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Sample concentration by sample stacking and sweeping using a microemulsion and a single-isomer sulfated β-cyclodextrin as pseudostationary phases in electrokinetic chromatography

Joselito P. Quirino[a,*], Shigeru Terabe[a], Koji Otsuka[a], J. Bryan Vincent[b], Gyula Vigh[b]

[a]*Faculty of Science, Himeji Institute of Technology, Kamigori, Hyogo 678-1297, Japan*

[b]*Department of Chemistry, Texas A&M University, College Station, TX 77842-3012, USA*

Abstract

Two of the powerful on-line sample concentration techniques, sample stacking and sweeping under pH-suppressed electroosmotic flow, have been evaluated using a microemulsion and a single-isomer sulfated β-cyclodextrin derivative in electrokinetic chromatography. Several clinically relevant steroids have been separated and concentrated using a microemulsion consisting of 100 m*M* sodium dodecyl sulfate, 41 m*M* *n*-heptane and 700 m*M* 1-butanol in 50 m*M* phosphoric acid (pH 1.9). Three environmentally relevant phenoxy acid herbicides and their enantiomers have been separated and concentrated using a background electrolyte consisting of 20 m*M* hepta-6-sulfato-β-cyclodextrin in 15 m*M* phosphoric acid (pH 1.9). Significant detector response improvements have been achieved and utilized for analysis of a relatively clean matrix, lake water.

Keywords: Sample stacking; Electrokinetic chromatography; Sweeping; Sample handling; Water analysis; Pseudo-stationary phases; Pesticides; Phenols; Steroids

1. Introduction

Electrokinetic chromatography (EKC) was invented to extend the utility of capillary electrophoresis (CE) and permit the separation and analysis of neutral analytes [1]. In EKC, separation can be achieved when the distribution coefficients of the analytes between the pseudostationary phase and the aqueous phase are different from each other. In addition to conventional micelles (usually formed of sodium dodecyl sulfate), microemulsions [2–4], macrocyclic and macromolecular phases [5,6], polymers [7], resorcarenes [8], and dendrimers [9] have also been used as advantageous, alternative pseudostationary phases for EKC.

EKC, just like the other modes of CE, is hampered by the low concentration sensitivity of the UV detector, a consequence of the short optical pathlength of the cell. Thus, trace analysis by EKC requires time consuming, pre-separation, off-line sample concentration steps (e.g., solid-phase extraction). In capillary zone electrophoresis (CZE), concentration detection limits have been improved few hundred-folds by on-line sample concentration methods, most notably by transient isotachophoresis [10] and sample stacking [11]. Unfortunately, these techniques are only applicable to ionizable analytes, because the neutral analytes are unaffected by the electric field and they do not follow the Kohlrausch rules. In order to eliminate this limitation and permit

*Corresponding author. Tel.: +81-791580173 fax: +81-791580132.

E-mail address: jquirino@sci.himeji-tech.ac.jp (J.P. Quirino)

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the separation and detection of complex samples at lower concentration levels, two on-line analyte concentration approaches have been developed for neutral solutes, which are analyzed by EKC [12–16].

The first analyte concentrating approach is sample stacking [12–15]. Sample stacking occurs as ions cross a boundary that separates regions of high and low electric field strength [11]. Since the electrophoretic velocity of ions in the high electric field strength region (in the less conducting sample zone) is higher than in the low electric field strength region (in the better conducting separation zone), ions slow down when they cross the boundary. This results in the narrowing of the analyte zone. In EKC, the charged pseudostationary phases provide the neutral analytes with the effective electrophoretic mobilities necessary for focusing to occur. However, the stacking techniques in EKC are not as simple as in CZE.

