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Journal of Chromatography A, 822 (1998) 281–290

JOURNAL OF CHROMATOGRAPHY A

Separation of ergot alkaloids by micellar electrokinetic capillary chromatography using cationic Gemini surfactants

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Received 2 June 1998; received in revised form 11 August 1998; accepted 12 August 1998

Abstract

A micellar electrokinetic capillary chromatographic method using novel cationic dimeric (Gemini) surfactants has been developed for the separation, in less than 8 min, of 17 dihydroergotoxines, aci-alkaloids and oxidation products. Both 1,3-bis(dodecyl-*N*,*N*-dimethyl ammonium)-2-propanol (20 m*M*) and 1,3-bis(tetradecyl-*N*,*N*-dimethyl ammonium)-2-propanol (40 m*M*) surfactants in 50 m*M* phosphate buffer, pH 3.0, at 20°C produced separations that could not be achieved using single chain tetradecyl- or hexadecyl-trimethyl ammonium micelles. The retention factors (k') increased linearly with surfactant concentration in accordance with theory. Similarly, the net mobilities generally decreased nonlinearly with surfactant concentration. The smallest alkaloids, i.e. the 6-methylergoline-8-carboxylic acids, and the more polar aci-alkaloids with a *Z*-type conformation, have the smallest k' values. For the larger alkaloids, the k' values with the Gemini surfactants were smaller than with the single chain species, possibly because the more rigid dimeric molecules hinder the partitioning of the alkaloids into the micelle as well as the greater hydrophilicity of the hydroxylated micelles. Increasing buffer pH mainly affects the more strongly acidic and basic alkaloids, causing a marked increase and decrease in net mobilities, respectively. k' values generally increased with phosphate buffer concentration because of the decreasing solubility of the hydrophobic alkaloids with increasing ionic strength.

Keywords: Ergot alkaloids; Alkaloids; Surfactants; Gemini surfactants

1. Introduction

The ergot alkaloids comprise a large group of structurally related, pharmacologically active compounds [1]. They are naturally produced primarily by species of the fungus *Claviceps*, which parasitizes grasses and cereals. Although severely toxic at higher concentrations, at low levels, certain natural ergot alkaloids are effective therapeutic substances for the treatment of various disorders [1,2]. There are four main structural groups of the naturally occurring ergot alkaloids [3]: clavine alkaloids, lysergic acids, simple lysergic acid amides and ergopeptines. The ergopeptines, or peptide ergot alkaloids, are hydrogenated to form a group of pharmaceutically important compounds, the dihydroergotoxines, which are generally used as their methanesulfonic acid salts

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0021-9673/98/$ – see front matter
PII: S0021-9673(98)00672-4

(ergoloid mesylates) [4]. They also undergo acid-catalyzed rearrangement to form the pharmacologically inactive aci-alkaloids [1], and are prone to light-enhanced oxidative degradation in aqueous solution [4]. The supply of natural product ergot alkaloids is insufficient for commercial distribution; a number of FDA-approved synthetic ergot alkaloids have been developed [5].

Derivatives of certain ergot alkaloid enantiomers have been resolved by capillary electrophoresis (CE) [6], but neither high-performance liquid chromatography (HPLC) nor CE methods for indicating purity and stability or for impurity testing of synthetic ergot alkaloids have been published in the United States Pharmacopoeia. We have developed a micellar electrokinetic capillary chromatography (MECC) method for the separation, in less than 8 min, of 17 dihydroergotoxines, aci-alkaloids and oxidation products using novel double alkyl chain (Gemini) surfactants as buffer additives. Both the di-dodecyl and di-tetradecyl (propanol spacer) quaternary ammonium Gemini surfactants gave separation of all 17 alkaloids. The MECC separation could not be achieved using single tetradecyl or hexadecyl chain cationic surfactants.

Gemini or dimeric surfactants contain at least two hydrophilic and two hydrophobic groups connected by a linkage (spacer) that may be rigid or flexible, hydrophilic or hydrophobic; both cationic and anionic Geminis have been synthesized [7,8]. These surfactants have unusually high surface activity and low critical micelle concentration values [9,10], and exhibit unusual micellar structures [11,12]. Two reports have been published on the use of anionic dimeric surfactants in MECC, in which anionic sulfonated compounds gave separations of naphthalene and benzene derivatives that were superior to those achieved with single chain surfactants [13,14].

