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Determination of cationic surfactants by capillary zone electrophoresis and micellar electrokinetic chromatography with deoxycholate micelles in the presence of large organic solvent concentrations

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Abstract

Mixtures of the cationic surfactants benzalkonium chloride (BKC) and cetylpyridinium chloride (CPC) were quickly resolved and reproducibly and reliably determined by using background electrolytes (BGEs) containing 80 m*M* borate, pH 8.5, bile salts and large concentrations of an organic solvent. When the bile salt is present, the separation mechanism changes from capillary zone electrophoresis (CZE) to a mixed micellar electrokinetic chromatography (MEKC)–CZE, with predominant MEKC interactions, which lead to an excellent resolution of all the solutes, including the C_{12}–C_{18} homologues of BKC and CPC. A BGE containing 50 m*M* sodium deoxycholate and 30% ethanol for an extreme resolution, or 20% tetrahydrofuran for an adequate resolution within a much shorter analysis time, is recommended. The procedure was applied to the determination of the surfactants in industrial and household formulations, with excellent resolution between the homologues, detection limits of a few $\mu g\ ml^{-1}$ and reproducibilities below 2%.

Keywords: Background electrolyte composition; Bile salts; Benzalkonium chloride; Cetylpyridinium chloride

1. Introduction

Cationic surfactants are frequently used as antimicrobial, emulsifying, anticorrosion and softening agents in pharmaceutical preparations, cosmetic formulations, cleaning products, disinfectants and other industrial manufactures [1]. Appropriate analytical procedures for their determination in these products, as well as in urban sewers, industrial effluents and in the environment, are required. Further, some cationic surfactants are industrially produced as mixtures of several homologous compounds, thus, the characterization of samples by resolving the surfactant individual components is of interest in industrial quality control and environmental studies. The determination of cationic surfactants is usually performed by two-phase titration [2,3], high-performance liquid chromatography (HPLC) [4–8], gas chromatography (GC) [9–11] and thin-layer chromatography (TLC) [12–14]. However, owing to the problems associated to strong sorption and peak tailing, resolution is frequently poor, and in many cases homologues cannot be distinguished.

An alternative to chromatography is capillary electrophoresis (CE). The determination and characterization of cationic surfactants by capillary zone

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electrophoresis (CZE) can be conveniently performed by using a background electrolyte (BGE) with a large concentration of an organic solvent which hinders adsorption to the capillary walls, and also prevents the formation of mixed micelles, thus forcing the different chemical species to migrate individually. For this purpose, tetrahydrofuran (THF) [15–17], methanol [18], acetonitrile and acetone [19–21] have been used.

In micellar electrokinetic chromatography (MEKC) uncharged compounds are separated by adding an ionic surfactant at a concentration above its critical micellar concentration (CMC) to the BGE [22,23]. The uncharged solutes are discriminated by the different strength of the solute–micelle interactions. In addition, ionic micelles can also modify the selectivity in the separation of charged solutes via solute–micelle electrostatic and hydrophobic interactions. Thus in MEKC, ionic solutes can be separated by a mixed CZE–MEKC mechanism.

The most widely used surfactant in MEKC has been sodium dodecyl sulfate (SDS) [24,25], but bile salts including sodium cholate (SC), sodium deoxycholate (SDC) and others [26–29], and mixtures of bile salts with SDS [30], or with polyoxyethylene ethers such as polyethylene glycol (PEG) [31,32], are also frequently used. Bile salts having a large, rigid and planar hydrophobic moiety of a steroid nucleus, with two or three hydroxyl groups, are a special group of biosurfactants, whose properties differ considerably from most other surfactants [33]. Bile salts form small helical micelles, with low aggregation numbers, and with the hydrophobic portions of the monomer facing the aqueous solution, while the hydrophilic portions turn inward [34–39]. Bile salts provide different selectivities than SDS, and exhibit several useful chromatographic properties such as the ability to recognize enantiomeric conformations [26,40–42] and increased micelle polarity [27,41]. Further, the micelles formed by bile salts can tolerate large concentrations of organic solvents (e.g., 30% ethanol) without disrupting their structural integrity. This is particularly remarkable, since the micelles of most surfactants are destroyed in water–organic media containing much lower organic solvent concentrations.

