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Investigation of the effects of cyclodextrins and organic solvents on the separation of cationic surfactants in capillary electrophoresis

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Abstract

The use of α- and β-cyclodextrin (CD) to understand and to improve the separation of a series of cationic surfactants, homologues of alkylbenzyldimethyl ammonium compounds (ABDACs) with an alkyl chain of varying length (C_{10}–C_{18}), in capillary electrophoresis (CE) is reported for the first time. Similar to the effects of organic solvents, the presence of α- or β-CD in the running buffer was found to reduce peak tailing/loss for the longer-chain ABDACs. Based on fluorescence measurements, it was found that formation of host–guest complexes occurred between α- or β-CD and various ABDACs, with the likelihood that the hydrophobic alkyl chain including into the CD cavity and the positively charged ammonium group remaining outside the cavity. The effects of α- or β-CD can be interpreted in terms of a shift away from the formation of (1) micelles in the buffer system and (2) surfactant aggregates at the fused-silica capillary walls, as a result of the formation of inclusion complexes between α- or β-CD and ABDACs. © 2000 Elsevier Science B.V. All rights reserved.

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1. Introduction

Cationic surfactants, such as unbranched alkyl homologues of alkylbenzyldimethyl ammonium compounds (ABDACs), are widely used in industrial and consumer applications, including antistatic agents in textile-softener formulations and antiseptic/antibacterial agents in pharmaceutical preparations [1]. The analysis of cationic surfactants by capillary electrophoresis (CE) is faced with a number of difficulties, most of which arose from the ability of cationic surfactants to sorb onto the capillary surface and/or to form micelles at low concentrations, leading to peak loss/tailing and, thus, poor resolution and detection sensitivity. To overcome these problems, the introduction of organic modifiers such as methanol, acetonitrile and tetrahydrofuran (THF) in the CE buffer system and/or sample solution in order to disrupt micelle formation and/or to reduce the ability of surfactants to sorb strongly onto the capillary surfaces has been proven to be quite effective [2–11]. Recently, attempts have been made in using coated capillaries to prevent surface adsorption of cationic surfactants; unfortunately, no advantages and, in some cases, deterioration in separation performances were obtained [11].

Weiss and coworkers [5] were the first in reporting the successful CE separation of ABDACs and have clearly demonstrated that the use of an organic solvent (THF) as buffer additive is essential in obtaining efficient separations. The improvement

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was attributed in large part to the effective disruption of micelle formation in the running buffer. Similar argument was also made by Lin et al. [7,8], who concluded that the formation of micelles in the buffer system was largely responsible for the severe peak loss/tailing of long-chain ABDACs. In their reports, the addition of certain organic solvents (acetonitrile, THF, acetone and/or methanol) in the sample solution as well as in the running buffer was needed to achieve good separations of these cationic surfactants. In contrast, Piera et al. [9] and Heinig et al. [3,10] have concluded in their CE separation of ABDACs that the reason for the improvements was primarily due to the ability of the organic solvents in reducing adsorption of these surfactants onto the capillary walls. Therefore, it appears the effectiveness of certain organic solvents as buffer additives for improving CE separation of cationic surfactants is clearly demonstrated; however, the basic processes by which these organic solvents operate in reducing peak loss/tailing for the longer-chain ABDACs (carbon number $\geq$12) are ambiguous.

Although it is common knowledge that cyclodextrins (CDs) are effective additives for the chiral separation of a wide range of enantiomers in CE [12], very few papers reported the use of CDs for the improved CE separation of surfactants. For examples, Heinig and co-workers [3,11] reported the use of CDs for the isomeric separation of anionic surfactants (linear alkylbenzenesulfonates). On the other hand, Gareil and coworkers [13–15] have reported the use of CDs for the CE separation of another series of anionic surfactants (C_2–C_{18} linear saturated free fatty acids), and they have emphasized the ability of CDs in enhancing the solubility and separation selectivity of the free fatty acids due to the formation of inclusion complexes with CDs.

In this paper the addition of α-/β-cyclodextrins (CDs) and organic solvents (acetonitrile and methanol) in the running buffer as organic modifiers to improve the CE separation of a series of cationic surfactants, ABDACs having carbon atoms number in the long chain alkyl group which extends from ten to eighteen (C_{10}–C_{18}), is reported. Furthermore, new insight into the basic processes by which CE separation of these cationic surfactants could be improved are gained by studying (1) the effects of organic modifiers (CDs and organic solvents) on the CE peak profiles of these ABDACs and (2) the formation of host–guest complexes between CDs and ABDACs using a pyrene derivative as the fluorescent probe. To the best of our knowledge, the use of CDs to reduce/disrupt surfactant aggregation/micellization in the buffer system as well as at the capillary surface, with the aim of understanding and improving the CE separation of cationic surfactants, has never been reported.