Sweeping is the second approach which, in theory, provides for an almost unlimited improvement in concentration detection sensitivity for analytes that have high affinities toward the pseudostationary phase: 5000-fold improvements have been demonstrated experimentally [16]. Sweeping results when the pseudostationary phase, which penetrates the sample zone entraps and accumulates the analytes. The sample is prepared in a matrix that is free of the pseudostationary phase and has a conductivity similar to (or higher than) that of the background solution (BGS).

In this work, a microemulsion and a single-isomer sulfated cyclodextrin derivative were evaluated as pseudostationary phases for on-line sample concentration in EKC. (In this paper, the acronym MEEKC will be used for microemulsion EKC and CDEKC for sulfated cyclodextrin EKC.) Low pH buffers were used to suppress the electroosmotic flow. In the stacking techniques, samples were prepared in low conductivity matrices, which were either free of the pseudostationary phase (permitting stacking with a reverse-migrating pseudostationary phase, SRMP [13]) or contained a pseudostationary phase and permitted (a) field enhanced sample injection with a reverse-migrating pseudostationary phase, FESI–RMP [14] or (b) stacking that relied on a reverse-migrating pseudostationary phase and a water plug, SRW [15]. In order to describe the techniques in a general manner, the term ‘pseudostationary phase’ is used instead of the earlier ‘micelle’ term. In the sweeping technique, samples were prepared in a phosphoric acid matrix whose conductivity was similar to that of the BGS.

2. Experimental

2.1. Apparatus

All separations were carried out with a Hewlett-Packard 3D Capillary Electrophoresis System (Waldbronn, Germany) using 50 μm I.D.×375 μm O.D. fused-silica capillaries (Polymicro Technologies, Phoenix, AZ, USA) that were 64.5 cm long (56 cm to the detector) and were thermostated at 20°C. Pressure injections were carried out at 50 mbar or 1 bar. An optimum detection wavelength was selected for each analyte based on the spectra recorded by a diode array detector. Conductivities were measured with a Horiba ES-12 conductivity meter (Kyoto, Japan). The pH of solutions was adjusted and measured with the aid of a Beckman Φ 34 pH meter. Water was purified with a Milli-Q system (Millipore, Bedford, MA, USA).

2.2. Reagents and solutions

Most reagents (highest grade available) were purchased from Nacalai Tesque (Kyoto, Japan). Phenoxy acid herbicides were obtained from Dr. Donald G. Patterson (Centers for Disease Control and Prevention, Atlanta, GA, USA). Stock solutions of the phenol derivatives [pentylphenol, 4-(1,1-dimethylethyl)phenol, 2,3,5-trimethylphenol, 4-ethylphenol, 3-chlorophenol, 2-chlorophenol, 3-methylphenol, 4-methylphenol, 2-methylphenol, 3-nitrophenol, 4-nitrophenol, 2-fluorophenol, and phenol] were prepared with purified water or 10% aqueous ethanol. Stock solutions of phenoxy acid herbicides (fenoprop, mecoprop, and dichlorprop) and steroids (testosterone, progesterone, hydrocortisone, cortisone, fluocinolone acetonide, betamethasone, and triamcinolone) were prepared with 95% ethanol. Appropriate amounts of the sample stock solutions were combined and diluted with the sample matrix to obtain sample solutions (S) in which the analytes had comparable peak heights.

For the MEEKC experiments, the BGSs contained 100 m*M* sodium dodecyl sulfate (SDS), 41 m*M* *n*-heptane, 700 m*M* butanol, and 50 m*M* phosphoric acid. They were made with stock solutions of 500 m*M* SDS and 500 m*M* phosphoric acid, which were prepared weekly. The clear solution had a 'pH' (apparent or pseudo pH) of around 1.9 and was found to be stable during the experiments. For the CDEKC experiments, the BGSs were prepared by weighing the calculated amount of hepta-6-sulfato-β-cyclodextrin and dissolving it in the appropriate phosphoric acid solutions (see figures). For the MEKC experiments, the BGSs were prepared by mixing stock solutions of SDS and phosphoric acid, followed by dilution with water. Urea, methanol or γ-cyclodextrin was added to the system to tune the separation selectivities. All BGSs were freshly prepared. All solutions were filtered through 0.45 μm filters (Toyo Roshi, Japan) prior to use.

2.3. Procedure

The capillary was flushed prior to use with 1 *M* NaOH (20 min), followed by methanol (20 min), 0.1 *M* NaOH (20 min), purified water (20 min) and, finally, with the BGS (5 min). To ensure repeatability, the capillary was flushed between consecutive analyses with 0.1 *M* NaOH (1 min), methanol (1 min), purified water (2 min) and, finally, with the BGS (3 min).

For SRMP, test analytes prepared in water were injected into the capillary at the cathodic end using pressure. The injection times were much longer than what is usual (i.e. 1 s) for hydrodynamic injection. The electrode vials were filled with the BGS and the negative potential was applied at the injection-end of the capillary. For FESI–RMP, short water plugs were injected at the cathodic end, followed by electrokinetic injection of the test analytes, prepared in low conductivity matrices, at negative polarity. The low conductivity solutions do contain the proper pseudostationary phase, but at concentrations much lower than the BGS. When a certain percentage of the original current was reached, the BGS vials were placed at both ends of the capillary and the separation potential was applied with negative polarity at the injection-end. For SRW, a long water plug was injected at the cathodic end, followed by a long injection of S (the same S as in FESI–RMP), then the BGS-containing vials were connected to both ends of the capillary and the separation potential was applied with negative polarity at the injection-end. For the sweeping experiments, test analytes prepared in phosphoric acid previously adjusted to the conductivity of the BGS were pressure injected into the capillary at the cathodic end. The injection times were quite long, depending on the affinity of the analyte for the pseudostationary phase (i.e., retention factor, *k*). Then, the BGS-containing vials were connected to both ends of the capillary and the separation potential was applied with negative polarity at the injection-end. A more detailed discussion of each technique can be found in the respective previous publications [13–16]. Other experimental conditions are stated in the figures or in the text.

3. Results and discussion

3.1. MEEKC and the on-line concentration techniques

Oil-in-water (o/w) microemulsions have been shown to be good pseudostationary phases for EKC [2–4]. Though the exact structure of o/w microemulsions has not been fully elucidated, it is thought to resemble swollen micelles. Here, we were able to separate, with great ease, seven steroids in a low 'pH' o/w microemulsion (see Fig. 1A). Moreover, we were able to achieve on-line sample concentration under 'pH'-suppressed electroosmotic flow (Fig. 1B–E) using several of the techniques discussed above. Stacking and sweeping enhancement factors are listed in Table 1 for this particular analysis. One to more than two orders of magnitude improvement in concentration detection limits is then affordable.

The peak shapes and separation efficiencies are fairly well preserved with all of the on-line concentration methods. Analyte migration times in SRMP (Fig. 1B), SRW (Fig. 1C), and sweeping (Fig. 1E) are longer compared to what were observed with conventional sample injection (Fig. 1A). Note that the time scale of all electropherograms in Fig. 1 is the same. This is due to the matrix removal phase during focusing. Analyte migration times in FESI–

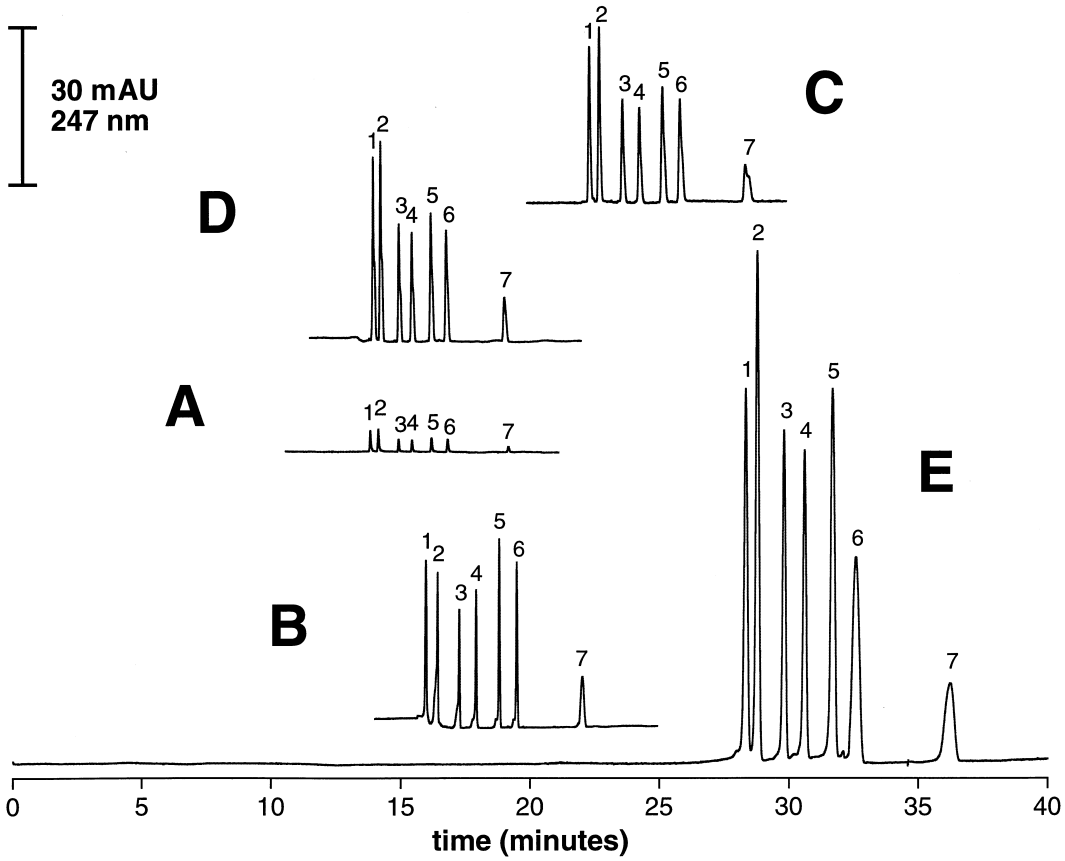

Fig. 1. Sample stacking and sweeping of test steroids in MEEKC. BGS: 100 m*M* SDS, 41 m*M* *n*-heptane, 700 m*M* 1-butanol in 50 m*M* phosphate buffer ('pH' 1.9). Steroids in sample (S): progesterone (1), testosterone (2), fluocinolone acetonide (3), betamethasone (4), hydrocortisone (5), cortisone (6), triamcinolone (7). Sample concentrations: ~90 ppm in the BGS (A), ~9 ppm in water (B), ~9 ppm in 1/10 dilution of the BGS (C, D), ~9 ppm in phosphoric acid solution which has the same conductivity as the BGS. Injection: 1-s or 0.64 mm (A, conventional injection), 7.04 cm (B, SRMP), 5.44 cm water and 6.4 cm S (C, SRW); 5.76 cm of water followed by electrokinetic injection at −18 kV until 80% of the original current is reached (D, FESI–RMP); 12.35 cm (E, sweeping). Separation conditions: applied voltage, −18 kV; injection pressure, 50 mbar (A, B, C, D), 1 bar (E); time scale of all electropherograms is the same.

RMP (Fig. 1D) are comparable to what were observed with conventional sample injection. This is because the water plug is short and it is partially removed from the capillary during electrokinetic injection and focusing.

3.2. CDEKC and the on-line concentration techniques

Hepta-6-sulfato-β-cyclodextrin, a new, hydrophilic, single-isomer charged cyclodextrin proved useful for the separation of the enantiomers of noncharged, acidic, basic, and zwitterionic analytes [5]. Here, we used it to separate six phenol derivatives (Fig. 2) as well as three phenoxy acid herbicides and their enantiomers (Fig. 3)

Since the test phenols bind weakly to hepta-6-sulfato-β-cyclodextrin, low (about 10-fold) detector response improvements were observed, both with the stacking techniques and the sweeping technique (see Fig. 2B–E). Again, the samples used for Fig. 2B–E were 1/10 dilutions of the sample used for conventional injection (Fig. 2A). The separation efficiencies are also low (the peaks are broad), especially for

Table 1
Sample stacking and sweeping enhancement factors obtained from the test analytes[a]

Name	SRMP	FESI–RMP	SRW	Sweeping
MEEKC of several clinically relevant steroids[b]				
(1) Progesterone	76	87	92	179
(2) Testosterone	66	88	93	235
(3) Fluocinolone a.	91	94	92	266
(4) Betamethasone	116	97	92	278
(5) Hydrocortisone	136	95	87	276
(6) Cortisone	129	89	80	163
(7) triamcinolone	87	79	63	138
CDEKC of several environmentally relevant phenoxy acid herbicides[c]				
(1) Fenoprop (1st peak)	158	94	49	5
(2) Fenoprop (2nd peak)	145	75	40	5
(3) Mecoprop (1st peak)	90	72	41	5
(4) Mecoprop (2nd peak)	98	82	43	6
(5) Dichlorprop (1st peak)	96	70	37	7
(6) Dichlorprop (2nd peak)	109	79	41	7

[a] Enhancement factor=peak height obtained with sample stacking or sweeping/peak height obtained with usual injection.
[b] Conditions are the same as those found in Fig. 1.
[c] Conditions are the same as those found in Fig. 3.

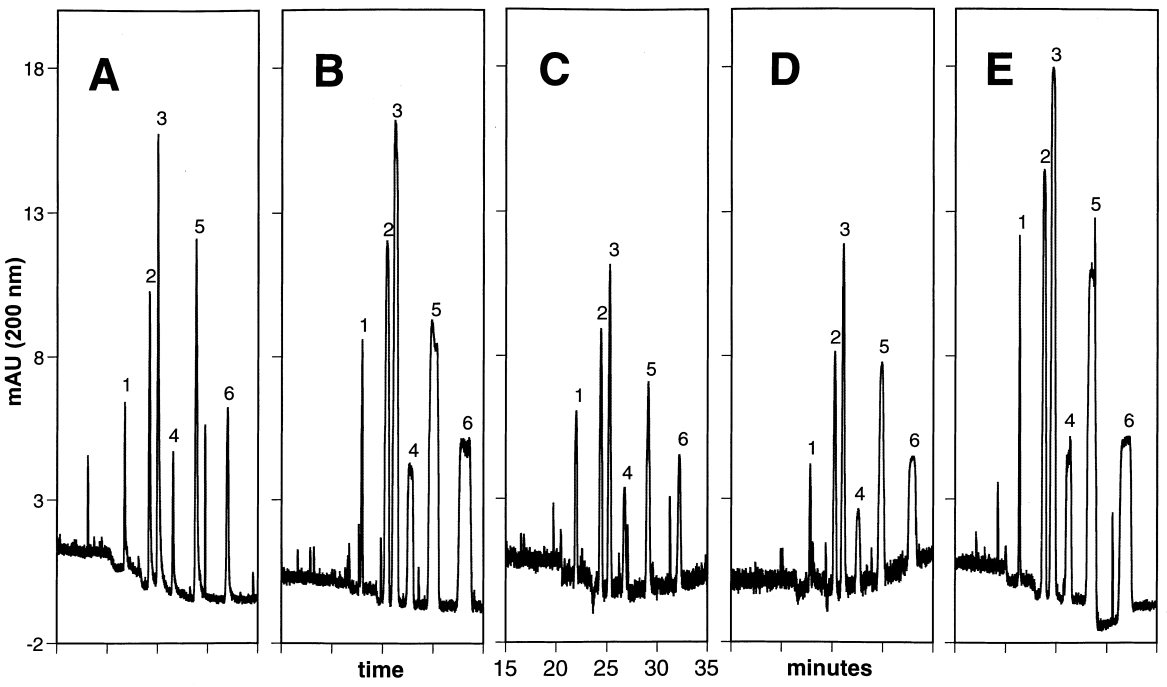

Fig. 2. Sample stacking and sweeping of test phenols in CDEKC. BGS, 40 m*M* hepta-6-sulfato-β-CD in 30 m*M* phosphoric acid (pH 2). Phenols and their concentration in sample S: 24.6 ppm pentylphenol (1), 32.7 ppm 4-ethylphenol (2), 27.1 ppm 3-nitrophenol (3), 27.7 ppm 3-chlorophenol (4), 32.3 ppm 3-cresol (5), 34.5 ppm phenol (6). Injected samples: S in water (A, B), 1/10 dilution of S in 1/10 dilution of BGS (C, D), 1/10 dilution of S in phosphoric acid having the same conductivity as the BGS (E). Injection: 0.64 mm (A), 3.84 cm (B, SRMP), 1.92 cm of water followed by electrokinetic injection at −10 kV until 60% of the original current was reached (C, FESI–RMP), 1.92 cm water followed by 1.92 cm S (D, SRW), 3.84 cm (E, sweeping). Pressure, 50 mbar; applied potential −10 kV.

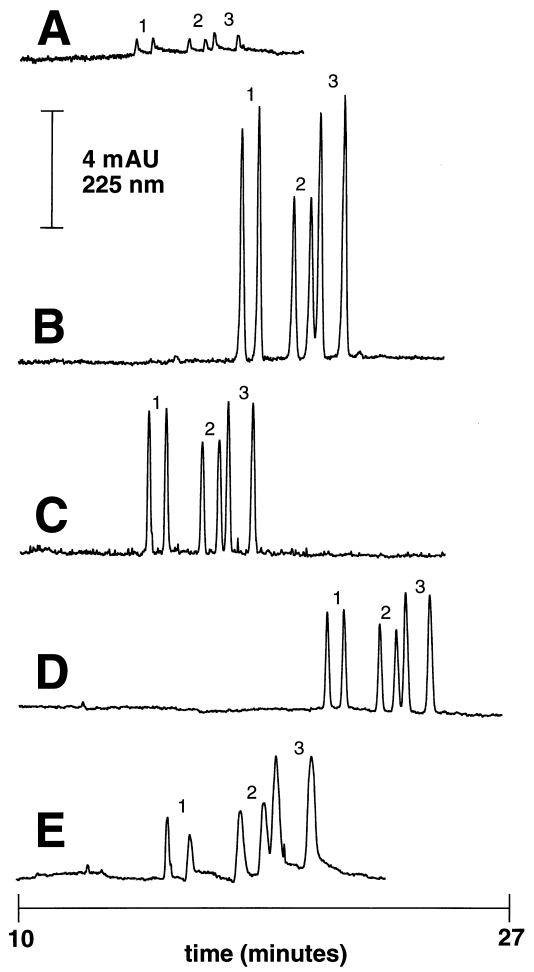

Fig. 3. Sample stacking and sweeping of phenoxy acid herbicides in CDEKC. BGS, 20 m*M* hepta-6-sulfato-β-CD in 15 m*M* phosphoric acid (pH 1.9). Sample S: phenoxy acid herbicides in water (A, B), in 1/40 dilution of the BGS (C, D), in phosphoric acid which has the same conductivity as the BGS (E). Peaks, fenoprop (1), mecoprop (2), dichlorprop (3). Concentration of analytes in A and E, ~50 ppm; concentration of analytes in B, C, and D, 1/10 diluted compared to A. Injection: 0.64 mm (A), 7.04 cm (B, SRMP), 0.96 cm of water followed by electrokinetic injection at −11 kV until 60% of the original current was reached (C, FESI–RMP), 2.88 cm water followed by 2.88 cm S (D, SRW), 0.45 cm (E, sweeping). Pressure, 50 mbar; applied potential, −11 kV.

analytes with small *k* values. Better stacking enhancement factors were obtained for the phenoxy acid herbicides (see Fig. 3). Once again, the samples used for Fig. 3B–D are 1/10 dilutions of the sample used for Fig. 3A (conventional injection). Stacking and sweeping enhancement factors are listed in Table 1 for this particular analysis. With sweeping (Fig. 3E), since the analytes bind weakly to hepta-6-sulfato-β-cyclodextrin, the enhancement factors are low (only around 5). Note that the analyte concentrations in Fig. 3A and E are the same.

3.3. Effect of the sample matrix on the on-line concentration techniques

In order to avoid concentration of the matrix constituents (or contaminants) – and their masking of the analyte peaks – it is often advisable to complete a single, preferably selective, clean-up step prior to the on-line sample concentration step. On the contrary, relatively clean liquid matrices may be analyzed after minimum pretreatment. For example, it might be enough to add a simple filtration step prior to the on-line concentration step and use SRMP if there are no surfactants in the matrix and the conductivity of the matrix is low, or mix the liquid matrix with several parts of the BGS prior to FESI–RMP or SRW, or adjust the conductivity of the liquid matrix with a concentrated buffer prior to sweeping. We investigated these possibilities by spiking test analytes into lake water.

In SRMP, test phenols were spiked in lake water to a concentration of around 0.7 ppm and then directly injected into the capillary. In SRW and FESI–RMP, test phenols were spiked in lake water to a concentration of around 1.5 ppm. This sample solution was then mixed with the BGS, 5:1 ratio of sample solution and BGS, before injection. In sweeping, fenoprop was spiked in lake water to a concentration of 28 ppb (total of both enantiomers) and then injected into the capillary after adjusting the conductivity with phosphoric acid solution (electropherogram obtained from Ref. [16]). The results are shown in Fig. 4. These indicate that the on-line concentration techniques described here can be accomplished without an off-line pretreatment step, provided that the concentration of the analytes is within the working range of the technique. No interference emanating from the sample matrix was observed, as verified from blank experiments.

In conclusion, we have shown that microemulsions and hepta-6-sulfato-β-cyclodextrin can be used