In this work, a CE procedure for the separation of the cationic surfactants benzalkonium chloride (BKC) and cetylpyridinium chloride (CPC) was developed. These surfactants are widely used as antimicrobial agents in the treatment of common infections of the mouth and throat, and BKC is also used as a vaginal spermicide and as a disinfectant in working environments, including hospitals. Therefore, rapid and reliable procedures for the determination of these surfactants, including the BKC homologues, in pharmaceuticals and other industrial products is of utmost interest. The surfactants, including the homologues of BKC, were separated by CE in presence of large concentrations of several organic solvents. In addition, SC and SDC were used as selectivity modifiers in the same media, to separate pairs of overlapping peaks, and to improve efficiency, via a mixed CZE–MEKC mechanism. The procedure was applied to the determination of cationic surfactants in industrial and household formulations, with excellent resolution between the homologues.

2. Experimental

2.1. Instrumentation

A HP^{3D} CE system (Hewlett-Packard, Palo Alto, CA, USA), equipped with a diode-array spectrophotometric detector, and a fused-silica capillary (Supelco, Bellefonte, PA, USA) of 33.5 cm (25 cm effective length)$\times$50 μm I.D. (363 mm O.D.) was used. The absorption spectra used for the determination of CMC were measured with two spectrophotometers, i.e., a Lambda-16 (Perkin-Elmer) and an HP8453 diode-array.

2.2. Reagents and samples

The BKC homologues benzyldimethyldodecyl ammonium chloride (C_{12}), benzyldimethyltetradecyl ammonium chloride (C_{14}), benzyldimethylhexadecyl ammonium chloride hydrate (C_{16}) (Fluka, Buchs, Switzerland) and benzyldimethyloctadecyl ammonium chloride (C_{18}) (Aldrich, Milwaukee, WI, USA), and CPC (Sigma, St. Louis, MO, USA), were used as standards. A commercial sample of BKC (containing 62.3% C_{12}, 34.7% C_{14} and 0.4% C_{16}, contents determined by HPLC, Fluka, Quality Con-

trol Department), SC, SDC (Fluka) and SDS (Merck, Darmstadt, Germany), were also used. Optimization studies were performed using 2 m*M* solutions of commercial BKC and CPC in water.

The two buffers used were prepared by adding a diluted sodium hydroxide solution to a sodium dihydrogenphosphate or to a boric acid solution to reach pH 7 or 8.5, respectively; analytical-grade reagents from Panreac (Barcelona, Spain) were used. Methanol, ethanol, acetonitrile, *n*-propanol and THF were from Scharlau (Barcelona). Rhodamine 6G (Sigma) was used for the CMC determinations. All solutions were prepared with deionized water (Barnstead deionizer, Sybron, Boston, MA, USA). Before injection, all solutions were filtered through a 0.45-μm pore size nylon filter (MSI, Westboro, MA, USA).

2.3. Procedures

Daily before use and between runs, the capillary was successively rinsed with 0.1 *M* NaOH (5 min), water (1 min) and the running buffer (5 min). After each working session the capillary was flushed with 0.1 *M* NaOH and water for 10 min each. The hydrodynamic injection mode at 50 mbar, 2 s was used. Unless otherwise indicated, the applied voltage was 15 kV of positive polarity. Detection wavelengths were 214 nm for BKC, and both 214 and 254 nm for CPC. The samples were directly dissolved in water, except some pharmaceutical preparations which were previously diluted with some ethanol. When necessary, peak identification was made by adding standards to the samples. The wavelength shift of the absorption maximum of rhodamine 6G (as the average of the values obtained with the two spectrophotometers) upon increasing the surfactant concentration was used to determine CMC values [28].

3. Results and discussion

3.1. CZE studies

Attempts to separate the homologues of BKC and CPC were initially performed at 25 kV using two BGEs: (i) 80 m*M* borate, pH 8.5, and (ii) 80 m*M* phosphate, pH 7. In both cases, a solution containing CPC and commercial BKC gave a single broad peak with a tail. As expected, the peak was located at a migration time below the electroosmotic flow (EOF) time. The low efficiency and lack of resolution were attributed to the formation of mixed micelles, and tailing to adsorption on the capillary walls. Then, the addition of increasing amounts of either ethanol and *n*-propanol to the BGEs as adsorption competitors and micelle disruptors, was investigated.

