

Note

Capillary zone electrophoretic separation of chlorophenols in industrial waste water with on-column electrochemical detection

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(First received January 25th, 1990; revised manuscript received April 18th, 1990)

Phenolic compounds, in particular chlorinated phenols as pollutants in drinking water, cause concern because of their high toxicity and wide distribution. The release of these chlorophenols through waste water has led to the need for methods for monitoring these compounds in industrial effluents and natural waters.

A variety of methods such as liquid-liquid extraction^{1–3} ion-exchange techniques^{4,5}, gas chromatography (GC) with different derivatization procedures^{6–8}, spectrophotometry^{9,10}, colorimetry¹¹ and high-performance thin-layer chromatography¹² have been used for the determination chlorophenols in waters. However, these were either tedious and time consuming or often tiresome. Reversed-phase high-performance liquid chromatography (HPLC) as applied to isomeric chlorophenols in waste waters with UV^{13–16}, fluorescence¹⁷ and electrochemical^{18–20} detection has already been reported. Electrokinetic chromatography with micellar solutions for the separation of chlorophenols as reported by Terabe *et al.*²¹ and Otsuka *et al.*²² employed UV detection, providing detection levels in the nanogram range.

The objective of this work was to study the suitability of capillary zone electrophoresis (CZE) for the separation of chlorinated phenols in industrial waste waters. A fused-silica capillary was employed with an applied potential of 20 kV. Detection was performed with an on-column electrochemical detector modified with carbon fibres. Detection levels in the picomole range were achieved with separation efficiencies of the order of 320 000 theoretical plates.

EXPERIMENTAL

Reagents and chemicals

The standards of chlorinated phenols (2-chloro-, 4-chloro-, 2,4-dichloro-, 2,6-dichloro-, 2,4,6-trichloro-, 2,3,4,6-tetrachloro- and pentachlorophenol) were obtained from Sigma (St. Louis, MO, U.S.A.) and phenol, *o*-phenylphenol, catechol

and 4,5,6-trichloroguaiacol from S. D. Fine Chemicals (Boisar, Tarapur, India). They were dissolved in methanol–water (1:1) to give a concentration of 5 ng/l of each of the components. Sodium dihydrogenphosphate and sodium borate were obtained from E. Merck (India) (Worli, Bombay, India). Other reagents were of analytical-reagent grade from S. D. Fine Chemicals and were used as received.

Industrial waste water samples containing isomeric chlorophenols and neutral phenols were the gifts from Dharamsi Morarji Chemicals (Ambarnath, India). Millipore Milli-Q water was used wherever necessary and to prepare sodium dihydrogenphosphate (0.045 *M*) and sodium borate (0.015 *M*) buffers for mixing in an appropriate ratio to give pH 8.0.

Extraction and CZE apparatus

Waste water samples (5 ml) were shaken with 5 ml of chloroform–diethyl ether (1:1) in a separating flask. An aliquot (1 ml) of the organic layer was diluted with sodium dihydrogen orthophosphate (0.045 *M*)–sodium borate (0.015 *M*) (1:1) and the pH was adjusted to 8.0. This was used directly for CZE.

All CZE experiments were performed using a 650 mm × 25 μ m I.D. fused-silica capillary (Scientific Glass Engineering). On-column measurements were carried out 300 mm from the negative end of the capillary using an electrochemical detector modified using carbon fibres (10- μ m diameter) as described by Knecht *et al.*²³. The connections of the capillary to the electrochemical detector were the same as employed by Ewing *et al.*²⁴, except for the porous-glass joint assembly connector. Detection was performed in the amperometric mode with an oxidation potential of +1.4 V vs. SCE. The temperature in the separation capillary unit was maintained at 20–25°C during the separation using a thermostated oven. A capillary was immersed in a mixture of buffer