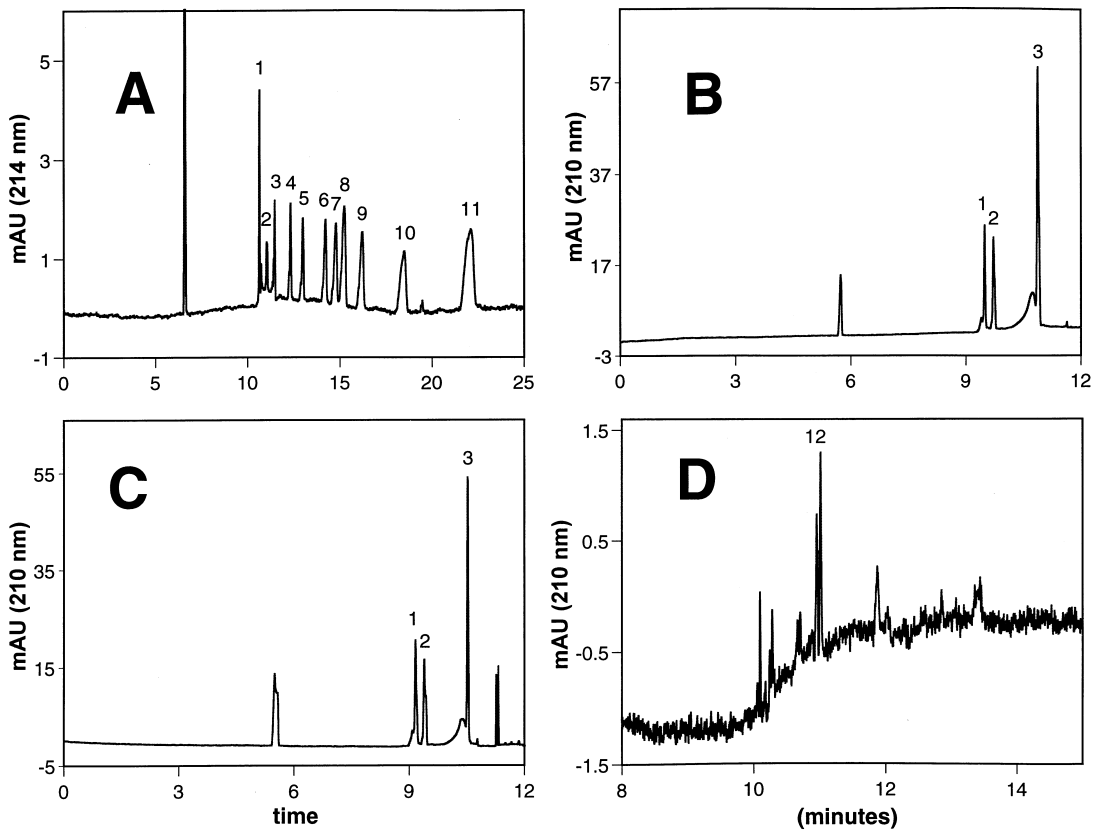

Fig. 4. On-line concentration of test analytes spiked in lake water without off-line pre-treatment in MEKC. A: SRMP; injection: 3.84 cm, BGS: 100 m*M* SDS and 1 *M* urea in 50 m*M* phosphate buffer (pH2.5), sample: phenols (~0.7 ppm) spiked in lake water, applied potential, −22kV. B: SRW; injection: 3.84 cm water plug and 3.84 cm of S, BGS: 0.05 *M* SDS in 0.05 *M* phosphate buffer (pH 1.9)/5% methanol, S: 8 parts phenols (~1.5 ppm) in lake water in 2 parts BGS; applied potential: −20 kV. C: FESI–RMP; injection: 3.84 cm of water plug followed by electrokinetic injection at −20 kV until 75% of the original current was reached, other conditions are the same as in B. D: sweeping; injection: 20.80 cm, BGS: 50 m*M* SDS and 15 m*M* γ-cyclodextrin in 40 m*M* phosphate buffer (pH 1.9), S: fenoprop racemate (28 ppb total, ~71% enantiomeric ratio) in lake water with a conductivity similar to that of the BGS (adjusted with 500 m*M* phosphoric acid), applied potential: −18 kV, peak identity: pentylphenol (1), 4-(1,1-dimethylethyl)phenol (2), 2,3,5-trimethylphenol (3), 4-ethylphenol (4), 3-chlorophenol (5), 2-chlorophenol (6), 4-methylphenol (7), 2-methylphenol (8), 4-nitrophenol (9), 2-fluorophenol (10), phenol (11), fenoprop (12), injection pressure: 50 mbar. (Fig. 4D. Reprinted with permission from Science 282 (1998) 465–468. ©1998 American Association for the Advancement of Science).

as pseudostationary phases for on-line sample concentration. Other pseudostationary phases are expected to be useful as well. By tuning the *k* values of the analytes (i.e., by choosing a pseudostationary phase that interacts with the analytes strongly), the concentration detection levels can be improved as needed. Moreover, fast real-world analysis can be achieved by using minimal sample pretreatment and the aforementioned on-line sample concentration techniques.

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