Three effects were observed: (i) a reduction of the EOF, as expected from the viscosity increase and adsorption of the alcohol to the capillary walls, (ii) a progressive resolution increase between the peak of the C_{12} homologue of BKC with respect to a single broad peak given by the C_{14} homologue and CPC,

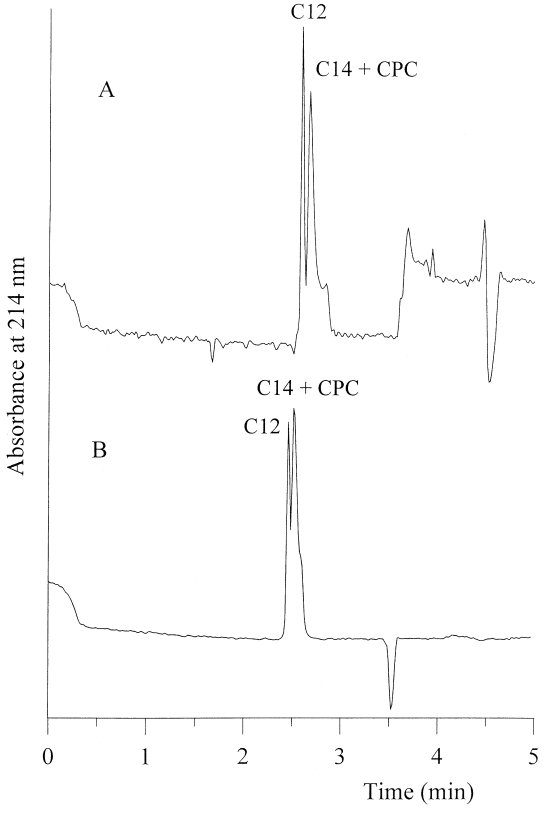

Fig. 1. Electropherograms of a mixture containing 2 m*M* commercial BKC (with 62.3% C_{12} and 34.7% C_{14}) and 2 m*M* CPC. Conditions: 25 kV, positive polarity, BGE containing 80 m*M* phosphate, pH 7.0 and 40% ethanol (A) or *n*-propanol (B). The height of the largest peak corresponds to 15 mAU.

and (iii) an improvement in the efficiency and symmetry of the peaks. The effects can be explained on the basis of the reduction of adsorption and the breakdown of micelles, which allows the migration of the surfactant molecules on individual basis rather than as mixed micelles. As shown in Fig. 1, an incipient separation of the C_{14} and CPC peaks was observed at pH 7 with 40% alcohol. Resolution was better at this pH than at pH 8.5, and tailing was also lower, which can be explained on the basis of the protonation of the silanol groups, and their strong interaction with phosphate.

It should be noted that CPC and the C_{14} homologue of BKC, which have different molecular masses, overlapped, whereas CPC and the C_{12} homologue, which have exactly the same molecular mass, i.e., 304.3 g mol^{-1}, gave a separate peak. The larger migration time of CPC in relation to the C_{12} homologue can be explained as a consequence of the lower electrical density on the CPC aromatic nitrogen, with a charge which is partially neutralized by the π electrons, which does not occur with the alkyl substituted nitrogen of the BKC homologues. This leads to a lower electrophoretic mobility in absolute terms for CPC than for the C_{12} homologue which appears at a longer distance from the EOF time.

Further studies were performed with *n*-propanol at pH 7. Resolution of the C_{12} peak with respect to the peak given by the other two solutes increased up to 40% *n*-propanol, and no further changes other than the progressive reduction of the EOF were observed at higher alcohol concentrations up to 60%. The influence of the buffer concentration was studied using from 20 to 120 m*M* phosphate and 40% *n*-propanol. Maximum resolution was obtained with 80 m*M* phosphate. Similar values of the efficiency and resolution were obtained by decreasing the applied voltage to 15 kV.

