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Polymeric Anionic Surfactant for Electrokinetic Chromatography: Separation of 16 Priority Polycyclic Aromatic Hydrocarbon Pollutants

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Electrokinetic chromatography (EKC) with poly(sodium undecylenic sulfate) (poly-SUS) is utilized to separate environmental pollutants such as 2−6-ring polycyclic aromatic hydrocarbons (PAHs). Parameters such as pH, concentration of polymeric surfactants, and the use of organic modifiers were investigated to follow the retention trends of PAHs. A baseline separation of all 16 PAHs in about 30 min, using 0.50% (w/v) of poly-SUS/12.5 mM sodium phosphate−borate buffer (pH 9.2) with 40% (v/v) acetonitrile, was possible for the first time in EKC by a single-surfactant system.

After the first publication on micellar electrokinetic chromatography (MEKC) more than 10 years ago,[1] many researchers have explored various types of monomeric surfactants above their critical micelle concentrations (cmc's) as pseudostationary phases for the separation of both ionic and nonionic compounds.[2−6] Among the pseudostationary phases investigated, sodium dodecyl sulfate (SDS) has been successful in the MEKC separation of many water-soluble solutes.[7,8] However, in the case of highly hydrophobic analytes such as polycyclic aromatic hydrocarbons (PAHs), the binding with SDS micelle is often too strong to permit adequate resolution of these compounds.[9,10]

Polymerized surfactants bearing both chiral[11−14] and achiral[15−18] ionic headgroups have been proposed as alternative pseudostationary phases in electrokinetic chromatography (EKC). This technique has several potential advantages over the use of the normal micelles generated from monomeric surfactants. First, polymerized surfactants have no cmc. In this respect, polymerized surfactants can be effective as pseudostationary phases over a wide range of concentrations. In contrast, normal micelles require higher surfactant concentrations (at least 2−10 times the cmc) for effective separations. Thus, Joule heating is expected to be more serious in micellar-mediated MEKC than in EKC with polymerized surfactants. Second, the elimination of dynamic equilibrium between monomer and micelle, as well as the presence of covalent bonds between these surfactant aggregates, provides enhanced stabilities, enhanced rigidities, and controllable sizes to polymerized surfactants. Third, in buffers modified with a higher fraction of organic solvents, the chromatographic selectivity with polymerized surfactant is superior to that with SDS micelle. For example, acetonitrile and methanol can be used at higher concentrations, ∼65−75% (v/v) with polymerized surfactants,[19,20] whereas the SDS micelle can only tolerate ∼30−40% (v/v) of these solvents.[20,21] Fourth, EKC with polymerized surfactants offers a wider elution window than MEKC, resulting in higher peak capacity.

Poly(sodium-10-undecylenate) was the first polymerized achiral surfactant used in EKC.[15,16] Although this surfactant provided high-performance separation of a wide range of neutral compounds including some PAHs, its application is limited by the carboxylated headgroups, whose ionization influences the electrophoretic mobility and solubility of the polymer at acidic or neutral pH values. Furthermore, problems such as erratic migration time and cloudiness of the anodic buffer vials after several runs have been reported.[16] To overcome these difficulties, our research group[19,22] and Palmer and Terabe[18,20] recently synthesized a polymerized surfactant with a sulfate headgroup, namely, poly(sodium undecylenic sulfate) (poly-SUS). However, the latter

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studies used potassium persulfate as a free radical initiator for the polymerization process, whereas we used ^{60}Co γ-irradiation. Two major limitations reported by Palmer with the chemical method of polymerization for poly-SUS were (1) low synthetic yields and (2) contamination of the product with sodium sulfate.[23] Our earlier EKC studies with poly(sodium *N*-undecylenyl-L-valinate)[11] and this study with poly-SUS indicate that these problems can be avoided if γ-irradiation is used to initiate polymerization.

The present studies report the application of poly-SUS for EKC separation of 16 PAHs categorized by the U.S. Environmental Protection Agency (EPA) as priority pollutants. To the best of our knowledge, this is the first report on the simultaneous separation of all 16 PAHs in a single EKC run. Poly-SUS and the nonpolymerized SUS as well as SDS are compared under similar conditions. Because of the high purity (97−99%), poly-SUS is stable even at higher concentration of organic solvents, making this methodology particularly useful for the separation of highly hydrophobic compounds.

EXPERIMENTAL SECTION

Materials. The 16 polycyclic aromatic hydrocarbons (PAHs) were obtained from the following suppliers: (1) naphthalene (NAPH), (2) acenaphthylene (ACY), (3) acenaphthene (ACE), (4) fluorene (FLU), (5) phenanthrene (PHEN), (6) anthracene (ANTH), (7) fluoranthene (FLT), (8) pyrene (PYR), (9) benz[*a*]-anthracene (BaA), (10) chrysene (CHRY), (11) benzo[*b*]fluoranthene (BbF), (12) benzo[*k*]fluoranthene (BkF) and (13) benzo-[*a*]pyrene (BaP) from Aldrich (Milwaukee, WI); (14) dibenz-[*a*,*h*]anthracene (DIBahA), (15) benzo[*ghi*]perylene (BghiP), and (16) indeno[1,2,3-*cd*]pyrene (INPY) from ChemService (West Chester, PA). HPLC grade acetonitrile (ACN) was obtained from Burdick and Jackson (Muskegon, MI). Disodium tetraborate ($Na_2B_4O_7$), disodium hydrogen phosphate (Na_2HPO_4), and sodium carbonate were of analytical grade and were purchased from EM Science (Gibbstown, NJ). The undecylenyl alcohol, alkyl aryl ketone homologues, C_4-C_{14} phenones, chlorosulfonic acid ($ClSO_3H$), sodium dodecyl sulfate, and pyridine (PY) were of analytical reagent grade and were obtained from Aldrich.

Synthesis of Poly(sodium *N*-undecylenic sulfate). The sodium undecylenic sulfate (SUS) monomer was prepared according to Bergstrom's procedure.[24] A schematic of the synthesis of poly(sodium undecylenic sulfate) (poly-SUS, **4**) is shown in Figure 1. To sulfate the alcohol, 113.8 mmol (7.5 mL) of $ClSO_3H$ was added dropwise to 75 mL of PY in a 250-mL round-bottom flask placed in an ice bath, and the mixture was stirred vigorously. Similarly, a solution of 82.3 mmol (16.5 mL) of ω-undecylenyl alcohol **(1)** and 75 mL of PY was slowly added to the above solution, and cooling and stirring were continued. The contents of the flask were refluxed with heat (heating mantle with transformer set on 40 V) for about 3 h until a clear yellow solution was formed. The product was undecylenic sulfuric acid (USA) (**2**).

The sodium salt of USA (i.e., SUS, **3**) was formed by adding USA solution to 600 mL of deionized water containing 4 g of NaOH and about 80−100 g of sodium carbonate. The solution was stirred overnight. The resulting SUS surfactant solution was

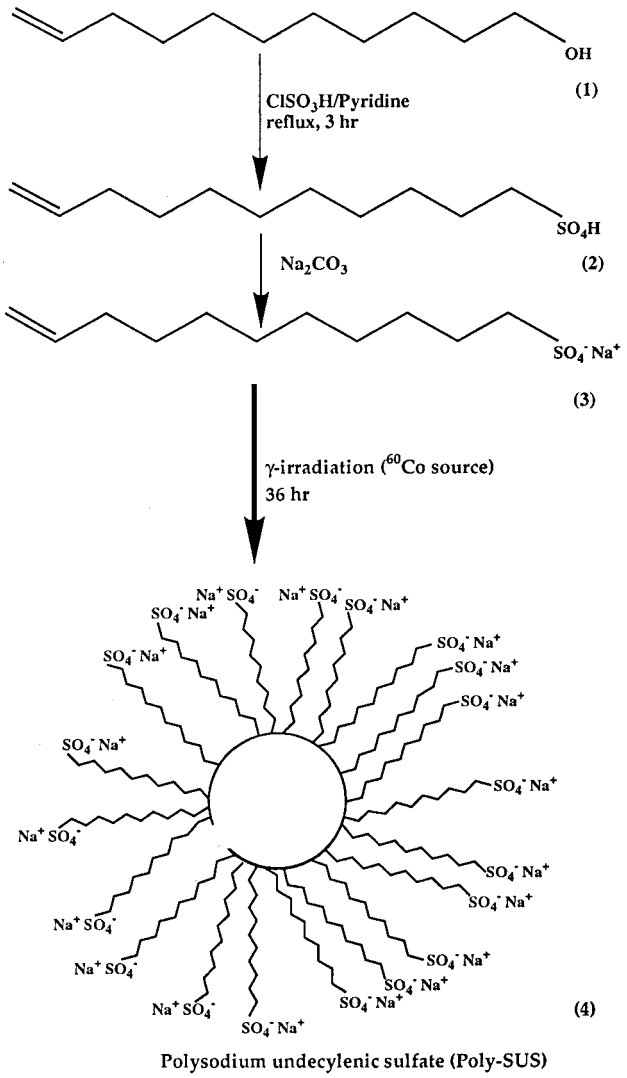

Figure 1. Synthetic scheme for the poly(sodium undecylenic sulfate) (poly-SUS).

extracted twice using *n*-butanol in a separatory funnel. The organic phase on the top contained the product. Evaporating the organic solvents (PY, butanol) by rotary evaporation followed by vacuum desiccation, produced a dry product. Purification of SUS surfactant was performed by dissolving the product in water and extracting with ethyl ether. This was followed by distillation and lyophilization which resulted in a dry white powder. Recrystallization was performed by dissolving the dry powder in 2-propanol using heat. The product was filtered, cooled to room temperature, and refrigerated for recrystallization. The crystals were dried in a vacuum desiccator overnight. The final product was SUS monomers.

A 100 mM aqueous solution of SUS monomers was exposed to a ^{60}Co γ-ray source for 92 h for polymerization in a micellar form. After irradiation, the poly-SUS **(4)** was dialyzed against bulk H_2O using a regenerated cellulose membrane with 2000 Da molecular mass cutoff. The purified solution was lyophilized and dried under a vacuum. The various batches of polymers were found to have 97−99% purity, as calculated from elemental analysis. Further characterization, such as molecular weight and partial specific volume of the polymer, is under study in our laboratory.

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Safety Precautions. Transfer of solid PAHs from the reagent bottle in a volumetric flask and dilution of the stock solutions were performed in a ventilated hood. All PAH solutions were stored in closed containers in a refrigerator. Disposable latex gloves were worn and care was taken to dispose of PAH waste solutions appropriately. For polymerization of SUS monomer, the surfactant solutions prepared in a glass bottle were placed in a container (using protective gloves) and lowered to the 14-ft level for radiation using an electric winch. The γ-irradiation source is located under a 14-ft pool of water covered by an iron gate. Access to the ^{60}Co γ-ray source facility is controlled by key padlocks and a card-reader door. However, it should be noted that irradiation by γ-rays does not induce radioactivity in the samples.

Electrokinetic Chromatography Instrumentation. A Beckman (Fullerton, CA) P/ACE model 5510 capillary electrophoresis (CE) instrument was employed in EKC separation of PAHs. This CE instrument was equipped with (1) a 21-position inlet and 10-position outlet sample carousels for automatic sample/buffer change, (2) a 0−30-kV high-voltage built-in power supply, (3) 200-, 214-, 254-, and 280-nm selectable wavelength filters for UV detection, (4) a liquid thermostated capillary cartridge (capillary 50 μm i.d. × 375 μm o.d. × 47 cm total length, 40 cm to the detector), and (5) software System Gold for system control and data handling. The capillary in the Beckman instrument was thermostated by use of a fluoroorganic fluid. The detector time constant was 0.2 s.

Capillary Electrophoresis Procedure. All new capillaries were prepared by use of a standard wash cycle of 1 M NaOH for 1 h before use. Each day, operation was started by purging the capillary with 1 M NaOH (15 min), triply deionized water (2 min), and the running EKC buffer (10 min). Prerun rinsing consisted of 3.0 min of the EKC buffer. Unless otherwise noted, the time for pressure injection was 3 s for most separations. Postrun rinse consisted of a 2.0-min flush with 0.5 M NaOH. These procedures resulted in improved peak shapes, minimized analyte adsorption on the capillary wall, and a good migration time reproducibility range of 2.0−2.5% RSD, $n = 3$.

Preparation of EKC Buffers and Standard Solutions. For all EKC experiments, the final background electrolyte (BGE) consisted of a 12.5 mM mixture of Na_2HPO_4 and Na_2B4O_7 buffered at pH 9.2. Appropriate percentages of poly-SUS surfactant (w/v) and of ACN (v/v) were added to the BGE, and then the final volume was adjusted with triply deionized water. After a thorough mixing in a sonicator for 10 min, the final running buffers were filtered through a 0.45-μm syringe filter (Nalgene, Rochester, NY) by creating a vacuum inside the syringe. All stock standard PAH solutions were prepared in 80/20 (v/v) ACN/H_2O at concentrations of about 3−5 mM each, except for BghiP, BaP, and INPY, which were dissolved in 80/20 (v/v) ACN/CH_2Cl_2. Molar concentrations of the injected test mixture of 16 PAHs ranged from 0.2 to 0.5 mM.

Calculations. The migration factor, k', of a neutral solute was measured according to the formula[25]

$$k' = \frac{t_r - t_0}{t_0(1 - t_r/t_{mc})}$$

where t_r is the migration time of a neutral retained analyte, t_0 is the migration time of a neutral unretained analyte, and t_{mc} is the migration time of the micelles. The void time, t_o, was determined by a first solvent disturbance due to a refractive index change. The value of t_{mc} was determined by using the procedure proposed for a series of homologous compounds by Bushey and Jorgenson.[26] This procedure consists of five steps: (1) migration times of some homologous series of alkyl aryl ketones (C_6−C_{14}) were measured at various percentages (20−50% v/v) of ACN; (2) using the longest migration time of C_{14} phenone as a measured (assumed) t_{mc} value, the k' values of C_6−C_{12} phenones were calculated using the above-mentioned equation; (3) from the plot of log k' versus the carbon number, a new k' value for C_{14} phenone was calculated; (4) a new t_{mc} was then found by rearranging the above equation and substituting the values of new k' and measured t_r for the C_{14} phenone; (5) all k' values (C_6−C_{12} phenones) are recalculated, and the procedure is reiterated ($n = 30$) until the t_{mc} converges to a value less than 0.1% from its previous iteration.

RESULTS AND DISCUSSION

PAHs are ubiquitous organic pollutants with at least two aromatic rings in their basic structure. They are widely distributed in the environment due to incomplete combustion processes.[27] The chemical structures of the 16 priority PAHs employed in this study are shown in Figure 2. These PAHs range from two to six fused rings, with widely different hydrophobic properties. As discussed in our previous paper,[28] an equimolar mixture of Na_2HPO_4 and $Na_2B_4O_7$ buffered at pH 9.2 is an effective BGE for the electrokinetic separation of PAHs. However, the solubility of most of the PAHs in a purely aqueous micellar solution is poor, owing to the strong hydrophobic properties of the former. For this reason, ACN was added as an organic modifier to the BGE containing poly-SUS for PAHs separation.

Comparison of Monomeric and Polymeric Surfactants as Pseudostationary Phases. The selectivity differences for monomeric (SDS and SUS) and polymeric (poly-SUS) surfactants for the separation of 16 EPA priority pollutants are shown in Figure 3. Although all three electrokinetic chromatograms were run under similar BGE conditions (i.e., 12.5 mM each of $Na_2B_4O_7$/Na_2HPO_4, at pH 9.2), the analyte peak shapes are significantly different. With SDS, poor selectivity and peak broadening are evident. Under equivalent buffer and surfactant concentrations, the PAHs showed some improvement in selectivity with monomeric SUS. These improvements in separation with SUS over SDS are probably due to π−π interaction between the PAHs and the terminal double bond of the SUS surfactant. However, the enhanced separations of PAHs with excellent peak shapes using poly-SUS are clear indicators that the structural integrity of poly-SUS is maintained, even at a very high concentration of organic solvent (e.g., 57% (v/v) ACN used to generate electrokinetic chromatogram in Figure 3). In contrast, for nonpolymerized surfactants (SDS or SUS), the use of such a high content of organic solvents breaks up the micelle. These improved separations of PAHs with poly-SUS are consistent with our previous study on enantiomeric separations, in which the polymerized chiral

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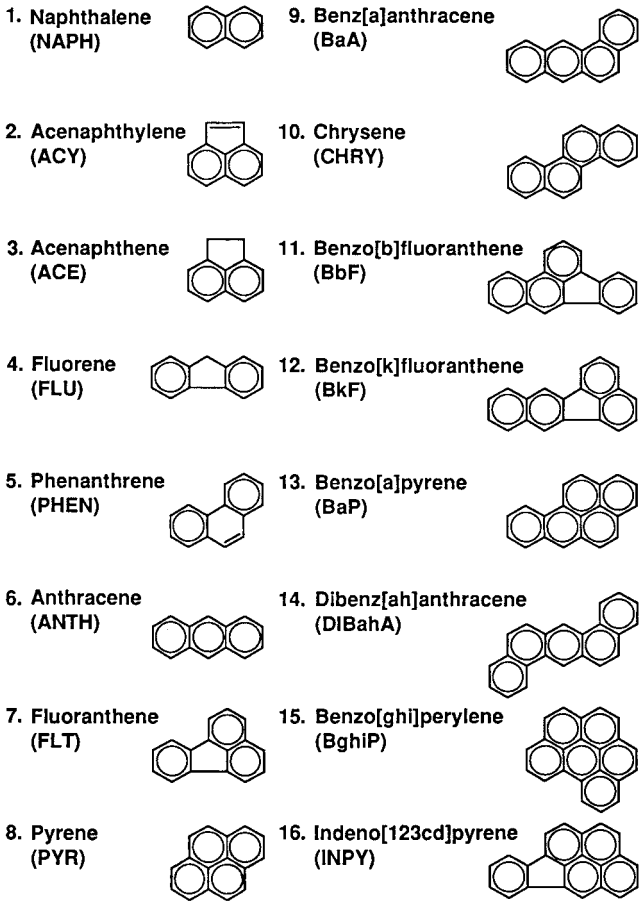

Figure 2. Structures of the 16 priority polycyclic aromatic hydrocarbon pollutants (PAHs).

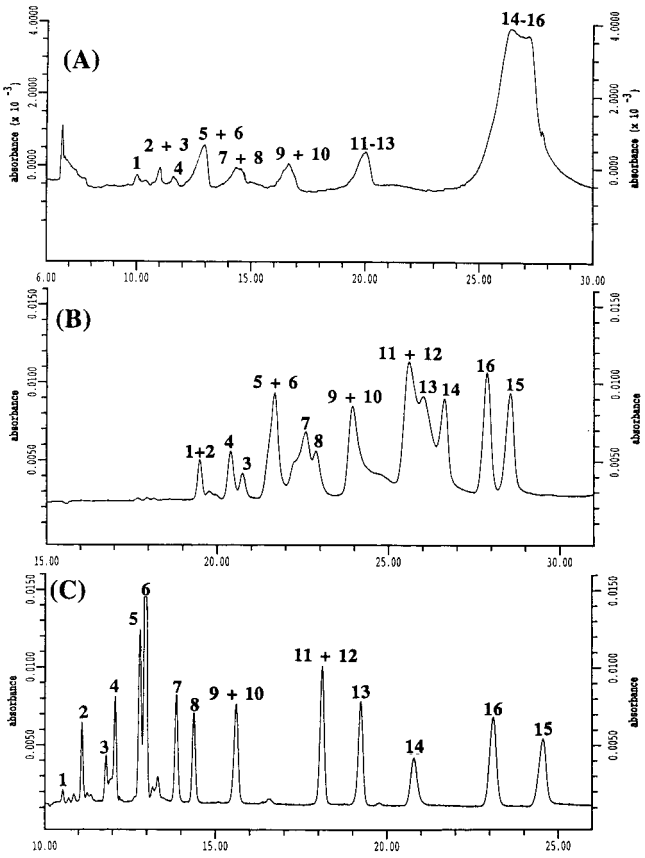

Figure 3. Comparison between (A) sodium dodecyl sulfate (SDS), (B) nonpolymerized sodium undecylenic sulfate (SUS), and (C) poly-SUS for the separation of PAHs. EKC conditions: 1.5% (w/v) each of SDS and SUS; 1.0% (w/v) of poly-SUS in 12.5 mM each of Na_2HPO_4 and Na_2B4O_7 buffered at pH 9.2 with 57% v/v of ACN; pressure injection for 3 s; +30 kV applied for separation; current, 55 μA for SDS, 50 μA for SUS, and 38 μA for poly-SUS; UV detection at 254 nm. For peak identification, see Figure 2.

surfactant poly(sodium *N*-undecylenyl-L-valinate)[11] showed superior electrokinetic separations over the corresponding monomeric surfactant. In addition, the spectroscopic data reported by Paleos et al.[29] indicated that the hydrophobic analyte does not penetrate deeply into the core of polymerized surfactant as it does into normal micelle. Thus, an increase in the mass transfer rate of the PAH to and from the polymerized pseudostationary phase indeed improves the separation efficiency and selectivity. However, certain critical pairs of analytes (mostly isomers), e.g., BaA−CHRY and BbF−BkF, remain unresolved. In addition, partial resolution was obtained for ANTH and PHEN. Thus, the optimization of an EKC method that can separate rapidly and efficiently the isomers of the above-mentioned peak pairs in the test mixture of 16 PAHs in a single run was necessary.

Effect of Poly-SUS Concentration. The purpose of varying the surfactant concentration is to adjust the k' values to obtain a compromise between resolution and analysis time. Figure 4 shows the effect of changing the concentration of the poly-SUS on the k' of 16 PAHs. It is noted that the k' values of PAHs increased proportionally with the poly-SUS concentration from 0.1 to 0.75% (w/v). As expected, the k' values and the slopes of the linear plots increase with increases in the ring size and hydrophobicities of different PAHs. In addition, the linear plot for each PAH passed close to the origin. This observation confirms the fact that the cmc of poly-SUS is zero. Moreover, it can be seen from Figure 4 that the separation of all and even the faster-eluting PAHs (e.g., NAPH, ACY, ACE, FLU, Figure 4 inset) is still possible at 0.25% (w/v) poly-SUS (equivalent to 9.2 mM SUS monomer). Thus, the separation with a micelle polymer is feasible even at lower concentrations much below the cmc (the cmc of SUS is ~32 mM). In contrast, for nonpolymerized micelles, the concentration of the surfactant has to be higher than the cmc in order for it to function as a pseudostationary phase. Table 1 (system 1) shows the values of the elution window defined here as the ratio of t_{mc}/t_o. As expected, the elution window became wider with increasing concentration of poly-SUS. On going from 0.10 to 0.70% (w/v) of poly-SUS, the elution range increased by a factor of ~13, and the migration window became infinite, i.e., a true stationary phase was approached when the concentration of poly-SUS was raised to 0.75% (w/v). With poly-SUS concentrations ≥0.75% (w/v), the first eight PAHs that elute earlier or in the middle of the chromatogram showed very high resolution. Unfortunately, this gain in resolution with an infinite elution window was accompanied by long analysis times (>150 min) for more hydrophobic PAHs, in particular DIBahA, BghiP, and INPY. Furthermore, no peak was observed for C_{14} phenone (this analyte elutes after BghiP and was used as a t_{mc} marker), even after 5 h

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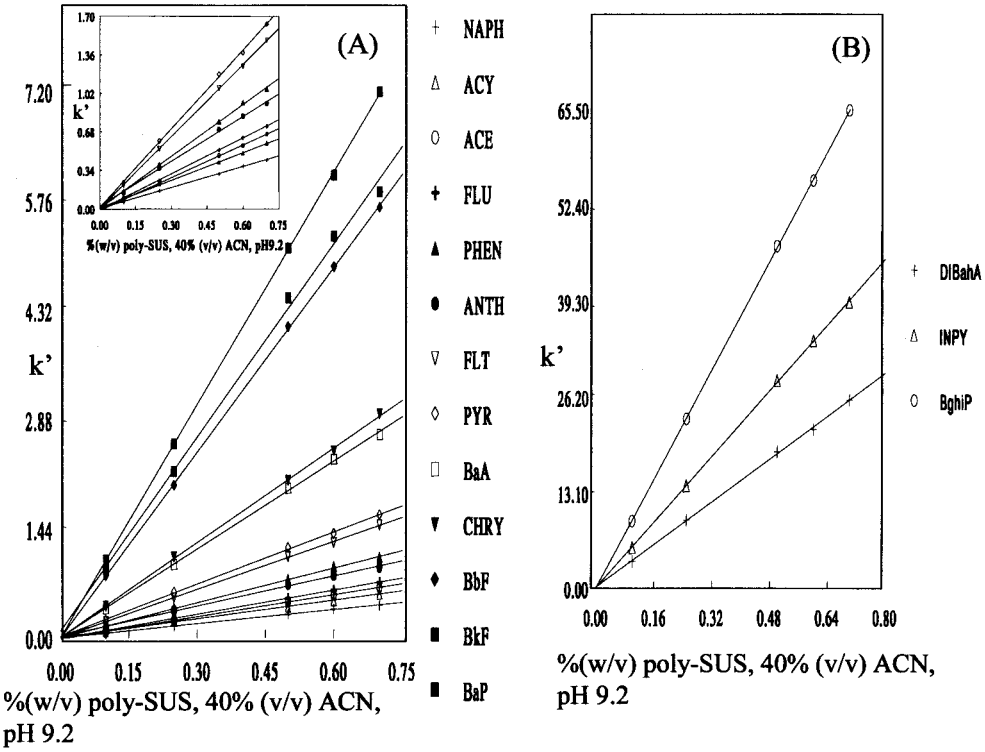

Figure 4. Retention factors of the 16 PAHs plotted as a function poly-SUS concentration. The inset plot in A shows an expanded view of retention factors for the first eight PAHs (peak 1−8). EKC conditions: 12.5 mM each of Na_2HPO_4 and Na_2B4O_7 buffered at pH 9.2 with 40% (v/v) of ACN; Separation voltage, +30 kV; current, 29−52 μA.

Table 1. Data Showing the Effect of Polymerized Anionic Surfactant and Acetonitrile Concentrations on the Elution Window in EKC

System 1[a]			
% (w/v) poly-SUS	t_{mc}/t_o	% (w/v) poly-SUS	t_{mc}/t_o
0.10	1.60	0.60	10.60
0.25	3.50	0.70	21.32
0.50	4.30	0.75	α

System 2[b]			
% (v/v) ACN	t_{mc}/t_o	% (v/v) ACN	t_{mc}/t_o
20	37.30	45	2.70
30	9.80	50	2.60
40	4.30		

[a] 40% (v/v) ACN/12.5 mM $Na_2B_4O_7$−Na_2HPO_4, pH 9.2. [b] 0.5% (w/v) poly-SUS/12.5 mM $Na_2B_4O_7$−Na_2HPO_4, pH 9.2.

of electrokinetic run. In general, 0.50% (w/v) of poly-SUS was chosen as the optimum concentration, as this was a best tradeoff between resolution and analysis time for 16 PAHs.

Effect of Acetonitrile Concentration. The use of polymerized surfactants as a pseudostationary phase provides an opportunity to investigate the role of organic solvents over a wide range of concentrations. The primary role of organic solvents such as ACN in MEKC or EKC is to shorten the k' values of highly hydrophobic solutes. Dependencies of the k' values of 16 PAHs on the fraction of ACN measured at the optimized poly-SUS concentration (0.5% w/v) are shown in Figure 5. The k' values for the first 11 PAHs (Figure 5A) decreased sharply as the ACN was raised from 20 to 30% (v/v) and then decreased gradually in the range 30−40% (v/v) ACN, and finally they leveled off and became very small at 50% (v/v). However, in Figure 5B, it can be seen that certain solutes (e.g., DIBahA, BghiP, and INPY) are predominantly hydrophobic and show large k' values. For such lipophilic PAHs, the k' shows a sharp drop at a much higher range of ACN (i.e., 40−50% v/v). In addition, note that, due to very strong surfactant−analyte interactions, no reliable k' values can be obtained for BkF and BaP at 20% (v/v) ACN, as well as for DIBahA, BghiP, and INPY at < 40% (v/v) ACN. Moreover, it is worth noting that the ACN content in the polymerized surfactant has a distinguished effect on the EOF. The electroosmotic mobility decreases from 2.26×10^{-4} to 1.01×10^{-4} cm^2 V^{-1} s^{-1} with an increase in ACN content from 20 to 50% (v/v). However, despite an increase in t_o values, the migration time and k' values of PAHs showed a continuous drop in the same range. This trend of converging k' with a decrease in polarity of the aqueous phase is very similar to the retention mechanism of reversed-phase HPLC. Since increasing the fraction of ACN in the poly-SUS does not break up the micelle polymer, a progressive decrease in k' values of PAHs is probably related to a synergistic effect of reduced partition coefficient and a change in shape of the polymerized surfactant. Table 1 (system 2) summarizes the effect of ACN content on the elution window. As shown, the elution range became narrower with an increase in the volume fraction of ACN. This is attributed to a decrease in EOF and an increase in apparent electrophoretic mobility of the micelle polymer. As t_o rises and t_{mc} drops, a decrease in t_{mc}/t_o values must occur with an increase in ACN content from 20 to 50% (v/v). A reasonable compromise is found at 40% (v/v) of ACN, since early-eluting PAHs were baseline resolved and relatively narrow peak spacings were observed for PAHs with large k' values.

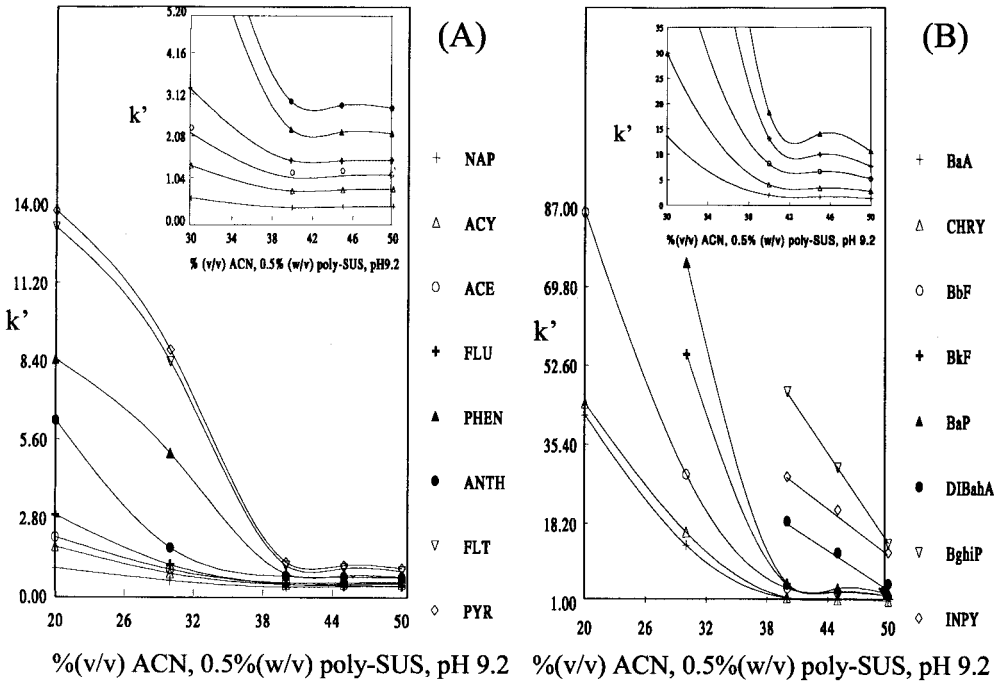

Figure 5. Retention factors of the 16 PAHs plotted as a function ACN concentration. The insets in both A and B show an expanded view of retention trends. Separation voltage, +30 kV; current, 58−35 μA. Other conditions are the same as in Figure 4.

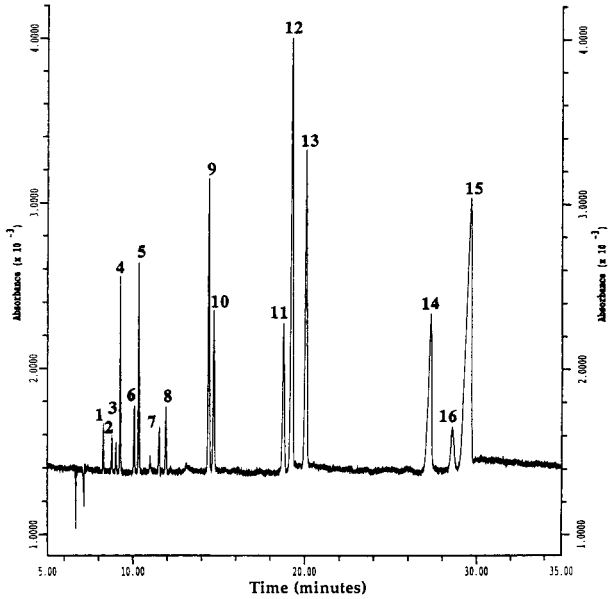

Figure 6. Optimized electrokinetic chromatogram for the separation of 16 PAHs. EKC conditions: 0.5% (w/v) of poly-SUS in 40% ACN; Separation voltage, +30 kV; current, 42 μA. Other conditions are the same as in Figure 4.

Optimized Separation. Figure 6 shows the separation of the 16 PAHs (EPA priority pollutants) in about 30 min with optimized poly-SUS and ACN concentrations. The elution orders of most PAHs generally follow an increasing length-to-breadth ratio,[30] with ANTH and INPY as the only two exceptions. For even faster separation (15 min), the percentage of ACN in the poly-SUS can be raised to as high as 65% (v/v) (data not shown). However, under such conditions, the signals for first eight PAHs of the chromatogram were a little compressed, but the last three PAHs (with high k' values) showed higher efficiencies with improved resolutions.

CONCLUSIONS

A high-purity T-type polymerized surfactant having an undecenyl (C_{11}) and a sulfate group was prepared from ^{60}Co γ-irradiation. This polymer was then used for the EKC separation of 16 PAHs. The methodology offers a valid alternative to gradient high-performance liquid chromatography (HPLC) and capillary electrochromatography (CEC). The former requires gradient and large amounts of organic solvents for PAHs eluting from HPLC columns; the latter still needs extensive studies on reproducible column preparation and optimization of conditions before it is ready for practical application. In contrast, with EKC, a simple manipulation of organic solvent composition and the concentration of polymerized surfactant in the running buffer enables one to realize the inherent benefits of EKC, that is, large number of peaks can be resolved at small k' values and relatively narrow peak spacings are observed at large k' values. This advantage is clearly demonstrated in the separation of 16 PAHs with varying hydrophobicities in a single run.

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