2. Experimental

2.1. Apparatus

MECC experiments were carried out using a Beckman P/ACE 5010 CE system (Beckman Instruments, Palo Alto, CA, USA) equipped with a UV absorbance detector set at 214 nm and liquid coolant circulation for temperature control at 20±0.1°C. Control of the CE system and data handling were carried out with a Waters/VAX 4000-50 system through an LAC/E interface module (Waters Associates, Milford, MA, USA) using Waters ExpertEase version 3.1 software. An Orion Model 290A pH meter was used to measure pH. Fused-silica separation capillaries (50 μm I.D.×375 μm O.D.) were obtained from Polymicro Technologies (Phoenix, AZ, USA) and were cut to a total length of 37 cm, with an effective length of 30 cm from the injection end to the detector window. The detector window was made by burning off a small length of the polyimide coating with a butane lighter and was cleaned with Kimwipe dampened with methanol. New capillaries were conditioned by purging, in order, for 15 min each, with 1 *M* KOH; 0.1 *M* KOH; degassed, filtered deionized water; and buffer with no surfactant, at a concentration that was three times greater than that of the running buffer. Samples were injected hydrodynamically at 0.5 p.s.i. (1 p.s.i.= 6894.76 Pa) for 2 s. All experiments were performed at a constant voltage of 13.5 kV, with the detector electrode positive.

2.2. Chemicals

The 17 ergot alkaloids used in this study have the structures shown in Fig. 1, and were obtained from Sandoz Pharma (Basel, Switzerland). They were dissolved in HPLC-grade methanol to give a mixed stock sample solution at a concentration of approximately 5 mg/ml of each alkaloid. The micelle marker was pentachlorophenol (PCP), obtained from Aldrich (Milwaukee, WI, USA). Sodium phosphate, 85% *o*-phosphoric acid and potassium hydroxide were purchased from Fisher Scientific (Fairlawn, NJ, USA); tetradecyltrimethylammonium bromide (TTAB) and hexadecyltrimethylammonium bromide (CTAB) surfactants were from Aldrich; and HPLC-grade methanol and acetonitrile were from Burdick and Jackson (Muskegon, MI, USA). All aqueous solutions were prepared using distilled water that had passed through a Millipore Milli-Q system (Millipore, Bedford, MA, USA).