3.2. Micellization and MEKC studies using bile salts

To modify selectivity increasing resolution a different approach was tried, i.e., the addition of SC or SDC to the BGE. All micelles are disrupted by large organic solvent concentrations, but the micelles of bile salts can stand much larger solvent concentrations than most other surfactants. Thus, in the presence of adequate concentrations of organic solvents, bile salt micelles can still interact strongly with the free molecules of other surfactants, thus modifying the selectivity of the electrophoretic separations via an additional MEKC retention mechanism. Further, several authors [29,43–45] have recommended the use of basic conditions for the analysis of cationic solutes by CE. However, in basic media cationic surfactants can strongly interact with the extensively ionized silanol groups of the capillary wall. Solute–wall interactions can be avoided by adding a competing base to the buffer [43], e.g., Quang et al. [44] observed a dramatic improvement in peak shapes of organic amines when the concentration of SDS was increased, which was attributed to the effective competition of the anionic micelles with the silanol groups on the capillary wall. Therefore, the presence of SC or SDC in the BGE can also help to improve efficiency and to reduce tailing owing to competition with the solutes for the silanol groups.

When MEKC with anionic micelles and positive polarity are used solute peaks appear after the EOF time and, thus, a basic medium can also be important to reduce analysis time by increasing the EOF.

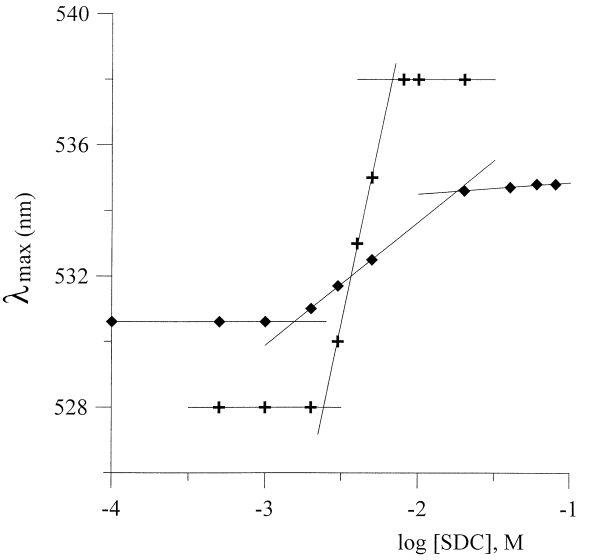

Fig. 2. Wavelength of the absorption maximum of rhodamine 6G plotted against the SDC concentration in a medium containing 80 m*M* borate, pH 8.5 in the absence of ethanol (+) and with 30% ethanol (♦).

Further, the solubility of anionic bile salts such SC and SDC increase in basic media. For these reasons, and to confirm the points discussed above, the CMC of SDC in a series of media containing 80 m*M* borate buffer of pH 8.5, and increasing amounts of ethanol (0, 10, 20, 30, 32, 35 and 40%) was determined. For this purpose, and for each ethanol concentration, a series of solutions with increasing SDC concentrations and containing 2.5 μ*M* rhodamine 6G, was prepared. In all cases, the wavelength of the absorption maximum of the dye, λ_{max}, was measured. For each ethanol concentration, the CMC of SDC was obtained from the inflection point of the corresponding λ_{max} against log [SDC] plot (Fig. 2). As shown in Fig. 3, the CMC decreased slightly when the ethanol concentration increased up to a 32%, and exhibited a sharp increase over this value. Thus, in the 80 m*M* borate buffer of pH 8.5, the SDC micelles stand up to 32% ethanol.

Next, electropherograms of the BKC and CPC mixture were obtained using the same series of 80 m*M* borate buffers, pH 8.5, containing 50 m*M* SDC and increasing amounts of ethanol up to 40%, as BGEs. As can be observed in Fig. 4, in the presence of SDC but without the organic solvent, a single narrow peak located after the EOF time was obtained; however, when the percentage of ethanol increased, the band was resolved in a series of peaks. In contrast to what was observed in the absence of SDC (Section 3.1), all the solutes, including the CPC with respect to the C_{14} homologue of BKC, were resolved over 10% ethanol. The resolution between the C_{14} and CPC peaks was plotted against the ethanol concentration. As observed in Fig. 3, resolution was low at ethanol concentrations below 20%, increased rapidly at higher concentrations and decreased over 35% ethanol, which coincided with the disruption of the SDC micelles.

