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Nonionic surfactants for improving resolution of the priority pollutant phenols by micelle-modified capillary electrophoresis

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Abstract

Nonionic surfactants used as buffer additives in capillary electrophoresis improve the separation of the eleven US EPA priority pollutant phenols. A borate/phosphate buffer (pH 9.8) containing 0.5–2% (w/v) Tween 40 or Brij 35 surfactant gives better separation of the phenols than the same buffer with no surfactant, especially for the solute pairs 2,4-dichlorophenol/2-methyl-4,6-dinitrophenol; 2-chlorophenol/2,4-dinitrophenol; and 4-nitrophenol/2-nitrophenol. Pentachlorophenol behaves anomalously with increasing concentration of surfactant in the 0 to 0.5% range. Its net mobility is sharply reduced, and peak shape changes from the typically narrow and symmetrical CE peak to a broad, electrophoretically tailing band at a surfactant concentration near the critical micelle concentration (CMC), and then reverts to normal shape at concentrations above the CMC. Compared with CE with buffer only, the small concentrations of surfactant required to produce significant changes in selectivity have little effect on the magnitude of the electroosmotic flow or the runtime. Nonionic surfactants neither increase conductivity nor contribute to Joule heating.

Keywords: Phenols; Surfactants, nonionic

1. Introduction

Separation by capillary electrophoresis (CE) is based on differences in the electrophoretic mobilities of solutes. Although CE is inherently a highly efficient separation technique, it is necessary to evaluate experimental conditions carefully to achieve maximum separation selectivity. Capillary length and diameter, applied voltage, buffer concentration, and temperature are important factors in separation efficiency and analysis time, but in general they are not of primary concern in improving separation selectivity. For weakly acidic or basic solutes, buffer pH selection is probably the most important among the experimental variables. The effective electrophoretic mobilities can be modified by optimizing buffer pH to maximize the mobility differences and achieve highest resolution between closely migrating solute pairs. For solutes with similar pK_a values, pH control alone cannot improve resolution.

A simple and effective method of modifying solute mobilities is the use of buffer additives. Surfactants at concentrations higher than the critical micelle concentration (CMC) were first applied in capillary electrophoresis by Terabe and co-workers [1,2], and have become widely used buffer modifiers for separation improvement in CE. The ionic surfactant micelles act as a sort of pseudophase which can solubilize hydrophobic compounds, and can provide a means of transport and separation of neutral

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molecules by micellar electrokinetic capillary chromatography (MECC). Anionic surfactants, especially sodium dodecylsulfate (SDS), are the most widely used additives in MECC. The anionic micelles migrate electrophoretically in the direction opposite that of electroosmotic flow (EOF) and do not adsorb onto the negatively charged wall of a fused-silica capillary. Although MECC is generally not quite as efficient a technique as normal CE, MECC extends many of the advantages of CE to the separation of neutral molecules. Consequently most work in micellar-assisted CE has focused on ionic surfactants. However, neutral molecules can migrate and be separated only within a separation window defined by the electroosmotic migration time and the migration time of the micelle [2]. Anionic micelles may interact strongly electrostatically with basic solutes, and cationic micelles can suppress or reverse the EOF. At high concentrations, ionic surfactants contribute to Joule heating and loss of efficiency [3].

