# Nonaqueous Capillary Electrophoresis of Chlorinated Phenols

**Bojeong Kim, Man-Seog Chun, Sangmoo Shin,* and Doo Soo Chung***

*Department of Chemistry, Seoul National University, Seoul 151-742, Korea*
*[Department of Textile Engineering, Soongsil University, Seoul 156-743, Korea*
*Received August 30, 1999*

The contents of pentachlorophenol (PCP) and 2,3,5,6-tetrachlorophenol (TeCP) in textile products are regulated for safety. Since an organic solvent such as 2-methoxyethanol is needed to extract chlorinated phenols from textile samples, nonaqueous capillary electrophoresis has been applied to achieve the separation of PCP and isomers of TeCP. The run buffer was 100 mM Tris/acetate in methanol whose pH was adjusted to 8.0. All of the analytes were negatively charged at pH 8.0 and their electrophoretic velocities were higher than the electroosmotic flow of the methanol buffer. A reverse voltage of -20 kV was applied along a 27-cm fused silica capillary with ID of 50 $\mu$m, and PCP and 3 TeCP isomers were separated based on the difference in p$K_a$ values in less than 4 min. The limits of detection ($S/N = 3$) were about 0.02 $\mu$M. By varying pH of the methanol run buffer, p$K_a$ values of the 4 chlorinated phenols were also estimated.

## Introduction

With the advent of a new generation of highly efficient and sensitive measuring instruments, the connection between various illnesses and pollutants in all areas of life is increasingly recognized. Some textiles were also found to contain high levels of pollutants and international efforts have been directed towards laying down safe limits for harmful substances in textile products of all kinds in an objective and scientifically verifiable manner. As a result, the Oeko-Tex Standard 100 was first issued in 1992 and has been updated in line with the latest scientific knowledge by the International Association for Research and Testing in Textile Technology.[1] It provides the criteria, limiting values and evaluation methods for the testing of harmful substances in textiles. Among many chemical species, it has listed pentachlorophenol (PCP) and 2,3,5,6-tetrachlorophenol (TeCP) as harmful chlorinated phenols which have been found to be carcinogens.[2,3] Their concentration levels in textile products need to be monitored constantly.

Determination of chlorinated phenols has been based mainly on gas chromatography, using a wide variety of derivatization methods and detectors.[4-6] Chlorinated phenols are soluble in aqueous as well as in organic solvents and both normal- and reverse-phased high-performance liquid chromatography (HPLC) can also be employed as a complementary analytical method.[7-11] However, in both techniques, complicated sample derivatization procedures are necessary and large amounts of reagents are consumed.

In the past decade, capillary electrophoresis (CE) has emerged as a fast and an efficient separation technique for a variety of compounds.[9,10,12-16] After a slow start, the number of studies on the use of CE for determining various hazardous substances is now growing rapidly. So far, a number of reports have been published in the area of CE using capillary zone electrophoresis and micellar electrokinetic capillary chromatography modes for the separation of chlorinated phenols in aqueous solution.[17,18] Since an organic solvent such as 2-methoxyethanol is needed to extract chlorinated phenols from textile products, we developed a separation method for PCP and isomers of TeCP in the nonaqueous capillary electrophoresis mode instead of typical aqueous CE modes.

## Experimental Section

**Chemicals.** PCP and TeCPs were obtained from Supelco (Bellefonte, PA, USA). Tris(hydroxymethyl)aminomethane (Tris) was obtained from Sigma. HPLC grade methanol was from Merck (Darmstadt, Germany) and glacial acetic acid from Duksan Pure Chemicals (Ansan, Kyunggi, Korea). 2-Methoxyethanol was from Aldrich. Water was purified with a NANOpure II System (Barnstead, Dubuque, IA, USA) and a 0.22-$\mu$m Millipak 40 filter (Millipore, Bedford, MA, USA). All other chemicals were of reagent grade and used without further purification.

