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Use of different surfactants (sodium dodecyl sulfate, bile salts and ionic polymers) in micellar electrokinetic capillary chromatography Application to the separation of organophosphorus pesticides

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Abstract

Sodium dodecyl sulfate (SDS) is the most widely used surfactant in micellar electrokinetic capillary chromatography (MECC). The separation of highly hydrophobic compounds by MECC can be difficult because they will completely associate with the micelles and will elute at the end of the elution window. The addition of organic solvents to the running buffer may enhance the separation, but they can also inhibit micelle formation. An alternative is the use of different surfactant systems such as bile salts and ionic polymers. In this work, four different surfactants (SDS, sodium cholate, sodium deoxycholate and Elvacite 2669) have been studied by using two organophosphorus pesticides (chlorpyriphos-methyl and -ethyl) as model compounds. © 1997 Elsevier Science B.V.

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1. Introduction

Micellar electrokinetic capillary chromatography (MECC) involves the addition of a surfactant above the critical micelle concentration (CMC) to the running buffer that allows the separation of neutral analytes which have to be separated within a finite elution window [1]. The existence of this elution window is a major limitation because resolution in MECC is optimal for a capacity factor (k') in the range 1–5 [1].

Sodium dodecyl sulfate (SDS) is the most widely used surfactant in MECC. Hydrophobic solutes will completely associate with the micelles and will elute at the end of the elution window (t_m), so its separation with MECC can be difficult.

Several approaches have been reported for the

improvement of separation of hydrophobic compounds. The use of cyclodextrins (CDs) as running buffer additives has been reported to enhance the separation of polycyclic aromatic hydrocarbons (PAHs), polychlorinated biphenyls (PCBs) and tetra-chlorodibenzo-p-dioxins in MECC [2]. Another method is the addition of organic solvents to the running buffer. Addition of these solvents extends the elution window and alters k' by decreasing the polarity of the running buffer and has been employed to enhance separations of hydrophobic compounds [3–7]. Unfortunately high concentrations of organic solvent (>30%, v/v) seriously inhibit micelle formation. An alternative is the use of different surfactant systems such as bile salts [8] and ionic polymers [9].

Bile salts are biological surfactants produced in the liver. Some studies suggest that they form helical micelles with the hydrophobic portions facing the aqueous solution [10]. Due to their unique structure,

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bile salt surfactants are more tolerant to the addition of organic solvents than SDS micelles and form aggregates more polar than those formed with *n*-alkyl surfactants leading to a general reduction of k' in MECC [11,12].

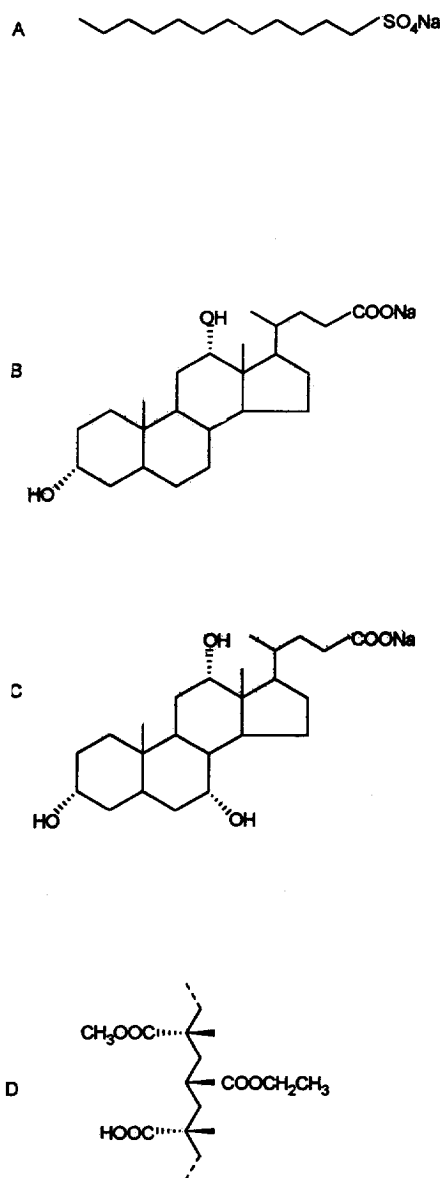Another approach is the use of ionic polymers as

Fig. 1. Structures of the different surfactants used in this work. (A) Sodium dodecyl sulphate (SDS), (B) sodium deoxycholate (NaDCh), (C) sodium cholate (NaCh), (D) ionic polymer (Elvacite 2669).

pseudo-stationary phases. The high-molecular-mass surfactant is considered to form the micelle from a single molecule, which essentially means there is no CMC. Therefore, we can expect a constant concentration of the micelle irrespective of the experimental conditions. This means that a high content of organic solvent will not break down the micelle; very low concentrations of the micelle will be available, and no monomeric surfactant that does not contribute to the separation will be present [13]. Different ionic polymers have been used to separate highly hydrophobic compounds by MECC [8,14,15]. In this work, the MECC separation of two organophosphorus pesticides (chlorpyriphos-methyl and -ethyl) with different surfactants [SDS, the bile salts sodium cholate (NaCh) and sodium deoxycholate (NaDCh) and poly (methyl methacrylate–ethyl acrylate–methacrylic) acid (Elvacite 2669)] have been studied. The structures of the different surfactants are shown in Fig. 1.

2. Experimental

2.1. Apparatus and reagents

The apparatus and experimental conditions for the analysis with SDS micelles were as described previously [7]. For bile salts and Elvacite 2669 analysis, a Hipotronics (Brewster, NY, USA) Model 340A high-voltage power supply was used. Inlet and outlet reservoirs were comprised of microcentrifuge tubes with platinum wire electrodes. Detection was carried out by on-column measurements at 200 nm on a Linear (Reno, NV, USA) Model 204 spectrophotometric detector. Hydrostatic injections were performed by a common siphoning procedure [16] using an inlet elevation of 10 cm and an injection time of 10 s. The effective void time (t_0) was determined by a solvent disturbance. Micellar time (t_m) was determined by injection of pyrene in the case of SDS and bile salts micelles, and by injecting a fullerene mixture (C_{60} and C_{70}) in the case of Elvacite 2669.

The copolymer, poly(methyl methacrylate–ethyl acrylate–methacrylic acid) (Elvacite 2669), was from ICI Acrylics (St. Louis, MO, USA) and a mixture of C_{60}, C_{70} and other fullerenes was pur-

chased from Fluka (Ronkonkoma, NY, USA). All other reagents were as described previously [7,8].

k' was determined by the equation [1]: $k' = (t_r - t_0)/t_0$ $(1 - t_r/t_m)$ and number of theoretical plates (N) and resolution (R_s) were calculated as $N = 16 t_r^2 w^{-2}$ and $R_s = 2(t_{r2} - t_{r1})(w_1 + w_2)^{-1}$, respectively, as in liquid chromatography, where t_r is the migration time of the analyte and w is the peak width at the baseline.

3. Results

Attempts in separating chlorpyriphos-methyl and -ethyl by using SDS in an aqueous running buffer gave a low signal and the peaks practically overlapped at micellar time $(k' = \infty)$ due to their strong interaction with the micelle. To increase the solubility of the solutes in the mobile phase and decrease their interaction with the micelle [6] methanol and acetonitrile have been added to the running buffer. In both cases, by the addition of high concentrations of each organic solvent (24% acetonitrile and 40% methanol), the separation of chlorpyriphos-methyl and -ethyl was achieved $(R_s = 1.3–2.0)$ (Fig. 2a) but they still exhibited very high values of k' (70 and 235 for chlorpyriphos-methyl and -ethyl, respectively using acetonitrile, and 32 and 46 for chlorpyriphos-methyl and -ethyl, respectively using methanol). However, the addition of organic solvents to the buffer is limited because of an increase in CMC with the organic solvent concentration.

The MECC separation of chlorpyriphos-methyl and -ethyl was studied by using the bile salts NaCh and NaDCh in a 10 mM phosphate–6 mM borate buffer at pH 9.3. Since these analytes are not very soluble in such buffers, methanol or acetonitrile as organic modifier was added. The results of this study are summarized in Table 1 where it can be seen that k' decreases and elution window (t_0/t_m) increases with increasing amounts of organic modifier.

Looking at the surfactant structures, it can be seen that NaCh micelle system is more polar than NaDCh, consequently, the k' values for the NaCh micelles are lower. For the same amount of added solvent, by working with NaCh micelles the pesticides retention times are lower, which means the analysis time is
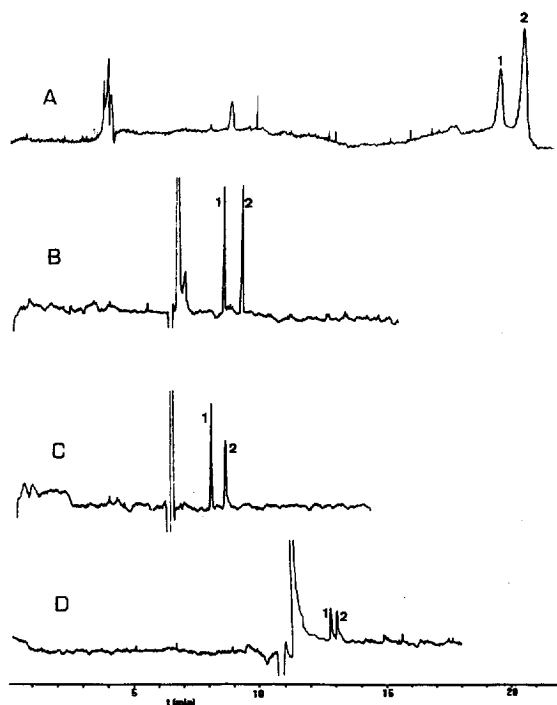

Fig. 2. MECC chromatograms of chlorpyriphos-methyl (1) and -ethyl (2) obtained with different micellar systems. Buffer A: 0.01 M Na$_2$HPO$_4$, 0.006 M Na$_2$B$_4$O$_7$ (pH 9.3). Buffer B: 0.1 M CAPS (pH 10). (A) Buffer A with 0.05 M SDS and 24% AcN. (B) Buffer A with 0.05 M NaDCh and 24% ACN. (C) Buffer A with 0.05 M NaCh and 24% ACN. (D) Buffer B with 2% Elvacite 2669, 50% MeOH and 24% ACN.

shorter, whereas by working with NaDCh better resolution values were achieved (see Fig. 2b Fig. 2c).

Finally, the copolymer Elvacite 2669 has been used for the analysis of chlorpyriphos-methyl and -ethyl. Buffer solutions of Elvacite 2669 were prepared in a 0.1 M 3-cyclohexylamino-1-propanesulfonic acid (CAPS) pH=10 buffer and different percentages of organic solvents were added. A basic medium (pH≥10) or the presence of organic solvents are necessary in order to dissolve this system.

The effect of the addition of different amounts of organic solvents (methanol and acetonitrile) to an initial buffer 1% Elvacite 2669, 0.05 M CAPS pH= 10, 50% MeOH, was studied. As it is shown in Table 2, the pesticides showed a good separation using only the initial buffer. With the addition of an organic solvent to the initial buffer, chlorpyriphos-methyl and -ethyl began to overlap, eluting near or at

Table 1
Capacity factor (k'), resolution (R_s) and elution window (t_0/t_m) values for chlorpyriphos-methyl (Clp-m) and -ethyl (Clp-e) using bile salts with different amounts of organic modifiers

Bile salt	Organic modifier	k' (Clp-m)	k' (Clp-e)	R_s	t_0/t_m
NaCh	none	59.4	–	1.16	0.36
	+12% MeOH	13.1	–	2.3	0.39
	+24% MeOH	1.9	4.6	5.1	0.43
	+36% MeOH	1.4	2.6	4.1	0.52
	+12% ACN	3.1	12.0	1.8	0.75
	+24% ACN	2.0	4.5	2.2	0.66
	+36% ACN	4.6	–	0.8	0.87
NaDCh	none	–	–	1.45	0.34
	+12% MeOH	34.4	–	4.0	0.31
	+24% MeOH	4.0	13.7	9.2	0.31
	+36% MeOH	1.8	4.2	10.6	0.36
	+12% ACN	7.6	16.1	6.1	0.36
	+24% ACN	3.0	11.0	4.6	0.64
	+36% ACN	6.5	–	0.9	0.84

ACN=Acetonitrile.

t_0. This overlap is greater with the addition of acetonitrile than with the addition of methanol. The same effect has been obtained by working with the initial buffer with a 50% of acetonitrile instead of a 50% of methanol. When Elvacite 2669 is added to the running buffer, we observed that retention times for all the compounds increase, as well as the resolution between the solutes. Fig. 2d shows a MECC chromatogram for the latter conditions.

Comparing the k' values obtained working with the three kinds of MECC systems (Table 3), it can be concluded that analysis with SDS shows higher k' values. Even with the addition of the maximum amount of organic solvent tolerated by the micelles,

there is a strong interaction with the micelle so retention times near t_m are obtained.

The analysis with the ionic polymer Elvacite 2669 showed the contrary effect. In the best analysis conditions k' values of approximately 0.5 were observed.

When bile salts are used the compounds elute in the middle of elution window with high resolutions. This confirms the results obtained by Terabe et al. [1] where resolution is maximum for k' values between 1 and 5.

Efficiency is another important aspect in any separation. Efficiency is lower for analysis with Elvacite 2669 and SDS. Two of the causes of band

Table 2
Retention times, efficiencies (N) and resolution (R_s) values for chlorpyriphos-methyl (Clp-m) and -ethyl (Clp-e) using buffers with different amounts of organic solvents and Elvacite (Elv) polymer

Buffer	t_0	t_{Clp-m}	t_{Clp-e}	N_{Clp-m}	N_{Clp-e}	R_s
1%Elv, 0.05 M CAPS pH=10, 50% MeOH	11.6	14.3	14.6	36 303	37 843	1
Idem+10% ACN	10.3	11.9	12.0	36 252	18 805	0.33
Idem+25% ACN	9.0	10.1	10.1	–	–	–
Idem+10% MeOH	12.6	14.8	14.95	87 616	39 707	0.6
Idem+25% MeOH	12.3	13.9	13.9	–	–	–
Idem+10% ACN+1% Elv	12.5	14.5	14.8	53 824	56 074	1.2
Idem+25% ACN+1% Elv	10.4	12.1	12.2	37 171	38 103	0.4
1%Elv, 0.05 M CAPS pH=10, 50% ACN	4.3	4.8	4.85	–	–	0.3

Table 3
Capacity factor (k'), resolution (R_s) and efficiency (N) values for chlorpyriphos-methyl and -ethyl using different micellar systems

Micellar system	Buffer	Organic modifier	$k'_{Clp\text{-}m}$	$k'_{Clp\text{-}e}$	R_s	$N_{Clp\text{-}m}$	$N_{Clp\text{-}e}$
0.05 M SDS	0.01 M Na$_2$HPO$_4$, 0.006 M Na$_2$B$_4$O$_7$, pH=9.3	24% ACN	68.3	234.6	1.3	20 736	9489
0.05 M NaDCh	0.01 M Na$_2$HPO$_4$, 0.006 M Na$_2$B$_4$O$_7$, pH=9.3	24% ACN	3	11	4.6	78 400	63 216
0.05 M NaCh	0.01 M Na$_2$HPO$_4$, 0.006 M Na$_2$B$_4$O$_7$, pH=9.3	24% ACN	2	4.5	4.3	57 600	23 716
Elvacite 2669 (2%)	0.1 M CAPS, pH=10	50% MeOH, 24% ACN	0.48	0.57	1.0	24 336	25 600

broadening are longitudinal diffusion and electrophoretic dispersion of micelles. Longitudinal diffusion is inversely proportional to electroosmotic flow (v_{eo}). Terabe et al. [17] suggested working with v_{eo} values higher than 1 mm/s would reduce band broadening due to longitudinal diffusion. In the case of Elvacite 2669 the obtained v_{eo} value was 0.3 mm/s. Efficiency depends also on electrophoretic dispersion of micelles. In the case of Elvacite 2669, as the "micelles" are formed by an unic polymer of similar molecular mass but not always identical, electrophoretic dispersion of their mobilities is important. Looking at capacity factors, resolution or efficiency, when comparing the three kinds of micellar phases for the separation of chlorpyriphos-methyl and -ethyl, the use of bile salts is desirable.

Acknowledgments

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