The influence of the SDC concentration, which

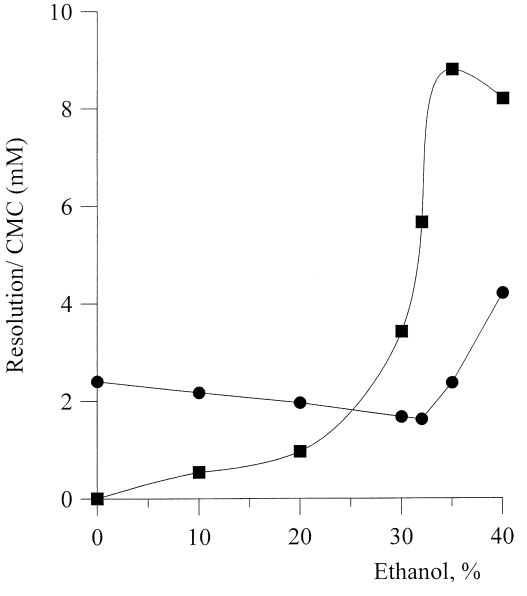

Fig. 3. CMC of SDC (●) and resolution for the C_{14} homologue of BKC and CPC (■) plotted against the ethanol concentration. Conditions: 15 kV, positive polarity, BGE containing 80 m*M* borate, pH 8.5, 50 m*M* SDC and increasing ethanol concentrations.

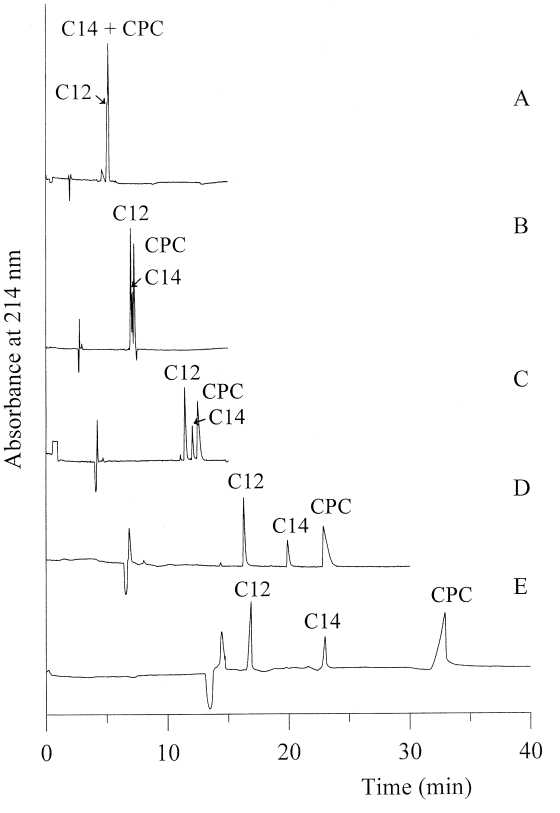

Fig. 4. Electropherograms of a mixture containing 2 m*M* commercial BKC (with 62.3% C_{12} and 34.7% C_{14}) and 2 m*M* CPC. Conditions as in Fig. 3; ethanol concentrations from A–E: 0, 10, 20, 30 and 40%, respectively. The height of the largest peak corresponds to 30 mAU.

was studied with 30% ethanol, is shown in Fig. 5, left part. Resolution and analysis time increased with the bile salt concentration. As also shown in Fig. 5, right part, the same behavior was obtained using SC instead of SDC; however, when compared at the same concentrations, SC gave lower resolution than SDC.

3.3. Comparison with other solvents and use of other additives

BGEs containing 80 m*M* borate, pH 8.5 and 50 m*M* SDC, but substituting ethanol by increasing amounts of methanol, *n*-propanol, acetonitrile or THF were tried. In all cases, and as already observed in Fig. 4 with ethanol, resolution increased up to a maximum when the percentage of organic solvent increased. The optimum found was located ca. 20% for *n*-propanol and THF, 30% for acetonitrile and 40% for methanol.