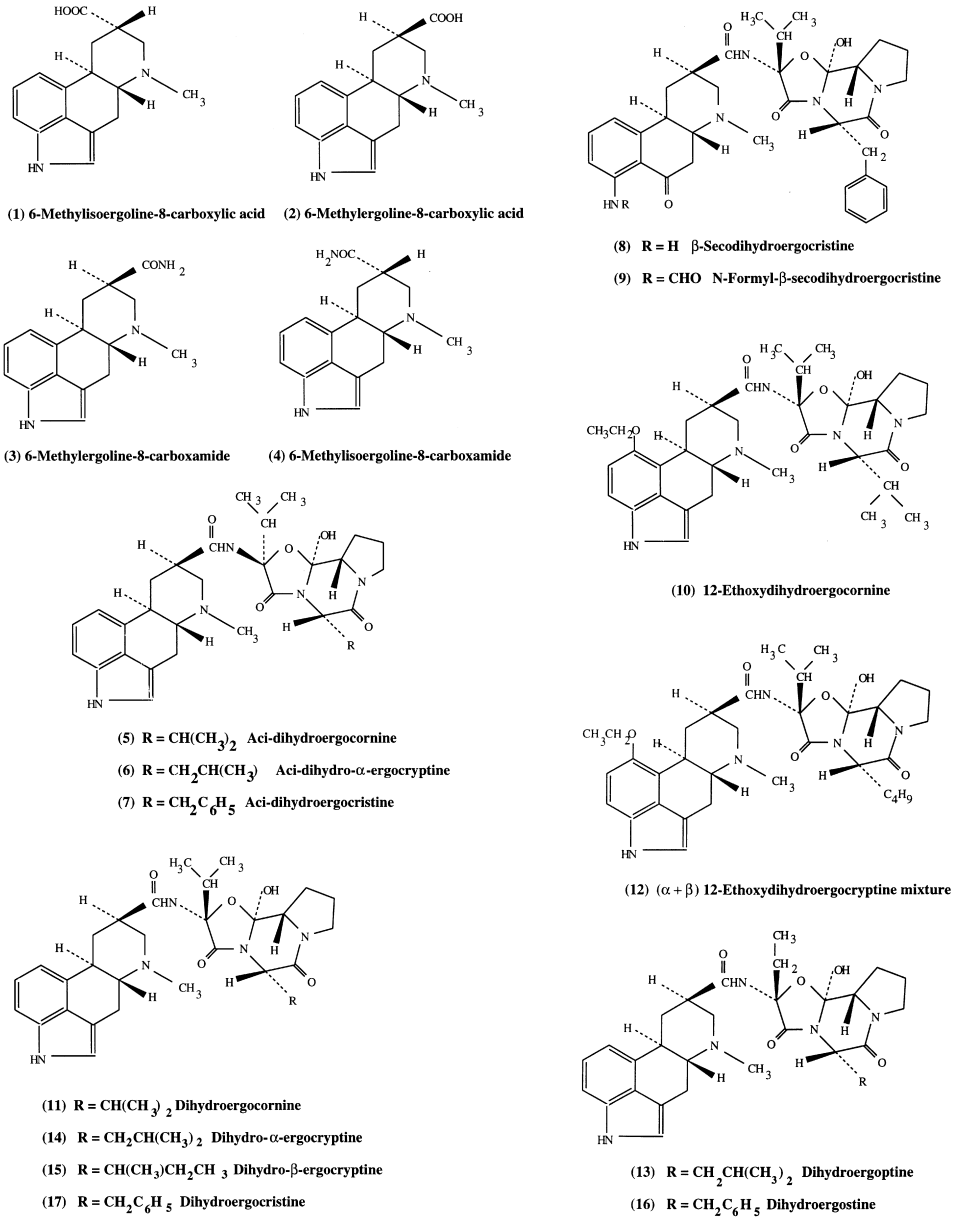

Fig. 1. Structures of the ergot alkaloids studied.

2.3. Solutions

Stock buffer solutions were prepared by mixing equimolar concentrations of H_3PO_4 and NaH_2PO_4 and adjusting the pH to the required value using a concentrated KOH solution. Phosphate concentrations were varied from 10 to 75 m*M*, and the pH was varied from 2.5 to 5.0. Most work was done with a 50-m*M* phosphate buffer, pH 3.0. Aqueous surfactant solutions were prepared in the range from 10 to 40 m*M*. Running buffers (background electrolyte, BGE) were prepared by mixing the stock buffer solution with the stock aqueous surfactant solution at each concentration studied, and then degassed and filtered

through 0.45 μm syringe filters. The sample solution was prepared by 20-fold dilution of the mixed stock sample solution in methanol with tenfold diluted running buffer without surfactant.

2.4. Synthesis of cationic Gemini surfactants

The procedure of Rosen and Song [15] was followed to prepare 1,3-bis(dodecyl-*N*,*N*-dimethyl ammonium)-2-propanol [Gemini-C_{12}] and 1,3-bis-(tetradecyl-*N*,*N*-dimethyl ammonium)-2-propanol [Gemini-C_{14}]. 1,3-Dichloro-2-propanol (22.5 ml, 0.235 mol; Aldrich) was refluxed with dodecyl-*N*,*N*-dimethylamine or tetradecyl-*N*,*N*-dimethylamine (135 ml, 0.488 mol; Albemarle, Baton Rouge, LA, USA) in 95% ethanol for 4 h. Ethanol and excess alkyldimethylamine were removed from the product in a rotary evaporator under reduced pressure. The crude surfactant products were dissolved in a minimum volume of acetone, recrystallized three times from $CHCl_3$–acetone, and the solvent was evaporated under vacuum to give a solid white powder. The structures of the Gemini surfactants were confirmed using ^1H and ^{13}C NMR spectroscopy. Elemental analysis results were: (a) theory for Gemini-C_{12} monohydrate: C, 65.52; H, 12.32; N, 4.83; Cl, 12.35; found: C, 64.81; H, 12.85; N, 4.82; Cl, 12.37; and (b) theory for Gemini-C_{14} monohydrate, C, 66.74; H, 12.48; N, 4.45; Cl, 11.26; found: C, 66.85; H, 13.06; N, 4.38; Cl, 11.24. HPLC analysis with a differential refractive index detector and a 30 cm×4 mm I.D., 10 μm Waters μBondapak CN column using acetonitrile–aqueous acetate buffer, pH 5.0 (55:45, w/w) at 2.0 ml/min showed that the purity of each surfactant was >99%.

3. Results and discussion

Separation of solutes by MECC is affected by the type and concentration of surfactant, the pH and ionic strength of the BGE, the type and concentration of any organic additive, and the temperature. It was determined early in this study that organic additives diminished the resolution of the alkaloids and that as low a temperature as possible was desired; 20°C with our instrument. The effects of surfactant type and concentration, and pH and phosphate concentration of the BGE on the migration behaviour of the 17 alkaloids were studied.

Retention factors (k') were determined from the migration times (s) of the micelle marker (pentachlorophenol), t_{mic}; the electroosmotic flow (EOF) marker (methanol; MeOH), t_{eof}; and the solute, t_s, from [16,17]

$$k' = (t_s - t_{eof})/t_{eof}(1 - t_s/t_{mic}) \quad (1)$$

The net mobility of the solute, μ_{net}, is the difference between its apparent mobility, μ_{app}, and the EOF mobility, μ_{eof}, i.e.,

$$\mu_{net} = \mu_{app} - \mu_{eof} = lL/Vt_s - lL/Vt_{eof}$$
$$= (lL/V)[(1/t_s) - (1/t_{eof})] \quad (2)$$

where l is the length (cm) of the capillary from the injection end to the detector window, L is the total length (cm) between the electrodes, and V is the applied voltage (volts). Because the EOF is larger than, and in the direction opposite to, the electrophoretic mobility of the micelles, the absolute value of μ_{net} is less than zero. Formation of a bilayer of cationic surfactant on the capillary wall results in an EOF caused by migration of hydrated anions, which is in the direction opposite to that of the normal EOF [18]. However, the cationic micelles try to move towards the cathode but are overcome by the EOF.

3.1. Effect of surfactant type and concentration

The k' values of the 17 ergot alkaloids increased linearly with the concentration, C_{surf}, of each of the four surfactants evaluated, as expected from the relationship [16]

$$k' = K\Phi_v(C_{surf} - CMC) \quad (3)$$

where K is the distribution coefficient of the solute between the micelle phase and the BGE, Φ_v is the partial specific volume of the micelle, and CMC is the surfactant's critical micelle concentration. CMC values for the gemini surfactants are of the order of 10 μM in 0.1 M NaCl at 25°C [15] and are negligible compared to C_{surf}. The slopes of these plots are given in Table 1. The intercepts were all small numbers, so the k' values can be easily reconstructed. The net mobilities, on the other hand, generally decreased nonlinearly with C_{surf}, except for

Table 1
Slopes (M^{-1}) of plots of k' vs. surfactant concentration

Ergot alkaloid[a]	Gemini-C_{12}	Gemini-C_{14}	TTAB	CTAB
1	10.5	8.02	10.1	8.82
2	15.0	12.3	13.5	12.5
3	34.6	20.9	65.5	67.9
4	40.8	29.0	83.4	88.6
5	12.0	7.03	8.93	7.23
6	3.86	6.61	4.88	3.03
7	36.8	69.9	179	224
8	39.8	33.5	113	127
9	51.1	46.3	116	134
10	104	88.8	261	300
11	127	86.8	246	253
12	158	112	404	447
13	161	134	305	312
14	188	144	391	412
15	236	182	488	488
16	269	222	609	637
17	313	294	777	821

Conditions: 50 m*M* phosphate BGE; pH 3.0; Surfactant: 10, 20, 30 and 40 m*M*.
[a]The number in this column corresponds to the alkaloid number, name and structure given in Fig. 1.

aci-dihydroergocornine (**5**) and α-aci-dihydro-ergocryptine (**6**), whose μ_{net} and k' values did not vary greatly with concentration. Illustrative of the μ_{net} vs. C_{surf} curves is the one for Gemini-C_{14} shown in Fig. 2. The stronger the degree of interaction of solute with micelle, the larger the k' and the effect of increasing C_{surf}, and the smaller the μ_{net}.