2. Experimental

2.1. Apparatus

All CE experiments were performed on a Thermo Capillary Electrophoreis System (Crystal 310, Franklin, MA, USA) using software PC1000 for data acquisition and a Spectra 100 variable UV–Vis CE detector for absorbance measurement at 210 nm. Uncoated fused-silica capillaries were purchased from Polymicro Technologies (Phoenix, AZ, USA) with 75 μm inner and 359 μm outer diameters. The effective length of the capillary was 44 cm and the detection window was created at 13.5 cm from the cathodic end of the capillary.

Fluorescence measurements were performed on a Perkin-Elmer LS 50B luminescence spectrometer. Corrected spectra were obtained by setting both excitation and emission slits at 2.5 nm. Absorbance measurements were carried out using a Gary 100 Scan UV spectrophotometer. A 1-cm path length quartz cell was used for both absorbance and fluorescence measurements. An Orion meter (model 720A) was used for all pH measurements.

2.2. Chemicals

Standards of ABDACs were obtained from Fluka (Buchs, Switzerland): benzyldimethyldecylammonium chloride (>97%), benzyldimethyldodecylammonium bromide (>99%), benzyldimethyltetradecylammonium chloride (>99%), benzyldimethylhexadecylammonium chloride (>96%) and from Aldrich (St. Louis, MO, USA): benzyldimethylstearylammonium chloride (>90%). HPLC grade acetonitrile and methanol were purchased from Arcros (Geel, Belgium). All other chemicals are of

analytical or reagent grade: α- and β-cyclodextrins, 1-pyrenebutyric acid, sodium hydroxide, phosphoric acid, sodium dihydrogen phosphate and sodium phosphate, were obtained from Aldrich (St. Louis, MO, USA). All solutions were prepared with doubly de-ionized water (DDI) from a Milli-Q system (Millipore, Bedford, MA, USA).

2.3. Procedures

For CE experiments buffer solutions of various pHs were prepared by mixing 20 m*M* sodium dihydrogen phosphate with appropriate amounts of sodium phosphate or phosphoric acid. To prepare the running buffers, various amounts of organic solvents (v/v) and/or CDs were added to the buffer solutions. Sample solutions containing the five homologues of ABDACs (C_{10}–C_{18}) were prepared by dissolving equal number of moles of each homologue into appropriate volumes of either DDI or DDI containing 60% methanol (v/v). All solutions were filtered through 0.45 μm membrane filter and degassed for 5 min. using sonication before usage.

Conditioning of new capillaries was performed by flushing firstly with DDI for 10 min, followed by 30 min each with 1.0 *M* NaOH and 0.1 *M* NaOH, and finally with DDI for 60 min and the running buffer for 10 min. In between runs, the capillary was flushed with 0.1 *M* NaOH for 1 min, followed by 2 min with DDI and 5 min with the running buffer. All separations were carried out by operating the power supply at 18 kV and samples were injected into the capillary hydrodynamically (3 s injection time with 50 mbar pressure).

For the fluorescence experiments, 1 m*M* solutions of C_{16} and C_{18} standards were prepared with 20 m*M* phosphate buffer at pH 5.0. To study the effect of CDs on the fluorescence intensity of the probe, α- or β-CD was added in an equal (1:1) or twice (2:1) the amount of the standard solutions of C_{16} and C_{18} together with the probe molecule. A 1 m*M* stock solution of the fluorescent probe: 1-pyrenebutyric acid (PBA) was prepared by dissolving appropriate amounts of PBA in acetonitrile. The excitation wavelength of PBA was set at 340 nm and emission spectra were obtained by scanning from 300 to 900 nm.

3. Results and discussion

3.1. Effect of organic solvents in the running buffer and/or sample solution

Fig. 1(A–C) and (D–F) show the effect of the addition of acetonitrile and methanol in the running buffer, respectively, on the separation of the five even-numbered alkyl homologues of ABDACs (C_{10}–C_{18}). Fig. 1A shows that without the presence of any organic modifier in the running buffer and/or sample solution, only three peaks were obtained in the electropherogram. The C_{10} homologue (highest charge-to-mass ratio) migrated ahead of the C_{12} and C_{14} homologues, with severe peak distortion/loss observed for the C_{14} peak and no signals for the C_{16} and C_{18} homologues.

Fig. 1(B–C) show that with the presence of an increasing amount of acetonitrile in the running buffer, increase in signal intensities were clearly observed for the shorter-chain ABDACs; however,

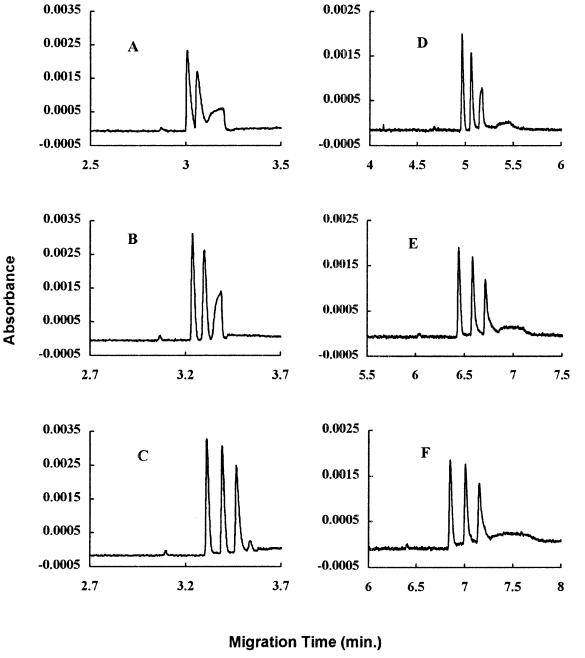

Fig. 1. Effect of organic solvents in the running buffer. The running buffer conditions were 20 m*M* phosphate at pH 5.0 with: (A) 0%, (B) 20% and (C) 40% acetonitrile; (D) 20%, (E) 40% and (F) 50% methanol. The sample solutions contained a mixture of 0.05 m*M* each of the five ABDACs dissolved in DDI.

peak tailing and peak loss can still be observed for the longer-chain homologues (especially C_{16} and C_{18}) when compared to an equal amount of C_{10} injected into the CE system. Fig. 1(D–F) show that similar observations were also obtained for the addition of various amounts of methanol in the running buffer; however, significant increase in migration times were obtained for all ABDACs with an increasing percentage of methanol, in large part due to a more pronounced effect of methanol on the magnitude of the electroosmotic flow [13,16].

To further improve upon the CE separation of the longer-chain ABDACs, Lin and co-workers [7,8] were the first in demonstrating the importance of adding organic solvents in the sample solution. They concluded that the presence of a minimal of 60% methanol (v/v) in the sample solution was needed for the efficient separation of 0.01 m*M* each of the C_{12}–C_{18} homologues when using a phosphate buffer (20 m*M*) containing 30–40% acetonitrile (v/v) at pH 5.0. Using similar experimental conditions, we also observed the effectiveness of adding organic solvents in the sample solution on the separation of the C_{10-18} homologues. Fig. 2(A–B) clearly show the improvement of peak intensity and peak shape for all five homologues as a result of the presence of methanol in the sample solution and an increasing amount of acetonitrile in the running buffer; however, these effects were less dramatic when using methanol as the organic modifier in the running buffer and sample solution as shown in Fig. 2(C–D).

3.2. Effect of α-cyclodextrin and organic solvents

Fig. 3(A–C) show the effect of the presence of α-CD in the running buffer on the separation of the C_{10-18} homologues. With the addition of only α-CD in the running buffer (without organic solvent) as shown in Fig. 3A, the area of the third peak (most likely arose from C_{14} and longer-chain homologues) is significantly larger than that of the one obtained without the presence of CD in the running buffer (Fig. 1A), accompanied by an increase in migration times for all peaks. These results indicate that the presence of α-CD clearly has a positive effect on reducing peak losses for the longer-chain homologues.

Fig. 3(B–C) show that with the presence of both

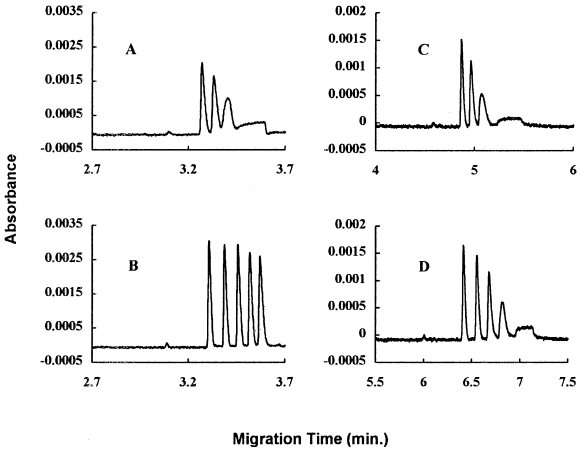

Fig. 2. Effect of organic solvents in the running buffer and sample solution. The running buffer conditions were 20 m*M* phosphate at pH 5.0 with: (A) 20% and (B) 40% acetonitrile; (C) 20%, and (D) 40% methanol. The sample solutions contained a mixture of 0.05 m*M* each of the five ABDACs dissolved in 60% methanol.

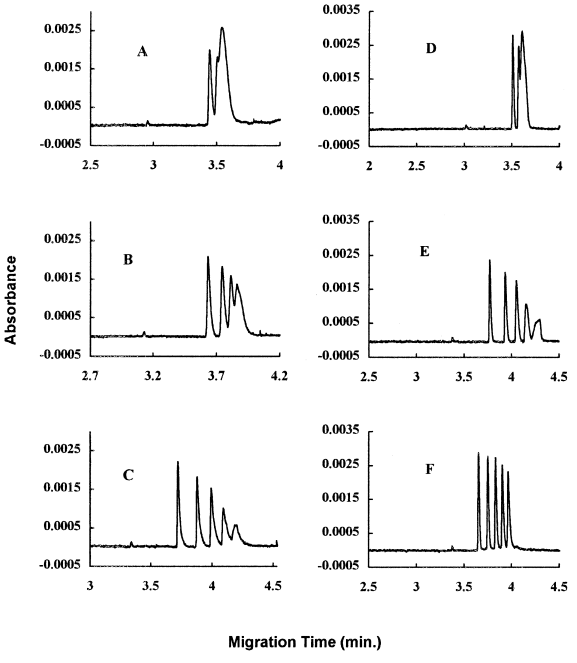

Fig. 3. Effect of α-CD and acetonitrile. The running buffers contained 20 m*M* phosphate at pH 5.0 with 5 m*M* α-CD and with: (A) and (D) 0%, (B) 10%, (C) and (E) 20%, (F) 40% acetonitrile. The sample solutions contained a mixture of 0.05 m*M* each of the five ABDAC dissolved in: (A–C) DDI and (D–F) 60% methanol.

α-CD and acetonitrile in the running buffer, more peaks appeared in the electropherogram when compared to having just α-CD or acetonitrile alone in the running buffer. For example, as shown in Fig. 3C, the presence of 5 m*M* α-CD and 20% acetonitrile in the running buffer allowed for all five ABDACs to be obtained; however, as shown in Fig. 1B, without the addition of any CDs, only three peaks were obtained for the same amount of acetonitrile in the running buffer.

It should be noted that by increasing the α-CD concentration in the running buffer to 10 m*M*, slight decrease in resolution was obtained for the C_{16} and C_{18} peaks when compared to 5 m*M* α-CD (Fig. 3C), while decreasing the concentration to 2.5 m*M* resulted in peak loss/tailing for these longer-chain homologues. Similar observations have also been made on the CE separation of anionic surfactants (free fatty acids (FFA)) in the presence of CDs in the running buffer [13]. Roldan-Assad and Gareil [13] have suggested that the formation of an inclusion complex between FFA and CD causes the effective mobility of the FFA to decrease with an increase in CD concentration, resulting in a loss in resolution when the CD concentration is increased to a point which causes the completion of complex formation (i.e., the effective mobilities of the FFA homologues become almost identical at high CD concentrations).

Fig. 3(D–F) show that even when the sample solution contained an organic solvent (60% methanol), improvement in resolution due to the presence of both α-CD and acetonitrile in the running buffer can also be observed. For example, Fig. 3E shows that with 20% acetonitrile and 5 m*M* α-CD in the running buffer, less peak tailing and more intense peaks were clearly obtained for the C_{16} and C_{18} homologues when compared to the electropherogram shown in Fig. 2A (without α-CD). However, the effect of α-CD became less obvious when the amount of acetonitrile in the running buffer was increased to 40% (comparing Figs. 2B and 3F), most likely due to a decrease in the extent of interaction between α-CD and the ABDACs at higher acetonitrile concentrations.

Identical series of experiments as shown in Fig. 3(A–F) were repeated to study the effect of α-CD on the separation of all five ABDACs by using methanol rather than acetonitrile as the organic solvent in the running buffer. When compared to acetonitrile, similar trends were also observed for methanol in that the presence of α-CD clearly helped in reducing peak loss/tailing for the longer-chain homologues. For example, when compared to Fig. 1(D–E), Fig. 4(A–B) show that the presence of 5 m*M* α-CD in the running buffer provided significant reduction in peak loss for the longer-homologues. Interestingly, unlike the case of acetonitrile, when the sample was prepared in a sample solution containing 60% methanol (rather than just DDI), significant improvement in separation performance was also observed. For example, by comparing Figs. 2(C–D) and 4(C–D), it can be seen that the presence of 5 m*M* α-CD in the running buffer clearly increased peak areas for the longer-chain homologues, even when the running buffer contained 40% methanol and the sample solution contained 60% methanol.

Fig. 5A shows that when the sample concentration of each homologue was increased to 0.5 m*M* each, severe peak distortion/losses were obtained for most of the ABDACs when CD was not added into the running buffer. It should be noted that the separation of a lower concentration sample (0.05 m*M* each homologue) showed that all five homologues were well-resolved using the same amount of acetonitrile in the running buffer at pH 5.0 (Fig. 2B). With the presence of 5 m*M* of α-CD in the running buffer,

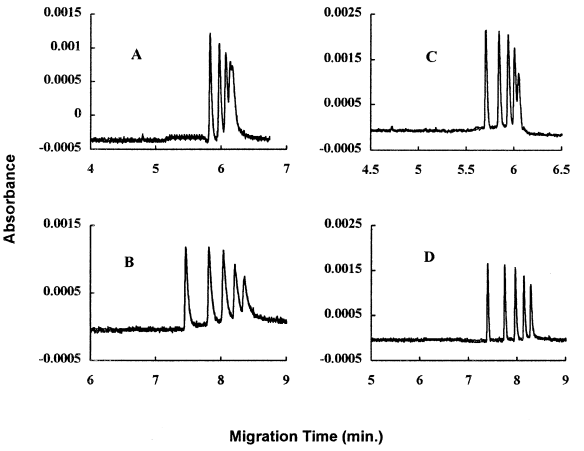

Fig. 4. Effect of α-CD and methanol. The running buffers contained 20 m*M* phosphate at pH 5.0 with 5 m*M* α-CD and with: (A) and (C) 20%, (B) and (D) 40% methanol. The sample solutions contained a mixture of 0.05 m*M* each of the five ABDACs dissolved in: (A–B) DDI and (C–D) 60% methanol.

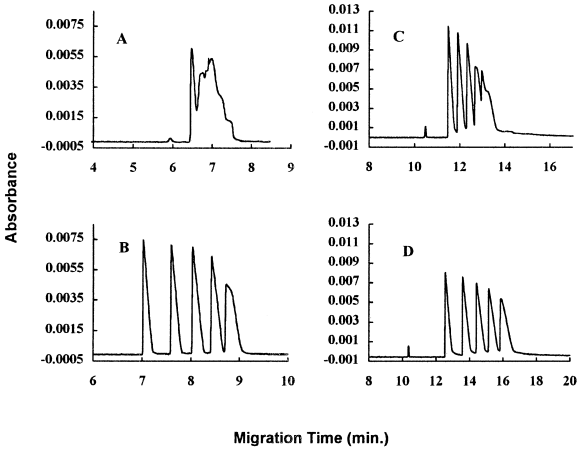

Fig. 5. Effect of sample concentration. The run buffers contained 20 m*M* phosphate at pH 2.5 with: (A–B) 20% acetonitrile and (C–D) 50% methanol; (A) and (C) without α-CD, (B) and (D) with 5 m*M* α-CD. The sample solutions contained: (A–B) 0.5 m*M* and (C–D) 1.0 m*M* each of the five ABDACs dissolved in 60% methanol.

dramatic improvements in peak areas and separation resolution were obtained for all five homologues (Fig. 5B). By increasing the sample concentration of each homologue to 1.0 m*M*, similar observations were also obtained when the running buffer contained methanol instead (Fig. 5C–D). It should be emphasized that other researchers found methanol (in the running buffer) to be an unsuitable solvent for the optimal CE separation of these ABDACs, even at low sample concentrations [7,10]. The data presented in Fig. 5(A–D) clearly demonstrated the effectiveness of using α-CD to improve the CE separation of these ABDACs, for both the shorter- and longer-chain homologues.

3.3. Effect of β-cyclodextrin and organic solvents

To examine the effect of the presence of β-CD on the separation performance, the same series of electropherograms as shown in Figs. 3–5 were obtained using identical experimental conditions, except for the replacement of α-CD with β-CD in the running buffer. In general, the effect of β-CD on the separation of the five ABDACs was similar to those of α-CD, but improvements in terms of reduction in peak tailing and peak loss for the longer-chain homologues appeared to be less pronounced for β-CD when present together with various amounts organic solvents in the running buffer.

3.4. Spectroscopic evidence for the formation of host–guest complexes between CDs and ABDACs

It is known that CDs form relatively strong complexes with surfactant molecules having an ionic head group as well as a long alkyl chain of varying hydrophobicity, and it is generally believed that these surfactant molecules form 1:1 or 2:1 CD/surfactant complexes, at a concentration below or above the critical micelle concentration (CMC) [17–20]. In the presence of micelles, the addition of CDs is believed to cause a break down of the micelles and the CMC is shifted towards higher concentrations [21–23].

Pyrene and its derivatives have been widely employed for the characterization of micellar assemblies of surfactants [24]. Recently, Lin et al. [18] have taken advantages of the special properties of 1-pyrenebutyric acid (PBA) as a probe to study the 1:1 inclusion complex of β–CD with an unbranched cationic surfactant consisting of a long hydrophobic tail: cetyltrimethylammonium bromide (CTAB). In the present study, we also used PBA as a probe to study the possible formation of host–guest complexes between various CDs and ABDACs.

Fig. 6A(a) shows a plot of the fluorescence intensity ratio of the PBA excimer to monomer (*I*e/*I*m) as a function of the C_{16} homologue concentration. The rising portion of the plot observed to the left of the maximum can be interpreted as the premicellar region in which an equilibrium is formed between the free monomer (C_{16} homologue) and the premicellar aggregates (containing certain number of PBA and C_{16} molecules). Near the maximum, the premicellar aggregates begin to turn into ''true'' micelles; after the maximum (decaying portion of the plot), most of the PBA molecules can be envisaged to be incorporated in the micellar psuedophase, causing a decrease in the excimer emission intensity and a concomitant increase in the monomer emission [24].

Fig. 6A(b) shows a plot of the *I*e/*I*m as a function of the 1:1 α-CD/C_{16} solution concentration (i.e., an

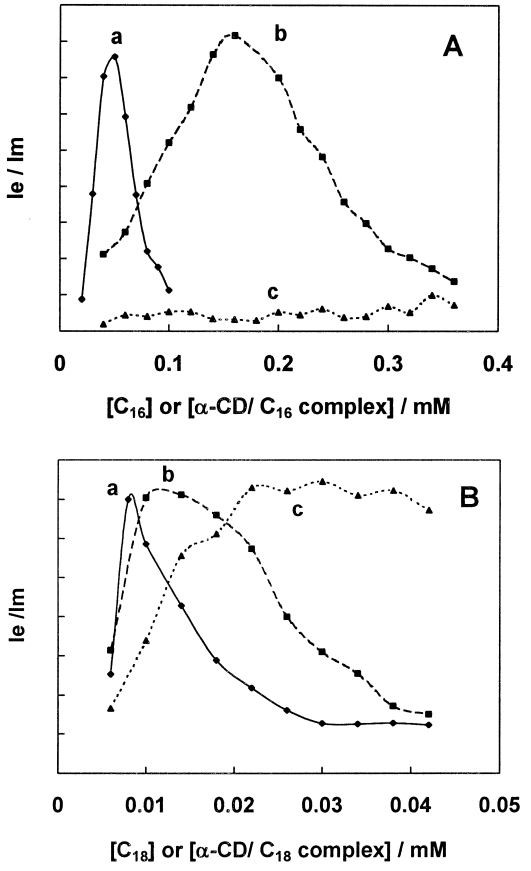

Fig. 6. (A) Plot of the fluorescence intensity ratio (*I*e/*I*m) of the PBA excimer (485 nm) to monomer (375 nm) emission as a function of: (a) C_{16}, (b) 1:1 α-CD/C_{16} and (c) 2:1 α-CD/C_{16} sample solution concentration; (B) Plot of the fluorescence intensity ratio (*I*e/*I*m) of the PBA excimer (485 nm) to monomer (375 nm) emission as a function of: (a) C_{18}, (b) 1:1 α-CD/C_{18} and (c) 2:1 α-CD/C_{18} sample solution concentration. The PBA concentration was 0.001 m*M* and the sample solutions were prepared in 20 m*M* phosphate at pH 5.0.

equal amount of α-CD and C_{16} in the sample solution). It is clear that when compared to Fig. 6A(a) the peak maximum for *I*e/*I*m is shifted to a higher concentration due to the presence of α-CD. Since the position of the maximum in the plot is an indicator of the approximate concentration at which ''true'' micelles are formed, the shift in the peak maximum indicates that the presence of α-CD in the solution altered the aggregation behavior of the C_{16} homologue (i.e., an increase in the CMC) and suggests that some type of host–guest complex was formed between the C_{16} homologue and α-CD [18,24]. Fig. 6A(c) shows a plot of *I*e/*I*m as a function of 2:1 α-CD/C_{16} solution concentration. It is interesting to note that by increasing the concentration of α-CD two-folds relative to C_{16}, the *I*e/*I*m decreased dramatically when compared to the C_{16} or 1:1 α-CD/C_{16} solutions. It is also interesting to note that the *I*e/*I*m maximum for the C_{16} solution as shown in Fig. 6A(a) occurred at a concentration of about 5.0×10^{-5} *M*, which is very close to the CMC value of C_{16} reported in the literature [5,9].

Fig. 6B(a–b) show that similar *I*e/*I*m plots can also be obtained for the C_{18} homologue in the absence and presence of an equal amount of α-CD, respectively. It is interesting to note that the *I*e/*I*m maximum for the C_{18} solution as shown in Fig. 6B(a) indicates that the C_{18} monomer and premicellar aggregates (C_{18}/PBA neutral complex) turned into ''true'' micelles at a concentration of about 8×10^{-6} *M*, which is in good agreement with the CMC value reported for the C_{18} homologue [5,9]. Also, although there is a difference in concentration at which the maximum occurred for the C_{18} (Fig. 6B(a)) and 1:1 α-CD/C_{18} (Fig. 6B(b)) sample solutions, the extent of this difference is much smaller when compared to the plots shown in Fig. 6A(a–b) for the C_{16} and 1:1 α-CD/C_{16} sample solutions, respectively. Additionally, Fig. 6B(c) shows that the *I*e/*I*m did not decrease dramatically for the 2:1 α-CD/C_{18} sample solution, which is in contrast to the 2:1 α-CD/C_{16} sample solution as shown in Fig. 6A(c).

The results shown in Fig. 6(A–B) suggest that the interaction between α-CD and C_{16} or C_{18} homologue is dependent on the length of the alkyl chain, and thus, it is likely that the interaction of these homologues with α-CD involves the inclusion of the hydrophobic alkyl chain into the α-CD cavity. This is consistent with observations made for CTAB using the same fluorescence method [18] and for other surfactants using different measurement methods [17,25]. Furthermore, the fact that excimer emission was obtained for PBA in the presence of the C_{16} or C_{18}/α-CD complex indicates that the charge of the complex lies outside the α-CD cavity, allowing for the formation of a neutral association complex between negatively charged PBA and the positively

charged C_{16} or C_{18}/α-CD species [18,24]. It should be noted that in our experiments similar fluorescence plots as shown in Fig. 6(A–B) for α-CD were also obtained when β-CD was used as the host molecule instead.

3.5. A proposed model for the effect of CDs on the improved CE separation of ABDACs

Fig. 7(A–C) show the change in peak profiles of an equal amount of the five homologues as a function of pH. At pH 3.0, Fig. 7A shows that peak intensity decreased according to the length of the alkyl chain, and the C_{18} peak is barely distinguishable from baseline at about 6.2 min. At pH 5.0, an increase in EOF was accompanied by a loss in signal intensity for all peaks (Fig. 7B). Further increase in pH resulted in additional peak losses, as observed at pH 7.0 in Fig. 7C. These observations indicate that increasing the number of negative charges at the capillary walls decreased the overall signal intensities for all five homologues, most likely as a result of increased electrostatic interaction between ABDACs and the capillary surface. However, peak losses were more severe for the longer chain homologues, as observed for the C_{16} and C_{18} peak areas relative to those of $C_{10,}C_{12}$ and C_{14} as the pH was increased from 3.0 to 5.0. This phenomenon cannot be explained by increased electrostatic interaction alone. It is likely that for the longer-chain homologues, electrostatic interaction is coupled with additional forces, such as hydrophobic interaction, which would result in better surface coverage of the capillary surface per unit concentration for these more hydrophobic homologues.

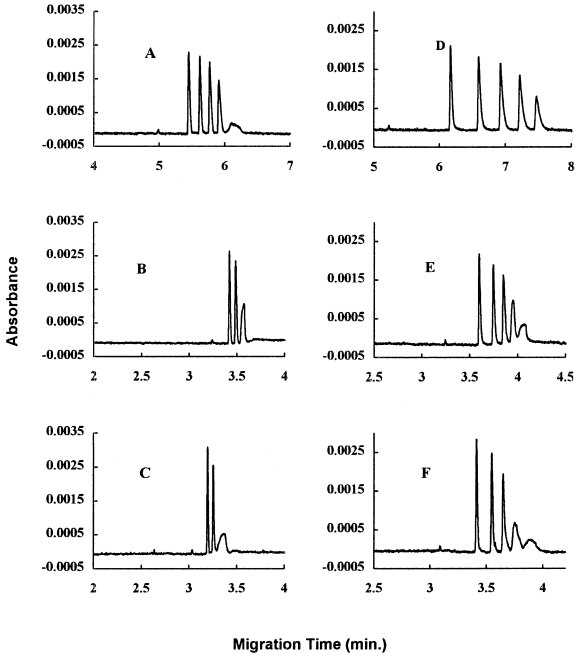

Fig. 7. Effect of pH as a function of α-CD. The running buffers contained 20 m*M* phosphate with 20% acetonitrile at pH: (A) and (D) 3.0, (B) and (E) 5.0, (C) and (F) 7.0. The sample solutions contained 0.05 m*M* each of the five ABDACs dissolved in DDI: (A–C) without α-CD and (D–F) with 5 m*M* α-CD.

Fig. 7(D–F) show the effect of adding 5 m*M* of α-CD in the running buffer on the peak profiles of the five homologues as a function of pH. At pH 3.0, Fig. 7D shows that the presence of 5 m*M* α-CD clearly reduced peak loss for the C_{18} peak (compared to Fig. 7A), but not as much for the shorter-chain homologues. As pH was increased to pH 5.0, peak losses occurred for the C_{10} and C_{12} homologues but to a similar extent as those without the presence of α-CD; in contrast, peak losses for the C_{16} and C_{18} homologues were clearly reduced with the presence of α-CD as the pH was increased from 3 to 5 (comparing Fig. 7E and B). This same trend continued as the pH increased to 7.0 as shown in Fig. 7F and C. Table 1 lists the peak areas of the five homologues as a function of pH and α-CD.

The above pH effects observed for the five homologues in the presence of 5 m*M* of α-CD in the running buffer appear to be consistent with the structure proposed for the various ABDAC/CD host–guest complexes. Since we proposed that the positive charge of the ABDACs lies outside the CD cavity, the formation of the inclusion complex would not prevent peak losses due to electrostatic interaction between these cationic surfactants and the negatively charged capillary surface. On the other hand, the inclusion of the hydrophobic tail of the ABDACs into the CD cavity would lead to a change in the hydrophobic/hydrophilic microenvironments of the ABDACs and, importantly, could result in

Table 1
Peak areas of the five homologues as a function of pH and α-CD[a]

pH	Without α-CD					With 5 m*M* α-CD				
	C_{10}	C_{12}	C_{14}	C_{16}	C_{18}	C_{10}	C_{12}	C_{14}	C_{16}	C_{18}
3.0	4394± 108.23	4603± 85.06	4708± 123.47	4211± 98.47	2880± 296.80	4679± 9.46	5054± 48.87	5102± 68.20	4637± 50.68	3448± 67.46
5.0	2763± 6.60	2827± 62.23	2692± 35.6	0	0	2885± 7.13	3031± 49.94	2961± 49.18	2330± 41.00	1256± 70.80
7.0	2573± 21.30	2628± 36.63	2516 ± 37.51	0	0	2617± 25.82	2695± 38.79	2573± 81.14	2067± 32.17	1157± 19.01

[a] The uncertainties were calculated based on three replicate measurements. The running buffer contained 20 m*M* phosphate at various pH with 20% acetonitrile (with or without 5 m*M* α-CD), and the sample solution contained a mixture of 0.05 m*M* each of the five homologues dissolved in DDI.

reduced peak loss/tailing for the CE separation of the longer-chain homologues.

4. Conclusion

In the present work, it is reasonable to envisage that the effects of CDs on the improved CE separation of longer-chain ABDACs (above their CMC) is in large part due to initially the formation of inclusion complexes between the free monomeric surfactants and α- or β-CD, followed by the disruption/break up of the micelles in the buffer system and the reduction/elimination of surfactant aggregates at the capillary walls. In other words, the inclusion of the alkyl chain of the ABDACs into the CD cavity altered the aggregation behavior (i.e., increased the CMC) of the various ABDACs and increased the concentration of ABDAC monomers and/or ABDAC/CD complexes in the buffer system relative to the amount of surfactants adsorbed onto the capillary surface. These effects are especially important for the CE separation of ABDACs with very low CMCs, such as the C_{16} and C_{18} homologues. It should be noted that, however, the effects of CDs in enhancing analyte solubility and separation selectivity are also important factors for consideration in the CE separation of surfactants [13–15]. Work is in progress in our laboratory to optimize the analytical conditions (e.g., the type and concentration of CDs and organic solvents in the running buffer and in the sample solution) for the CE determination of ABDACs present in real analytical samples.

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