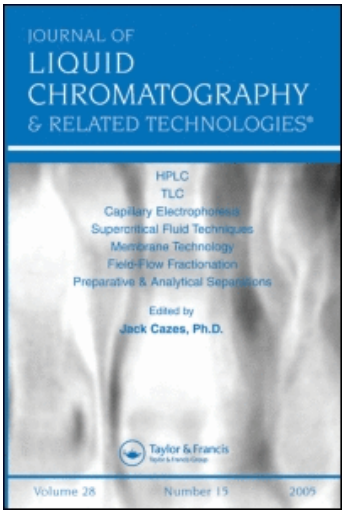

Journal of Liquid Chromatography & Related Technologies
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597273

Capillary Zone Electrophoresis Versus Micellar Electrokinetic Chromatography in The Separation of Sphenols of Environmental Interest

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To cite this Article Crego, A. L. and Marina, M. L.(1997) 'Capillary Zone Electrophoresis Versus Micellar Electrokinetic Chromatography in The Separation of Sphenols of Environmental Interest', Journal of Liquid Chromatography & Related Technologies, 20: 1, 1 — 20
To link to this Article: DOI: 10.1080/10826079708010632
URL: http://dx.doi.org/10.1080/10826079708010632

J. LIQ. CHROM. & REL. TECHNOL., 20(1), 1-20 (1997)

CAPILLARY ZONE ELECTROPHORESIS VERSUS MICELLAR ELECTROKINETIC CHROMATOGRAPHY IN THE SEPARATION OF PHENOLS OF ENVIRONMENTAL INTEREST

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ABSTRACT

The application of capillary electrophoresis techniques to the analysis of phenols is reviewed. Capillary Zone Electrophoresis and Micellar Electrokinetic Chromatography have been primarily employed. The experimental conditions used for determining phenols in environmental samples by these techniques are presented.

INTRODUCTION

Phenolic compounds are important environmental pollutants, due to their high toxicity even at low concentrations ($\mu g \cdot L^{-1}$ range) and common use. Therefore, their concentration in the environment requires constant monitoring. Many important phenolic compounds have nitro groups (NO_2) and halogen atoms (Cl) bonded to the aromatic rings. These substituents may strongly affect chemical and toxicological behavior.[1,2] These compounds originate from such diverse sources as pesticide application, industrial wastes, water supplies, and automobile exhausts. Chlorophenols as pollutants in drinking water, released

through waste water, have urged the need for methods to monitor these compounds in industrial effluents and natural waters. In addition, the US Environmental Protection Agency (EPA)[3] has listed eleven phenols as organic priority pollutants: phenol; 2-nitrophenol; 4-nitrophenol; 2,4-dinitrophenol; 2-chlorophenol; 2,4-dichlorophenol; 2,4-dimethylphenol; 4-chloro-3-methyl-phenol; 2-methyl-4,6-dinitrophenol; 2,4,6-trichlorophenol and pentachloro-phenol.

The analysis of phenols has been widely studied using Gas Chromatography (GC)[4-6] and High Performance Liquid Chromatography (HPLC).[7-11] The polarity of phenols and their low vapor pressure are factors that complicate GC analysis. In order to enhance the volatility and detectability of phenols, sample derivatization is typically necessary prior to GC analysis. This is why GC methods present some disadvantages, such as long sample preparation time and incomplete recoveries for many phenolic derivatives. On the other hand, the factors that complicate GC analysis do not have adverse effects on HPLC analysis. The mode utilized in HPLC is the reversed-phase mode with isocratic or gradient elution. However, owing to the inherent limited resolving power of conventional HPLC techniques, optimization of phenols separation often involves complex procedures or numerous experiments, especially gradient elution.

Presently, Capillary Electrophoresis (CE) is a major trend in analytical chemistry, and the number of publications has increased exponentially in recent years.[12-17] Initially, CE was primarily applied to the field of biochemical analysis, but it has also proved useful in the separation of pollutants. The need for optimized separations for a wide variety of compounds has promoted several working modes that can be used in CE. Capillary Zone Electrophoresis (CZE) and Micellar Electrokinetic Chromatography (MEKC) have become the most popular modes of CE in environmental applications. These techniques are a good alternative for pollutants unsuitable for GC, and affected by the poor efficiency of HPLC. For this reason, the review of CZE and MEKC capabilities for the analysis of phenolic compounds is the aim of this work. Articles which appeared on the subject from 1984 through February 1996 are included.

ANALYSIS OF PHENOLS BY MICELLAR ELECTROKINETIC CHROMATOGRAPHY

Micellar Electrokinetic Chromatography (MEKC) was developed by Terabe et al.[18-20] In this technique, an ionic surfactant is added to the CZE buffer at concentrations exceeding the critical micelle concentration (cmc) to form micelles, therefore expanding CE's enormous power to the separation of

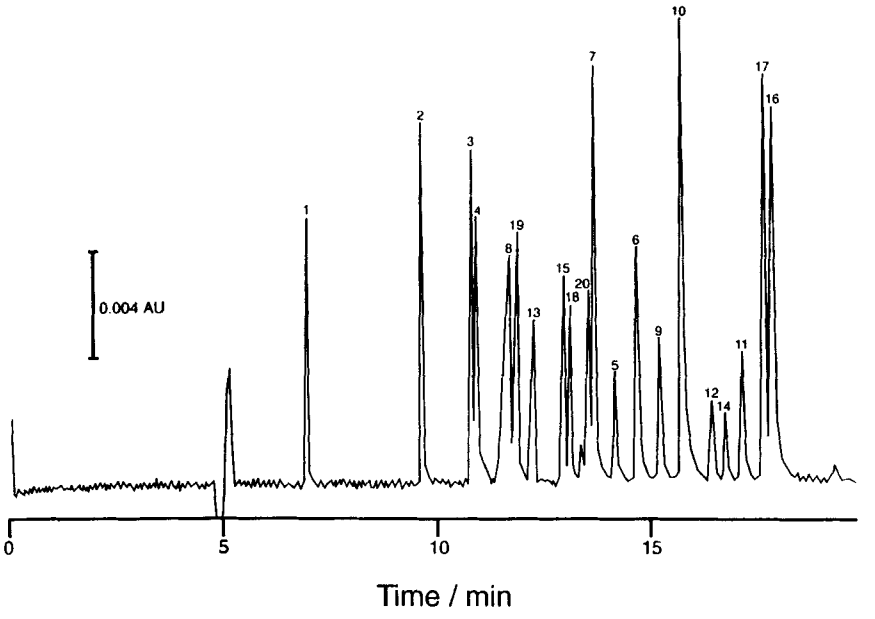

Figure 1. Elcetropherogram of a mixture of all the isomeric chlorinated phenols, including phenol by MEKC. Pcaks: (1) phenol; (2) 2-chloro; (3) 3-chloro; (4) 4-chloro; (5) 2,3-dichloro; (6) 2,4-dichloro; (7) 2,5-dichloro; (8) 2,6-dichloro; (9) 3,4-dichloro; (10) 3,5-dichloro; (11) 2,3,4-trichloro; (12) 2,3,5-trichloro; (13) 2,3,6-trichloro; (14) 2,4,5-trichloro; (15) 2,4,6-trichloro; (16) 3,4,5-trichloro; (17) 2,3,4,5-tetrachloro; (18) 2,3,4,6-tetrachloro; (19) 2,3,5,6-tetrachloro; (20) pentachloro. Conditions: micellar solution, 0.07 M SDS, in phosphate-borate buffer, pH 7.0; separation tube, 650 x 0.05 mm i.d.; length of the tube used for separation, 500 mm; total applied voltage, 15 kV; current 28 μA; detection wavelength, 220 nm; temperature, 35°C. Reproduced from (23) with permission of Elsevier Science Publishers.

both charged and uncharged solutes.[21-22] Although anionic surfactants are the most commonly used, especially sodium dodecyl sulphate (SDS), others such as cationic, non-ionic, and zwitterionic have been used too. The micelles are spherical aggregates the hydrophobic groups of which are oriented toward the center of the micelle, and polar or charged groups are along the sphere's surface. Anionic micelles are retarded in the electric field and move at slower velocity than the electroosmotic flow. In this instance, analytes are separated based on their differential partitioning between the buffer phase (which migrates with the velocity of the electroosmotic flow) and the hydrophobic interior of the micelles (micellar phase, which acts as a pseudo-stationary phase). Due to the fact that the micellar phase is moving toward the detector,

an elution window is created and bordered by a column void time (t_o, mobility of the electroosmotic flow) and a micelle migration time (t_{MC}). All analytes must elute between those two limits, t_o and t_{MC}, depending on their partition between the aqueous and micellar phases.

The use of CE for the separation of several *substituted phenols* was first reported by Terabe et al.[18] in 1984. In this initial work on the use of MEKC, up to fourteen phenols were completely resolved within 19 minutes using a borate-phosphate buffer at pH 7.0 (solute molecules were electrically neutral) and with SDS as micellar system.

These authors also studied the separation of all *isomers of chlorophenols* (nineteen) under various conditions of pH and SDS concentration.[23] Complete separation of all isomers was accomplished within 18 min (see Figure 1) under experimental conditions similar to those described previously. In both works, plate numbers ranged from 200,000 to 400,000 and detection limits in the $mg\,L^{-1}$ or nanogram range were obtained with UV detection. The reproducibility and quantitative aspects of the results obtained in the separation of chlorophenols by MEKC were studied.[24] Reproducibility of migration times ($RSD_{n=5}$: 0.3-1.2%) was commensurate with that obtained in HPLC. However, reproducibility of injected amount *(manual gravity flow injection)* was not good ($RSD_{n=5}$: 2-5%, for peak height; and $RSD_{n=5}$: 1-8%, for peak area). Correlation coefficients showed good linear correlations between peak area ($r \geq 0.999$) or peak height ($r \geq 0.99$) and concentrations under two orders of magnitude, when an internal standard calibration method was used.

Good results obtained in the separation of chlorophenols by MEKC were the basis for the first separation of eleven *EPA priority phenols* obtained by Ong et al.[25] in 1990 with MEKC. The authors used the electrophoretic medium described before, phosphate-borate buffer with neutral pH and SDS as surfactant. The separation was obtained within a high analysis time (45 min) with a relatively large inner diameter (180 μm). However, although the resolution was improved when a 50 μm i.d. capillary was used, the analysis time was not shorter.[26] The detection levels were in the nanogram range with UV detection.

Recently, the effects of organic additives (tetrahydrofuran, methanol or acetone) on separations by MEKC have been studied.[27] The results obtained were discussed in terms of MEKC applicability to field screening methods. Methanol and tetrahydrofuran tended to bunch peaks whereas acetone appeared to add selectivity. The best separation of seven priority phenols in less than 20 minutes was under acetone-cholate-borate buffer conditions. The micellar

agent chosen was sodium cholate because bile salts micelles are more stable than conventional SDS micelles in the presence of organic modifiers. Acetone allowed a better resolution by reducing the electroosmotic flow.

Table 1 groups the experimental conditions in which the separation of phenols by MEKC was achieved. It is observed that the electrophoretic medium used is similar in almost all applications, 50-100 mM SDS and borate-phosphate buffer (pH 7). However, sodium cholate with acetone and basic pH can be used for rapid separations. On the other hand, the instrumentation is the same: capillaries of 50 μm i.d. and an effective length of ~ 50 cm, at 10-15 kV, with hydrodynamic injection and on-column UV detector. Finally, it is important to note that all applications include demonstrations of standard separations but not real samples. The reason is the limited sensitivity of UV detectors (> mg L^{-1}).

PHENOLS ANALYSIS BY CZE

Capillary Zone Electrophoresis (CZE) is the most common and simple working mode in CE. The separation by CZE is carried out in a capillary filled with a continuous background electrolyte (buffer).[28-30] The direction and the migration velocity of the analytes are determined by both electrophoresis and electroosmosis phenomena. Analytes are separated based on the difference in their electrophoretic mobilities, which are related to their charge densities, mainly based on differences in solute size and charge at a given pH. Generally, the electroosmotic flow will be higher than the electrophoretic migration velocity of most anionic analyses. Consequently, both cations and anions will migrate in the same direction and can be separated in the same run.

With regard to CZE applicability in the analysis of phenols, it is very interesting to note that, if the suitability of CZE for the separation of the chlorophenols is compared with the results obtained by MEKC,[23] even though the separation by CZE was optimized in terms of pH, buffer concentration, and applied voltage to obtain maximum peak separation, all the isomers of chlorinated phenols could not be resolved by CZE.[31] Therefore, MEKC has greater selectivity than CZE, allowing the analysis of all chlorophenol isomers, as stated previously.

On the other hand, the electrophoretic behavior of the eleven *EPA priority phenols* was studied recently by Li and Locke[32] and a simple analytical method using CZE was established. The effects of pH, buffer concentration, and applied voltage on the separation were investigated, and the main conclusion