**Sample preparation.** Textile sample solutions were prepared following the official safety test procedure of the Korean Agency for Technology and Standards.[19] 5 g of ground textile sample in a Thimble filter was extracted into 80 mL of 2-methoxyethanol for 2 h using a Soxhlet extractor. After cooling, 5 mL of water and 4 g of KOH were added and the mixture was refluxed for 30 min. The cooled mixture was then neutralized with 6 mL of concentrated hydrochloric acid and 3 mL of acetic acid. The filtered mixture was diluted with 2-methoxyethanol to a volume of 100 mL. Standard solutions were prepared by dissolving analytes in 2-methoxyethanol which is used for extracting chlorinated phenols from textile samples in the Korean official method.[19]

**Capillary electrophoresis.** Capillary electrophoretic analyses were carried out using a P/ACE 5000 capillary electrophoresis system (Beckman, Fullerton, CA, USA) equipped

*To whom correspondence should be addressed. Phone: +82-2-880-8130; FAX: +82-2-877-3025; E-mail: dschung@snu.ac.kr

with a filter-selectable UV detector module. Absorbances at 214 nm were monitored for detecting the analytes. A bare fused-silica capillary (Polymicro Technologies, AZ, USA) with an effective/total length of 20/27 cm and a 50-$\mu$m ID was kept at 20 °C. The run buffer was 100 mM Tris/acetate in methanol. By using a pH meter calibrated for aqueous solutions at 25 °C, the run buffer pH was adjusted to 8.0 by titrating 100 mM Tris with 100 mM acetic acid both in methanol. Since all of the analytes are negatively charged at pH 8.0 and their electrophoretic velocities are faster than the electroosmotic flow (EOF) of the methanol run buffer, a reverse voltage of -20 kV was applied along the capillary. At the start of an experiment, the capillary was pretreated with methanol for 10 min and then with the run buffer for 10 min. Prior to each run, the capillary was rinsed with the run buffer for 5 min. Samples were introduced with a pressure of 0.5 psi nitrogen. The injection time was varied from 2 sec to 20 sec to improve the detection sensitivity by the sample stacking method.

## Results and Discussion

Chlorinated phenols in neutral form can be separated electrophoretically by being partitioned in a charged pseudostationary phase such as charged cyclodextrins or micelles of ionic surfactants such as sodium dodecyl sulfate.[20] Alternatively, chlorinated phenols, which are weak monoprotic acids, could be ionized in a basic buffer. The apparent mobility ($\mu_{APP}$) of an analyte is given by the sum of the electrophoretic mobility ($\mu_{EP}$) and electroosmotic mobility ($\mu_{EOF}$)

$$\mu_{APP} = \mu_{EP} - \mu_{EOF}. \quad (1)$$

Since EOF is usually much faster than electrophoretic velocities, anions as well as cations and neutrals migrate toward the cathode. However, $|\mu_{EP}|$ of the chlorinated phenols in our study were larger than $|\mu_{EOF}|$. The obtained values of $\mu_{EP}$ at pH 8.0 for PCP, 2,3,5,6-TeCP, 2,3,4,6-TeCP, and 2,3,4,5-TeCP were $-1.69 \times 10^{-4}$ cm$^2$/Vs, $-1.63 \times 10^{-4}$ cm$^2$/Vs, $-1.55 \times 10^{-4}$ cm$^2$/Vs, and $-1.31 \times 10^{-4}$ cm$^2$/Vs, respectively. $\mu_{EOF}$ of the 100 mM Tris/acetate buffer in methanol (pH 8.0) was found to be $+4.84 \times 10^{-5}$ cm$^2$/Vs from the migration time of a neutral marker, dimethyl sulfoxide or 10 mM Tris/acetate in methanol. Therefore, the negatively charged small analytes would not migrate from the anodic inlet to the cathodic outlet of the capillary under a normal positive electric field. Migration of the analytes toward the detector under a reverse potential of -20 kV was observed within a reasonable time at pH > 7. We chose pH 8.0 as an optimal value considering resolution and analysis time.

