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Capillary zone electrophoresis of eleven priority phenols with indirect fluorescence detection

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Abstract

A scheme for the separation and detection of eleven priority phenols using capillary zone electrophoresis (CZE) coupled with laser-induced indirect fluorimetry is described. With a 50 cm × 20 μm I.D. capillary at 9 kV and an electrophoretic buffer of 15 m*M* sodium borate (pH 9.9) containing 1 m*M* fluorescein, complete separation of the eleven compounds can be performed in less than 14 min. Linearity over two orders of magnitude of concentration was generally obtained and limits of detection for the priority phenols were in the ppb (10^{-6}–10^{-7} *M*) range. Quantitative applicability of CZE–indirect fluorimetry was demonstrated by analysing the standard reference material NIST SRM 1584 (priority pollutant phenols in methanol). The method was also applied to the determination of phenols in industrial wastewaters.

1. Introduction

Phenolic compounds are of great environmental concern owing to their high toxicity. The US Environmental Protection Agency (EPA) has listed eleven phenols as organic priority pollutants [1], *viz.*, phenol, 2-nitrophenol (2-NP), 4-nitrophenol (4-NP), 2,4-dinitrophenol (2,4-DNP), 2-chlorophenol (2-CP), 2,4-dichlorophenol (2,4-DCP), 2,4-dimethylphenol (2,4-DMP), 4-chloro-3-methylphenol (4-C-3-MP), 2-methyl-4,6-dinitrophenol (2-M-4,6-DNP), 2,4,6-trichlorophenol (2,4,6-TCP) and pentachlorophenol (PCP). Their concentration level in the environment needs to be constantly monitored.

Both gas chromatography (GC) and high-performance liquid chromatography (HPLC) have been the commonly used techniques for analyses for phenols. In order to enhance the volatility and detectability of phenols, sample derivatization is often necessary prior to GC analysis. In general, GC methods suffer from disadvantages such as lengthy sample preparation time and incomplete recoveries for many phenolic derivatives. Alternatively, HPLC is an advantageous technique and the polarity of phenols and their low vapour pressure, factors that complicate GC analysis, do not have adverse effects on HPLC analysis. HPLC with either isocratic [2,3] or gradient elution [4,5] has been widely used to separate substituted phenols. Ultraviolet (UV) detection is commonly employed as phenols possess strong absorption bands in the UV region. Better sensitivity can be achieved by employing electrochemical detection [2,6].

In the past few years, capillary electrophoresis (CE) has been shown to be a fast, powerful, and efficient separation technique for a variety of

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compounds [7]. These characteristics are the direct result of the use of a high separation voltage and rapid dissipation of Joule heat in a narrow capillary, typically 25–100 μm I.D. In capillary zone electrophoresis (CZE), a capillary is filled with a buffer solution for the separation of only charged analytes, and neutral substances cannot in principle be separated by conventional CZE. Micellar electrokinetic capillary chromatography (MECC), in which ionic surfactants are added to the CZE buffer at concentrations exceeding the critical micelle concentration (cmc), has extended the enormous power of CZE to the separation of both charged and uncharged solutes [8,9]. The analysis of a series of chlorophenols [8–10] and eleven priority phenols [11] using MECC with UV detection has been demonstrated. An analysis time of *ca.* 45 min was required to separate the eleven priority phenols and the detection levels were in the nanogram range. However, CE with on-column UV detection generally suffers from the disadvantages that the sensitivity is limited by the short optical path length as a result of the use of small I.D. capillaries and the need for chromophoric analyte molecules.

Recently, fluorescence detection, particularly laser-induced fluorimetry, has become popular mainly because of its capability to provide extremely high sensitivity. Detection limits of 10^{-21} mol of fluorescein isothiocyanate-labelled amino acids have been reported [12]. However, a drawback of this detection mode is the need to derivatize most analytes because only a few compounds show native fluorescence. An alternative to derivatizing non-fluorescent substances is the use of indirect detection techniques. The application of indirect fluorimetry in CZE has been reviewed recently [13]. In brief, a non-interacting, fluorescing ion is added 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. Indirect fluorimetry has been applied in CZE for the detection of metal ions [14,15], sugars [16], amino acids [17], peptides [18], nucleotides [19] and DNA restriction fragments [20], and in MECC for the detection of aliphatic alcohols and some phenolic compounds [21].

In this paper, a scheme for the separation and detection of the eleven priority phenols using free solution CZE coupled with indirect fluorescence detection is described. Fluorescein was added to the running buffer as the fluorescing ion and an argon ion laser was used to induce the fluorescence background. Detection limits, linearity and reproducibility were examined. Application of the method to the analysis of priority phenols in industrial wastewaters is also described.

2. Experimental

2.1. Apparatus

The CZE system was assembled in-house. A high-voltage power supply (Model PS/MJ30P0400-11; Glassman High Voltage, Whitehouse Station, NJ, USA) was used to generate the potential across the capillary. Fused-silica capillaries (Polymicro Technologies, Phoenix, AZ, USA) were of 50 cm total length × 20 μm I.D. × 360 μm O.D. Before use, the capillary was washed (pressurized flow) with 0.1 *M* NaOH for 15 min, followed by a 2-min rinse with water and a 2-min flush with the running buffer. The capillary was then equilibrated with the buffer under an electric field of 200 V cm^{-1} for 2 h. Samples were injected by either electromigration or gravity flow injection. Electromigration was used when obtaining migration times for peak identification. Gravity flow injection was used when quantitative measurements of peaks were made.

On-column detection was performed using indirect fluorimetry. A small region of the polymer coating was burned off 5 cm from the cathodic end of the capillary to form a detection window. An argon ion laser (Model ILT-5000; Ion Laser Technology, UT, USA) operating in the light-regulated mode at 10 mW for all lines was used for excitation. The 488-nm beam (about 40% of the total power) was selected with an interference filter (peak wavelength 488 nm,

effective bandwidth 10 nm) (Edmund Scientific, Barrington, NJ, USA). The laser light was focused into the capillary with a 1-cm focal length lens. The capillary was mounted at Brewster's angle to reduce scattered radiation. Background fluorescence emitted from the fluorophore in the CZE buffer was collected with a 10× microscope objective and passed through a 520-nm interference filter (effective bandwidth 10 nm; Edmund Scientific). The fluorescent image was focused on to a silicon photodiode (Model S2281-01; Hamamatsu, Hamamatsu City, Japan) connected with a current amplifier (Model S2719; Hamamatsu). The high background output from the detector was first lowered with a laboratory-built voltage offset circuit, followed by passing through a 1-s *RC* low-pass filter. The data were collected using a Macintosh SE computer connected with a MacLab/4 data acquisition interface (Analog Digital Instruments, NSW, Australia).

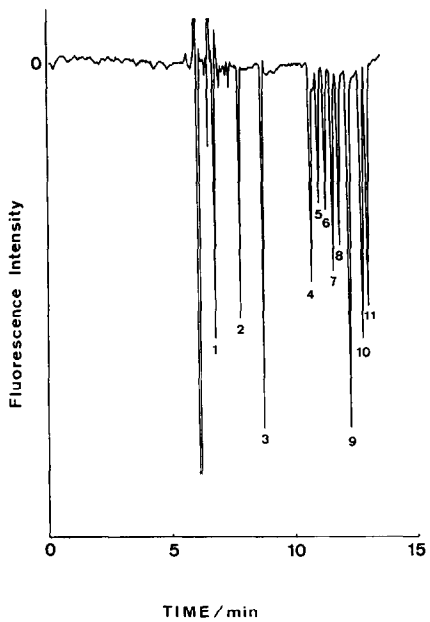

Fig. 1. Electropherogram of the eleven priority phenols with indirect fluorescence detection. Conditions are given in Table 1.

2.2. Chemicals

All phenols were purchased from Supelco (Bellefonte, PA, USA) and used as received. Laser-grade fluorescein was obtained from Eastman Kodak (Rochester, NY, USA). Standard reference material for priority pollutant phenols, SRM 1584, was purchased from the National Institute of Standards and Technology (NIST, Gaithersburg, MD, USA). All other chemicals were of analytical-reagent grade. Distilled water was further purified by passing it through a NANOpure II deionization system (Sybron-Barnstead, Boston, MA, USA). All solutions were filtered through a 0.45-μm pore-size membrane filter before use.

3. Results and discussion

3.1. Separation and detection of the eleven priority phenols

The electropherogram in Fig. 1 shows the separation and detection of the eleven priority phenols using CZE and indirect fluorescence detection. All components of the test mixture are baseline resolved. The large derivative peak at *ca.* 6 min was due to methanol, which was the solvent used for preparing the stock solutions of phenols. Note that all eleven compounds elute within 14 min of injection, which is to be compared to the 25 and 45 min typically needed using HPLC [3] and MECC [11], respectively. The concentrations of the eleven phenols in Fig. 1 are given in Table 1, together with their migration times. The repeatabilities of the migration times for the eleven phenols are good, with relative standard deviations (R.S.D.s) all less than 1%, based on fifteen replicate determinations.

The separation efficiency, represented by the number of theoretical plates, N, was calculated from the peak half-width for each phenol. The peak width and hence the efficiency were found to be governed by both the separation voltage and the duration of injection. With a separation voltage of 9 kV and an electromigration injection time of 2 s, N ranged from 187 000 for phenol to 99 000 for 2,4-DNP (see Table 1).

It is interesting that the elution order of the eleven phenols found in CZE is opposite to that

Table 1
Concentrations, migration times and efficiencies of the eleven phenols in Fig. 1

Peak No.	Compound	Concentration (μg ml^{-1})	Migration time (min)	R.S.D.[a] (%)	N ($\times 10^4$)
1	2,4-DMP	25.2	6.89	0.74	10.6
2	Phenol	0.2	7.94	0.69	18.7
3	4-C-3-MP	0.9	8.89	0.72	11.4
4	PCP	10.8	10.84	0.87	11.9
5	2,4,6-TCP	0.4	11.16	0.87	12.6
6	2,4-DCP	0.4	11.43	0.88	11.1
7	2-M-4,6-DNP	3.6	11.72	0.99	10.3
8	2-CP	0.4	11.98	0.87	12.5
9	2,4-DNP	7.2	12.36	0.90	9.9
10	4-NP	7.2	12.88	0.90	10.8
11	2-NP	10.8	13.09	0.95	11.2

Conditions: column, 50 cm total length (45 cm to the window) × 20 μm I.D.; buffer, 15 mM sodium borate (pH 9.9) containing 1 mM fluorescein; injection, 2 s at 9 kV; electrophoresis, 2.8 μA at 9 kV.
[a] Relative standard deviation of migration times ($n = 15$).

obtained by Ong *et al.* [11] using MECC with a neutral buffer (pH 6.6). For example, 2,4-DMP, phenol and 4-C-3-MP, which are the first three peaks in Fig. 1, were eluted last by MECC, while 4-NP and 2-NP, which are the last in Fig. 1, were eluted first by MECC. This is understandable because the separation mechanisms of CZE and MECC are basically different. The separation in CZE is based on the differences in the electrophoretic mobilities resulting in different velocities of migration of ionic species in the electrophoretic buffer contained in the capillary. Therefore, the separation mechanism in Fig. 1 is mainly based on differences in size and charge of the eleven phenols at a given pH. On the other hand, the main separation mechanism in MECC is based on solute partitioning between the micellar phase and the solution phase. A combination of charge/mass ratios, hydrophobicity and charge interactions at the surface of the micelles combine to affect the separation of the analytes.

In order to improve the efficiency of charge displacement and therefore the sensitivity in indirect fluorescence detection, a buffer with low ionic strength, or preferably a low concentration of fluorescing ion as part of the buffer, has been recommended [13]. However, we found that, in addition to the buffer pH, a relatively high concentration (>10 mM) of borate in the electrophoretic buffer was crucial in the separation of the eleven phenols. With lower borate concentrations, complete separation of the eleven phenols was not possible. This is probably because the electroosmotic velocity is inversely proportional to ionic concentration [22]. Decreasing the buffer concentration induces an increase in electroosmotic velocity, which is generally detrimental to the CZE separation of analytes with similar electrophoretic mobilities. On the other hand, the increase in the concentration of background electrolyte will have an adverse effect on the sensitivity of indirect detection. In principle, a highly sensitive indirect fluorescence signal requires a high transfer ratio (TR, defined as the number of background fluorophore ions displaced by one analyte ion [13]). Unfortunately, borate anions also can be displaced along with fluorescein, which results in a loss of sensitivity. The electrophoretic buffer in Fig. 1 was a solution of 15 mM sodium borate (pH 9.9) containing 1 mM fluorescein as the background fluorophore, which represents a compromise between optimum peak resolution and satisfactory detection sensitivity.

During the investigation of the optimum fluorophore concentration, we also found that the direction of some peaks was affected by both

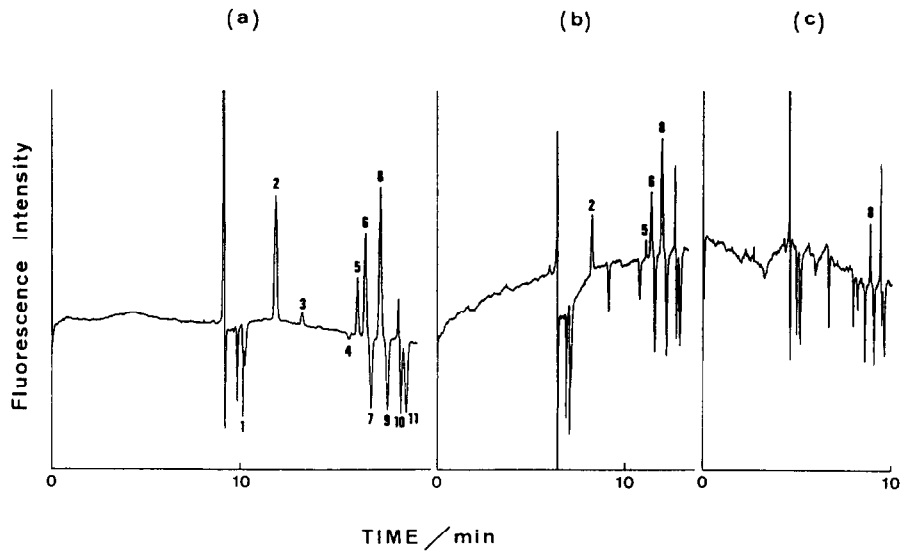

Fig. 2. Electropherograms of phenols obtained under different electric fields with a buffer containing 0.1 m*M* fluorescein: (a) 120; (b) 180; (c) 240 V cm^{-1}. Peak identities and conditions as in Table 1.

electric field and fluorescein concentration. These phenomena are illustrated in Figs. 2 and 3. With a fluorescein concentration of 0.1 m*M* and an electric field of 120 V cm^{-1}, five phenols, *viz.*, phenol, 4-C-3-MP, 2,4,6-TCP, 2,4-DCP and 2-CP, showed positive peaks (Fig. 2a). An increase in field strength caused a gradual reverse of peak position (Fig. 2b), and only the 2-CP

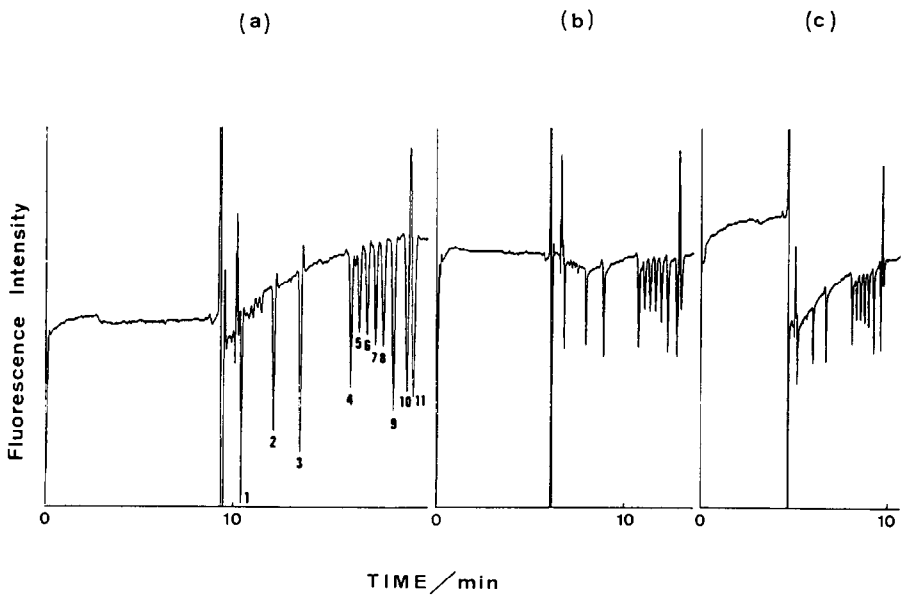

Fig. 3. Electropherograms of phenols obtained under different electric fields with a buffer containing 1 m*M* fluorescein: (a) 120; (b) 180; (c) 240 V cm^{-1}. Peak identities and conditions as in Table 1.

peak was positive-going at a field strength of 240 V cm^{-1} (Fig. 2c). This trend was not observed if the fluorescein concentration was raised to 1 m*M* (Fig. 3). Positive peaks indicate an increase in the concentration of the background fluorophore present at the detector, while negative peaks indicate a decrease. Negative peaks in indirect detection (positive displacement) have positive *TR* values [13], and therefore *TR* values for positive peaks (negative displacement) must carry a minus sign. The negative *TR* values found for those phenols cannot be accounted for by the simple indirect detection theory. Negative *TR* values have also been reported by Williams *et al.* [23] in the indirect absorbance detection of tetraalkylammonium compounds. However, the detailed mechanism behind negative displacement (positive peaks) in indirect detection is not fully understood and further study in this respect is needed.

3.2. Calibration graphs for the eleven priority phenols

A series of solution mixtures containing known amounts of the eleven phenols were prepared using the electrophoretic buffer as the solvent. These standard solutions were used for the construction of calibration graphs. Samples were injected by gravity flow at a 12-cm height for 20 s. The results are summarized in Table 2. Within the concentration range studied, a good linear correlation ($r \geq 0.99$) between peak height and concentration was obtained for each species. The linear dynamic range generally covered more than two orders of magnitude of concentration. The linearity obtained by electromigration injection (2 s at 9 kV) was poorer, covering less than two orders of magnitude of concentration. Similar results were obtained if peak areas were used for regression analysis. From the slopes of the regression lines listed in Table 2, considerably higher sensitivity was obtained for phenol, 4-C-3-MP, 2,4,6-TCP, 2,4-DCP and 2-CP. This is probably due to fluorescence quenching by phenol and chlorophenols (via the external heavy atom effect [24]) on the fluorescein, which further decreases the background fluorescence. Phenols have been shown to have a strong quenching effect on quinine fluorescence [21].

The reproducibility was examined by seven replicate injections of each compound at a concentration corresponding to the lower limit of the calibration line. The R.S.D.s on peak heights

Table 2
Calibration data for the eleven priority phenols

Peak No.	Compound	Linear range ($\mu g\ ml^{-1}$)	Slope (mV ml μg^{-1})	Intercept (mV)	r^a	R.S.D.[b] (%)	LOD[c] ($\mu g\ ml^{-1}$)
1	2,4-DMP	1.61–57.60	2.7	+3.5	0.997	3.2	0.75
2	Phenol	0.01– 3.69	195.8	+14.3	0.986	2.7	0.01
3	4-C-3-MP	0.06– 9.22	64.0	+7.5	0.988	4.3	0.02
4	PCP	0.69–57.60	4.5	+2.9	0.999	3.7	0.48
5	2,4,6-TCP	0.02– 3.69	73.0	+8.2	0.992	6.3	0.02
6	2,4-DCP	0.02– 3.69	67.6	+11.7	0.993	4.3	0.02
7	2-M-4,6-DNP	0.23–57.60	13.9	+3.6	0.999	4.9	0.17
8	2-CP	0.02– 3.69	137.2	+4.0	0.996	4.7	0.02
9	2,4-DNP	0.46–36.00	8.3	+13.1	0.990	5.9	0.15
10	4-NP	0.46–57.60	4.6	+3.3	0.998	5.2	0.38
11	2-NP	0.69–57.60	4.2	+1.4	0.992	2.8	0.33

Gravity flow injection at 12-cm height for 20 s; other conditions as in Table 1.
[a] Correlation coefficients ($n = 7$).
[b] Based on seven measurements with replicate injections of each compound at the concentration corresponding to the lower limit of the calibration line.
[c] Signal-to-noise ratio = 3.

ranged from 2.7% for phenol to 6.3% for 2,4,6-TCP. The R.S.D.s on peak areas were slightly larger (4.7–9.2%), which was probably due to the lower accuracy of the integration data at concentrations near the detection limits. The limits of detection (LOD) listed in Table 2 were calculated based on a ratio of the signal to the background noise level of 3, which range from 0.01 μg ml^{-1} (0.11 μM) for phenol to 0.75 μg ml^{-1} (6.14 μM) for 2,4-DMP. These values are in agreement with the LOD levels achievable with CZE–indirect fluorimetry [13], and are much better than those with UV detection [11]. An electropherogram of the eleven phenols at concentrations close to their respective LODs are given in Fig. 4.

3.3. Applications

In order to validate the method, the concentrations of the eleven phenols were determined in a standard reference material, NIST SRM 1584 (priority pollutant phenols in methanol). For the determination of phenol, 4-C-3-MP, 2,4,6-TCP, 2,4-DCP and 2-CP, which possess a higher detection sensitivity, the sample was diluted 250-fold with the electrophoretic buffer before analysis. For determining the others, the sample was diluted tenfold prior to analysis. Quantification of each compound was performed using the calibration lines constructed simultaneously with the analysis of sample. The results are given in Table 3, along with the certified values for the eleven phenols. The experimental results are in good agreement with the certified values for most phenols, but not phenol, 2,4-DCP and 2-CP. One of the reasons for the larger deviations of these three is probably the larger dilution factor, which amplifies the experimental errors. Nevertheless, the results in Table 3 clearly demonstrate the applicability of CZE–indirect fluorimetry to the determination of phenols.

The method was applied to the determination of priority phenols in two industrial wastewater samples taken from a coke plant, one an untreated wastewater and the other an effluent

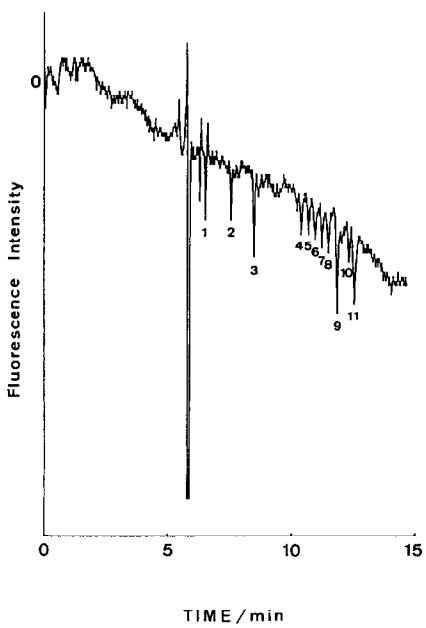

Fig. 4. Electropherogram of the eleven priority phenols at trace levels. Gravity flow injection at a 12-cm height for 20 s; the concentration of each compound corresponds to the lower limit of the respective calibration line in Table 2. Other conditions as in Table 1.

Table 3
Determination of the eleven phenols in NIST SRM 1584 by CZE–indirect fluorimetry

Compound	Concentration (μg ml^{-1})	
	Experimental[a]	Certified
2,4-DMP	51.1 ± 2.9	51.6 ± 0.2
Phenol	33.2 ± 1.4[b]	29.7 ± 0.9
4-C-3-MP	26.8 ± 0.9[b]	27.4 ± 0.9
PCP	16.8 ± 0.9	15.4 ± 1.1
2,4,6-TCP	19.8 ± 0.5[b]	20.4 ± 1.9
2,4-DCP	31.9 ± 0.9[b]	35.6 ± 1.3
2-M-4,6-DNP	23.2 ± 1.0	20.1 ± 0.9
2-CP	73.8 ± 1.0[b]	64.4 ± 1.4
2,4-DNP	23.9 ± 1.2	22.4
4-NP	20.9 ± 2.7	20.7 ± 0.7
2-NP	23.9 ± 2.6	25.2 ± 0.7

Conditions as in Table 2.

[a] Uncertainties are given as 95% confidence intervals about the mean ($n = 5$). Before analysis, sample was diluted tenfold with the electrophoretic buffer.

[b] The sample was diluted 250-fold with the electrophoretic buffer.

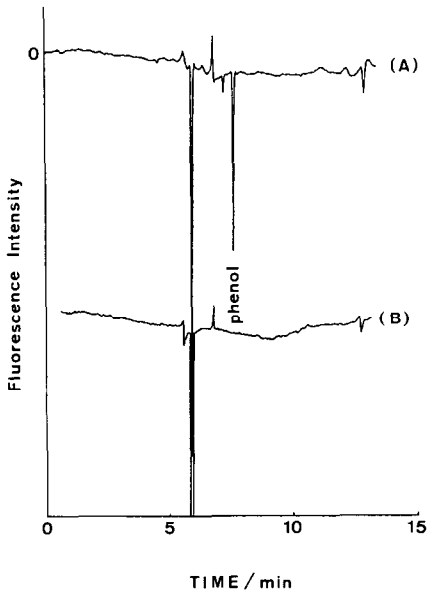

Fig. 5. Electropherograms of (A) an untreated industrial wastewater sample (500-fold dilution) and (B) a treated wastewater sample (tenfold dilution). Conditions as in Table 1.

from the microbiological treatment plant. Both samples were first filtered through a 0.45-μm membrane filter. For the untreated wastewater sample, a 500-fold dilution with the electrophoretic buffer was made before analysis. A typical electropherogram obtained for the untreated wastewater is shown in Fig. 5A. Only phenol could be found in this sample. No interferences from the impurities present in the sample were observed. The concentration of phenol in this untreated wastewater was determined to be 269 ± 8 μg ml^{-1}, based on five replicate analyses. Fig. 5B shows the electropherogram obtained from the treated wastewater sample. This sample was diluted tenfold with the electrophoretic buffer before analysis. No phenol was found in the effluent after microbiological treatment.

4. Conclusions

CZE with laser-induced indirect fluorimetry can provide rapid separation and sensitive detection of the eleven priority phenols. Complete separation of the eleven compounds can be achieved in *ca.* 14 min using an electrophoretic buffer of 15 m*M* sodium borate (pH 9.9) containing 1 m*M* fluorescein. Linearity of the calibration graphs over two orders of magnitude of concentration was generally obtained and the limits of detection were in the ppb (10^{-6}–10^{-7} *M*) range. Quantitative applicability of the CZE–indirect fluorimetry method was demonstrated by analysing the standard reference material NIST SRM 1584. The method was successfully applied to the determination of priority phenols in industrial wastewaters.

5. Acknowledgement

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6. References

[1] *Sampling and Analysis Procedures for Screening of Industrial Effluents for Priority Pollutants*, US Environmental Protection Agency, Environment Monitoring and Support Laboratory, Cincinnati, OH, 1977.
[2] C.W. Whang, *J. Chin. Chem. Soc. (Taipei)*, 34 (1987) 81.
[3] H.K. Lee, S.F.Y. Li and Y.H. Tay, *J. Chromatogr.*, 438 (1988) 429.
[4] S. Hussain and M. Kifayatulla, *J. Chromatogr.*, 168 (1979) 517.
[5] D.N. Armentrout, J.D. McLean and M.W. Long, *Anal. Chem.*, 51 (1979) 1039.
[6] R.E. Shoup and G.S. Mayer, *Anal. Chem.*, 54 (1982) 1164.
[7] W.G. Kuhr and C.A. Monnig, *Anal. Chem.*, 64 (1992) 389R.
[8] S. Terabe, K. Otsuka, K. Ichikawa, A. Tsuchiya and T. Ando, *Anal. Chem.*, 56 (1984) 111.
[9] S. Terabe, K. Otsuka and T. Ando, *Anal. Chem.*, 57 (1985) 834.
[10] M.G. Khaledi, S.C. Smith and J.K. Strasters, *Anal. Chem.*, 63 (1991) 1820.
[11] C.P. Ong, C.L. Ng, N.C. Chong, H.K. Lee and S.F.Y. Li, *J. Chromatogr.*, 516 (1990) 263.
[12] S. Wu and N.J. Dovichi, *J. Chromatogr.*, 480 (1989) 141.
[13] E.S. Yeung and W.G. Kuhr, *Anal. Chem.*, 63 (1991) 275A.

[14] L. Gross and E.S. Yeung, *Anal. Chem.*, 62 (1990) 427.
[15] L. Gross and E.S. Yeung, *J. Chromatogr.*, 480 (1989) 169.
[16] T. Garner and E.S. Yeung, *J. Chromatogr.*, 515 (1990) 639.
[17] W.G. Kuhr and E.S. Yeung, *Anal. Chem.*, 60 (1988) 1832.
[18] B.L. Hogan and E.S. Yeung, *J. Chromatogr. Sci.*, 28 (1990) 15.
[19] W.G. Kuhr and E.S. Yeung, *Anal. Chem.*, 60 (1988) 2642.
[20] K.C. Chan, C.W. Whang and E.S. Yeung, *J. Liq. Chromatogr.*, 16 (1993) 1941.
[21] L.N. Amankwa and W.G. Kuhr, *Anal. Chem.*, 63 (1991) 1733.
[22] T. Tsuda, K. Nomura and G. Nakagawa, *J. Chromatogr.*, 248 (1982) 241.
[23] S.J. Williams, E.T. Bergström, D.M. Goodall, H. Kawazumi and K.P. Evans, *J. Chromatogr.*, 636 (1993) 39.
[24] J.D. Ingle, Jr., and S.R. Crouch, *Spectrochemical Analysis*, Prentice-Hall, Englewood Cliffs, NJ, 1988, Ch. 12.