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Effect of short-chain alcohols on surfactant-mediated reversed-phase liquid chromatographic systems

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ABSTRACT

The behaviour of β-blockers in a reversed-phase liquid chromatographic (RPLC) column with mobile phases containing a short-chain alcohol (methanol, ethanol or 1-propanol), with and without the surfactant sodium dodecyl sulphate (SDS), was explored. Two surfactant-mediated RPLC modes were studied, where the mobile phases contained either micelles or only surfactant monomers at high concentration. Acetonitrile was also considered for comparison purposes. A correlation was found between the effects of the organic solvent on micelle formation (monitored by the drop weight procedure) and on the nature of the chromatographic system (as revealed by the retention, elution strength and peak shape of β-blockers). When SDS is added to the mobile phase, the free surfactant monomers bind the C18 bonded chains on the stationary phase, forming an anionic layer, which attracts strongly the cationic β-blockers. The retention is modified as a consequence of the solving power of the organic solvent, micelles and surfactant monomers. The molecules of organic solvent bind the micelles, modify their shape, and may avoid their formation. They also bind the monomers of surfactant, desorbing them from the stationary phase, which affects the retention. The remaining surfactant covers the free silanols on the siliceous support, avoiding the interaction with the cationic solutes. The retention of β-blockers results from a combination of electrostatic and hydrophobic interactions, the latter being weaker compared to the hydro-organic system. The peak efficiencies and asymmetries are excellent tools to probe the surfactant layer on the stationary phase in an SDS/organic solvent system. The peaks will be nearly symmetrical wherever enough surfactant coats the stationary phase (up to 60% methanol, 40% ethanol, 35% 1-propanol, and 50% acetonitrile).

1. Introduction

In 1980, a reversed-phase liquid chromatographic (RPLC) mode was first reported, where the mobile phases consisted of aqueous solutions of a surfactant at concentration levels above the critical micellar concentration (CMC) [1]. Owing to the existence of micelles in the mobile phase, the technique was called micellar liquid chromatography (MLC). The addition of an organic solvent to the mobile phase was, however, soon suggested in order to enhance the low efficiencies and weak elution strength associated to the mobile phases that contained only micelles [2].

In MLC, solute separation is achieved on the basis of the differential partitioning between the bulk aqueous phase and the micellar aggregates in the mobile phase, and the bulk aqueous phase and the monomers of surfactant coating the stationary phase [3]. Several authors have shown that organic solvents affect these partitioning equilibria [4], and strongly influence micelle formation [5]. Such is the enhancement of the chromatographic performance (i.e. elution strength, efficiency, selectivity and resolution) in the presence of organic solvents that most analyses in MLC are performed with mobile phases containing both surfactant and organic solvent.

Although surfactants of different nature (with either ionic and non-ionic head groups) can be used in MLC, the most common in analytical procedures is the anionic sodium dodecyl sulphate (SDS) [4,6], which attracts positively charged solutes. This surfactant allows the use of organic solvents that are normally not considered in classical hydro-organic RPLC [7,8]. Aliphatic alcohols are the most frequent. Short-chain alcohols (ethanol and propanol) interact with the micelle surface, reducing the repulsions among the ionic heads of the monomers of surfactant, whereas more hydrophobic alcohols (butanol and pentanol) are inserted in the non-polar micelle core [9]. Only few references have been reported in MLC on the analytical use of the most common RPLC solvents, acetonitrile and methanol [4,6].

In practice, the amount of organic solvent that can be added to a micellar mobile phase is limited by its solubility and micelle

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disaggregation. It is well accepted that micelles are disrupted at concentrations (v/v) above 40% methanol, 30% ethanol, 22% 1-propanol, and 30% acetonitrile [10,11], but these values are not conclusive. Also, the absence of a sudden breakdown of micelles, and of remarkable differences in the chromatographic retention behaviour, below and above these limits, do not allow to know if micelles still exist.

If the added organic solvent induces micelle breakdown, ion–pair interactions between charged solutes and free surfactant monomers (instead of micelles) in the bulk solvent will coexist with the ion-exchange on the still surfactant-modified stationary phase. This gives rise to another chromatographic mode, recently referred to as high submicellar chromatography (HSC) [11]. This chromatographic mode was first used to separate aromatic compounds [12,13]. Later, it showed attractive advantages with respect to MLC for a group of basic drugs (β-blockers), in terms of analysis time, selectivity and peak shape [11,14].

In previous work, the examination of the chromatographic behaviour (retention and peak shape) of several β-blockers in RPLC with mobile phases containing SDS and acetonitrile revealed information on micelle formation and stationary phase coating with surfactant [11,14]. The anionic surfactant adsorbed on the stationary phase interacts strongly with the cationic basic drugs increasing their retention, and masks the silanol groups that are the origin of the poor efficiencies and tailing peaks for these drugs in hydro-organic RPLC with conventional columns. The strong attraction between the cationic solutes and anionic SDS micelles or monomers in the mobile phase and stationary phase suggested a direct transfer mechanism of β-blockers between mobile and stationary phase [14].

In the pioneering report by Dorsey et al. [2] on the effect of several organic solvents (methanol, ethanol, 1-propanol and acetonitrile) on the efficiency in MLC with SDS, the probe compounds were acetone, methyl ethyl ketone, toluene, benzene, acetophenone, phenol, nitrobenzene and anisole. By observing the peak shape of these compounds, the authors concluded that 1-propanol was the best modifier. Later research with other analytes also recommended the use of 1-propanol due to the enhanced resolution, although apolar solutes require 1-butanol or even 1-pentanol, instead, to decrease the retention times [4,6,15].

In this work, the chromatographic behaviour of β-blockers in SDS-mediated mobile phases in the presence of short-chain alcohols (methanol, ethanol and 1-propanol) is explored. In the studied conditions, the mobile phases contained either micelles or only surfactant monomers at high concentration. Acetonitrile is also considered for comparison purposes. The changes in the nature of the chromatographic system are interpreted considering the changes in retention, elution strength and peak shape.