Table 1

Experimental Conditions for Separation of Phenols by MEKC

Compound Type*	Buffer**	ΔV	Capillary*** System	Injection Syst/Limits	Detection	Notes	Ref.
Subst'd Phenols							
P; 2-CR; 3-CR; 4-CR 2-CP; 3-CP; 4-CP; 2,3-XY 2,4-XY; 2,5-XY; 2,6-XY; 3,4-XY; 3,5-XY; 4-EP	50 mM SDS 25 mM $Na_2B_4O_7$/ 50 mM NaH_2PO_4 (pH 7.0)	15 kV	50(45) cm x 50 μm	Hydrodynamic (gravity) 4 cm x 5s	UV-270 nm 1-10 mg/L	Sep'ns of std solutes	18
Chlorophenols							
P; 2-CP; 3-CP; 4-CP; 2,3-DCP; 2,4-DCP; 2,5-DCP; 2,6-DCP; 3,4-DCP;; 3,5-DCP;	70 mM SDS 25 mM $Na_2B_4O_7$/ 50 mM NaH_2PO_4 (pH 7.0)	15 kV	65(50) cm x 50 μm	Hydrodynamic (gravity) 4.5 cm x 5s	UV-220 nm 1-10 mg/L	Optimized sep'n of std solutes	23
2,3,4-TCP; 2,3,5-TCP; 2,3,6-TCP; 2,4,5-TCP; 2,4,6-TCP; 3,4,5-TCP; 2,3,4,5-TeCP; 2,3,5,6-TeCP; and PCP	100 mM SDS 25 mM $Na_2B_4O_7$/ 50 mM NaH_2PO_4 (pH 7.0)	15 kV	65(50) cm x 50 μm	Hydrodynamic (gravity) 4.5 cm x 5s	UV-220 nm 1-10 mg/L	Quantitation reproducibility of std solutes	23
Priority Phenols							
P; **2-NP; 4-NP 2,4-DNP**; 2-CP; **2,4-DCP**; 2,4-DMP; **4-C-3-MP**; 2-M-4,6-DNP; 2,4,6-TCP and **PCP**	50 mM SDS 100 mM $Na_2B_4O_7$/ 50 mM NaH_2PO_4 (pH 6.6)	10 kV	100(85) cm x 180 μm	Hydrodynamic (gravity) 5 cm x 5s	UV-254 nm 1-10 mg/L	Retention behav. of std solutes	25

(continued)

Table 1 (continued)

Experimental Conditions for Separation of Phenols by MEKC

Compound Type*	Buffer**	ΔV	Capillary*** System	Injection Syst/Limits	Detection	Notes	Ref.
Priority Phenols (cont)							
P; **2-NP; 4-NP** **2,4-DNP**; 2-CP; **2,4-DCP**; 2,4-DMP; **4-C-3-MP**; 2-M-4,6-DNP; 2,4,6-TCP and **PCP**	50 mM SDS 100 mM $Na_2B_4O_7$/ 50 mM NaH_2PO_4 (pH 6.6)	15 kV	60(50) cm x 50 μm	Hydrodynamic (gravity) 5 cm x 5s	UV-254 nm 1-10 mg/L	Retention behav. of std solutes	26
P; **2-NP; 4-NP** **2,4-DNP**; 2-CP; **2,4-DCP**; 2,4-DMP; **4-C-3-MP**; 2-M-4,6-DNP; 2,4,6-TCP and **PCP**	100 mM SDS 50 mM boric acid/ (pH 8.35) 10% (v/v) acetone	25 kV	57(50) cm x 50 μm	----	UV-214 nm	Rapid sep'n of std solutes****	27

*P: Phenol; CR: Cresols; XY: Xylenols; EP: Ethylphenol; CP: Chlorophenols; DCP: Dichlorophenols; TCP: Trichlorophenols; TeCP: Tetrachlorophenols; PCP: Pentachlorophenol; CMP: Chloromethylphenol; NP: Nitrophenols; DNP: Dinitrophenols; DMP: Dimethylphenol; C-MP: Chloro-methylphenol; M-DNP: Methyl dinitrophenol.

** Sodium dodecyl sulfate.

***x(y). x: total length, y: effective length

****Application including the separation of solutes in negrite.

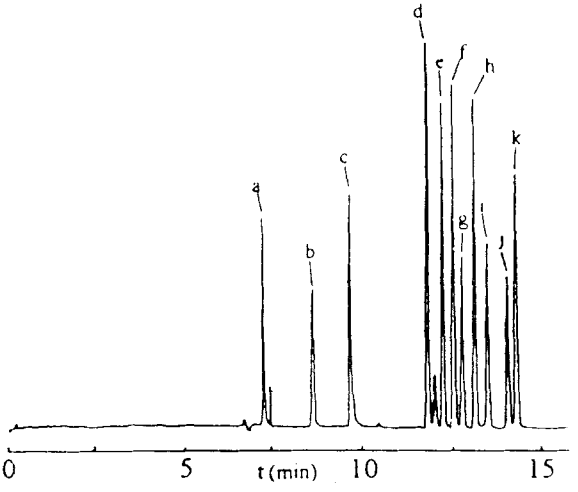

Figure 2. Electropherogram of eleven priority phenols (solute concentration 25 mg L^{-1}) by CZE. Peaks: (a) 2,4-dimethyl-phenol; (b) phenol; (c) 4-chloro-3-methylphenol; (d) pentachlorophenol; (e) 2,4,6-trichloro-phenol; (f) 2,4-dichloro-phenol; (g) 2-methyl-4,6-dinitrophenol; (h) 2-chlorophenol; (i) 2,4-dinitro-phenol; (j) 4-nitrophenol; and (k) 2-nitrophenol. Conditions: phosphate-borate buffer, pH 9.8; separation tube, 100 cm x 75 μm i.d.; length of the tube used for separation, 65 cm; total applied voltage, 22.5 kV; current 53 μA; detection wavelength, 210 nm; vacuum injection time 10 s. Reproduced from (32) with permission of Elsevier Science Publishers.

was that the most critical parameter controlling resolution and separation time was the pH. In this case, CZE provided better results than MEKC, because the eleven phenols can be completely resolved in less than 15 min (Figure 2) analysis time, noticeably shorter compared to the 45 min obtained by MEKC,[26] or the 25 min typically required by HPLC.[33] Optimum conditions included a smaller concentration of the same buffer utilized in MEKC (10 mM phosphate-borate), and basic pH (9.8), for ionization of all phenols except one. Detection was performed with an on-column UV detector and good linearities ($r \geq 0.999$) were obtained for concentrations up to at least 50 mg L^{-1}, with detection limits less than 1 mg L^{-1}.

Comparing the retention behavior between CZE and MEKC, it is interesting to note that the elution order of the eleven phenols found in CZE with a basic buffer (pH ≈10) is opposite to that obtained using MEKC with a neutral buffer (pH ≈7). This is understandable because the separation

mechanisms in CZE and MEKC are basically different. CZE separation is only based on the phenols difference in size and charge at a given pH, whereas in MEKC, it is based on a combination of effects, such as charge/mass ratios, hydrophobicity and charge interactions at the surface of the micelles. In both techniques, the most critical parameter in the separation is the pH, because phenols are weakly basic solutes and the extent of their dissociation, which determines the overall electrical charge of the solute, is governed by the buffer pH.

A new method for the rapid analysis of phenols by CZE was developed in 1995 by Masselter and Zemann.[34,35] In this method, the direction of the electroosmotic flow in a fused silica capillary is reversed by dynamically coating the negatively charged inner surface of the capillary with a layer of either positively charged hemimicelles or polycations, which is formed by adding either a cationic surfactant (cetyltrimethylammonium bromide, CTAB) or a polycation (1,5-dimethyl-1,5-diazaundecamethylene polymethobromide, HDB) to the buffer. A reversal of the electroosmotic flow reduces the analysis time by migration of the anionic analytes in the same direction as the electroosmotic flow *(Coelectroosmotic Capillary Electrophoresis)*. The best separation of several *isomers of alkyl-phenols*, in less than 6 min, is performed using a buffer of low concentration and at high pH value (pH 11, above the pK_A value of the solutes) to achieve the complete dissociation of phenols,[34] with 2-propanol as organic modifier to improve, significantly, peak shape and separation.[35] Other organic solvents (methanol, ethanol, 1-propanol and acetonitrile) have also been studied.[35] The only advantage of this method is the ability to achieve rapid separations of anions at the expense of selectivity and resolution and, although it has been applied only to the separation of several isomers of alkyl-phenols, none of which are priority pollutants, its possibilities could be employed for the rapid analysis of phenols to field-screening methods in simple samples.

The detection system used in all the above-mentioned works has been on-column UV detection, generally employed in CE.[36] This detector is commonly employed in the analysis of phenols because these compounds possess strong absorption in the UV region (210-280 nm). However, despite this detector's acceptable absolute detection limits (in the range of ng solute), the concentration in the peak is relatively high (more than 1 mg L^{-1} for a common solute), because the injection volume in CE is often several nanoliters. This concentration detection ability is not sensitive enough to determine phenols in environmental samples, in which pollutants exist at μg L^{-1} level or lower. Therefore, the use of CE for the analysis of phenols in real samples will not be possible unless enrichment procedures or improved detection systems are employed.

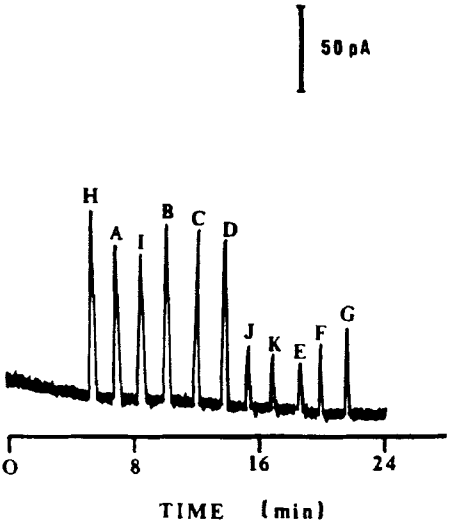

Figure 3. Elcetropherogram of an industrial waste water sample with (A) a 2-chlorophenol concentration of 50 μg L^{-1} by CZE. Peaks: (H) phenol; (B) 2-chlorophenol; (I) 4-chlorophenol; (B) 2,4-dichlorophenol; (C) 2,6-dichlorophenol; (D) o-phenyl phenol; (J) catechol; (K) 2,4,6-trichlorophenol; (E) 2,3,4,6-tetrachlorophenol; (F) 4,5,6-trichloroguiacol; (G) pentachlorophenol. Conditions: 45 mM orthophosphate-15 mM borate buffer, pH 8.0; separation tube, 65 cm x 25 μm i.d.; length of the tube used for separation, 35 cm; total applied voltage, 20 kV; amperotric detection using carbon fibers at +1.4 V versus SCE. Reproduced from (54) with permission of Elsevier Science Publishers.

Several enrichment procedures are being exploited in CE: solid phase extraction with membrane disk[37-39] or in-capillary,[40,41] field amplification injection[42-45] and others based on isotachophoresis.[46-48] Two reviews have been reported recently on referenced procedures.[49,50]

On the other hand. the fact that phenols respond to a sensitive detection method such as electrochemical detection with a microelectrode[51-53] has allowed the separation of *chlorinated phenols in industrial waste* by CZE with on-column electrochemical detection.[54] Seven chlorophenol isomers and three neutral phenols were completely resolved within 24 min (Figure 3) using similar conditions to those described before. Detection was performed in the amperometric mode using a microelectrode (carbon fiber of 10 μm diameter) with an oxidation potential of + 1.4 V vs. SCE. Levels in the μg L^{-1} or picomole range were achieved thermostating the separation capillary. Efficiencies of about 320.000 theoretical plates were obtained, and no

interferences from the impurities present in industrial waste water samples were observed, using only a simple liquid-liquid extraction with chloroforrn-diethyl ether. Therefore, the use of an on-line electrochemical detector provides excellent sensitivity and selectivity without derivatization.

Chen and Whang[55] also obtained the separation of eleven EPA priority phenols by CZE with on-column amperometric detection. This method has been successfully applied to the analysis of *priority phenols in industrial waste water*. Initially, sodium borate was used as the background buffer (according to previous results). However, large electrophoretic currents (10-100 μA) generated large detector noise, which seriously interfered with amperometric detection (phenomenon reported by other workers[56,57]). In order to minimize this effect, Cyclohexylaminoethanesulfonic acid (CHES) was used as the operating buffer. Due to its zwitterionic nature, electrophoretic currents were only about 1-4 pA. On the other hand, the work electrode potential must be +1.50 V vs. SCE to detect the eleven phenols, although the background stability was poorer than that obtained at +1.10 V, and the carbon fiber electrode durability decreased significantly. But only nine phenols were detected with +1.10 V. The separation of all phenols, obtained within 17 min, presented a number of theoretical plates in the range from 87,000-114,000. Reproducibility results showed satisfactory values in migration times ($RSD_{n=5} < 2$ %), but not good reproducibilities in the injected amount *(manual gravity flow injection)* with values of $RSD_{n=5}$: 2-9 % for the peak height. However, the results showed good linear correlation ($r \geq 0.99$) between peak height and concentration (over two orders of magnitude), and with concentration detection limits in the $\mu g\,L^{-1}$ level (10^{-5} - 10^{-7} M). These values were better than those obtained with UV detection but poorer than those of HPLC-amperometric detection.[58]

Finally, laser-induced fluorescence based detection systems have become popular mainly because of their capability to provide extremely high sensitivity (10^{-12} M). However, phenols, as many other compounds, cannot give response because only a few compounds show native fluorescence. In these cases, there are two alternatives: to derivatize non-fluorescent substances[59] or use indirect detection techniques.[60] Briefly, indirect detection consists in the addition of a non-interacting and fluorescing ion to the running buffer to create a constant fluorescence background. When a charged analyte is present, it displaces the fluorescing ion of the same charge due to local charge neutrality, resulting in a decreased background signal even though the analyte does not absorb or fluoresce. This technique was applied by Chao and Whang[61] to the analysis of eleven priority phenols by CZE in NIST standard reference materials and industrial waste waters. In this method, a compromise between optimum peak resolution and satisfactory detection sensitivity must be considered.

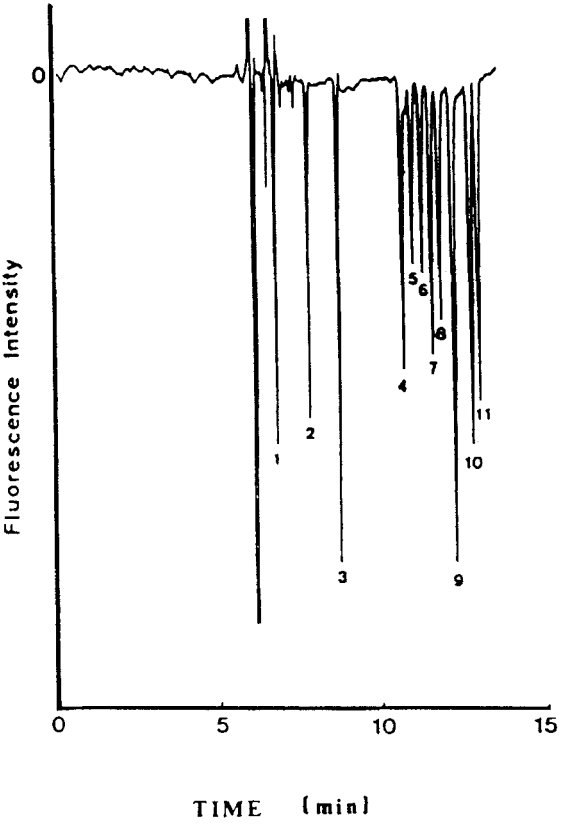

Figure 4. Electropherogram of eleven priority phenols by CZE with indirect fluorescence detection. Peaks: (1) 2,4-dimethylphenol; (2) phenol; (3) 4-chloro-3-methylphenol; (4) pentachlorophenol; (5) 2,4,6-trichlorophenol; (6) 2,4-dichlorophenol; (7) 2-methyl-4,6-dinitrophenol; (8) 2-chlorophenol; (9) 2,4-dinitrophenol; (10) 4-nitrophenol; and (11) 2-nitrophenol. Conditions: buffer, 15 mM borate (pH 9.9) with 1 mM fluorescein; separation tube, 50 cm x 20 μm i.d.; Length of the tube used for separation, 45 cm; total applied voltage, 9 kV; current 2.8μA. Reproduced from (61) with permission of Elsevier Science Publishers.

Firstly, the authors found that a relatively high concentration of electrophoretic buffer (>10 mM) was crucial in the separation of the eleven phenols (the electroosmotic velocity is inversely proportional to ionic concentration[62]), but the increase in the buffer concentration had an adverse effect on the sensitivity of indirect detection. On the other hand, results showed

that the direction of some peaks (positive or negative) was affected by both electric field and background fluorophore concentration. Once the optimal concentrations for the buffer and the fluorophore were chosen (see Table 2), complete separation of the eleven compounds could be achieved in less than 14 min (Figure 4) using a sodium borate buffer at basic pH, as in previous works. The results obtained showed lower analysis time, with better resolution and a higher number of theoretical plates (in the range 99,000-187,000) than those obtained by amperometric detection.[55] The results on reproducibility and quantitative aspects are similar or slightly better, values of $RSD_{n=15} < 1$ % in migration times, $RSD_{n=7}$ 2.7-6.3 % for peak height, and linear correlations ($r \geq 0.99$) between peak height and concentration over two orders of magnitude were obtained, with detection limits in the $\mu g \cdot L^{-1}$ range (10^{-6}-10^{-7}M).

Table 2 groups the experimental conditions in which the analysis of phenols by CZE was performed. It is observed that the pH chosen for the electrophoretic medium depends on the type of compounds. The analysis of chlorophenols need a pH between 7 and 8 but, for priority phenols, is more basic (pH ~ 10). Another possibility is a pH 11 when a new method of CZE is used *(Coelectroosmotic Capillary Electrophoresis).* On the other hand, although in general terms, the buffer used is borate/phosphate, CHES can be utilized. As for the instrumentation, there are several options: capillaries with inner diameter between 20-75 μm, at 9-30 kV, with different injection (hydrodynamic or electrokinetic) and detection systems (UV, amperometric or indirect fluorimetry). It is important to note that detection systems other than UV detectors help to obtain adequate detection limits (in the $\mu g \cdot L^{-1}$ range) to analyze phenols in real samples (industrial waste water), being the most adequate the amperometric to chlorophenols and indirect fluorimetry detection to priority phenols. Finally, the sensitivity obtained by CZE with UV detection is better than that obtained by MEKC (see Table 1), but it is still inadequate for trace analysis of real samples.

CONCLUSION

MEKC techniques were widely used in the analysis of phenols in the past. However, in the last five years, CZE has received more attention. In fact, the theoretical plate number obtained with CZE is higher than with MEKC due to the mass transfer resistance caused by solute partitioning between the bulk buffer and the micelles. Consequently, the sensitivity in MEKC is lower than in CZE.[63] On the other hand, micellar systems are less stable than CZE systems because of the temperature effect on the equilibrium involved. In addition, MEKC optimization is more complicated than in CZE. Two important experimental parameters, pH and micelle concentration, have a great

Table 2

Experimental Conditions for Separation of Phenols by CZE

Compound Type*	Buffer	ΔV	Capillary**** System	Injection Syst/Limits	Detection	Notes	Ref.
Substituted Phenols							
2-MP; 3-MP; 4-MP; 2,3-DMP; 2,6-DMP; 3,4-DMP; 2,3,5-TMP; 2,4,6-TMP	1.25 mM $Na_2B_4O_7$ 15 mM NaH_2PO_4 (pH 11) with 0.001% (v/v) HDB** 40% (v/v) 2-PrOH	30kV	32(24) cm x 50 μm	Hydrodynamic gravity (10 cm x 5s)	UV-254 nm	Sep'ns of std solutes	34 35
Chlorophenols							
2-CP; 2,3-DCP; **2,4-DCP**; 2,5-DCP; **2,6-DCP**; 3,5-DCP; 2,3,4-TCP; 2,3,5-TCP; 2,3,6-TCP;	50 mM Na_2HPO_4/ NaH_2PO_4 (pH 6.9)	18 kV	57(50) cm x 75 μm	Hydrodynamic (Pressure)	UV-214 nm 0.06-0.1 mg/L	Optimized sep'n of std solutes	31
2,4,5-TCP; **2,4,6-TCP**; **2,3,5,6-TeCP**; and **PCP** (Phenol; Catechol; 4-CP; 2-PHP; 4,5,6-TCG)	15 mM Na_3BO_3/ 45 mM NaH_2PO_4 (pH 8.0)	20 kV	65(35) cm x 25μm	Electrokinetic (20kV x 30s)	Amperometric +1.4V *vs.* SCE 1-2 μg/L	Real sample of industr. waste water*****	54
Priority Phenols							
Phenol; 2-NP; 4-NP; 2,4-DNP; 2-CP; 2,4-DCP; 2,4-DMP; 4-C-3-MP; 2-M-4,6-DMP; 2,4,6-TCP; and PCP	10 mM $Na_2B_4O_7$/ Na_3PO_4 (pH 9.8)	22.5 kV	100(65) cm x 75μm	Hydrodynamic (vacuum x 10s)	UV-210 nm 0.3-0.6 mg/L	Optimized sep'n of std solutes	32

(continued)

Table 2 (continued)

Experimental Conditions for Separation of Phenols by CZE

Compound Type*	Buffer	ΔV	Capillary**** System	Injection Syst/Limits	Detection	Notes	Ref.
Priority Phenols (cont)							
Phenol; 2-NP; 4-NP; 2,4-DNP; 2-CP; 2,4-DCP; 2,4-DMP; 4-C-3-MP; 2-M-4.6-DMP; 2,4.6-TCP; and PCP	20 mM CHES*** (pH 10.1)	9 kV	62.5(50) cm x 50μm	Electrokinetic (9kV x 2s)	Amperometric +1.5V *vs.* SCE 0.03-7 mg/L	Real sample of indust waste water	55
	15 mM Na_3BO_3 (pH 9.9) with 1mM fluorescein	9 kV	50(45) cm x 20 μm	Electrokinetic (9kV x 2s)	Indirect Fluorimetry 0.01-0.75 mg/L	Real sample of indust. waste water	61

* MP: Methylphenol; DMP: Dimethylphenol; TMP: Trimethylphenol; PHP: Phenylphenol; TCG: Trichloroguaiacol; CP: Chlorophenols; CMP: Chloromethylphenol; DCP: Dichlorophenols; TCP: Trichlorophenols; TeCP: Tetrachlorphenol; PCP: Pentachlorophenol; NP: Nitrophenols; DNP: Dinitrophenols; MDNP: Methyldinitrophenol.

** HDB: Hexadimethrine bromide.

*** CHES: Cyclohexylaminoethanesulfonic acid.

**** x(y), x: total length, y: effective length.

*****Application including the separation of solutes in negrite and those included in parentheses.

influence on the migration behavior and selectivity in MEKC;[64] but only one important experimental parameter, pH, has a great influence in CZE.[65,66] Despite these drawbacks, MEKC, as opposed to CZE, allows the separation of ions with very similar electrophoretic mobilities, as chlorophenol isomers, because the partition between the aqueous and micellar phases increases the selectivity.

In summary, CZE in conjunction with laser-induced indirect fluorimetry can provide rapid separation and sensitive detection of the eleven priority phenols in real samples. On the other hand, a sensitive detection of chlorophenols can be obtained with amperometric detection, but the separation of all chlorophenols isomers that is possible by MEKC, cannot be achieved by CZE. Finally, it is interesting to note that the separations can be compared to GC separations in terms of resolving power, efficiency, and run time. Moreover, CE techniques do not show peak tailing with the polar nitrophenols and pentachlorophenol, but this appears to be a recurring problem with GC when real sample extracts are injected.

ACKNOWLEDGMENTS

The authors thank the Comunidad Autonoma de Madrid (Spain) for project COR0010/94.

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Received April 3, 1996
Accepted April 23, 1996
Manuscript 4135