Excellent resolution was achieved with THF, the analysis time being almost half that when using ethanol (Fig. 6). The peaks were also well resolved in a short time (less than 10 min) with *n*-propanol, but an impurity of CPC appeared too close to the peak of the C_{14} homologue of BKC. Finally, resolution was lower with acetonitrile or methanol. Using these solvents, each one at its respective optimum concentration, resolution and analysis time increased in the order: methanol<acetonitrile<*n*-propanol<THF<ethanol.

The effect of using mixtures of 50 m*M* SDC with other surfactants and PEG, in the presence of 20% *n*-propanol, was tried. Significant modifications of the electropherograms were not observed when the non ionic surfactants Tween 20 or Triton X-100

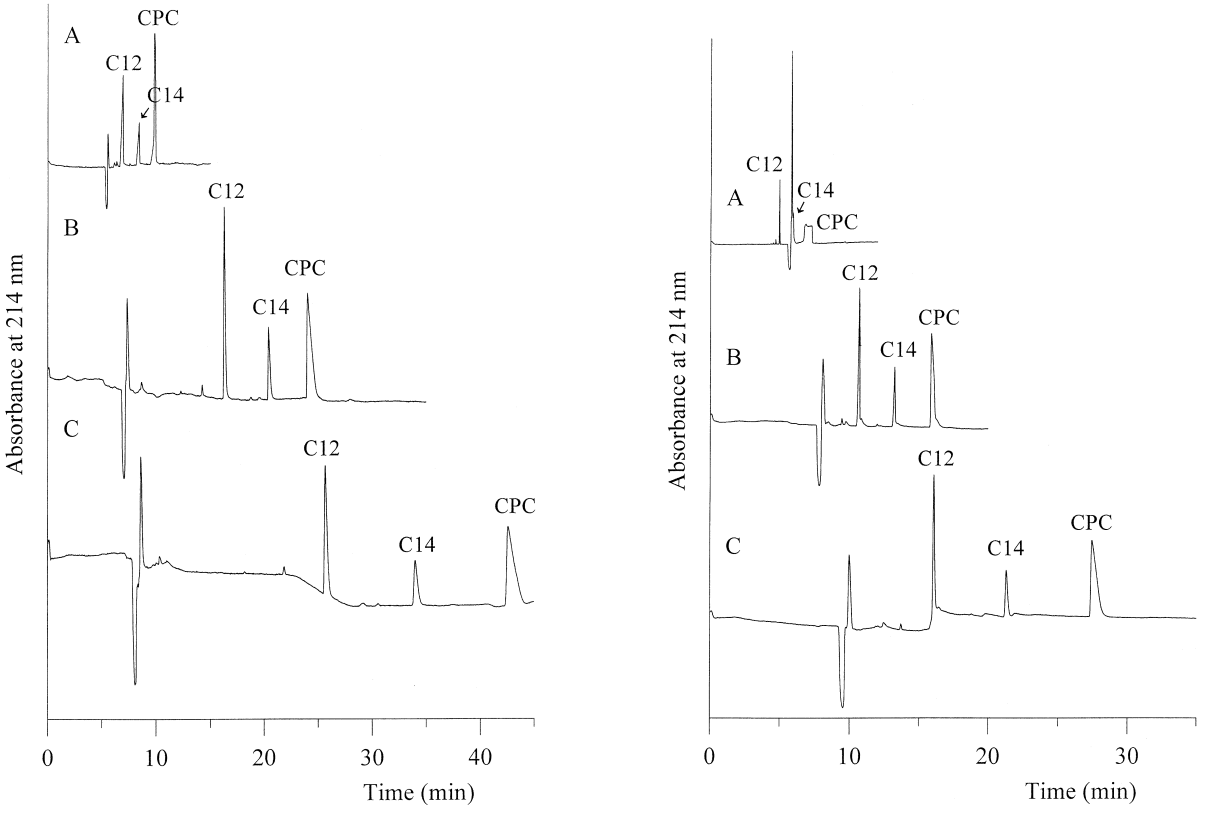

Fig. 5. Electropherograms of the mixture indicated in Fig. 4. Conditions: 15 kV, positive polarity, BGE containing 80 m*M* borate, pH 8.5, 30% ethanol and different bile salt concentrations: 15 (A), 50 (B) and 75 m*M* (C) of SDC (left part) or SC (right part). The height of the largest peak corresponds to 30 mAU.