2. Experimental

2.1. Reagents

The probe compounds were seven β-blockers: acebutolol (Italfarmaco, Alcobendas, Madrid, Spain), atenolol, pindolol, timolol (Sigma, St. Louis, MO, USA), celiprolol (Rhône-Poulenc Rorer, Alcorcón, Madrid), metoprolol and oxprenolol (Ciba-Geigy, Barcelona, Spain). The drugs were dissolved in a small amount of the organic solvent used as modifier in the mobile phase and diluted with water. The concentration of the stock and injected solutions was 100 and 20 μg/mL, respectively.

The mobile phases were prepared with sodium dodecyl sulphate (99% purity, Merck, Darmstadt, Germany), and methanol, ethanol, 1-propanol or acetonitrile (Scharlab, Barcelona), and 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).

2.2. Apparatus and column

A liquid chromatograph (Agilent, Waldbronn, Germany) equipped with an isocratic pump (Series 1200), an autosampler and a UV-visible detector (Series 1100) set at 225 nm was used. Data acquisition was obtained through an HPChemStation (Agilent, B.02.01). The analytical separation was accomplished using a Kromasil C18 column (5 μm particle size, 150 mm × 4.6 mm i.d., Análisis Vínicos, Ciudad Real, Spain), connected to a 30 mm guard column of similar characteristics. The flow-rate was 1 mL/min and the injection volume, 20 μL. Duplicate injections were made. An analytical balance (±0.0001 g, Precisa, Dietikon, Switzerland) was used to monitor micelle changes according to the drop weight procedure.

3. Results and discussion

In spite of the name usually given to the RPLC mode with mobile phases of a surfactant above the CMC (MLC), it should be remarked that the surfactant molecules do not only form micelles in the mobile phase. They are also adsorbed on the stationary phase surface, giving rise to an open micelle-like structure, with a high protagonism in the observed behaviour [16]. In RPLC with organic solvent–water mixtures, the β-blockers (which are positively charged at the usual pH of the mobile phases, pH < 7) associate with the stationary phase due to a combination of hydrophobic interactions with the alkyl-bonded chains (usually C18), and electrostatic interactions with residual silanols. When SDS is added to the mobile phase, the free surfactant monomers bind the C18 bonded chains on the stationary phase, forming a layer [17]. The stationary phase adopts, thus, a negative charge that attracts the cationic β-blockers. This attraction increases so remarkably the retention times, that for relatively low polar β-blockers, these are easily beyond 100 min in mobile phases containing only the surfactant. In fact, this significant increased retention indicates the coating of the stationary phase by the anionic SDS. It should be noted that SDS binding to the C18 chains takes place at concentrations below the CMC. Thus, for mobile phases containing 0.02 M SDS, which is not far from the CMC in water (ca. 8 mM [4]), the retention of polar β-blockers is significantly larger in MLC than in classical RPLC with organic solvent–water mixtures.

A chromatographic system with mobile phases containing SDS and an organic solvent is more complex than a hydro-organic system without additives. Besides the described effects, several simultaneous changes take place upon addition of an organic solvent on: (i) the amount of surfactant adsorbed on the stationary phase; (ii) the elution strength of the micelles, which are modified by the organic solvent, or eventually disrupted giving rise to surfactant monomers, and (iii) the elution strength of the organic solvent, which is partially associated to the micelles or surfactant monomers. The absolute retention is a result of the combination of all these effects. This can make the interpretation of the modification in the nature of the system extremely difficult. However, as we will comment below for mobile phases of SDS in the presence of acetonitrile, or the short chain alcohols methanol, ethanol and 1-propanol, the retention, efficiency and asymmetry of chromatographic peaks reveal details on the nature of the stationary phase, and the elution strength gives information on the nature of the mobile phase.

3.1. Probe compounds and concentration ranges of the organic solvents

The chromatographic behaviour of seven β-blockers, with pK_a values in the range 8.9–9.2, was examined, which ordered according to their polarity (octanol–water partition coefficient as log $P_{o/w}$ are given) are: atenolol (−0.026), acebutolol (1.19), pindolol (1.48), metoprolol (1.69), timolol (1.75), oxprenolol (1.82), and celiprolol (1.93) [18]. We have selected, however, only pindolol and celiprolol for the discussion below on the effects of organic solvents added to the surfactant-mediated RPLC system, since similar effects were observed for the other five β-blockers.

The retention times and peak profiles of the β-blockers were obtained in mobile phases containing 0.075 M and 0.15 M SDS, and methanol, ethanol or 1-propanol in the ranges 50–60%, 5–40% and 5–35%, respectively. For 1-propanol, the peaks for 0.02 M and 0.04 M were also examined in the range 5–35%. These data were compared with those obtained in previous work with mobile phases containing 0.075 M or 0.15 M SDS, and 5–50% acetonitrile [11]. According to the reported concentration limits that guarantee micelle formation, the selected ranges in the experimental design covered domains from micellar to high submicellar RPLC, where micelles and free surfactant monomers dominate in the bulk solvent, respectively. The usual cautions required when working with micellar mobile phases in RPLC were followed [19].

The concentration ranges for the different modifiers were selected to achieve enough retention for the most polar β-blockers, and not excessive retention for the most apolar ones. The pump back-pressure at increasing concentration of the short-alcohols, acetonitrile or SDS limited also their maximal content in the mobile phase. The weak elution strength of methanol, ethanol and acetonitrile forced the use of concentrations of SDS ≥ 0.075 M. Hydro-organic mobile phases (without surfactant) in the ranges 20–50% methanol, 10–25% ethanol, 5–15% 1-propanol, and 15–30% acetonitrile were used for comparison purposes.

3.2. Effect of alcohols on the properties of SDS micelles

The presence of micelles explains partially the elution strength in MLC. This is also modified by the added organic solvent, which associates to the micelles. Alcohols induce several changes in the properties of SDS micelles, such as the CMC, the aggregation number and the micelle structure [10]. These changes may have an important role in MLC and other separation techniques, such as micellar extraction and micellar electrokinetic chromatography, and should be considered to select the concentrations of surfactant and alcohol that allow micelle formation. However, surprisingly, there is a wide range of reported values for the CMC and aggregation number in SDS solutions prepared in the presence of alcohols. For example, in SDS/1-propanol systems, the CMC for SDS was estimated to be:

5.75, 4.65, 3.4 and 2.7 mM in 5, 10, 15 and 20% 1-propanol, respectively [5]
4.4, 5.2, 8.3 and 13.5 mM in 5, 10, 15 and 20% 1-propanol [20]
7.2, 6.3, 6.0 and 5.9 mM in 1.5, 3.0, 4.5 and 6% 1-propanol [21]

and the aggregation number:

10, 9, 8 and 7 in 5, 10, 15 and 20% 1-propanol [20]
65, 56, 47 and 44 in 1.5, 3, 4.5 and 6% 1-propanol [21]
76 and 67 in 1 and 2% 1-propanol [22]

The disagreement can be explained mainly by the large variety of physical methods used to make the measurements, and the different concentrations of SDS in the solutions. Also, the estimations were performed for narrow SDS concentration ranges, which are usually out of the practical values in MLC. Therefore, it is difficult to get conclusions from this information. On the other hand, there is little information on the micelle changes in the presence of other organic solvents, such as acetonitrile [5,11].

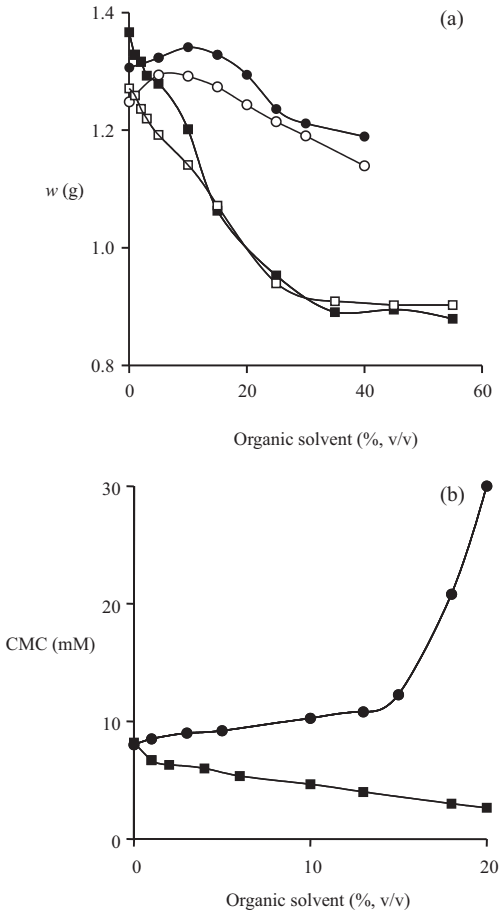

Fig. 1. (a) Weight of 50 drops of solutions containing 1-propanol and 0.02 M (■) or 0.15 M (□) SDS, or acetonitrile and 0.05 M (●) or 0.15 M (○) SDS. (b) Critical micellar concentration (CMC) of SDS in solutions containing 1-propanol (■) and acetonitrile (●).

In order to monitor the changes in the SDS micelles upon addition of 1-propanol at concentrations usual in MLC for both modifiers, an experimental study was performed by weighting 50 drops (delivered from a burette) of solutions containing SDS and 1-propanol. The results were compared with those obtained in a previous work with SDS and acetonitrile in the range 0–40% [11]. The drop weight procedure is based on the influence of the surface tension of a liquid on the size of a drop formed when the liquid is suspended from a glass tip. Working solutions contained 0.02 M or 0.15 M SDS, and variable contents of 1-propanol in the range 0–55%.

The results are plotted against the organic solvent content in Fig. 1a. As observed, the addition of 1-propanol to the SDS micellar solution decreased the drop weight up to 20–25% 1-propanol. Also, the weight did not depend on the surfactant concentration above 15% 1-propanol. Note that the addition of acetonitrile results in a different behaviour (Fig. 1a): initially, the drop weight increased up to a maximum close to 10% acetonitrile, and then decreased gradually up to 30%. This reveals that micelle perturbation is different for both solvents. In previous work, we checked that the addition of acetonitrile to SDS aqueous solutions increases the CMC (which was determined by the drop weight procedure) with an abrupt change

above 12% acetonitrile, whereas 1-propanol reduces this property (Fig. 1b) [5].

As we will comment below, the changes in the drop weight upon addition of an organic solvent correlate with the changes in retention and peak shape of β-blockers, which reveals some micelle perturbation and possible disaggregation. The behaviour depicted in Fig. 1a suggests a progressive reduction in the aggregation number of micelles with a final micelle breakdown that takes place in the ranges 15–20% for 1-propanol and 20–30% for acetonitrile.

3.3. Retention of β-blockers as indicator of the surfactant layer on the stationary phase

The organic solvent added to a surfactant-mediated chromatographic system reduces the amount of surfactant monomers coating the surface of the stationary phase. Berthod and Roussel reported a linear decrease in the adsorbed amount of SDS upon addition of several organic solvents, including methanol and 1-propanol [23]. The desorption rate of SDS for methanol was 9-folded smaller compared to 1-propanol. The maximal concentration of both modifiers examined for these authors was 5% methanol and 3% 1-propanol. If the linear pattern were followed at larger concentrations, the surfactant would be completely desorbed for 95% methanol and 10% 1-propanol. The assumption of a linear decrease of adsorbed surfactant with increasing modifier content beyond the studied range is questionable. The long retention times and high efficiencies of the β-blockers with mobile phases containing SDS and 50–60% methanol suggests that an important amount of surfactant still covers the stationary phase, whereas for 1-propanol at the maximal concentration examined in this work (35%), the surfactant layer was not desorbed totally.

