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Comparison of micellar electrokinetic chromatography (MEKC) with capillary gas chromatography in the separation of phenols, anilines and polynuclear aromatics Potential field-screening applications of MEKC

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Abstract

Capillary electrophoresis (CE) is known to be complementary to liquid chromatography, but comparison of CE with capillary gas chromatography (GC) for applicable analytes has not been extensive. Capillary GC has been the preeminent separation technique for environmental analysis, but CE has yet to be applied systematically to the determination of environmental analytes. We present data on separations of three classes of semivolatile analytes of interest to environmental analysis: phenols, anilines and polynuclear aromatic hydrocarbons (PNAs). Standard GC conditions were used to illustrate typical separations observed on 30-m and 40-m columns. Rapid analyses were addressed using a high-temperature 15-m column of thinner film. CE separations employed borate buffer with sodium cholate as the micellar agent in micellar electrokinetic chromatography (MEKC). The effects of organic additives were studied using methanol, acetone and tetrahydrofuran. γ-Cyclodextrin was also used in MEKC to enhance the separation of polynuclear aromatic hydrocarbons and to examine its effects on separations of phenols and anilines. Short capillaries effected very rapid (< 3 min) compound-class characterization, an approach which has potential use in site characterization/remediation (field-screening) studies

1. Introduction

Capillary gas chromatography (cGC) is the preeminent separation technique in environmental analysis, especially for volatile and semivolatile analytes. For example, US Environmental Protection Agency (EPA) Methods 625 [1] and 8270 [2] use cGC for separation of analytes. The Environmental Monitoring Systems Laboratory in Las Vegas has a continuing interest in improving methodology based on cGC–mass spectrometry (MS) [3,4] and in investigating new analytical techniques such as high-performance capillary electrophoresis (HPCE) for target analytes [5–7].

Analytical interest in the environmental field has also been focused on liquid chromatographic techniques because of the need to determine non-volatile analytes or very polar compounds. Some results of this interest include EPA Methods 553 [8] and 8321 [2]. Sometimes reversed-

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phase high-performance liquid chromatography (HPLC) offers unique selectivity for compounds usually separated by cGC such as polynuclear aromatic hydrocarbons (PNAs) [2].

Liquid chromatographies are the more universally applicable separation techniques since they do not depend on volatility and have no molecular mass limitations. In addition, coextractives, metabolites and alteration products of analytes that increase polarity or molecular mass are less likely to create problems for subsequent sample runs because they can be, ideally, washed off the column between runs. Advances in the design of LC–MS interfaces have hastened the application of LC to environmental analysis [9–13].

Recently, interest has heightened in the area of very rapid analyses, in "quick turnaround methods", and in field-screening methods [14]. The emphasis on speed is driven on one hand by the requirements of real-time multidimensional analysis. Monnig and Jorgenson [15] have reported analysis times in the seconds using HPCE. Another consideration is the economics of large volume sampling and analysis in Superfund cleanup efforts. Field-screening methods are one approach to this problem, and they have been the subject of an international symposium held every other year since 1988 in Las Vegas [16].

The high efficiency of micellar electrokinetic chromatography (MEKC) makes it attractive for a variety of separations and suggests its applicability to high-speed analyses. Environmental applications of HPCE are on the increase [17–25].

As a capillary column electrophoretic technique, HPCE employs complete column rinsing between sample runs. Even the pseudochromatographic phase (micelles) in MEKC is regenerated before each run by way of buffer rinsing and equilibration. The low cost of fused silica and the rapid changeouts thus make MEKC attractive for field-screening applications.

In this work we compare the separations obtained using MEKC with those obtained using cGC for three representative compound classes of environmental interest: phenols, anilines and PNAs. Very rapid separations are effected on short capillaries under MEKC. These results are discussed in terms of the applicability of MEKC to field screening methods in environmental analysis.

2. Experimental[2]

2.1. Chemicals

All compounds were obtained from Aldrich (Milwaukee, WI, USA) unless otherwise indicated. Solutions of analytes were made up to appropriate concentrations in methanol or tetrahydrofuran (THF). Acetone, THF and methanol were obtained from Burdick & Jackson (Muskegon, MI, USA). Deionized water (ASTM Type II) was produced (Barnstead/Thermolyne, Dubuque, IA, USA) for all aqueous solutions.

2.2. Soil extraction

A creosote-contaminated soil from Spotsylvania, VA, USA was extracted by sonication extraction using standard EPA methodology [2]. This soil had been previously characterized for PNAs and nitrogen-containing aromatic compounds [3].

2.3. HPCE

A Beckman P/ACE 5000 was used for obtaining separations by MEKC. Capillaries were 57 cm or 27 cm × 0.050 mm I.D. (50 cm or 20 cm to the detector, respectively). Buffer systems used were either a 50 m*M* boric acid/sodium borate (pH 8.35), 100 m*M* sodium cholate, 10% acetone, THF or methanol system; or a 50 m*M* boric acid/sodium borate, 100 m*M* sodium cholate, 30 m*M* γ-cyclodextrin system. Voltage was 25 kV under MEKC for 57-cm capillaries and 20 kV for 27-cm capillaries. UV detection was at 214 nm, and acridine was used as an internal standard for migration-time corrections.

2.4. cGC

A Hewlett-Packard (Avondale, PA, USA)

[2] Mention of trade names or commercial products does not constitute endorsement or recommendations for use.

5890 Series II gas chromatograph with flame ionization detector and electronic pressure control was used. Separations were obtained using a DB-5 capillary column of 30 m × 0.25 mm I.D. (0.25 μm film thickness) and a DB-5HT capillary column of 15 m × 0.25 mm I.D. (0.10 μm film thickness) (J & W, Folsom, CA, USA). Temperature program for DB-5 was 60°C for 3 min followed by a rate of 20°C/min to 300°C. Temperature program for DB-5HT was 60°C for 3 min followed by a rate of 30°C/min to 380°C. All injections were on-column using a retention gap of 3 m × 0.53 mm I.D.

2.5. GC–MS

Additional separations were obtained using a Finnigan-MAT TSQ-45 mass spectrometer operated in the electron impact mode and fitted with a DB-5MS capillary column of 40 m × 0.18 mm I.D. (0.1 μm film thickness). The temperature program was 60°C for 3 min followed by a rate of 20°C/min to 300°C. All injections were on-column with a retention gap of 3 m × 0.53 mm I.D. MS operating parameters included an emission current of 0.27 mA, source temperature of 180°C, and multiplier, pre-amplifier and conversion dynode at −1100 V, 10^{-8} A/V, and −3000 V respectively. The instrument was scanned repetitively at 0.5 s/scan under computer control of an INCOS 2300 data system (Nova 4×, software rev. 6.6).

Table 1
Corrected MEKC migration times for phenols (10% acetone–cholate–borate buffer)

Compound	t_m (min)
Resorcinol	5.98
o-Cresol	6.89
m-Cresol	6.92
p-Cresol	7.16
Catechol	9.77
2-Nitrophenol	10.18
4-Chloro-3-methylphenol	10.54
4-Nitrophenol	10.58
2,4-Dichlorophenol	10.88
2,4,6-Trichlorophenol	11.91
2,4,5-Trichlorophenol	12.05
2,4-Dinitrophenol	12.27
Pentachlorophenol	12.83
Acridine (IS)	10.50

Table 2
Corrected MEKC migration times for anilines (γ-CD–cholate–borate buffer)

Compound	t_m (min)
m-Toluidine	4.68
N-Methylaniline	4.71
p-Toluidine	4.98
3,5-Dimethylaniline	4.99
o-Toluidine	5.01
o-Chloroaniline	5.06
3,4-Dimethylaniline	5.29
4-Methyl-3-nitroaniline	5.58
2-Chloro-4-methylaniline	5.65
2-Methyl-6-nitroaniline	5.76
2-Methyl-4-nitroaniline	5.78
3-Chloro-2-methylaniline	5.91
5-Chloro-2-methylaniline	5.95
4-Methyl-2-nitroaniline	6.01
2,6-Dichloro-3-methylaniline	6.95
2,4,5-Trichloroaniline	7.45
Acridine (IS)	7.00

3. Results and discussion

3.1. MEKC conditions

Several conditions under MEKC were investigated for the separation of phenols, anilines and PNAs. In each case, the micellar agent chosen was sodium cholate. In our study, the sodium cholate concentration was fixed at 100 m*M* and organic additives of acetone, THF and methanol were added to a 10% (90% water) level to examine their effects on separations. Methanol and THF tended to bunch peaks, whereas acetone appeared to add selectivity. An alternative additive investigated was γ-cyclodextrin (γ-CD) at 30 m*M*. Terabe [26] previously indicated the use of this additive for highly hydrophobic compounds.

Tables 1–4 tabulate observed corrected [5] migration times relative to the internal standard (IS) acridine. Figs. 1 –4 illustrate electropherograms of the three classes of compounds and an extract of a creosote-contaminated soil.

3.2. Phenols

Phenols represent a class of compounds that are weakly acidic. They were best separated under 10% acetone–cholate–borate buffer conditions (Fig. 1). The use of γ-CD–cholate buffer also gave good separations. Acetone, by reducing electroosmotic flow (EOF), allowed a better definition between the EOF peak and the very polar, early eluting cresols.

Fig. 1 may be compared to cGC separations found in the literature [27] in terms of resolving power, efficiency and time of run. Although the separation mechanism of cGC depends on both volatility and polarity, there are some similarities in the order of elution in comparing MEKC with cGC. For example, cresols elute first in both MEKC and cGC, and pentachlorophenol is last. The efficiency of both techniques is high, but MEKC does not suffer peak tailing with the polar nitrophenols and pentachlorophenol, a problem that is often evident with cGC when real sample extracts are injected.

It is evident from the migration times in Table 1 that not all phenols are resolved under these conditions. In our hands, not all of the phenols were resolved on a 40-m capillary column as well. Incomplete resolution was observed between *m*- and *p*-cresol, resorcinol and 2,4-dichlorophenol, 2,4,6-trichlorophenol and 2,4,5-

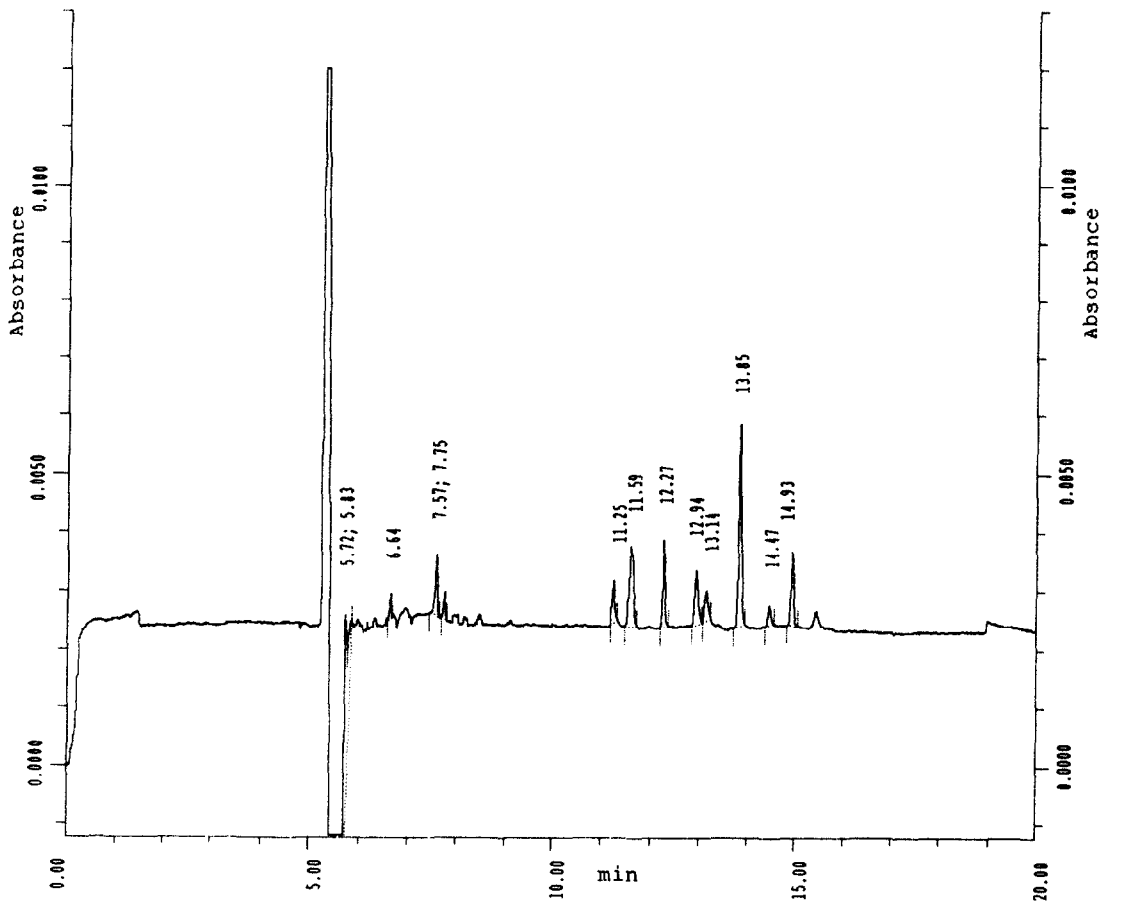

Fig. 1. Electropherogram of 13 phenols under MEKC (10% acetone–cholate–borate buffer). Acridine (IS), migration time (t_m = 11.59 min).

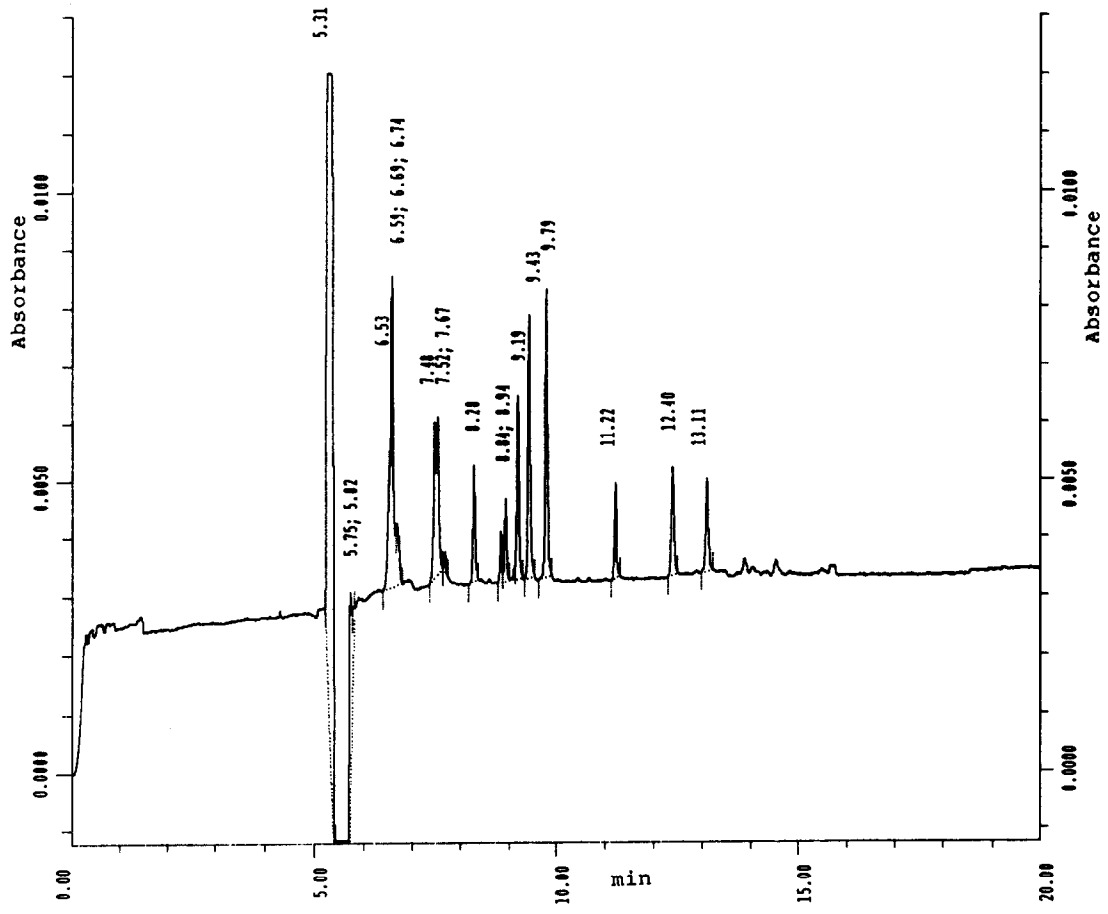

Fig. 2. Electropherogram of 16 anilines under MEKC (10% acetone–cholate–borate buffer). Acridine (IS), $t_m = 11.22$ min.

trichlorophenol, and 4-nitrophenol and 2,4-dinitrophenol.

3.3. Anilines

Anilines were well separated under 10% acetone–cholate–borate (Fig. 2) and CD–cholate–borate conditions. Symmetrical peaks were obtained for these relatively polar compounds under MEKC. As an illustration of selectivity changes as a function of additives, Tables 2 and 3 tabulate corrected migration times for both MEKC conditions. There are obvious changes in elution order in comparing the results in the two tables. Such changes may be useful in particular separation problems. It is also evident from the tables that not all of the compounds are resolved under the conditions studied.

Comparison of MEKC to cGC (Fig. 5) separation indicates relatively high efficiency and selectivity for both techniques. The presence of numerous impurities and alteration products in the solution of anilines was detected by MS, and they appear on the chromatogram as peaks beyond trichloroanilines. Very polar polymerization products are unlikely to be detected by cGC and probably remain on the retention gap where they may be pyrolyzed. Such alteration products are probably observed as late-eluters (e.g., $> t_m = 14$ min) in MEKC, and are flushed from the capillary between runs if not eluted during determination.

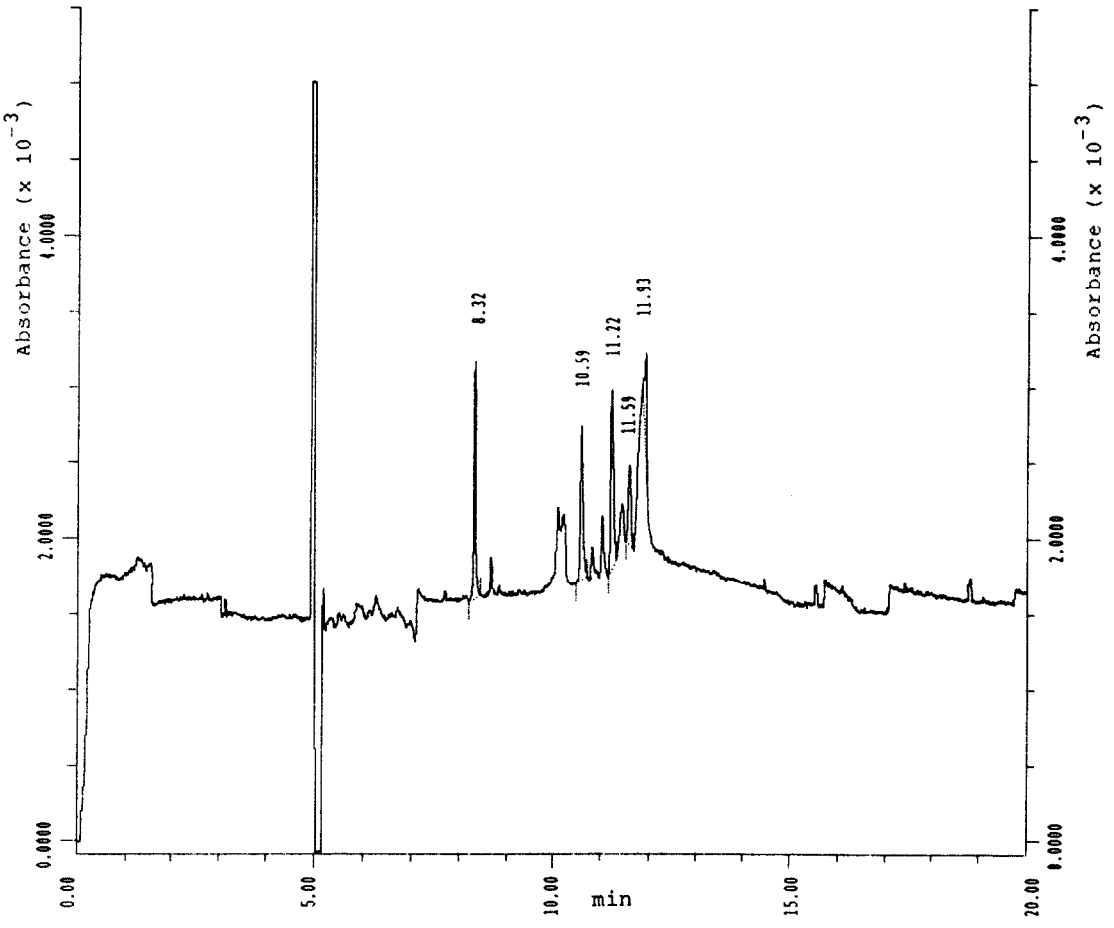

Fig. 3. Electropherogram of 16 PNAs under MEKC (CD–cholate–borate buffer). Acridine (IS), t_m = 8.32 min.

3.4. PNAs

PNAs were best separated under CD–cholate–borate conditions (Fig. 3). Some bunching of the higher-molecular-mass PNAs is evident in Fig. 4 and in Table 4. Additional separation of the late-eluting compounds may be effected by the addition of acetonitrile [28] or acetone to the γ-CD-containing buffer. Even so, with 15% acetonitrile, complete separation of all 16 compounds was not obtained under these conditions. Fig. 4 illustrates the electropherogram of an extract of a creosote-contaminated soil using the 10% acetone–CD–cholate–borate conditions. The complexity of this sample is reflected in the large number of polar and hydrophobic compounds present [3].

All 16 PNAs are completely or almost completely resolved by cGC [27]. Thus, for the MEKC conditions investigated, cGC exhibited better resolution for PNAs. However, the range of applicability of MEKC for weakly acidic, basic and neutral compounds was demonstrated in the data presented.

3.5. Short-column, rapid separations

One of the issues we wanted to address in our

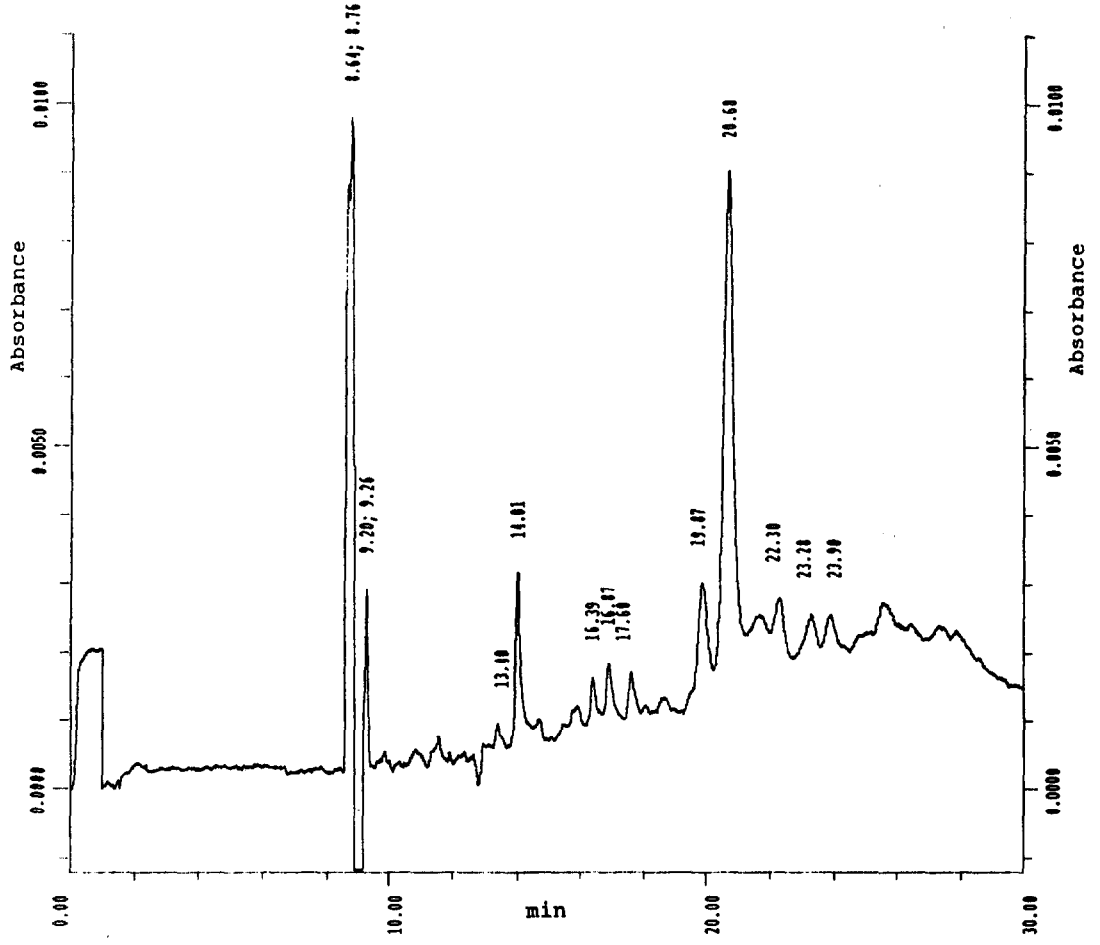

Fig. 4. Electropherogram of an extract of creosote-contaminated soil (10% acetone–CD–cholate–borate buffer). Acridine would elute at t_m = 12.99 min in this electropherogram.

work was the use of MEKC in very rapid compound-class separations. Very rapid determinations in conjunction with rapid extraction [e.g., via supercritical fluid extraction (SFE)] and rapid sample cleanup [e.g., via solid-phase extraction (SPE) cartridges] are of great interest in rapid site characterization/remediation studies and in field-screening methodology.

As an example of this potential, we illustrate a short capillary MEKC separation of PNAs (Fig. 6). Partial separations are obtained in 2–3 min for each compound class. