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Micellar electrokinetic chromatography of polychlorinated biphenyl congeners using a polymeric surfactant as the pseudostationary phase

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Abstract

Micellar electrokinetic chromatography (MEKC) of highly hydrophobic compounds is generally difficult using sodium dodecyl sulfate micellar solutions. The polymeric surfactant, polysodium undecyl sulfate (poly-SUS) has been used to separate moderately to highly hydrophobic polychlorinated biphenyl (PCB) congeners by MEKC in the absence of cyclodextrins. Parameters such as concentration of acetonitrile (ACN), polymeric surfactant concentration, and the effect of pH were examined. Optimum MEKC conditions to get baseline resolution of nine PCBs was 7.5 m*M* borate in 40% (v/v) ACN fraction buffered at pH 9.2 using 0.5% (w/v) poly-SUS. The applied voltage was 30 kV and the temperature was maintained at 25°C. Elution order for each PCB congener was found to be dependent on the degree of chlorination and hydrophobic character.

Keywords: Pseudo-stationary phases; Micellar electrokinetic chromatography; Polychlorinated biphenyls; Surfactants; Polysodium undecyl sulfate

1. Introduction

Biphenyl compounds with one or more of the hydrogens substituted with chloro groups are collectively called polychlorinated biphenyls (PCBs). There are about 209 PCB congeners. Commercial products of PCBs are mixtures of a large number of these congeners. Due to their remarkable stability, high electrical resistivity and low flammability [1,2], they are used extensively as heat transfer fluids, dielectrics for capacitors and transformers, hydraulic fluids, lubricants, additives in plastics and dyes [3]. However, this class of compounds is considered to be toxic. In fact, the use of PCBs has been forbidden for some applications due to their toxicity and carcinogenicity. Presently, they are identified as carcinogens by both the United States Environmental Protection Agency (EPA) and the International Agency for Research on Cancer (IARC). In addition, the EPA and the European Union also list them as priority pollutants [4]. Although, the use of PCBs has been reduced over the years, they are still produced as a consequence of different industrial and human activities. For example, they are produced as by products in various industrial chemicals processes such as: combustion of chloride containing waste [5],

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synthesis of azo dyes [6], phthalocyanine pigments, phenolic resins [7] etc. Their persistence as environmental pollutants has prompted research into the development of analytical methods for both qualitative and quantitative determination of PCB levels in the environment.

At present the two most common and reliable analytical methods for PCBs are gas chromatography (GC) with electron-capture detection [8–12] and GC–mass spectrometry [13]. In contrast, there are only a few investigations reported on their determination by high-performance liquid chromatography (HPLC) [14,15]. In recent years, micellar electrokinetic chromatography (MEKC) has emerged as a powerful separation mode of capillary electrophoresis (CE). This mode has the potential to complement GC and HPLC for the analysis of PCB congeners. Mixtures of trichlorobiphenyl isomers were first separated via cyclodextrin-modified micellar electrokinetic chromatography (CD-MEKC) by Terabe et al. [16]. Since triChlorobiphenyls were too hydrophobic to be separated by MEKC, a neutral cyclodextrin (CD) had to be present in the sodium dodecyl sulfate (SDS) micellar solution as additive. The authors reported separation of eleven isomers of triChlorobiphenyls using a 100 m*M* SDS and 60 m*M* γ-CD at pH 8.0 in ca 35 min. The technique of CD-MEKC has also been used for the chiral separation of atropisomeric PCB congeners using mixtures of β-, and γ-CD [17], hydroxypropyl-γ-CD [18], and γ-CD [19], in combination with SDS. In addition, chiral separations of three atropisomeric PCBs were achieved using sodium cholate as a single chiral selector in the MEKC buffer [20]. Recently CD-MEKC of fourteen PCB isomers using SDS in combination with β- or γ-CD have also been reported [21]. Better selectivity of PCBs were observed with γ-CD as the buffer additive to SDS than with β-CD.