As can be observed in Fig. 2a, the plots of the inverse of the retention factor ($1/k$) *vs.* the concentration of SDS, at varying 1-propanol contents in the mobile phase, are linear except for 35% 1-propanol at the smallest SDS concentrations assayed (i.e. below 0.04 M, the retention factors were smaller than expected). The linear behaviour indicates the saturation of the stationary phase by the surfactant. Meanwhile, departure from linearity suggests a reduction in the surfactant layer, owing to the small concentration of SDS in the mobile phase, combined with the high 1-propanol content.

The dashed lines in Fig. 2a depict the expected behaviour at concentrations of the surfactant close or below the CMC. In these regions, the concentration of micelles or surfactant monomers is small, and consequently, the elution strength associated to the surfactant. The added surfactant will be mainly bound to the stationary phase. When the surfactant layer decreases, the retention times tend to those observed in 1-propanol/water mixtures.

The extrapolation of the linear segments, where the classical MLC behaviour is observed, gives a measurement of the strength of the interaction between solute and stationary phase, expressed as the inverse of the intercept, according to:

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

where $[M]$ is the molar concentration of surfactant monomers forming micelles, and K_{AS} and K_{AM} are the solute-stationary phase and solute-micelle association constants.

The fitted Eq. (1) for pindolol and celiprolol, eluted with hybrid mobile phases containing SDS and increasing amounts of 1-propanol, is given in Table 1. Similar equations were obtained for the other β-blockers examined in this work. As observed, in mobile phases containing SDS, and 5 or 15% 1-propanol, the intercept is practically null (see also Fig. 2a), and consequently, K_{AS} should be large. The negative sign in the intercept in these conditions has no physical meaning and should be explained by the fitting error. This did not allow the calculation of K_{AS}. It was also not possible

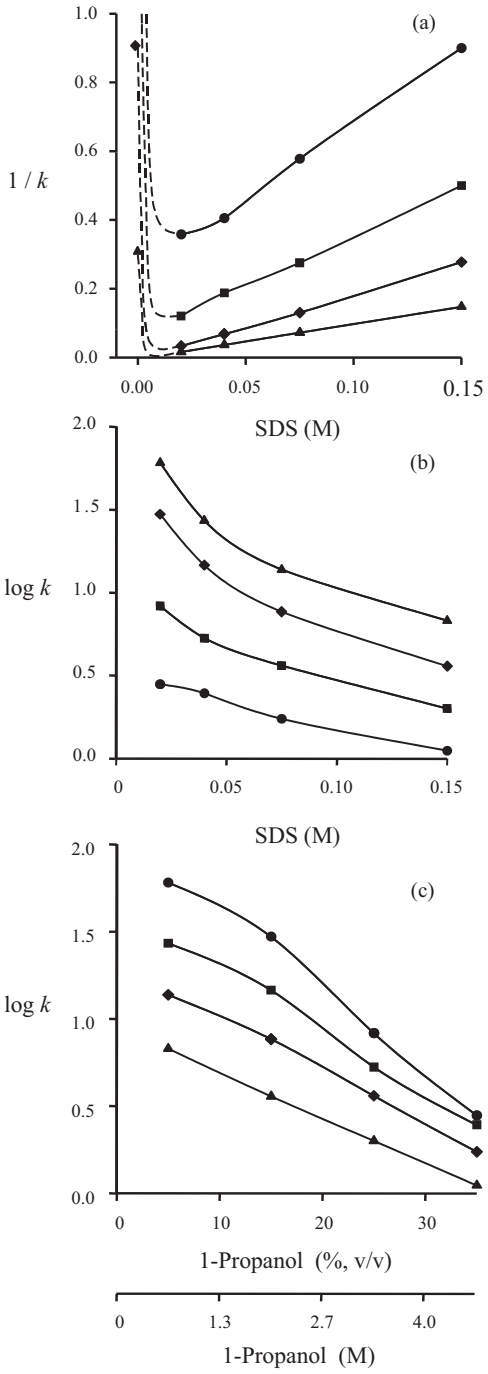

Fig. 2. Retention behaviour of pindolol in an RPLC system with mobile phases containing SDS and 1-propanol: (a) and (b) change in the retention factor with the surfactant concentration under different alcohol volume fractions: 5% (▲), 15% (♦), 25% (■), and 35% (●); (c) change in the retention factor with the 1-propanol content under different SDS concentrations (M): 0.02 (●), 0.04 (■), 0.075 (♦) and 0.15 (▲).

to evaluate the magnitude of K_{AM}, but the K_{AM}/K_{AS} ratio indicates that it should be also large. This result suggests the high affinity of the cationic β-blockers to the anionic surfactant, and the likely direct transference of these compounds between the micelle and the stationary phase modified by the surfactant.

The behaviour changes in mobile phases containing 25% 1-propanol (Fig. 2a), with positive intercepts (Eq. (1)), and K_{AS} = 15.2 and 24.9 for pindolol and celiprolol, respectively. A further decrease in K_{AS} is observed for 35% 1-propanol (K_{AS} = 3.9 and 4.4). The decrease in the association of solutes with the stationary phase

Table 1
Fitting to Eq. (1) of the retention data of two β-blockers eluted with hybrid mobile phases of SDS and 1-propanol.

Pindolol		
5% 1-propanol	$1/k = (-0.0035 \pm 0.0003) + (1.011 \pm 0.003)\,[M]$	$r^2 = 1.000$
15% 1-propanol	$1/k = (-0.007 \pm 0.003) + (1.88 \pm 0.04)\,[M]$	$r^2 = 0.9992$
25% 1-propanol	$1/k = (0.066 \pm 0.006) + (2.88 \pm 0.07)\,[M]$	$r^2 = 0.9988$
35% 1-propanol	$1/k = (0.233 \pm 0.015) + (4.46 \pm 0.15)\,[M]$	$r^2 = 0.9988$
Celiprolol		
5% 1-propanol	$1/k = (-0.0035 \pm 0.0011) + (0.792 \pm 0.013)\,[M]$	$r^2 = 0.9995$
15% 1-propanol	$1/k = (-0.0078 \pm 0.0015) + (1.481 \pm 0.018)\,[M]$	$r^2 = 0.9997$
25% 1-propanol	$1/k = (0.040 \pm 0.003) + (2.60 \pm 0.04)\,[M]$	$r^2 = 0.9995$
35% 1-propanol	$1/k = (0.1970 \pm 0.0007) + (4.227 \pm 0.007)\,[M]$	$r^2 = 1.000$

should be explained, at least partially, by the reduction of the SDS layer on the stationary phase. It should be noted that the longer retention times of the β-blockers in the hybrid mobile phases of SDS and ethanol, methanol or acetonitrile did not allow the examination of the chromatographic behaviour at concentrations of SDS below 0.075 M, and the calculation of K_{AS} values.

Fig. 3 shows the retention behaviour for pindolol and celiprolol in mobile phases containing different organic solvents (methanol, ethanol, 1-propanol or acetonitrile), in the presence and absence of SDS. The polar pindolol exhibits short retention times in the hydro-organic mobile phases (Fig. 3a). Therefore, the higher retention in the chromatographic system with surfactant is produced by the surfactant layer on the stationary phase, which retains this β-blocker mainly through electrostatic attraction. Observe

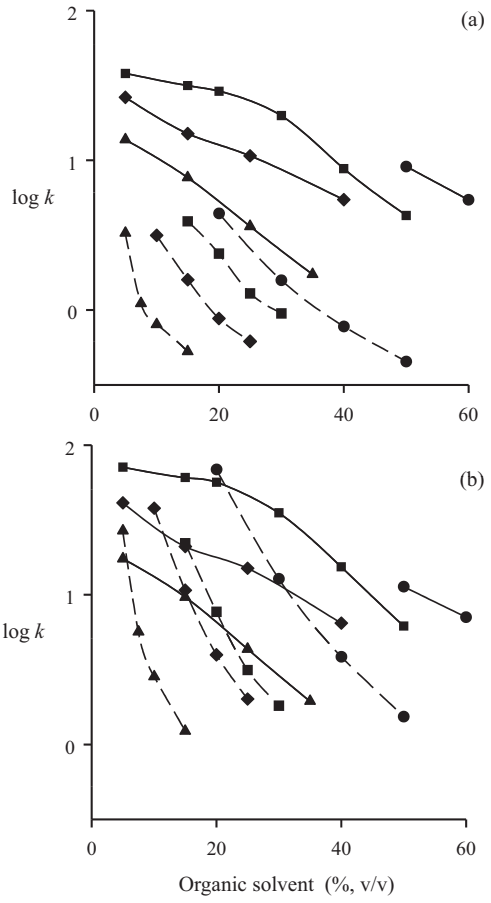

Fig. 3. Retention behaviour of: (a) pindolol, and (b) celiprolol eluted with hydro-organic mobile phases (dotted lines), and mobile phases containing 0.075 M SDS and organic solvent at increasing concentration (full lines). Organic solvents: methanol (●), ethanol (♦), 1-propanol (▲) and acetonitrile (■).

that, in contrast, the retention times for the less polar celiprolol with hydro-organic mixtures containing a small amount of the organic solvents (5%) were longer than those with the micellar mobile phases in the presence of 0.075 M SDS (Fig. 3b). Therefore, the hydrophobic interactions although still being important, are decreased in the presence of surfactant. On the other hand, the retention factors decreased upon the addition of the organic solvents in the order: methanol, acetonitrile, ethanol, and 1-propanol. This indicates the extent of surfactant desorption from the stationary phase, which is stronger for 1-propanol.

3.4. Efficiency and skewness of β-blockers as indicators of the surfactant layer on the stationary phase

The analysis of β-blockers and other basic drugs by classical hydro-organic RPLC is problematic due to the severely low efficiencies and tailed peaks, produced by the ionic interaction of the cationic solutes with the free silanols of the alkyl-bonded packings [24]. In previous work, we observed high efficiencies and symmetrical peaks for the β-blockers chromatographed with a C18 column, using hybrid mobile phases of SDS and acetonitrile [11]. The enhanced peak shape is explained partially by the reduction of the surfactant layer on the stationary phase in the presence of acetonitrile (i.e. a decrease in the carbon content). The remaining surfactant covers the free silanols on the siliceous support, avoiding the interaction with the cationic solutes. The mass transfer associated to the interaction of β-blockers with the anionic sulphate group in the surfactant seems to be more facile. Provided enough surfactant covers the silanol groups, the peak shape will be enhanced with respect to that obtained in the absence of surfactant.

The organic solvents examined in this work exhibit diverse ability to desorb the surfactant from the stationary phase, as indicated by the retention factors of the β-blockers (Fig. 3). However, the retention times do not describe exclusively the particular interaction of solutes with the stationary phase. These are also influenced by the elution strength of the modifiers in the mobile phase. In contrast, the peak shapes depend mainly on the kinetics of the interaction with the stationary phase. Therefore, the efficiencies and asymmetries of the β-blockers are excellent tools to probe the surfactant layer on the stationary phase in a hybrid SDS/organic solvent system [25].

The efficiencies, expressed as theoretical plates (N), were calculated according to the equation proposed by Foley and Dorsey [26], and the peak asymmetry as the tailing-to-fronting half-width ratio (B/A) at 10% peak height. The N and B/A values for 0.075 M SDS are plotted in Fig. 4a and b, respectively, but similar trends were observed for 0.15 M SDS. Smaller concentrations of SDS in the mobile phase yielded excessive retention for the assayed concentration ranges of the organic solvents.

As commented, the effect of the organic solvents on the peak shape is related to their ability to desorb SDS from the C18 stationary phase. This ability is greater for 1-propanol. The initial decrease in the amount of surfactant on the stationary phase facilitates solute diffusion and improves the efficiency. The peaks will be nearly symmetrical wherever enough surfactant coats the stationary phase. However, after reaching a plateau, further surfactant desorption allows the interaction of β-blockers with the unmasked ionized silanols on the C18 stationary phase, which results in poorer efficiencies and skewness. Interestingly, peak shape deterioration coincides with the region where the micelles disaggregate: 25–40% ethanol, 15–25% propanol and above 30% acetonitrile.