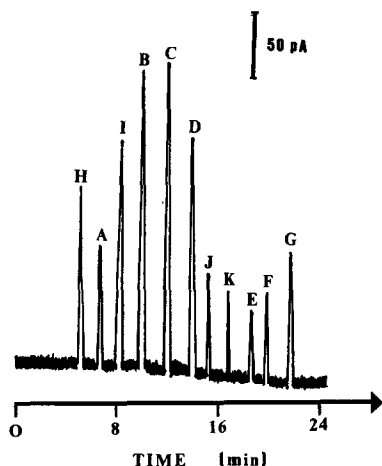


Fig. 1. Electropherogram of a standard mixture of isomeric chlorophenols, 4,5,6-trichloroguaiacol and neutral phenols (5–8 ng/l of each). Electrochemical detection at an applied voltage of 20 kV, carbon fibres, +1.4 V vs. SCE. Peaks: A = 2-chlorophenol; B = 2,4-dichlorophenol; C = 2,6-dichlorophenol; D = *o*-phenylphenol; E = 2,3,4,6-tetrachlorophenol; F = 4,5,6-trichloroguaiacol; G = pentachlorophenol; H = phenol; I = 4-chlorophenol; J = catechol; K = 2,4,6-trichlorophenol.

solution from the two buffer reservoirs which were kept at the same levels. Sample introduction was accomplished as described by Rose and Jorgenson²⁵, at a constant voltage (20 kV, high-voltage d.c. power supply, Siemens, Amberg, F.R.G.) at the anodic end for exactly 30 s. The detector signal was recorded on an Omniscribe Model 5000 strip-chart recorder (Digital Electronics, Andheri, Bombay, India).

RESULTS AND DISCUSSION

An electropherogram of a standard mixture of seven chlorophenols together with phenol, *o*-phenylphenol catechol and 4,5,6-trichloroguaiacol is shown in Fig. 1. The amount of each component in the mixture injected was estimated to be 5–8 ng/l. The applied voltage between the two ends of the 650-mm capillary was 20 kV. Detection was performed on-column using an oxidation potential of +1.4 V vs. SCE. All the compounds were completely resolved within 24 min, the minimum detectable amount being 0.001 ng/ml. The use of a small-diameter capillary (25 μm) enhances the detection with a signal-to-noise ratio of 4.5. Attempts were made to optimize the separation conditions by using different capillary lengths of 400, 500, 600, 650, 800 and 1000 mm and an applied voltage of 20 kV. The theoretical plate number (N) was calculated using the equation²⁴

$$N = \frac{(\mu_c + \mu_{eo})V}{2D}$$

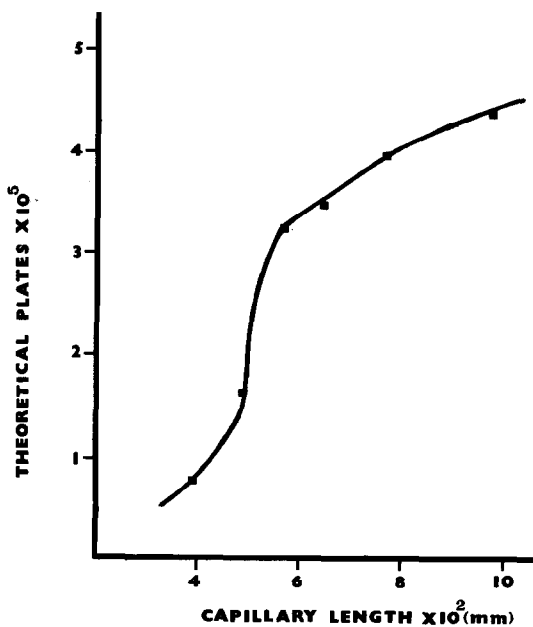


Fig. 2. Theoretical plate number as a function of capillary length (25 μm I.D.) with a buffer mixture of sodium dihydrogenphosphate (0.045 M)–sodium borate (0.015 M) adjusted to pH 8.0. Detection conditions as in Fig. 1. Sample, 2-chlorophenol.