The synthesis of the polymeric surfactant, polysodium undecyl sulfate (poly-SUS) was reported previously [22–24]. Its application to the separation of polycyclic aromatic hydrocarbons, benzenes and naphthalene derivatives [22,23] as well as isomers of monomethylbenz[*a*]anthracenes [24] showed significant advantages over the unpolymerized form of the surfactant. One of the major benefits of poly-SUS is its stability as a pseudostationary phase at high concentration of organic solvent in the CE buffer. In addition, this polymeric surfactant has zero critical micelle concentration (CMC) and therefore separations of neutral compounds can be achieved even at very low concentrations of polymer. In contrast, micelles that are generated from unpolymerized surfactant require higher surfactant concentration for effective separations.

The high efficiency and the versatility of the poly-SUS, makes its use in MEKC ideal for the separation of moderately to highly hydrophobic compounds such as PCBs. In this study, we report separation of a mixture of nine PCB congeners by MEKC using a single additive, the polymerized surfactant, poly-SUS as the pseudostationary phase. To the best of our knowledge, this is the first time that a polymeric surfactant has been used to resolve PCB congeners.

2. Experimental

2.1. Reagents and chemicals

All reagents used were of analytical grade. Sodium borate was purchased from Sigma (St. Louis, MO, USA). Acetonitrile (ACN) of HPLC grade was obtained from Burdick and Jackson (Muskegon, MI, USA). Sodium hydroxide was purchased from Fisher Scientific (Fairlawn, NJ, USA). All PCBs (99.5% purity) standards as represented in Table 1 were obtained from ChemService (Westchester PA, USA). The polymeric surfactant, poly-SUS, was prepared according to the procedure previously reported by Shamsi et al. [23].

2.2. MEKC Instrumentation

MEKC experiments were conducted using a Beckman P/ACE 5500 CE System (Beckman Instruments, Fullerton, CA, USA). This CE instrument was equipped with a P/ACE diode array detector along with 200-, 214-, 254-, and 280-nm selectable wavelength UV filters and high voltage power supply with a voltage range of 1.0–30 kV. The electropherograms were recorded on a personal computer with Beckman System Gold software. Fused silica capillaries externally coated with polyimide having the following

Table 1
General structure and IUPAC names and numbers for the nine polychlorinated biphenyls congeners used

Polychlorinated biphenyl	IUPAC No.	IUPAC Nomenclature
1	PCB 1	2-Chlorobiphenyl
2	PCB 5	2,3-Dichlorobiphenyl
3	PCB 29	2,4,5-Trichlorobiphenyl
4	PCB 47	2,2',4,4′-Tetrachlorobiphenyl
5	PCB 98	2,2',3′,4,6-Pentachlorobiphenyl
6	PCB 154	2,2',4,4′,5,6′-Hexachlorobiphenyl
7	PCB 171	2,2',3,3′,4,4′,6-Heptachlorobiphenyl
8	PCB 200	2,2',3,3′,4,5′6,6′-Octachlorobiphenyl
9	PCB 206	2,2',3,3′,4,4′,5,5′,6-Nonachlorobiphenyl

dimensions: 50 μm internal diameter, 361 μm external diameter, 47 cm total length (39.5 cm length to detector) were obtained from Polymicro Technologies (Phoenix, AZ, USA). The detection window for the capillary was prepared by burning a segment of the capillary (0.5 cm) to remove the polymer coating. The sample was pressure injected at 0.5 p.s.i. for a period of 1.5 s (1 p.s.i.=6894.76 Pa). The temperature was maintained at 25°C throughout the separation and the applied voltage was +30 kV.

2.3. Capillary conditioning

A new capillary was pre-conditioned for 6 h by first rinsing with 1 *M* NaOH, followed by flushing with deionized water for 0.50 h. At the start of each day the capillary was rinsed with 0.1 *M* NaOH for 15.0 min, followed by flushing for 10.0 min with the operating buffer. Between injections, the capillary was first rinsed with 0.1 *M* NaOH for 2.0 min followed by the operating buffer for 4.0 min. To ensure reproducible retention times and to avoid adsorption of the solute, the aforementioned rinsing procedure between each run was found to be essential.

2.4. Standard solutions and operating buffer preparation.

Stock solutions of all PCB congeners were prepared in ACN at concentrations of 3 mg/ml. The sample mixture for injection was prepared by diluting the stock solutions of each PCB to ca. 85 μg/ml using an ACN–water (60:40, v/v) mixture. A 7.5 m*M* sodium borate background electrolyte (BGE) solution was prepared by dissolving appropriate amount of sodium borate in triply deionized water (ca. 30 mL) in a 150 mL beaker. The desired pH values over the range of 7.2–10.2 were achieved using either 100 m*M* H_3BO_3 or 1 *M* NaOH while no acid or base was added to the pH 9.2 buffer solutions. The adjusted solutions were transferred to a 100 mL volumetric flask and appropriate volume fractions of ACN were added. To ensure proper mixing, the aqueous buffer solution was thoroughly degassed, and then triply deionized water was added diluting up to the 100 mL mark. To this BGE solution various amounts of poly-SUS was added and mixed thoroughly with sonication for 10.0 min. Prior to use, the running MEKC buffer solution were filtered through a 0.45 μm syringe filter (Nalgene, Rochester, NY, USA) by creating a vacuum inside the syringe.

3. Results and discussion

The general structure and IUPAC names for the nine PCB homologues studied are shown in Table 1. Note that these homologues differ from one another in the substitution pattern of the chlorine atoms in the biphenyl moiety. Varying the following MEKC parameters: ACN fraction, concentration of poly-SUS and pH, optimized separation of nine PCBs was achieved. The details of each parameter are discussed below.

3.1. The effect of acetonitrile concentration

Under purely aqueous conditions, the influence of both the hydrophobicity of PCBs and the hydrophobic nature of the micelle interior would promote stronger micelle–analyte interactions and thus stronger affinity of PCBs for the micellar interior. This results in poor resolution and long analysis times. The use of organic solvents is a useful parameter for improving the affinity of the analyte for the aqueous phase in MEKC. In particular, the use of ACN improves peak shape and solubility of the hydrophobic compounds in the aqueous buffer [22–24].

ACN concentration was varied over the range of 30 to 50% (v/v) to strike a balance between analysis time and resolution between individual peaks corresponding to each PCB congener, and to achieve baseline separation of all nine PCBs in the test mixture. As shown in Fig. 1, with increasing ACN fraction, there was a significant decrease in the retention values for all PCBs. However, the smallest and largest change in retention was observed for the least and most hydrophobic PCBs, respectively. The inset in Fig. 1 shows the variation of t_0 ($n=3$, RSD=0.19–2.5%) with the ACN fraction in the buffer. It was observed that the t_0 value first increased slightly between 30 and 35% (v/v) ACN then remained unchanged between 35 and 40% (v/v) ACN, however, it increases sharply between 40 and 50% (v/v) ACN. The t_0 trend is consistent with the mobility (μ_{eo}) which decreases from $4.247 \cdot 10^{-4}$ cm^2 s^{-1} kV^{-1} to $2.93 \cdot 10^{-4}$ cm^2 s^{-1} kV^{-1} with an increase in ACN fraction from 30 to 50% (v/v). However, despite increase in t_0 values, the t_R values of PCBs does not show a similar increase in the same range. Previous studies has shown that the use of ACN decrease the migration time of polycyclic aromatic hydrocarbons [22]. Our results are consistent with these studies.

At 40% (v/v) ACN fraction, all peaks were baseline resolved and analysis time was ca.10 min. In general, above this volume fraction, retention times of PCBs decreased. In particular, resolution values for moderately hydrophobic PCBs decreased significantly. For example, PCBs 29 (2,4,5-trichlorobiphenyl), 47 (2,2′,4,4′-tetrachlorobiphenyl) and 98 (2,2′,3′,4,6-pentachlorobiphenyl) eluted very close to each other. However, high ACN fraction [≥50% (v/v) ACN] favors faster elution of highly hydrophobic PCBs (PCBs 206, 200), hence improving the accuracy in the measurement of their retention times. In the present study, we aimed at establishing an MEKC system that could maximize the peak capacity to its greatest potential. To achieve this goal a successful separation of both moderately hydrophobic and the strongly hydrophobic PCBs is required. Therefore, a running MEKC buffer of 40% (v/v) ACN was chosen to further optimize the separation conditions.

3.2. The effect of poly-SUS concentration

It is now well established that the performance of any MEKC system relies very much on the appropriate surfactant concentration. Since poly-SUS concentration has a direct impact on the retention time, its effect on the separation of PCBs was studied over the range 0.075–0.5% (w/v) poly-SUS. As anticipated, initially retention values were low and resolution of early eluting PCBs (peak 1–5) were poor, but increased gradually as the poly-SUS concentration was increased (Fig.. 2). The changes in retention between 0.15 and 0.25% (w/v) poly-SUS was not as large as that between 0.075 and 0.15% poly-SUS. However a significant increase in retention values in the range of 0.25–0.50% (w/v) of poly-SUS was observed. Above 0.5% (w/v) poly-SUS concentration, the baseline became unstable and longer analysis times were realized. Note that Fig. 2A clearly shows that poly-SUS, due to its zero CMC, was able to resolve some of the PCBs (e.g. peaks 6–9) even at very low concentration [0.075% (w/v) poly-SUS] which is below the CMC (32 m*M*) of the monomer [23].

The inset in Fig. 2, shows the variation in t_0 values as a function of the polymer concentration. As observed the t_0 ($n=3$, RSD=0.22–1.59%) showed only a slight increase over the concentration range of 0.15–0.50% (w/v) poly-SUS. This suggests that retention trends for the PCB congeners are not only a consequence of variation in the electroosmotic flow (EOF) with polymerized surfactant concentration. Obviously, the binding of PCBs with the micelle is also a contributing factor that can account for the increase in the retention values.

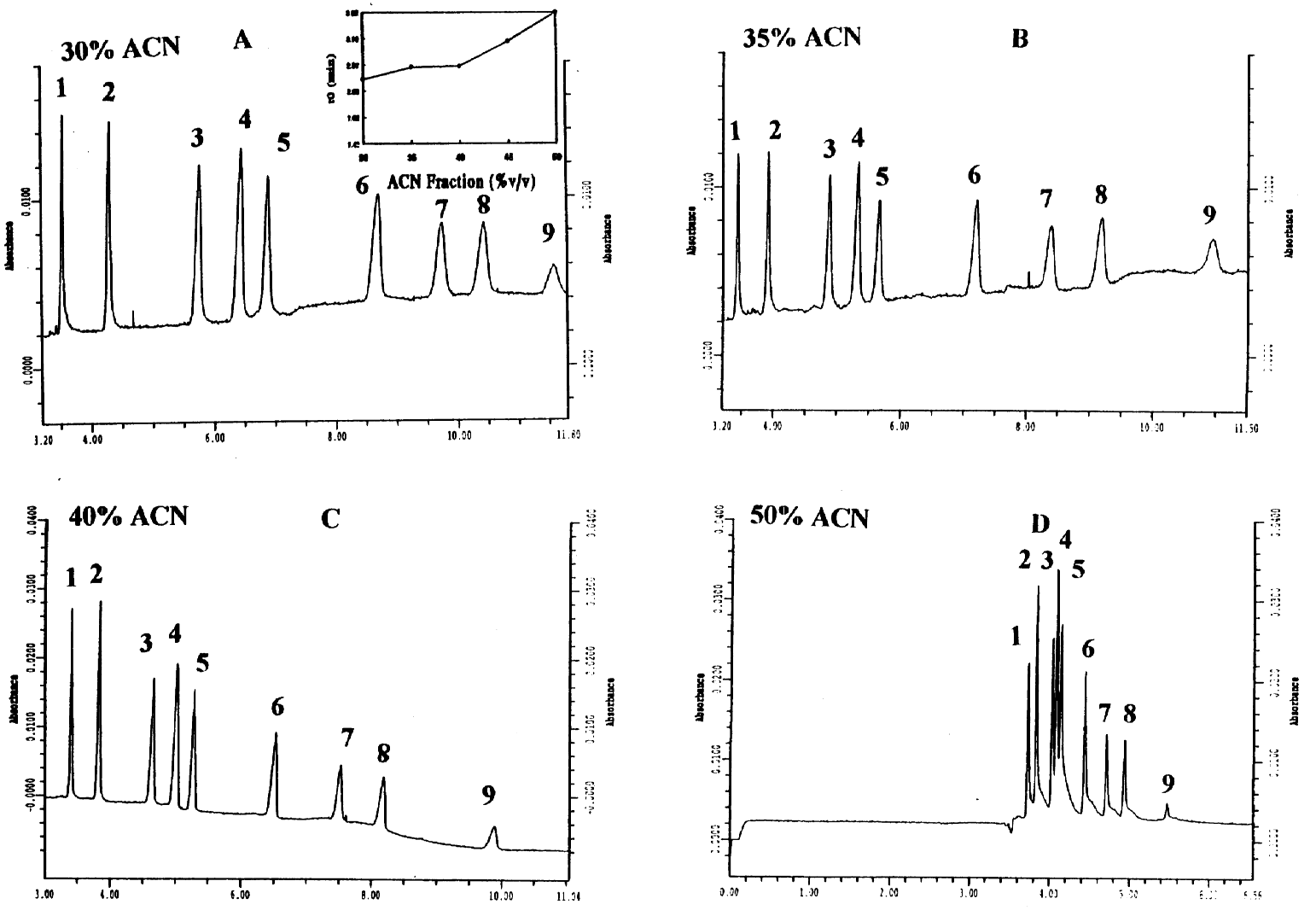

Fig. 1. Electropherograms showing the effect of percentage (v/v) of ACN on the separation of nine PCB congeners. The inset shows variation in the t_0 as ACN concentration is varied. EKC conditions: 7.5 m*M* borate buffer; pH 9.2; 0.5% (w/v) poly-SUS; applied voltage +30 kV. Capillary temperature was maintained at 25°C.

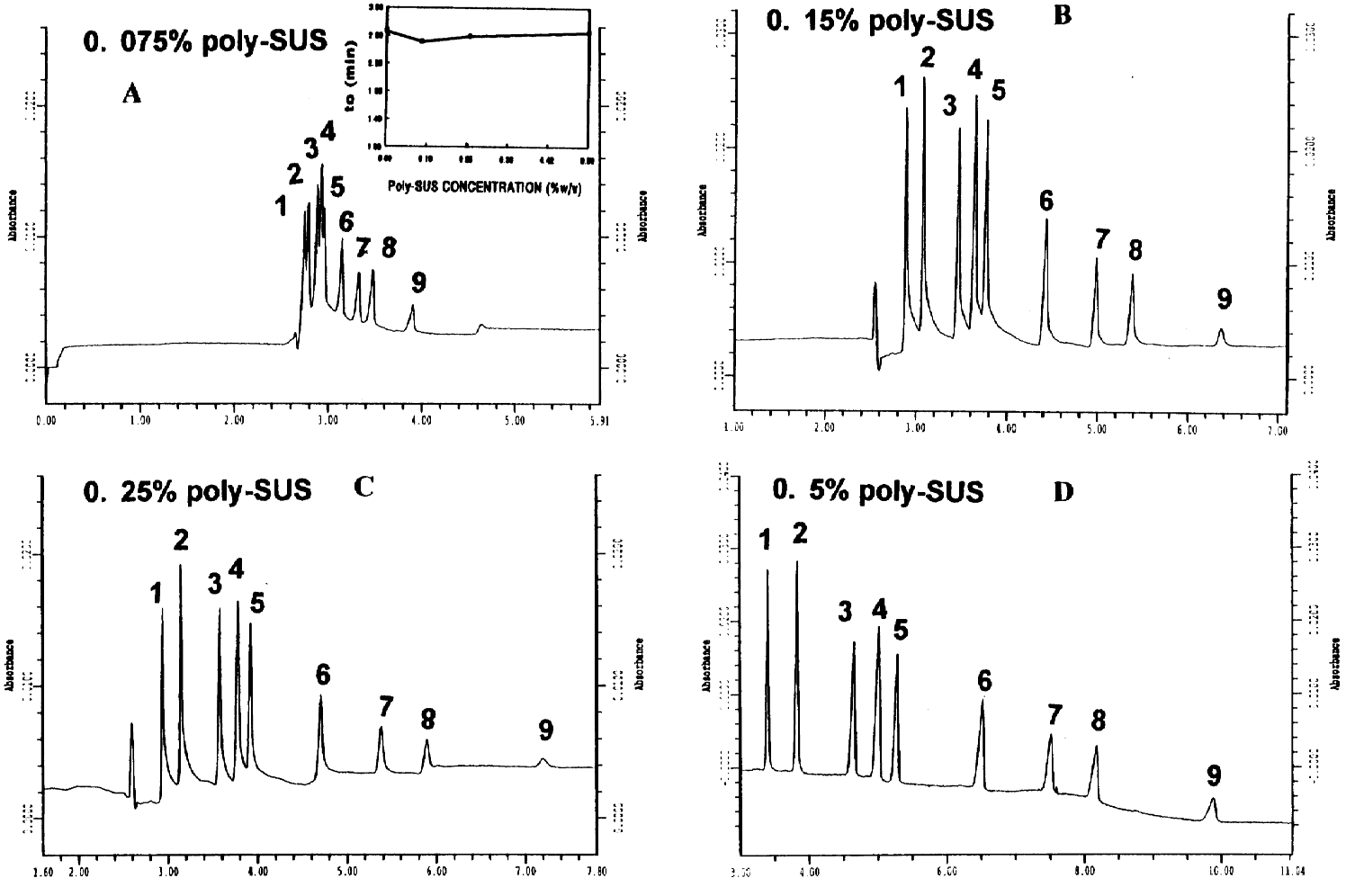

Fig. 2. Electropherograms showing the effect of percentage (w/v) of poly-SUS. The inset shows changes in the t_0 values with variation in surfactant concentration. EKC conditions same as Fig. 1 except the running buffer contains 40% (v/v) ACN with variable % (w/v) of poly-SUS.