Maximal efficiencies for the SDS system were obtained for 30% acetonitrile, with a median value of $N = 8200$. The best efficiencies were: $N = 4400$, 3200, and 2800 for 50% methanol, 25% ethanol and 15% 1-propanol, respectively. The asymmetry factors were in the range $B/A = 0.93$–1.34 for 50% methanol, 5–25% ethanol, 5–15%

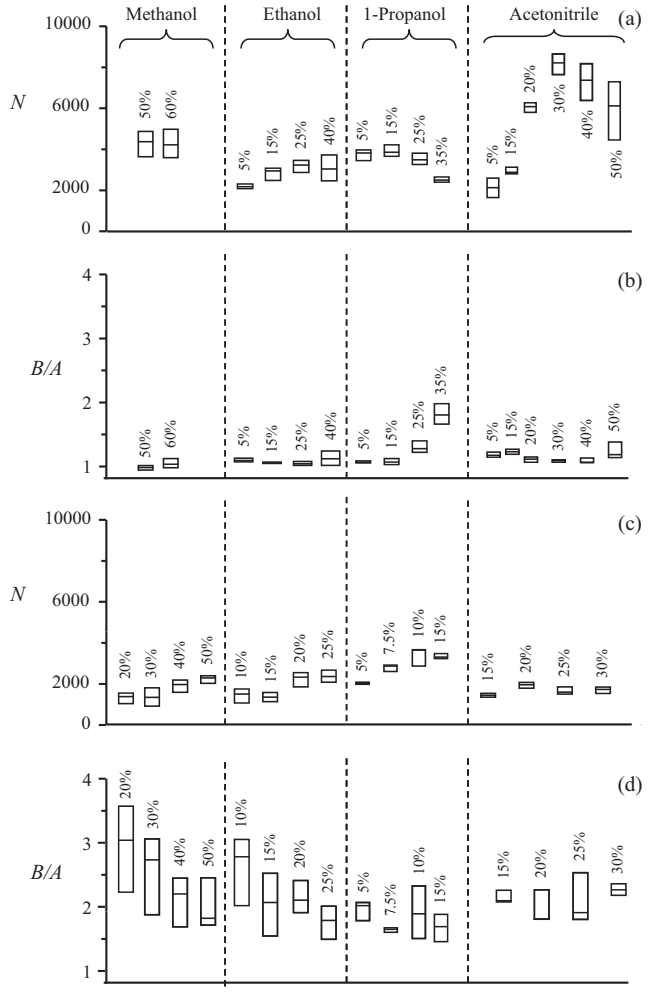

Fig. 4. Box-and-whisker plots depicting the efficiencies (N) and asymmetry factors (B/A) for the set of seven β-blockers, eluted with: (a) and (b) mobile phases containing 0.075 M SDS, and (c) and (d) hydro-organic mixtures. The volume fractions of the organic solvents in the mobile phases are indicated.

1-propanol and 5–30% acetonitrile. Fig. 4c and d depict the N and B/A values for hydro-organic mobile phases containing the four organic solvents. The trends are different from the SDS mobile phases, with poorer maximal median values of N = 1600, 1800, 1700, and 1300 for methanol, ethanol, 1-propanol and acetonitrile, respectively. The peaks were also significantly more asymmetrical, with median values usually above B/A = 2.

3.5. Role of the surfactant and organic solvent in the mobile phase

Solute retention in RPLC with surfactant-mediated mobile phases is decreased at increasing concentration of surfactant and organic solvent. The elution strength in RPLC is traditionally measured as the slope (S) of the plot of the logarithm of the retention factor ($\log k$) *vs.* the concentration of modifier. However, such plots are not really linear (i.e. the elution strength changes with the concentration of the modifier), except in narrow concentration ranges.

The retention behaviour in an SDS/1-propanol system is shown in Fig. 2b and c for pindolol, where $\log k$ is plotted against the molar concentration of SDS at four 1-propanol contents, or against the organic solvent content at four surfactant concentrations, respectively. As observed, the elution patterns for SDS and 1-propanol are different. The elution strength of the surfactant decreased gradually when its concentration increased (Fig. 2b). Since micelle disaggregation seems to occur in the range 15–25% (Fig. 1a), ion–pair interactions with surfactant monomers in the bulk mobile phase should replace those with micelles at 35% 1-propanol. Also as commented above, for 35% 1-propanol and SDS below 0.04 M, the stationary phase seems to be no more saturated.

Fig. 2c shows that at the smallest SDS concentrations assayed (0.02 and 0.04 M), the elution strength increased at increasing concentration of the organic solvent, as already observed for acetonitrile in a previous work [11]. The plots exhibit a change in the slopes around 15–25% 1-propanol, where according to the drop weight procedure (Fig. 1a), micelles probably disaggregate. This would indicate a change to a mobile phase containing free surfactant monomers. The relatively strong association of 1-propanol to the SDS micelles, with a binding constant, K = 3.5 at 25 °C (expressed as mole fraction ratio of the organic solvent per surfactant molecule) [27] can explain the smaller elution strength below 15–25% 1-propanol for 0.02 and 0.04 M SDS. The change in slope is less evident for 0.075 M and is not observed for 0.15 M SDS, where the organic solvent to surfactant concentration ratio is smaller.

Fig. 3 compares the retention behaviour in mobile phases containing methanol, ethanol, 1-propanol or acetonitrile in the presence (0.075 M SDS) and absence of surfactant, for pindolol and celiprolol. As commented above, owing to the weak elution strength of methanol in the presence of SDS, only a narrow range of concentrations could be examined for this alcohol (50–60%). On the other hand, the change in the slope in the presence of surfactant at increasing concentration of the organic solvent was more noticeable for acetonitrile, with a transition region between 20 and 30% acetonitrile, which as demonstrated in a previous work, is the region where micelles probably disaggregate [11]. This transition appears also for 0.15 M SDS. In contrast, the transition is less clear for ethanol. Note that the binding constant for this alcohol is K = 1.1 [28]. Unfortunately, we have not found this information for acetonitrile.

On the other hand, in all cases, there is a significant difference between the retention behaviour of the β-blockers in the presence and absence of SDS (Fig. 3). As commented, for pindolol, the retention times are appreciably longer with SDS mobile phases at all assayed concentrations of organic solvent, but the retention of celiprolol is larger using mobile phases with 5% organic solvent and without surfactant. Nevertheless, in both cases, the elution lines are concave in the absence of surfactant and convex for the mobile phases containing SDS. The presence of surfactant forming micelles seems to decrease the elution strength of the organic solvents. Therefore, in conditions where micelles probably do not exist and there is an excess of organic solvent (e.g. above 30% acetonitrile), the elution strength gets closer to that observed without surfactant. Thus, the slopes for 0.075 M SDS/50–60% methanol and 40–50% methanol–water, on the one hand, and for 0.075 M SDS/30–50% acetonitrile and 15–35% acetonitrile–water, on the other, are similar. Ethanol and 1-propanol were still stronger in the hydro-organic mobile phases, for the assayed ranges.

Finally, in the surfactant-mediated systems, the elution strength (S) of the organic solvent was smaller with respect to SDS. Thus, for pindolol, the S value for SDS in the region of smaller elution strength (Fig. 2b) was 4.11, 4.38, 3.44 and 2.56 for 5, 15, 25 and 35% 1-propanol, respectively; and the S value for 1-propanol in the region of larger elution strength (Fig. 2c) was 0.35, 0.25, 0.24 and 0.19 for 0.02, 0.04, 0.075 and 0.15 M SDS, respectively (expressed considering molar concentrations for both SDS and alcohol).

3.6. Implications on the selectivity

The additional interactions that take place inside a chromatographic column, in the presence of SDS, give rise to changes in the absolute and relative retention, and better peak profiles. This may enhance the resolution with respect to classical RPLC. Fig. 5

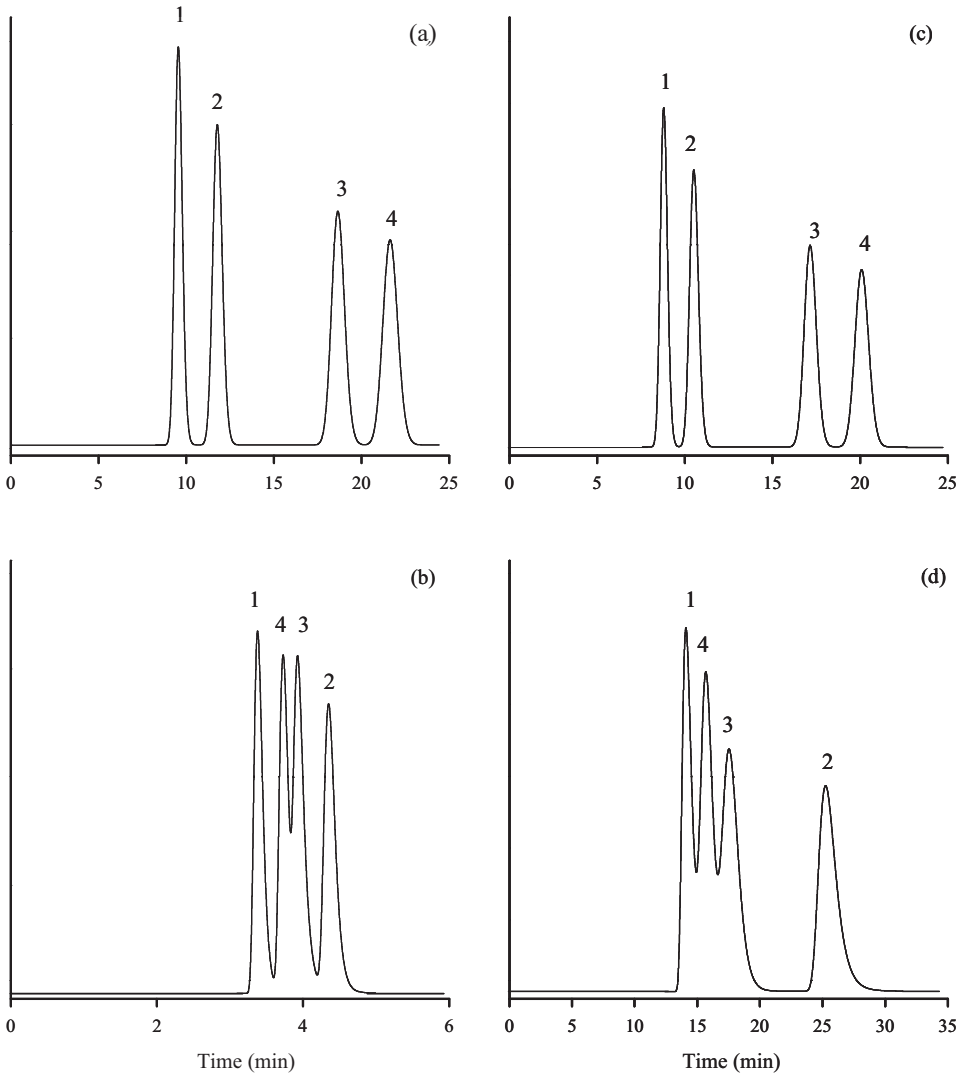

Fig. 5. Optimal chromatograms for a mixture of four β-blockers. Mobile phases: (a) 15% ethanol and 0.15 M SDS, (b) 24% ethanol, (c) 5% 1-propanol and 0.15 M SDS, and (d) 5.6% 1-propanol. Peak identities: (1) acebutolol, (2) celiprolol, (3) metoprolol, and (4) timolol.

shows as an example the optimal separation of a group of four β-blockers with mobile phases containing the alcohols ethanol and 1-propanol, in the presence (micellar conditions) and absence of SDS. In previous work, we described the enhanced separation of β-blockers with mobile phases of SDS and acetonitrile [14].

4. Conclusions

The chromatographic behaviour of basic drugs, as β-blockers, reveals the changes that take place in an RPLC system in the presence of an anionic surfactant, upon the addition of an organic solvent, but the interpretation is not simple due to the several simultaneous interactions that are established inside the chromatographic column. Changes in retention are yielded as a consequence of the solving power of three components: organic solvent, micelles and surfactant monomers. The organic solvent binds the micelles, modifies their shape, and finally avoids their formation. It also binds the monomers of surfactant, desorbing them from the stationary phase.

The retention and peak shape of β-blockers are indicators of the existence of a surfactant layer bound to the C18 groups, which covers the free silanol groups on the stationary phase. This gives rise to an enhanced peak shape in RPLC with hybrid surfactant/organic solvent mobile phases, in comparison to the hydro-organic mode, especially for acetonitrile. The results indicated that the stationary phase was still coated by SDS with mobile phases containing at least up to 60% methanol, 40% ethanol, 35% 1-propanol, and 50% acetonitrile.

Among the four organic solvents examined in this work, methanol showed the weakest interactions. Mobile phases with a large amount of this modifier (50–60%), where the existence of micelles is unlikely, were needed to get short retention times. Acetonitrile was also significantly weaker compared to ethanol and 1-propanol, which was the strongest modifier. The retention of β-blockers in the SDS system is a combination of electrostatic and hydrophobic interactions, the latter being weaker compared to the hydro-organic system. This is the reason that the requirement in conventional RPLC of performing gradient elution to achieve practical analysis times in a single run is not so imperative in the SDS-mediated systems.

In previous work, the advantage of using mobile phases of SDS containing a large amount of acetonitrile, where micelles are not formed (HSC mode), was shown. The combination of large efficiencies and enhanced selectivity yielded much better resolution and shorter analysis times, in comparison to MLC with SDS and acetonitrile, and to conventional RPLC with acetonitrile–water. In contrast,

this work shows that the best separation conditions for ethanol and 1-propanol corresponded to the MLC mode, which has the advantage of the small consumption of organic solvent. Finally, methanol cannot be recommended as modifier in combination with SDS for the analysis of basic drugs, owing to the long retention times and the need of high concentrations of this organic solvent. The practical implications of micellar and submicellar RPLC with short-chain alcohols in the resolution and analysis time of β-blockers are considered in further work in detail.

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References

[1] D.W. Armstrong, S.J. Henry, J. Liq. Chromatogr. 3 (1980) 657.
[2] J.G. Dorsey, M.T. DeEchegaray, J.S. Landy, Anal. Chem. 55 (1983) 924.
[3] D.W. Armstrong, F. Nome, Anal. Chem. 53 (1981) 1662.
[4] A. Berthod, M.C. García-Álvarez-Coque, Micellar Liquid Chromatography, Marcel Dekker, New York, 2000.
[5] S. López-Grío, J.J. Baeza-Baeza, M.C. García-Álvarez-Coque, Chromatographia 48 (1998) 655.
[6] M.J. Ruiz-Ángel, M.C. García-Álvarez-Coque, A. Berthod, Sep. Purif. Rev. 38 (2000) 45.
[7] S. López-Grío, M.C. García-Álvarez-Coque, W.L. Hinze, F.H. Quina, A. Berthod, Anal. Chem. 72 (2000) 4826.
[8] P. Loginova, E.Y. Yakovleva, M.N. Galat, A.P. Boichenko, J. Mol. Liq. 145 (2009) 177.
[9] K.L. Mittal (Ed.), Micellization, Solubilization and Microemulsions, Vol. 1, Plenum Press, New York, 1979.
[10] R. Zana, Adv. Colloid Interface Sci. 57 (1995) 1.
[11] M.J. Ruiz-Ángel, J.R. Torres-Lapasió, M.C. García-Álvarez-Coque, S. Carda-Broch, Anal. Chem. 80 (2008) 9705.
[12] X. Li, J.S. Fritz, J. Chromatogr. A 728 (1996) 235.
[13] P. Jandera, J. Fischer, H. Effenberger, J. Chromatogr. A 807 (1998) 57.
[14] M.J. Ruiz-Ángel, J.R. Torres-Lapasió, M.C. García-Álvarez-Coque, S. Carda-Broch, J. Chromatogr. A 1216 (2009) 3199.
[15] R.D. Caballero, M.J. Ruiz-Ángel, E. Simó-Alfonso, M.C. García-Álvarez-Coque, J. Chromatogr. A 947 (2002) 947.
[16] M.J. Ruiz-Ángel, S. Carda-Broch, J.R. Torres-Lapasió, M.C. García-Álvarez-Coque, J. Chromatogr. A 1216 (2009) 1798.
[17] A. Berthod, I. Girard, C. Gonnet, Anal. Chem. 58 (1986) 1356.
[18] S. Carda-Broch, A. Berthod, J. Chromatogr. A 995 (2003) 55.
[19] M.J. Ruiz-Ángel, M.C. García-Álvarez-Coque, LC–GC Eur. 21 (2008) 420.
[20] A.A. Rafati, H. Gharibi, M. Rezaie-Sameti, J. Mol. Liq. 111 (2004) 109.
[21] A.P. Romani, M.H. Gehlen, G.A.R. Lima, F.H. Quina, J. Colloid Interface Sci. 240 (2001) 335.
[22] E. Caponetti, D. Chillura-Martino, M.A. Floriano, R. Triolo, Langmuir 13 (1997) 3277.
[23] A. Berthod, A. Roussel, J. Chromatogr. 449 (1988) 349.
[24] J. Nawrocki, J. Chromatogr. A 779 (1997) 29.
[25] M.J. Ruiz- Ángel, S. Carda-Broch, M.C. García-Álvarez-Coque, J. Chromatogr. A 1217 (2010) 1786.
[26] J.P. Foley, J.G. Dorsey, Anal. Chem. 57 (1983) 730.
[27] L. Sepúlveda, E. Lissi, F.H. Quina, Adv. Colloid Interface Sci. 25 (1986) 1.
[28] R. De Lisi, S. Milioto, J. Solut. Chem. 17 (1988) 245.