Nonionic surfactants do not exhibit many of these limitations. Hjertén et al. [4] were the first to use nonionic surfactants as CE buffer additives, applying heptaoxyethylene lauryl ether and octyl glucoside to facilitate the separation of aromatic acids and basic pharmaceuticals. Swedberg [5] used the zwitterionic surfactant CHAPS (3-[3-(cholamidopropyl)dimethylammonio]-1-propanesulfonate) and the nonionic octyl glucoside for the separation of tricyclic antidepressants and of heptapeptides. Matsubara and Terabe resolved closely related peptides [6] and 24 dansylamino acids [7] with the aid of the nonionic surfactant Tween 20 in low pH buffers. Surfactant concentrations of 100 m*M*, far higher than the CMC, were required for separation. The migration order of the dansyl amino acids was that of increasing hydrophobicity, and since most of the solutes were positively charged at the pH used, the Tween 20 eluted last. Peptide separations based on hydrophobic selectivity were carried out by Greve et al. [8] who used a zwitterionic (DAPS, 3-(N-dodecyl-N,N-dimethyl-3-ammonio-1-propanesulphonate) or nonionic (Tween 20 or decanoyl-N-methyl glucamide) surfactant with organic solvent modifiers in a hydrophilically coated capillary. Basic drug substances of similar structure were separated by Hansen et al. [9] using zwitterionic (MAPS, 3-(N,N-dimethylmyristylammonio-1-propanesulphonate) or neutral (Tween 20) surfactant micelles in an acidic buffer.

Neutral surfactants have also been used in conjunction with ionic surfactants to enhance separations. Thus SDS plus the neutral surfactant Brij 35 allowed separation of benzene and benzaldehyde, which was not observed with SDS alone [10]. Ahuja and co-workers [11,12] have recently used this combination to extend infinitely the elution range in MECC. Mixed micelles were used by Song et al. [13] to separate herbicides, and by Erim et al. [14] to resolve all the underivatized saturated C_8–C_{20} fatty acids.

In this work, enhancement of the resolution of the eleven US EPA priority pollutant phenols was investigated using a nonionic surfactant, either Tween 40 or Brij 35, as a buffer additive. Ong and co-workers [15,16] investigated the separation of these eleven phenols by MECC using the anionic surfactant sodium dodecyl sulphate (SDS) in near-neutral pH buffers. The separation of these compounds by normal CE was studied by Li and Locke [17]. Although there are differences in analysis time and migration order between the results using SDS in MECC and the nonionic micelles reported here, both types of CE give good separation of these phenols.

2. Experimental

2.1. Apparatus

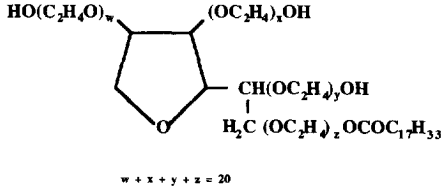
CE was carried out using an Isco Model 3850 electropherograph with an adjustable-wavelength UV detector set at 210 nm. An uncoated fused-silica capillary 100 cm long (65 cm from injector to detector) and 75 μm I.D. was used. Sample injection was done by applying a vacuum at the outlet buffer beaker for 10 s. The applied voltage was 20 kV. The electropherograms were recorded on either a Spectra-Physics SP-4600 or a Shimadzu CR-6A integrator.

2.2. Chemicals

The phenols were obtained from Aldrich (Milwaukee, WI, USA). Water purified using a Milli-Q

system (Millipore, Bedford, MA, USA) or HPLC-grade water (Fisher Scientific, Fair Lawn, NJ, USA) was used to prepare buffers and samples. The nonionic surfactants, polyoxyethylene(20)sorbitan monopalmitate (Tween 40) and polyoxyethylene(23)lauryl ether (Brij 35), were obtained from Sigma (St. Louis, MO, USA). The structures of the two nonionic surfactants are shown in Fig. 1. The stock buffer consisted of 25 m*M* Na_3PO_4 and 25 m*M* $Na_2B_4O_7$, pH 9.8. Running buffer containing nonionic surfactant was prepared by pipeting the appropriate amount of surfactant solution and diluting the buffer to a volume such that the concentrations of both electrolytes were 12.5 m*M*. Surfactant concentration was varied from 0% to 2% (w/v) in these experiments.

Stock solutions of the substituted phenols were prepared separately by weighing and dissolving individual compounds in HPLC-grade methanol. A mixture of the eleven phenols at a concentration of 100 mg/l of each was prepared by mixing aliquots of the stock solutions and diluting with methanol. This mixture was stored at 4°C. Test solutions for CE were prepared by further dilution of the mixture with the running buffer to 25 mg/l of each phenol. Buffer and sample solutions were filtered through 0.45-μm syringe filters.