The k' values and the net mobility curves in Fig. 2 can be rationalized as follows. The two compounds with the highest mobility are the isomers 6-methylergoline-8-carboxylic acid (**1**) and 6-methylisoergoline-8-carboxylic acid (**2**). By analogy to the structurally similar lysergic acid, whose $pK_A = 3.4$ [19], the carboxyl groups on compounds **1** and **2** should both be partially dissociated in the pH 3.0 buffer used here. In addition, the pK_B of lysergic acid is 7.68 [19] and, by the same analogy, the tertiary amines should be protonated in all of these alkaloids. Although one might expect the reduced positive charge to increase the ionic attraction between the micelle and these solutes to dominate, it is apparently outweighed by the combination of their smaller size and higher solubility in the BGE than those of the other alkaloids, which allows alkaloids **1** and **2** to migrate electrophoretically more rapidly. Also, unlike the non-aci-isomeric alkaloids, the k' values of **1** and **2** are similar to the Gemini-C_{12} and TTAB, and the Gemini-C_{14} and CTAB, further indicating generally weaker interactions. The other two alkaloids with small k' values, **5** and **6**, have intermediate net mobilities. These aci-alkaloids have a '*Z*'-type conformation compared with a 'U'-type arrangement for the non-aci-alkaloids. In the *Z*-conformation, these molecules are arguably more polar, which increases their affinity for the aqueous phase. As shown by molecular modeling, in the U-conformation, an intramolecular hydrogen bond between the hydroxyl group and the amide carboxyl oxygen reduces the overall polarity of the alkaloid, which results in a stronger hydrophobic affinity for the micelle phase. The other aci-alkaloid, aci-dihydroergocristine (**7**), has a benzyl substituent, which makes it relatively more hydrophobic and produces behaviour more similar to that of the non-aci compounds. This compound also has an anomalously larger k' with Gemini-C_{14} than with the Gemini-C_{12}. Since the k' values for **7** follow the pattern of the other alkaloids with TTAB and CTAB, there may be some shape-related specific interaction of **7** with the Gemini-C_{14}. For the other alkaloids, the k' values are significantly larger with the Gemini-C_{12} than with the C_{14}. The former is less hydrophobic, allowing stronger interactions with polar groups in the alkaloids. With the single chain surfactant micelles, the

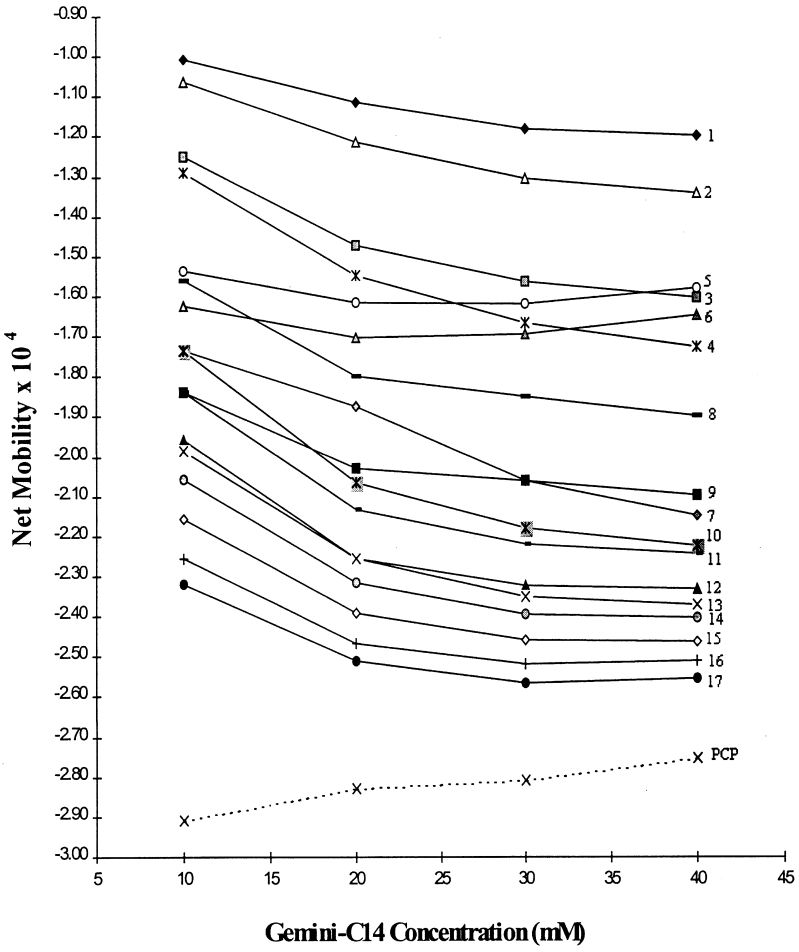

Fig. 2. Effect of Gemini-C_{14} concentration on net mobility ($cm^2/V \cdot s$). CE conditions: Phosphate buffer, 50 m*M*, pH 3.0; uncoated fused-silica capillary, 50 μm I.D., 30 cm effective length, 37 cm total length; 20°C; UV detector at 214 nm; applied voltage, −13.5 kV. The numbers on each curve correspond to those alkaloids in Fig. 1, and PCP is the EOF marker, pentachlorophenol.

alkaloid k' values are generally similar for the TTAB and CTAB. The k' values for the larger alkaloids (**7**–**17**) with the Gemini surfactants are generally smaller than with the single chain species. This could be attributed to the greater rigidity of the former, caused by the 2-propanol group connecting the two quaternary ammonium chains, which produces a steric hindrance to the free partitioning of these alkaloids into the micelle phase, and to the less hydrophobic character of the Gemini's imparted by their hydroxyl group.

In Fig. 3, the electropherograms of the 17 ergot alkaloids, with each of the four surfactants at a concentration of 20 m*M* in a BGE containing 50 m*M* phosphate at pH 3.0 are shown. At this concentration, only the Gemini-C_{12} allows complete separation, which was also obtained with 30 m*M* Gemini-C_{12}. With Gemini-C_{14}, separation of all of the compounds was achieved at a concentration of 40 m*M*. Use of higher concentrations runs the risk of Joule heating. Complete resolution could not be obtained with either of the single chain surfactants under any of the conditions studied.