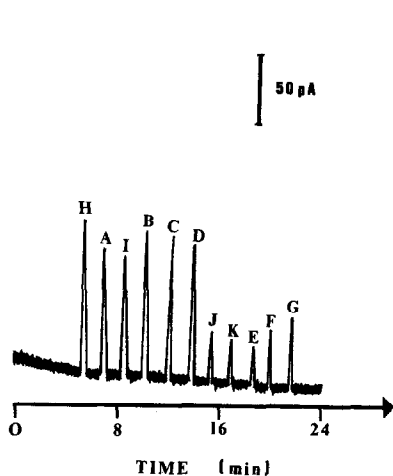


Fig. 3. Electropherogram of an industrial waste water sample with a 2-chlorophenol concentration of 50 ng/ml. Conditions and peaks as in Fig. 1.

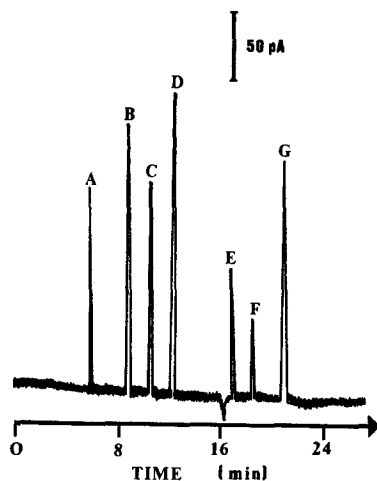


Fig. 4. Electropherogram of an industrial waste water sample with a 2-chlorophenol concentration of 100 ng/ml. Conditions and peaks as in Fig. 1.

where, μ_c and μ_{eo} are the electrophoretic and electroosmotic mobilities, respectively, at the applied voltage V and D is the diffusion coefficient.

The values of N were then plotted against capillary length for 2-chlorophenol as shown in Fig. 2. The results of this experiment clearly indicate that, as the length of the capillary increases beyond 650 mm, the theoretical plate number increases, thereby prolonging the separation of 2-chlorophenol (>60 min) from other components and causing peak broadening. This was also observed for other chlorophenols (results not shown). Important contributors to the peak broadening effect were found to be longitudinal diffusion resulting in a flat flow profile and interactions between the capillary walls and analytes, as noted by others²⁶.

TABLE I

RETENTION TIME DATA FOR THE COMPOUNDS ANALYSED BY CZE

Results obtained on a 650 mm \times 25 μ m I.D. capillary, 20 kV, +1.4 V vs. SCE.

Compound ^a	Retention time (min)	Compound ^a	Retention time (min)
Phenol (H)	5.8	Catechol (J)	15.6
2-Chlorophenol (A)	7.1	2,4,6-Trichlorophenol (K)	17.0
4-Chlorophenol (I)	8.1	2,3,4,6-Tetrachlorophenol (E)	19.1
2,4-Dichlorophenol (B)	10.1	4,5,6-Trichloroguaiacol (F)	20.0
2,6-Dichlorophenol (C)	12.0	Pentachlorophenol (G)	22.0
<i>o</i> -Phenyl phenol (D)	13.8		

^a Letters in parentheses indicate peaks in Figs. 1, 3 and 4.

Two samples of industrial waste water containing chlorophenol isomers and neutral phenols were subjected to CZE. The chlorophenols were well separated, as shown in Figs. 3 and 4. The limits of detection of each of these components in waste water were found to be 0.005 ng/ml and the analysable concentration range was the same as for model compounds. Table I gives retention time data for these compounds, demonstrating the effective resolution achieved with this system. No interferences from the impurities present in industrial waste water samples were observed.

CONCLUSION

The proposed method of CZE as applied to the separation of chlorophenols and other phenols in industrial waste water samples is more promising than techniques such as HPLC and GC. The use of an on-line electrochemical detector provides excellent sensitivity and selectivity without derivatization or tedious extraction of the sample.

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