(a) Tween 40

R $(OC_2H_4)_nOH$

R ≈ $C_{12}H_{25}$

n ≈ 23

(b) Brij 35

Fig. 1. Structures of (a) Tween 40 and (b) Brij 35.

2.3. Capillary conditioning

Each day the capillary was first conditioned by filling with 1 *M* NaOH and soaking for 10 min. The capillary was then washed with deionized water followed by running buffer. At the end of the day the column was washed with water followed by 0.1 *M* NaOH. The column was left filled with 0.1 *M* NaOH overnight.

3. Results and discussion

In our previous work [17], the separation of the eleven EPA priority pollutant phenols by CE was studied with emphasis on buffer pH selection. The pK_a values of these phenols range from 4 to 10.6 [17], so that in aqueous solution at alkaline pH values they are all at least partially dissociated. The overall electrophoretic mobility (μ_{ep}) of each phenol could be estimated using the following equation, which neglects activities [18]:

$$\mu_{ep} = \mu_A K_a/(K_a + [H^+]) \quad (1)$$

where μ_A is the electrophoretic mobility of the fully ionized species and K_a the dissociation constant of the acidic solute. To a first approximation, μ_{ep} is a function only of pH and K_a. Thus selectivities for compounds of different K_a values may be improved by adjusting buffer pH. It was found that all eleven phenols could be separated at pH 9.8 in about 15 min. However, the resolution of solute pairs with the longer migration times, e.g. 4-nitrophenol/2-nitrophenol, 2-chlorophenol/2,4-dinitrophenol, and 2,4-dichlorophenol/2-methyl-4,6-dinitrophenol was relatively small. For 4-nitrophenol/2-nitrophenol, both the μ_A and K_a values are very close, and their overall electrophoretic mobilities are also similar, so changing the pH cannot significantly improve resolution.

The association equilibrium of a solute, P, with a nonionic surfactant micelle, N, can be described by

$$P + N \rightleftharpoons PN \quad (2)$$

for which

$$K_1 = [PN]/[P][N] \quad (3)$$

If the electrophoretic mobility of P is μ_P and that of PN is μ_{PN}, the average mobility of P is

$$< \mu_P > = (\mu_P + K_1[N]\mu_{PN})/(1 + K_1[N]) \quad (4)$$

and since μ_{PN} is approximately the same as the electroosmotic mobility, μ_{eo}, which is generally larger than μ_P and in the opposite direction, solutes associating with the neutral micelles will move towards the anode. The migration order will be that of decreasing degree of association with the micellar phase.

Quite similar results were obtained using either Tween 40 or Brij 35; the discussion below specifies Tween 40 but it applies to both surfactants. The phenol mixture was separated in a series of phosphate/borate buffers (pH 9.8) that contained various concentrations of each surfactant. The electroosmotic mobility (μ_{eo}) and the net mobility (μ_{net}) of each solute were calculated from the migration time of methanol, t_{MeOH}, and the solute migration time, t, using the following equations

$$\mu_{eo} = (1/t_{MeOH}) l\, L/V \quad (5)$$

$$\mu_{net} = -[(1/t) - (1/t_{MeOH})] l\, L/V \quad (6)$$

where l is the column length from inlet buffer to detector, L is the total column length, and V is the applied voltage. The negative sign in Eq. 6 accounts for the fact that the direction of electrophoretic movement of anions is opposite that of electroosmotic flow. The designation μ_{net} is used to recognize the electrophoretic mobility of an ionic phenol solute is altered by its interaction with the neutral micelles, which move in the opposite direction, with the EOF.