The p$K_a$ values of a material in aqueous and organic solutions should differ. Table 1 lists the p$K_a$ values of PCP and TeCPs in the aqueous medium. Since the electrophoretic mobility of the neutrals are zero, $\mu_{EP}$ is given by the product of the fraction of dissociation and the electrophoretic mobility of the free anions, $\mu_0$:

$$\mu_{EP} = \frac{K_a}{[H^+] + K_a}\mu_0 . \quad (2)$$

**Table 1.** Electrophoretic mobilities of the free anions ($\mu_0$) and apparent p$K_a$ of chlorinated phenols in methanol

| Compound | $\mu_0$ ($10^{-4}$ cm$^2$/Vs) | p$K_a$ in methanol | p$K_a$ in water** |
|---|---|---|---|
| Pentachlorophenol | 2.3 ± 0.1* | 5.7 ± 0.3* | 4.74 |
| 2,3,5,6-tetrachlorophenol | 2.3 ± 0.1* | 6.1 ± 0.1* | 5.03 |
| 2,3,4,6-tetrachlorophenol | 2.2 ± 0.1* | 6.41 ± 0.07* | 5.22 |
| 2,3,4,5-tetrachlorophenol | 1.9 ± 0.3* | 7.01 ± 0.09* | 5.64 |

*95% confidence intervals. **Ref.[32].

Rearranging the above equation, one obtain

$$\frac{1}{\mu_{EP}} = \frac{1}{\mu_0 K_a}[H^+] + \frac{1}{\mu_0}. \quad (3)$$

The values of $\mu_0$ and p$K_a$ in a methanol buffer were estimated by monitoring $\mu_{APP}$ (< 0) and $\mu_{EOF}$ (> 0) at various pH values. By applying the pressure of 0.5 psi in addition to the normal electric field along the capillary, the peaks for the EOF marker and analytes were observed in the same run. As shown in Figure 1, fitting the data to Eq. (3) yielded p$K_a$ values in methanol of 5.7 + 0.3, 6.1 ± 0.1, 6.41 ± 0.07, and 7.01 + 0.09 for PCP, 2,3,5,6-TeCP, 2,3,4,6-TeCP, and 2,3,4,5-TeCP, respectively. The error ranges given are the 95% confidence intervals. Table 1 summarizes the fitting results for $\mu_0$ and p$K_a$. Note that the pH values of the methanol run buffer in this report were measured by a pH meter calibrated for aqueous solutions at 25 °C and the apparent p$K_a$ values in this report are not the thermodynamic ones which should include the activity corrections for the buffer ionic strength.[21-29]

Figure 2 shows that separation of the regulated substances PCP and 2,3,5,6-TeCP in a standard solution was achieved in less than 4 min. Furthermore, two additional isomers, 2,3,4,6-TeCP and 2,3,4,5-TeCP were baseline separated in the same run without any additives. Their migration order reflects their acidities in water, in ascending order of their aqueous p$K_a$ values. Efficiencies for the peaks ranged from

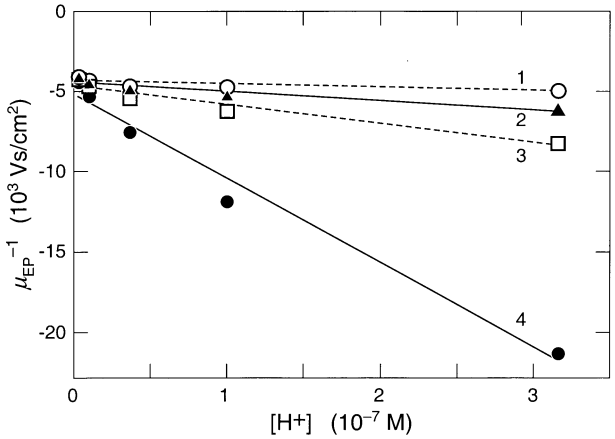


**Figure 1.** pH dependence of the electrophoretic mobilities of PCP (1. ○). 2,3,5,6-TeCP (2. ▲), 2,3,4,6-TeCP (3. □), and 2,3,4,5-TeCP (4. ●). Solid and dashed lines are the fits of data represented by closed and open symbols to Eq. (3), respectively.