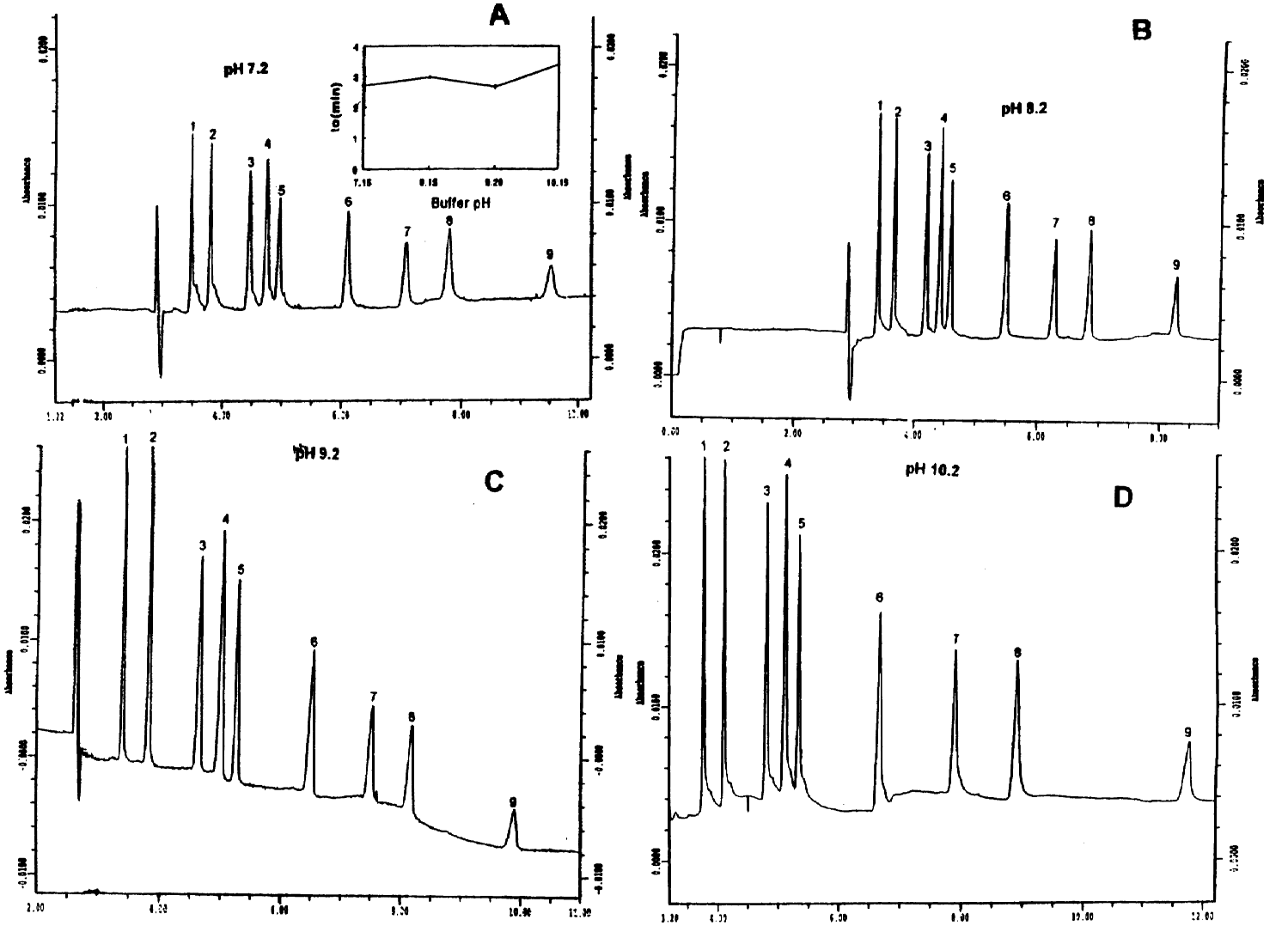

Fig. 3. Electropherograms showing a comparison of resolution and analysis times of PCBs at different pH: (A) 7.2; (B) 8.2; (C); 9.2; (D) 10.2. EKC conditions remained the same [0.5% (w/v) poly-SUS, 40% ACN] as Fig. 1 apart from the pH adjustments. For peak identification see Table 1. The inset shows the variation of t_0 with pH.

3.2.1. The effect of pH variation

Fig. 3 shows the effect of variation in pH on the separation of nine PCB congeners with 0.5% (w/v) poly-SUS in 40% (v/v) of ACN. Observed retention times for PCB congeners were strongly influenced by the pH of the MEKC solution. At pH 7.2 (Fig. 3A), the analysis time for separation of the nine congeners was under 10 min. Although baseline resolution of the latter eluting congeners was achieved at this pH, earlier eluting congeners for example, PCB 29, 47 and 98 (peaks 3, 4 and 5) were not completely baseline resolved. Increasing the pH to 8.2 (Fig. 3B) decreased the analysis time for the nine PCB congeners to well below 9.0 min. In addition, the resolution between individual congeners also improved at pH 8.2 compared to pH 7.2. The increase in pH from 7.2 to 8.2 causes a small increase in t_0 ($n=3$, RSD=0.34–0.57%) and a decrease in the EOF. In contrast to the increase in t_0 values the t_R values of all PCB congeners decreased. No ready explanation is available for this behavior.

Increasing the pH from pH 8.2 to pH 9.2 resulted in longer separation time and improved peak to peak resolution. All PCBs at pH 9.2 (Fig. 3C) were baseline resolved. Since the natural pH of 7.5 m*M* borate buffer was 9.2 and no acid or base was used to adjust the pH, there is a lowering of the overall ionic strength. Thus, t_0 decreases and EOF increases. Apart from own results, two other examples were found in the literature where similar trends observed [25,26].

An increase in pH from 9.2 to 10.2 (Fig. 3D) was effected using 1 *M* NaOH and accordingly the ionic strength of the BGE increased due to the presence of more borate, $OH^-$and Na^+ ions. This increase in ionic strength decreases the zeta potential and there-

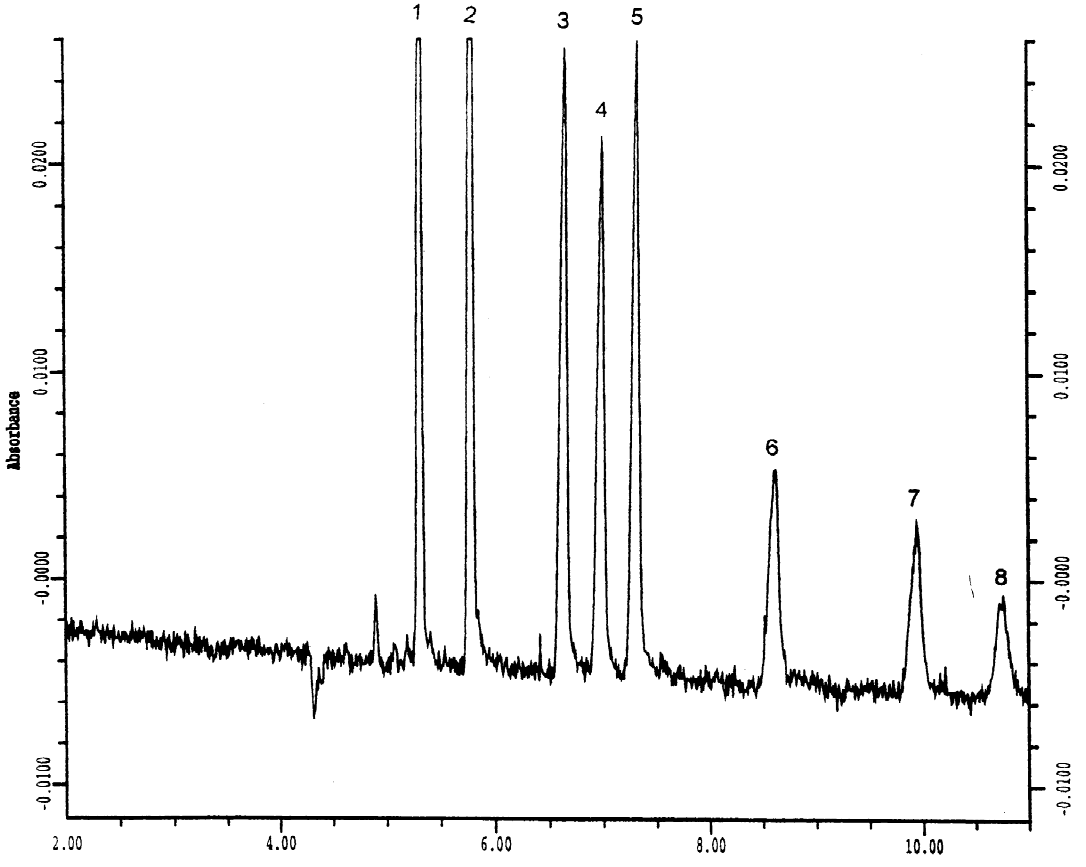

Fig. 4. Electropherogram for the separation of eight PCB congeners in EPA PCB 525.1 test mixture under optimized conditions.

fore the EOF is decreased resulting in the observed reduction of t_0 value at pH 10.2 [25,26]. These additional charged species therefore extended the analysis time and t_R increases for the PCBs. It is generally expected that increasing the ionic strength of capillary zone electrophoresis (CZE) [27] or MEKC [28] buffers often results in better efficiency due to stacking of the analyte zone. However, in this study the overall efficiency of the separation at pH 10.2 did not increase.

To perform analysis of PCBs by MEKC selection of the ideal pH for such analysis must be done. Studying the run time and the efficiency of the MEKC over the selected pH range from pH 7.2 to 10.2 revealed that best resolution and retention times were seen at pH 9.2, the natural pH of sodium borate in aqueous solution. It should be noted that peak distortions observed at pH 7.2, 8.2 and 10.2 might have been caused due to mobility mismatch between extraneous BGE ions (introduced upon pH adjustments) and the analyte.

3.3. Optimum conditions

To separate nine PCBs studied at optimum conditions in MEKC, the pH of the running buffer should be selected at about pH 9.2 (Fig. 3C) with 0.50% (w/v) poly-SUS and 40% (v/v) ACN with sodium borate as BGE. Under 30 kV applied voltage, baseline separation of all PCBs was achieved in ca.10.0 min with high resolution and theoretical plate numbers ranging from 33 000 to 49 000 (N_{avg} was ca. 43 000). In order to qualitatively validate the MEKC method, a standard mixture of eight PCBs (EPA method 525.1) was chromatographed at the optimum conditions (Fig. 4). The EPA test mixture originally contained ca. 100 μg/ml of each PCB in acetone. However, injection of PCB mixture in acetone was unsuccessful because of the peak splitting. Therefore, acetone was evaporated and the PCB mixture was reconstituted in ACN–water (75:25, v/v).

4. Conclusions

Nine PCB congeners were successfully separated by MEKC using only a single polymeric surfactant in a mild concentration of borate buffer. In addition, polymeric surfactant was able to perform this separation at higher ACN concentration without the aid of cyclodextrin as additive. Increasing the organic solvent concentration reduced the elution window but the resolution between each peak was not drastically affected. The elution order for the PCB congeners was found to be dependent on the hydrophobicity, size and degree of chlorination of the PCB congener.

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