The dependence of electroosmotic flow on the concentration of Tween 40 was measured using the methanol peak as an electroosmotic flow marker (Eq. 5). Fig. 2a shows that over the surfactant concentration range studied, the EOF is slightly reduced (less than 10%). This probably results from the effect of the surfactant on the buffer viscosity and the capillary wall zeta potential, which produce the decrease in the EOF. The effect of surfactant on the solute net mobility is more significant than its effect on the EOF. As described by Eq. 4 above, the average mobilities of weak acids such as phenols are determined by (a) the relative amounts of time solutes spend in the micellar phase and in the buffer and (b) their degree of dissociation, which depends on the phenol pK_a and the buffer pH. Undissociated solutes and anionic solutes with a high affinity for the micelle phase move most rapidly, with the EOF; fully dissociated solutes with low micelle affinity have the slowest migration times. The net mobilities, μ_{net}, of the phenols calculated using Eq. 6 from experimental migration times are plotted in Fig. 2b as a function of Tween 40 concentration. For all solutes the net mobility decreases upon addition of up to about 1% Tween 40 to the buffer. The initial sharp decrease for some solutes and change in migration order reflects the transition from normal CE to micelle-influenced CE as the surfactant concentration reaches and exceeds the CMC. The CMC of Tween 40 in water is reported [19] to be 0.0029% (w/v), although in the aqueous buffer the CMC is probably different [20]. The change is smallest for 2,4-dimethylphenol. The pK_a of this compound is 10.59, so that at the buffer pH used it is only partially dissociated, has a small μ_{net}, and moves at a rate close to the electroosmotic flow; addition of the surfactant consequently has little effect on its overall mobility. The behaviour of the other compounds can be accounted for in terms of a combination of pK_a and water solubility. Phenol and 4-chloro-3-methylphenol have similar pK_a values (9.89 and 9.54, respectively [17]) but the latter is less water-soluble and associates more strongly with the micelle than does phenol; the migration order reverses between 0 and 0.5% surfactant, and the μ_{net} of phenol becomes the larger. Compounds such as 2-nitrophenol, 4-nitrophenol, and 2,4-dinitrophenol are fully dissociated at the pH used (pK_a values 7.23, 7.15, and 4.00, respectively [17]), and show a trend of decreasing μ_{net} reflecting increasing affinity for the micellar phase because of decreasing water solubility. This drop is most pronounced for pentachlorophenol, whose net mobility at 1% Tween 40 is the about the same as the μ_{net} of 2,4-dimethylphenol, close to the μ_{eo}.

The net migration times plotted in Fig. 2c reflect the decreases in both the net and electroosmotic mobilities with increasing concentration of surfactant. Thus the migration times of pentachlorophenol and 2,4-dimethylphenol increase slightly with the percentage Tween 40 for concentrations >1% as a result of the decrease in the EOF. The migration times of 2- and 4-nitrophenol change little with