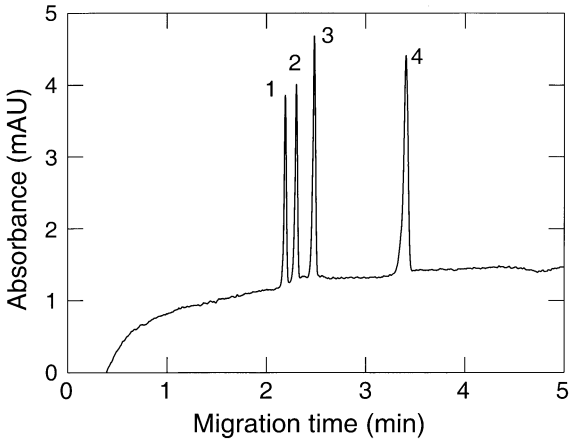


**Figure 2.** A typical electropherogram obtained from a standard solution of 20 $\mu$M each of PCP (1), 2,3,5,6-TeCP (2), 2,3,4,6-TeCP (3), and 2,3,4,5-TeCP (4) in 2-methoxyethanol. CE conditions: 100 mM Tris/acetate run buffer in methanol (pH 8.0); -20 kV applied along a 20/27-cm fused silica capillary of a 50-$\mu$m ID at 20 °C; hydrodynamic injection for 2 s at 0.5 psi nitrogen.

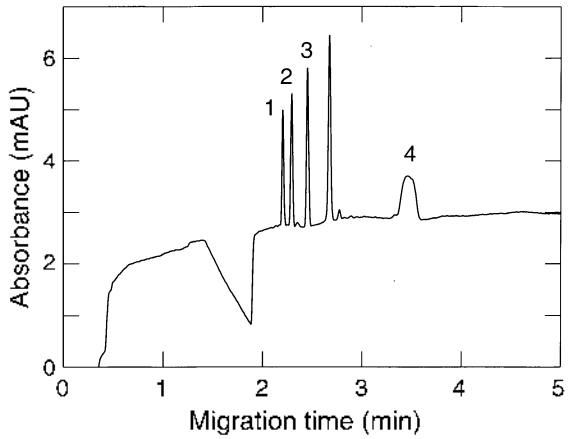


**Figure 4.** Electropherograms of a textile sample spiked with 20 $\mu$M each of the chlorinated phenols PCP (1) and 2,3,5,6-TeCP (2), 2,3,4,6-TeCP (3), and 2,3,4,5-TeCP (4). CE conditions: 100 mM Tris/acetate run buffer in methanol (pH 8.0); -20 kV applied along a 20/27-cm fused silica capillary of a 50-$\mu$m ID at 20 °C; hydrodynamic injection for 5 s at 0.5 psi nitrogen.

50000 to 60000.

The sample solution in 2-methoxyethanol has a lower conductivity than the 100-mM Tris/acetate run buffer in methanol. When a voltage is applied, a stronger electric field is produced in the dilute sample plug. The analytes rapidly migrate through the dilute sample plug until they reach the concentration boundary and slow down and form a narrow stacked zone. As the sample injection volume was increased from 2 sec to 20 sec, the peak heights were increased about 3 times (Figure 3).

The calibration curve was obtained by using the standard solutions of PCP and TeCPs in 2-methoxyethanol. In CE, the peak area is proportional to the migration time since later-eluting compounds move through the detector slower than earlier-eluting compounds. Thus any variations in peak area caused by changes in migration time can be compensated by using the peak area divided by the migration time, or equivalently the peak area multiplied by the electrophoretic velocity. The concentration *versus* the corrected peak area (= peak area multiplied by the electrophoretic velocity) was plotted, and its regression line was used for the determination of sample concentrations. The calibration curves were linear over the concentration range 0.1-200 $\mu$M. The relative standard deviations (RSD) of the migration time and the corrected peak area were obtained by analyzing the standard solution for six times. The limits of detection (LOD, $S/N = 3$) were about 0.02 $\mu$M in 2-methoxyethanol which corresponds to 0.1 ppm in a textile sample and is sufficient for detecting the regulated concentration of 0.5 ppm in the textile products classes II-IV of the Oeko-Tex Standard 100[1]. RSD of the migration time and the corrected peak area were in the ranges 0.6-1% and 2-4%, respectively. For CE analyses of aqueous samples, lower LODs were reported with extensive pretreatment and loading of the sample.[6,17,18,30,31] However, those are not suitable for textile samples dissolved in an organic solvent of 2-methoxyethanol prepared following the official test procedures.

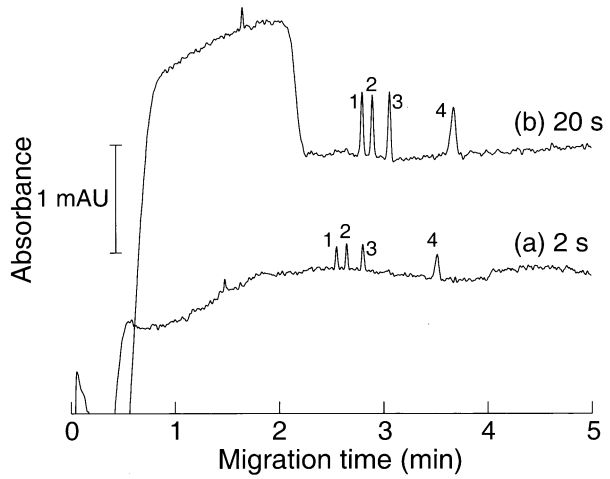


**Figure 3.** Sensitivity improvement with stacking. Electropherograms for (a) 2 s × 0.5 psi injection and (b) 20 s × 0.5 psi injection of 0.1 $\mu$M each of PCP (1), 2,3,5,6-TeCP (2), 2,3,4,6-TeCP (3), and 2,3,4,5-TeCP (4) in 2-methoxyethanol. Other CE conditions are the same as in Figure 2. The baseline fluctuations were due to the large injection volume of 2-methoxyethanol.

In order to verify that the efficiencies of our separation condition are sufficient for analyzing textile samples, the electropherogram of an actual textile sample prepared following the official preparation method is shown in Figure 4. The peaks of PCP and 3 TeCPs (20 $\mu$M each) were well separated from other peaks of the sample matrix. Under a reverse voltage, cations and neutrals including most anions could not migrate from the inlet to the outlet of the capillary. Only highly mobile anions such as chlorinated phenols could migrate against EOF toward the detector. Consequently, the analysis of chlorinated phenol standards as well as textile samples did not suffer much from interference. With further improvements of LOD through preconcentration, chlorinated

phenol levels in all textile product classes could be tested with the nonaqueous CE method.

## Conclusion

Chlorinated phenols in an organic solvent were determined by nonaqueous capillary electrophoresis with a basic methanol run buffer of pH 8.0. Since the anionic analytes were mobile enough to migrate against EOF, a reverse voltage was used and the resulting electropherograms displayed low interference from the textile sample matrix. LOD was about 0.02 $\mu$M using UV detection, which is about 2 orders of magnitude smaller than typical LOD of CE. The apparent $pK_a$ values of the chlorinated phenols in methanol were also estimated from the pH dependence of the electrophoretic mobilities.

**Acknowledgment**. This work was supported by the Korea Research Foundation Grant (KRF-97-003D00126) and the Korea Science and Engineering Foundation (KOSEF: 96-0501-08-03-3).

