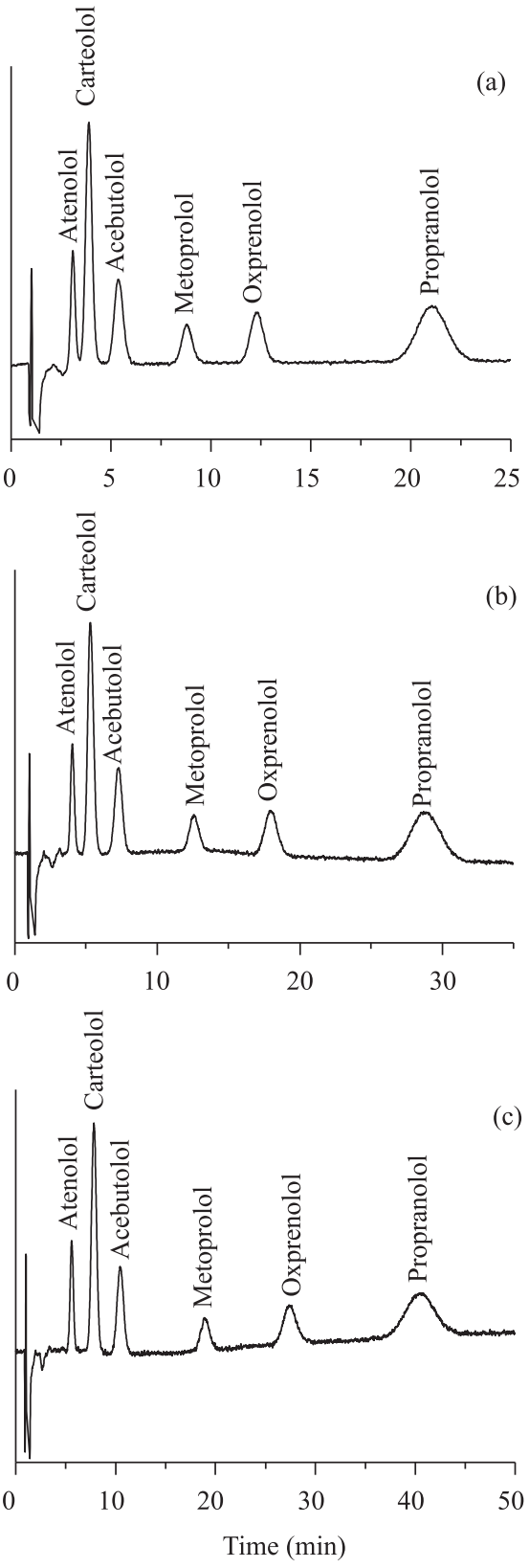

Fig. 7. Experimental chromatograms obtained at 254 nm for mixtures of the β-adrenoceptor antagonists with 0.15 M SDS and 0.02 M IL: (a) $[C_6C_1IM][Cl]$, (b) $[C_4C_1IM][Cl]$, and (c) $[C_2C_1IM][Cl]$. Column: XTerra-MS C18 (150 mm × 4.6 mm i.d., 5 µm).

3.4. Separation of mixtures of β-adrenoceptor antagonists

Fig. 7 depicts experimental chromatograms obtained for a mixture of the six β-adrenoceptor antagonists included in this work, using mobile phases buffered at pH ~ 3 containing 0.15 M SDS and 0.02 M $[C_6C_1IM][Cl]$, $[C_4C_1IM][Cl]$ or $[C_2C_1IM][Cl]$. The concentration of the β-adrenoceptor antagonists was 80 μg/ mL for atenolol, 10 μg/mL for carteolol, 3 μg/mL for acebutolol, and 100 μg/mL for metoprolol, oxprenolol and propranolol. It should be indicated that the optimal wavelength to detect the β-adrenoceptor antagonists is 225 nm. However, it was necessary to use longer wavelengths (254 nm), due to IL absorption at 225 nm.

The proposed mobile phases that combine SDS and IL are able to separate the mixture of six β-adrenoceptor antagonists with a basic cationic character, using liquid chromatography without the need of adding an organic solvent to the mobile phase. The chromatograms show peaks with good profiles and without significant overlapping. As observed, the analysis time depended on the adsorption capability of the added IL on the stationary phase, following the order $[C_6C_1IM]^+ < [C_4C_1IM]^+ < [C_2C_1IM]^+$. In fact, the elution order of β-adrenoceptor antagonists followed the capability of ILs to repel basic compounds.

4. Conclusions

In this work, a novel aqueous liquid chromatographic methodology is proposed. The elution of solutes is carried out with an aqueous solution containing an anionic surfactant (SDS) and an IL derived from 1-alkyl-3-methylimidazolium, both of them environmentally friendly. The mobile phases are completely transparent and have the right viscosity to be used with conventional pumps commonly used in liquid chromatography.

The retention of β-adrenoceptor antagonists is excessive when the mobile phases contain only SDS or a 1-alkyl-3-methylimidazolium IL. When both reagents are mixed, the anionic surfactant adsorbed on the stationary phase is able to attract the cationic solutes, whereas the IL cations repels them. The combination of both effects (attraction and repulsion) allows the modulation of retention, by varying the IL/SDS ratio. The nature of the IL cation (in this work, $[C_6C_1IM]^+$, $[C_4C_1IM]^+$ and $[C_2C_1IM]^+$) gives rise to different adsorption capabilities on the stationary phase, which produces changes in the analysis times. It should be noted that, given the significant adsorption of SDS and the negligible adsorption of the chloride anion in the IL, the effect of the studied ILs only depends on the nature of the IL cation. The longer the side alkyl chain of the imidazolium cation, the more strongly adsorbed on the stationary phase and therefore, the larger the repulsion of the cationic solutes.

The observed behavior seems to indicate that the anionic SDS shows more affinity for the stationary phase surface, compared to the ILs examined in this work. As the IL concentration in the mobile phase increases, its amount adsorbed on the stationary phase also increases. This compensates the attraction produced by the SDS anions over the cationic basic compounds. Therefore, both the absolute retention of the solutes and the elution strength decrease. The observed pattern also suggests that the electrostatic attraction between the basic cationic solutes and the anionic surfactant adsorbed on the stationary phase prevails over the repulsion produced by the adsorbed IL cations.

Finally, it is shown that it is possible to achieve the analysis of mixtures of acebutolol, atenolol, carteolol, metoprolol, oxprenolol, and propranolol, with practical analysis times and good resolution. In this work, we have only studied the separation of six β-adrenoceptor antagonists, but more complex mixtures are expected to be resolved in acceptable analysis times. The big advantage of the proposed methodology is the removal of the organic

solvent from the mobile phase, which gives rise to a new type of green liquid chromatography. It should be reminded that most reported procedures in the literature, with mobile phases containing SDS or IL, require the addition of organic solvent to achieve acceptable analysis times.

An additional advantage of our proposal is the possibility of direct injection of physiological samples, in the presence of SDS. This also avoids the use of organic solvents for the extraction of the analytes before their injection into the chromatograph. There is a great deal of reports on the development of direct injection methods in MLC with mobile phases containing SDS. However, there are no previous reports on the use of an IL to carry out direct injection in a chromatographic system.

Declaration of Competing Interest

None.

Data Availability

Data will be made available on request.

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