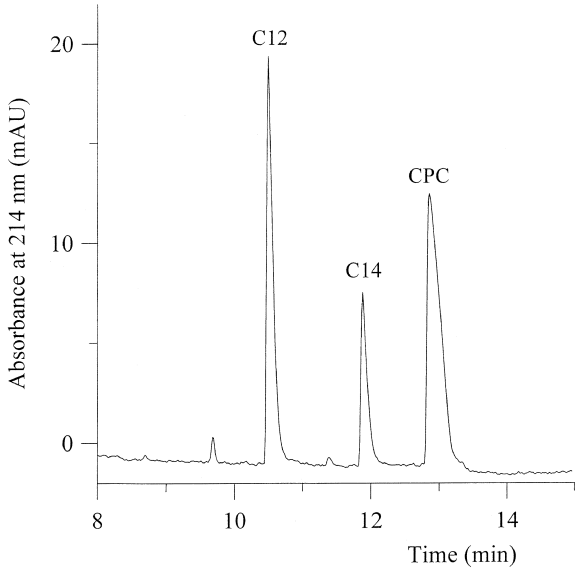

Fig. 6. Electropherogram of the mixture indicated in Fig. 4. Conditions: 15 kV, positive polarity, BGE containing 80 m*M* borate, pH 8.5, 50 m*M* SDC and 20% THF.

(1–10%), or when PEG-4000, PEG-6000 and PEG-10 000 (1, 5 and 10%), were added to the BGE. Resolution and efficiency decreased when SDS (10–40 m*M*) was added.

3.4. Figures of merit

The figures of merit for the determination of the surfactants, including migration time and peak area repeatabilities, relative sensitivities, efficiencies and detection limits at a signal-to-noise ratio of 3, were obtained with 80 m*M* borate, pH 8.5, 50 m*M* SDC and 30% ethanol. Intra- and inter-day repeatabilities were obtained by injecting the same 0.2 m*M* solution three times per day during 3 days. Calibration curves for the C_{12}, C_{14}, C_{16} and C_{18} homologues of BKC (measured at 214 nm), and for CPC (measured at 254 nm) were established by preparing six standard solutions of each solute at evenly spaced concentrations up to 0.6 m*M*. The curves were linear with $r>0.999$; other data are given in Table 1.

Table 1
Migration time (t_m) and peak area (A) repeatabilities[a], relative sensitivities (RSs)[b], efficiencies and detection limits for $S/N=3$

Solute	t_m (%)	A (%)	RS	$N\cdot10^{-5}$ (m^{-1})	DL ($\mu g\ ml^{-1}$)
C_{12}	0.7; 1.2	1.2; 1.7	1.00	4.9	3
C_{14}	0.8; 1.2	1.4; 1.7	1.01	3.4	4
C_{16}	1.0; 1.4	1.7; 2.1	0.65	2.5	8
C_{18}	1.2; 1.7	2.0; 2.5	0.57	2.05	25
CPC	0.8; 1.4	1.3; 1.9	0.70	2.3	4

[a] As intra- and inter-day relative standard deviations.
[b] As the ratio of the slopes of the calibration curves using concentrations in m*M*. Absorbances were measured at 214 nm for the BKC homologues and at 254 nm for CPC.

Table 2
Analysis of commercial samples

Samples (manufacturer), surfactant analyzed	Declared (%)	Found (%)
Industrial disinfectant for hospitals (Lab. Coloma), BKC	10	9.98
Commercial BKC concentrate (Guinama), BKC	50	49.8
Mini-óvulo, spermicide (Lab. Lanzas), BKC	1.18	1.13
Afta Juventus, mouth disinfectant (Labs. ERN), BKC	0.01	0.0098
Topicaína, oral disinfectant (Organon Teknica), BKC	0.5	0.50
Cuve, skin disinfectant (Labs. Pérez Giménez), CPC	0.1	0.096
Silidermil polvo, skin disinfectant (Fides-Rottapharm), CPC	0.5	0.48
Deli Plus, mouthwash (Faman Cosmetics), CPC	0.025	0.025
Deli Plus with fluoride, mouthwash (Faman Cosmetics), CPC	0.05	0.049