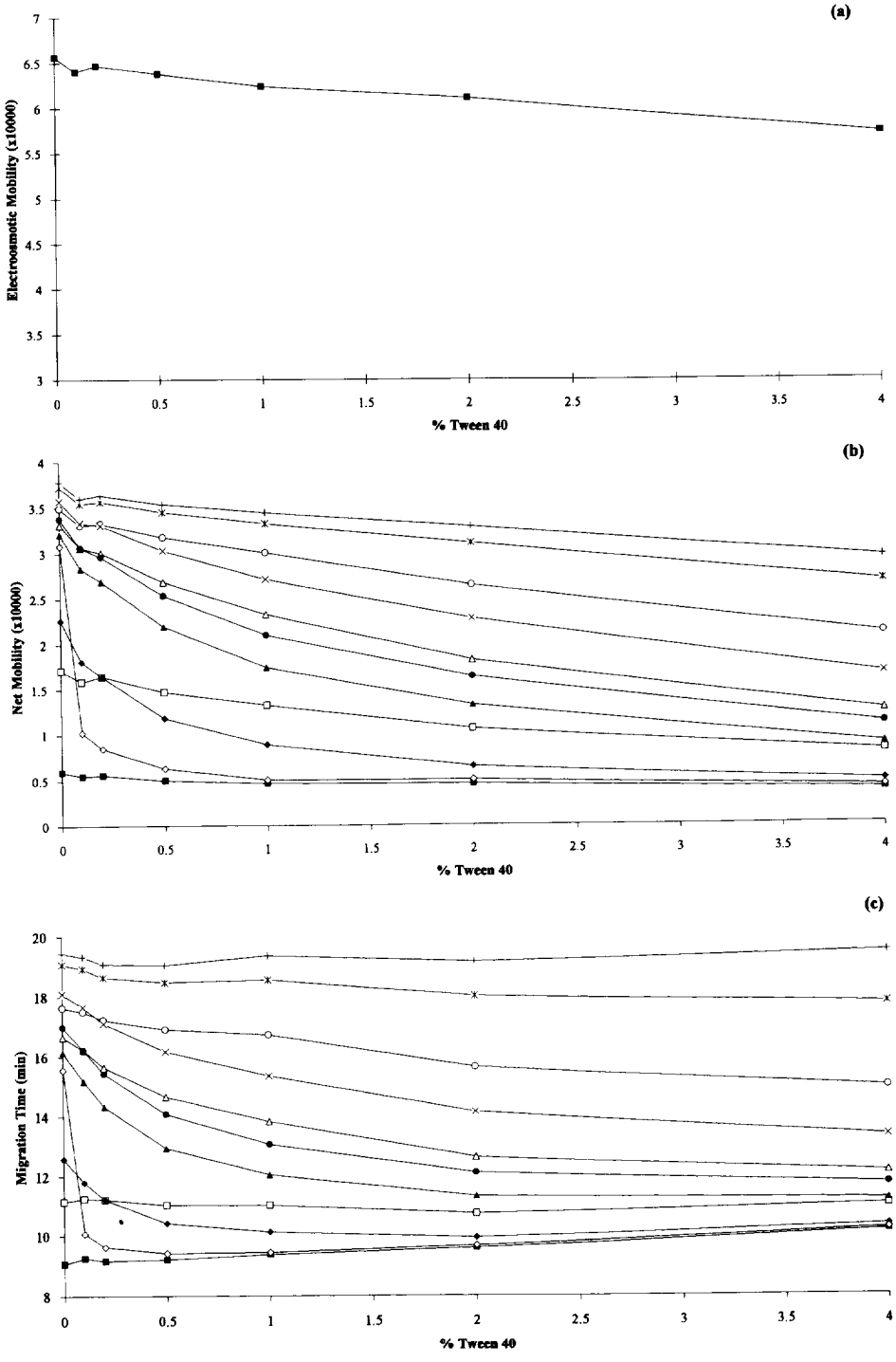


Fig. 2. The effect of Tween 40 concentration on (a) electroosmotic mobility (cm^2/Vs); (b) net solute mobility (cm^2/Vs); and (c) solute migration time (min). (■) 2,4-Dimethylphenol; (□) Phenol; (◇) 4-chloro-3-methylphenol; (◇) pentachlorophenol; (▲) 2,4,6-trichlorophenol; (△) 2,4-dichlorophenol; (●) 2-methyl-4,6-dinitrophenol; (○) 2-chlorophenol; (×) 2,4-dinitrophenol; (*) 4-nitrophenol; and (+) 2-nitrophenol.

surfactant concentration because the two effects of the surfactant work in opposite directions. Thus overall, addition of neutral surfactant does not change the total run time significantly relative to CE with buffer alone.

It is clear in Fig. 2b that at low concentrations of Tween 40, the net mobility decrease differs for each solute. This allows adjustment of selectivity and optimization of resolution by varying the amount of Tween 40 in the buffer solution. Resolution, R_s, of two solutes 1 and 2 is defined in the usual way by

$$R_s = 2(t_2 - t_1)/(w_2 + w_1) \quad (7)$$

where w is the peak baseline width. With no surfactant, the R_s values of the three slowest-migrating solute pairs, 2,4-dichlorophenol/2-methyl-4,6-dinitrophenol, 2-chlorophenol/2,4-dinitrophenol, and 4-nitrophenol/2-nitrophenol, are all small. The effect of the addition of Tween 40 on resolution is shown in Fig. 3. 4-Nitrophenol and 2-nitrophenol are poorly separated by CE without surfactant because of their very close pK_a and μ_A values. With surfactant in the buffer, their resolution improves by about 1 R_s unit per 1% increase in Tween 40 concentration. For the other two pairs, resolution goes through a minimum at about 0.1% Tween 40 because of the reversal in their migration order, and then increases significantly up to 1 or 2% Tween 40. Thus a good separation of all eleven phenols can be achieved using Tween 40 or Brij 35 in the range of 0.5% to 2%. Electropherograms are given in Fig. 4 for the separation

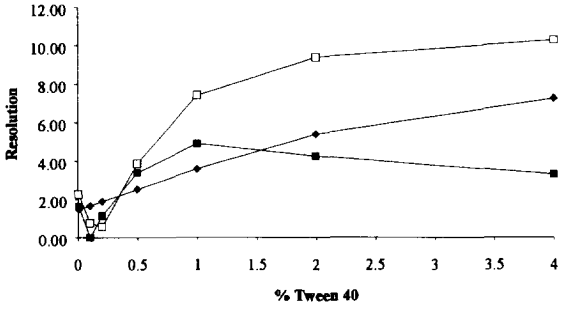


Fig. 3. The effect of Tween 40 concentration on the resolution of three solute pairs. (□) 2-Chlorophenol/2,4-dinitrophenol; (◇) 4-nitrophenol/2-nitrophenol; and (■) 2,4-dichlorophenol/2-methyl-4,6-dinitrophenol.

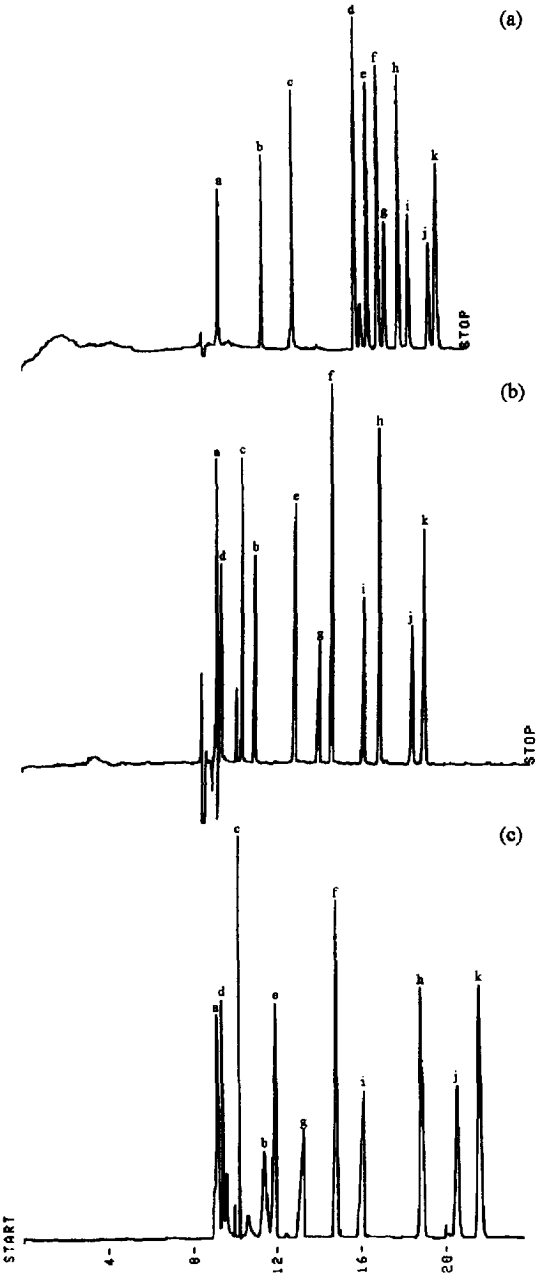


Fig. 4. Electropherograms of the EPA priority pollutant phenols at pH 9.8 (phosphate/borate buffer), 65 cm×75 μm I.D. capillary, 20 kV, with (a) no surfactant added; (b) 0.5% Tween 40; and (c) 0.5% Brij 35. Peak identifications: a = 2,4-dimethylphenol; b = phenol; c = 4-chloro-3-methylphenol; d = pentachlorophenol; e = 2,4,6-trichlorophenol; f = 2,4-dichlorophenol; g = 2-methyl-4,6-dinitrophenol; h = 2-chlorophenol; i = 2,4-dinitrophenol; j = 4-nitrophenol; and k = 2-nitrophenol. x-Axis: time in min.

of the phenol mixture (a) without surfactant additive, (b) with 0.5% of Tween 40, and (c) with 0.5% of Brij 35, all in borate/phosphate pH 9.8 buffer. Although the chemical structures of the two surfactants are quite different, they produce similar separations and similar behaviour in general. It is the general micelle–solute interaction that is important.

In addition to its remarkable decrease in net mobility, pentachlorophenol also shows an anomalous peak distortion. The electropherograms of the pentachlorophenol (PCP) peak are reproduced in Fig. 5 for several Tween 40 concentrations between 0% and 0.2%. The peak migration times (min) are printed above each peak. The peak is initially narrow, but near the CMC the peak broadens with tailing (see below), and then becomes again characteristically narrow and symmetrical as the Tween 40 concentration passes from 0% through 0.02% to 0.2%. The same peak distortion of pentachlorophenol was also observed when Brij 35 was used as the surfactant additive, but among the eleven phenols studied, only PCP obviously showed this unexpected peak distortion. Careful examination of other hydrophobic phenol peaks may reveal similar but lesser distortion. The electrophoretic explanation for asymmetrical peaks in free zone electrophoresis is electromigration dispersion relating to the relative mobilities of the sample ion and the background electrolyte ion; tailing results when the conductivity within the zone is lower than in the electrolyte and peak fronting when the reverse obtains [21]. To avoid this type of distortion, solute concentration should be maintained approximately a factor of 100 smaller than the electrolyte concentration [22]. In the present case, although the conditions producing electrodispersion-tailing exist for the anionic PCP

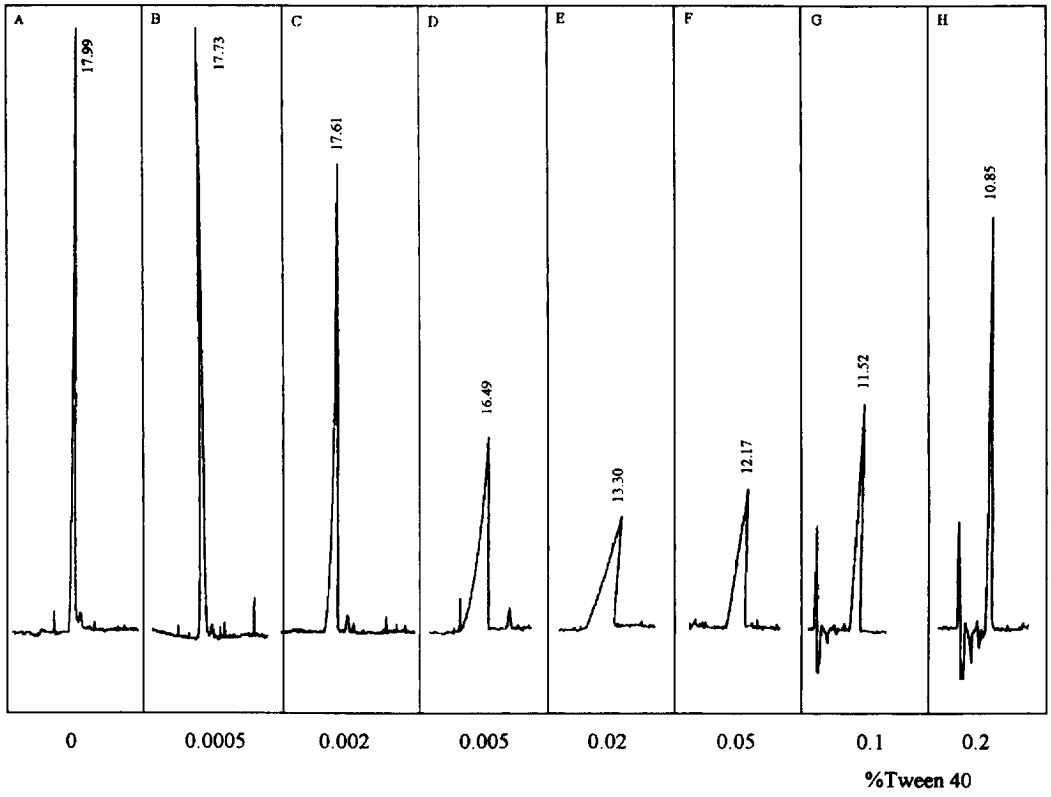


Fig. 5. Changes in the shape of the pentachlorophenol peak with increasing concentration of Tween 40. The peak migration times (min) are given above each peak.

associated with the surfactant, it cannot be the whole reason because tailing occurs only over a limited range of surfactant concentrations. In addition, dilution of the PCP by a factor of 10 produced the same peak tailing. The reason for this behaviour is not clear, but it is probably related to the extreme hydrophobicity of the pentachlorophenolate anion. Despite their charge, pentachlorophenolate anions will associate more strongly with neutral surfactant molecules and micelles than will the other anionic phenol solutes. Strong solute–surfactant interactions could increase the polydispersity of the degree of aggregation of the micelles at concentrations near the CMC, causing unfavorable solute–micelle phase mass transfer kinetics [23,24]. Further investigation of this phenomenon is planned.

It should be pointed out that a chromatographer would consider the peak asymmetry in Fig. 5 to be peak *fronting*, i.e. a peak with a sharp tail and a diffuse front, and would normally attribute this to an anti-Langmuir isotherm and sample overload. However, in capillary zone electrophoresis with the EOF suppressed, an anion of lower mobility than that of the background electrolyte would migrate in the direction of the positively polarized cathode, and a detector placed at that end would find a sharp leading edge and a diffuse tail. With EOF, which has no effect on peak shape per se, the stronger EOF drives anionic peaks backwards towards the anode, and to a detector at that end an electrophoretically tailing peak looks like a fronting peak. This matter has not, to our knowledge, been addressed before, and bears further examination.

A point-by-point comparison of the separation of these phenols using the anionic surfactant SDS [15,16] and the present work is not warranted because the experimental conditions, especially the lower pH buffer used with SDS, are quite different. A pH value near neutral is required with SDS to suppress dissociation of these weak acids to allow greater interaction of the solutes with the anionic surfactant micelle. The separations achieved with both systems are good. With the neutral surfactants, resolution of substituted phenols moving at a rate close to the EOF is generally more difficult to achieve than for those with longer migration times; with the anionic surfactants, the converse obtains. In comparison with neutral surfactants, anionic surfactants have a generally greater potential for resolution of mixtures because the negatively charged micelles migrate more slowly than the EOF. In addition, as noted above, neutral solutes cannot be separated using a nonionic surfactant; but nonionic surfactants may be used to advantage for strongly dissociated, hydrophobic compounds. With both types of surfactant, the small concentrations required to produce significant changes in selectivity have little effect on the magnitude of the electroosmotic flow, and buffer compositions are not changed substantially. Nonionic surfactants neither increase conductivity nor contribute to Joule heating. Each type of surfactant has its own role to play in micelle-modified CE.

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