## References

1. http://www.oeko-tex.com/my_html/tab_e.html, Oeko-Tex Standard 100 (Accessed Oct 18 1999).
2. Chhabra, R.; Maronpot, R.; Bucher, J.; Haseman, J.; Toft, J.; Hejtmancik, M. *Toxicol. Sci.* **1999**, *48*, 14.
3. Ahlborg, U.; Larsson, K. *Arch. Toxicol.* **1978**, *40*, 63.
4. Boyd, T. J. *J. Chromatogr. A* **1994**, *662*, 281.
5. Renberg, L.; Lindström, K. *J. Chromatogr.* **1981**, *214*, 327.
6. Turnes, I.; Rodriguez, I.; Garcia, C. M.; Cela, R. *J. Chromatogr., A* **1996**, *743*, 283.
7. Mundy, D. F.; Machin, A. F. *J. Chromatogr.* **1981**, *216*, 229.
8. Daniels, C.; Swan, E. *J. Chromatogr. Sci.* **1979**, *17*, 628.
9. Ervin, H. E.; Meginnis, G. D. *J. Chromatogr.* **1980**, *190*, 203.
10. Martinez, D.; Pocurull, E.; Marce, R. M.; Borrull, F.; Calull, M. *Chromatographia* **1996**, *43*, 619.
11. Sajid, H.; Mohd, K. *J. Chromatogr.* **1979**, *168*, 517.
12. Song, L.; Xu, Z.; Kang, J.; Cheng, J. *J. Chromatogr., A* **1997**, *780*, 297.
13. Timerbaev, A. R. *Electrophoresis* **1997**, *18*, 185.
14. Dabek-Zlotorzynska, E. *Electrophoresis* **1997**, *18*, 2453.
15. Kang, S. H.; Kim, J.-W.; Chung, D. S. *J. Pharm. Biomed. Anal.* **1997**, *15*, 1435.
16. Kang, S. H.; Chang, S.-Y.; Chi, S.-C.; Chung, D. S. *J. Chromatogr. B* **1998**, *712*, 153.
17. Turnes, M. I.; Mejuto, M. C.; Cela, R. *J. Chromatogr., A* **1996**, *733*, 395.
18. Rodriguez, I.; Turnes, M. I.; Bollain, M. H.; Mejuto, M. C.; Cela, R. *J. Chromatogr., A* **1997**, *778*, 279.
19. *Safety Inspection Standards of Textile Goods Containing Harmful Substances 1996-322*; Agency for Technology and Standards, Ministry of Commerce, Industry and Energy: Seoul, 1996.
20. Groom, C. A.; Luong, J. H. T. *Electrophoresis* **1997**, *18*, 1166.
21. Gluck, S. J.; Cleveland, J. A., Jr. *J. Chromatogr., A* **1994**, *680*, 49.
22. Sarmini, K.; Kenndler, E. *J. Chromatogr., A* **1998**, *806*, 325.
23. Sarmini, K.; Kenndler, E. *J. Chromatogr., A* **1998**, *811*, 201.
24. Sarmini, K.; Kenndler, E. *J. Chromatogr., A* **1998**, *818*, 209.
25. Sarmini, K.; Kenndler, E. *J. Chromatogr., A* **1999**, *833*, 245.
26. Cleveland, J. A., Jr.; Benko, M. H.; Gluck, S. J.; Walbroehl, Y. M. *J. Chromatogr., A* **1993**, *652*, 301.
27. Fernando, R.; Marti, R.; Elisabeth, B. *Anal. Chim. Acta* **1998**, *374*, 309.
28. Barbosa, J.; Barron, E. *J. Chromatogr., A* **1999**, *839*, 183.
29. Mercier, J.-P.; Morin, P.; Dreux, M.; Tambute, A. *Chromatographia* **1998**, *48*, 529.
30. Praus, P. *Anal. Chim. Acta* **1995**, *302*, 39.
31. Martinez, D.; Pocurull, E.; Marce, R. M.; Borrull, F.; Calull, M. *J. Chrimatogr., A* **1996**, *734*, 367.
32. Ugland, K.; Lundanes, E.; Greibrokk, T.; Bjorseth, A. *J. Chromatogr.* **1981**, *213*, 83.