3.5. Application to real samples

The procedure was applied to identify and quantify BKC homologues and CPC in several pharmaceutical preparations, cosmetics, industrial disinfectants and cleaning products. The results are given in Table 2. As shown in Fig. 7 for two samples, well resolved peaks and flat backgrounds were obtained. In all cases, the CPC and BKC concentrations found, this later calculated as the sum of corresponding homologues, corresponded well with the declared composition. For the Guinama sample, whose declared BKC homologues composition was available, we found 67.6% C_{12}, 29.0% C_{14} and 3.4% C_{16}, the declared values being 68% C_{12}, 29% C_{14} and 3% C_{16}.

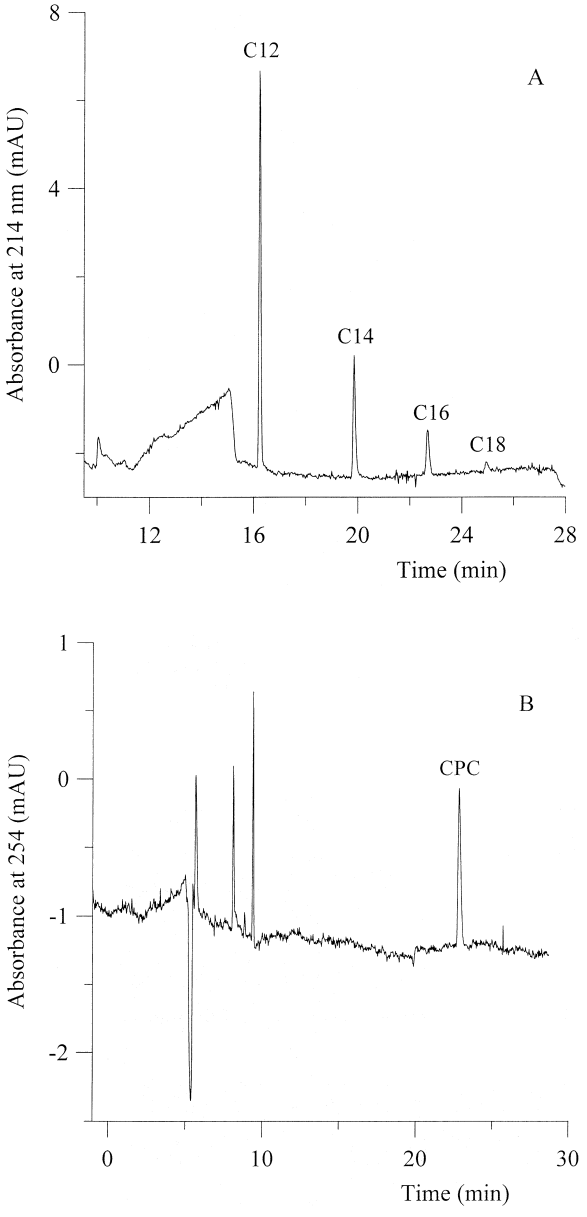

Fig. 7. Electropherograms of an industrial disinfectant for hospitals (A) and a mouthwash (B). Conditions as in Fig. 6, but using 30% ethanol instead of THF.

4. Conclusions

Mixtures of cationic surfactants can be quickly resolved and reproducibly and reliably determined by using BGEs containing a bile salt and large concentrations of an organic solvent. When the bile salt is present, the separation mechanism changes from CZE to a mixed MEKC–CZE, with predominant MEKC interactions. For BKC and CPC mixtures, a BGE containing 80 m*M* borate buffer, pH 8.5, 50 m*M* SDC and 30% ethanol for an extreme resolution, or 20% THF for an adequate resolution with a much shorter analysis time, is recommended. Mixtures of these and other cationic surfactants can also be resolved by using different bile salts at the adequate concentrations. The surfactants and their homologues can be determined with reproducibilities below 2% and detection limits of a few μg ml^{-1}. These limits can be lowered by using extended light path capillaries. The procedure is useful to determine cationic surfactants in a wide scope of industrial and household products.

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