3.2. Effect of the BGE's pH

The EOF, as measured by the migration time of

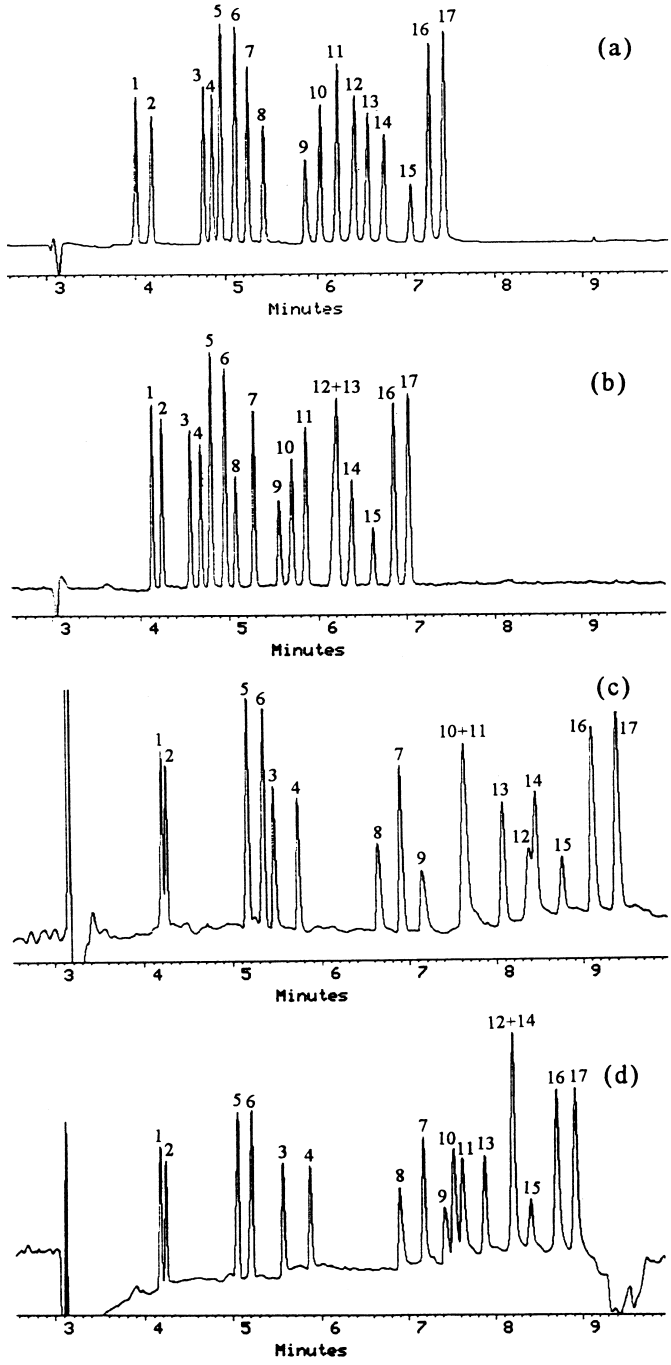

Fig. 3. Electropherograms of alkaloids using (a) Gemini-C_{12}, (b) Gemini-C_{14}, (c) C_{14}TAB and (d) C_{16}TAB surfactants. CE conditions: same as Fig. 2, except that each surfactant was at 20 m*M*. Peak numbers correspond to compounds in Fig. 1.

methanol, passes through a maximum at pH 4.2 as the pH is increased from 2.5 to 5.0. At low pH, the fused-silica is not dissociated so there is little tendency for the surfactant to adsorb. As the pH is increased to 4.2, a completed bilayer of cationic surfactant is established on the walls, which leads to an EOF towards the anode. At higher pH values, further dissociation of the silica reduces the net surface positive charge and thus the EOF [20]. pH has little effect on μ_{net} for most of the alkaloids, whose degree of protonation of the tertiary nitrogen should not vary much over the pH range studied. For the two acids, **1** and **2**, the typical S-shaped change in mobility with pH is observed in Fig. 4 with Gemini-C_{12} surfactant. Greater dissociation leads to reduced positive charge and higher electrophoretic mobility towards the anode. The β-secodihydro-ergocristines (**8** and **9**), which have a non-heterocyclic nitrogen, are more basic compounds than the others, since a marked decrease in μ_{net} is found over this pH range as they are increasingly neutralized.

3.3. Effect of phosphate concentration

As shown in Fig. 5, k' values generally increase with phosphate concentration. The effect is greater for the more hydrophobic alkaloids, one presumes through a reduction in the aqueous solubility of these compounds with increasing ionic strength. Again, the β-secodihydroergocristines behave somewhat anomalously.

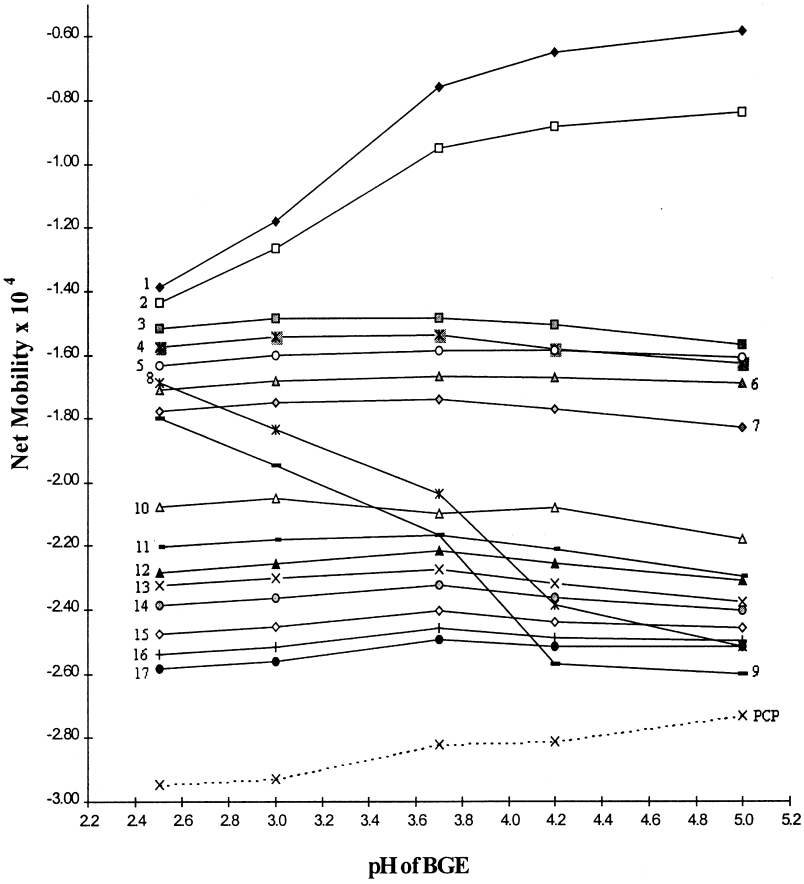

Fig. 4. Effect of buffer pH on net mobility ($cm^2/V \cdot s$). CE conditions: same as Fig. 2, except for pH.

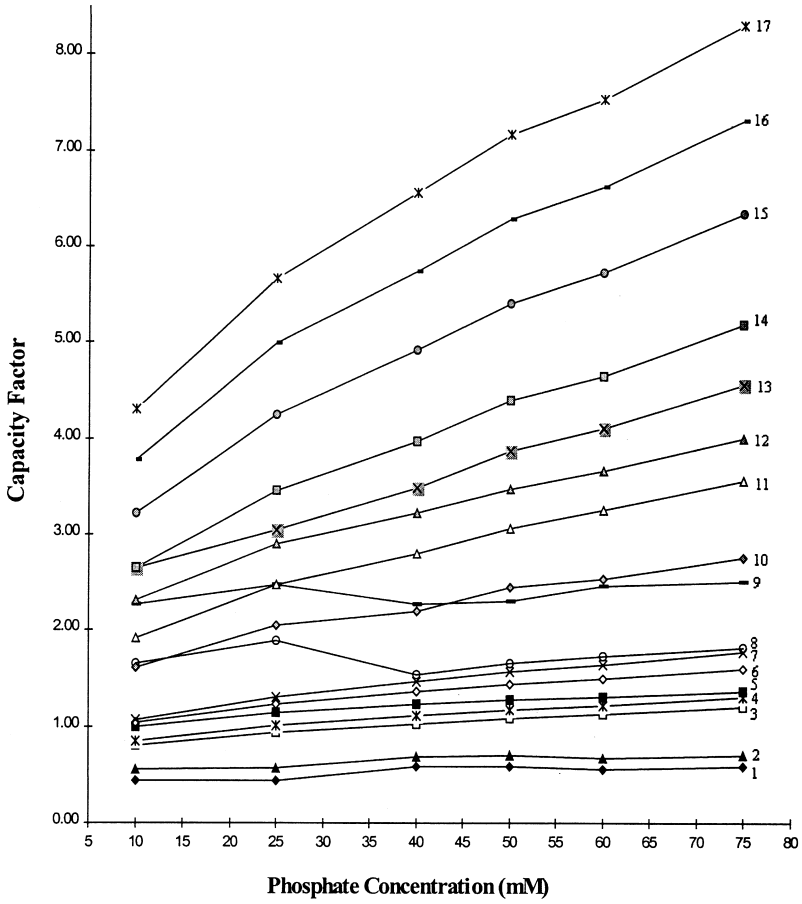

Fig. 5. Effect of phosphate concentration on retention factor. CE conditions: same as Fig. 2, except for phosphate concentration.

4. Conclusions

The cationic dimeric surfactant Gemini-C_{12} has been shown to provide the best MECC separation of 17 dihydroergotoxines and related compounds. The alkaloid k' values in the various micellar systems can be explained qualitatively in terms of relative polarities and different structures of the dimeric and single-chain surfactant micelles, but more studies would be required to pin down the precise mechanism of interaction. The pH of the BGE mainly affects the adsorption of the cationic surfactants to the fused-silica wall and reversal of the EOF direction from the cathode to the anode with increasing pH. The μ_{net} values of most of these alkaloids are little affected by the pH of the BGE, over the range from 2.5 to 5, but the more strongly acidic and basic compounds behave in the usual fashion.

Acknowledgements

We thank Prof. Milton J. Rosen, Brooklyn College, CUNY, and Prof. William F. Berkowitz, Queens College, CUNY, for helpful discussions, and to the reviewers for useful comments.

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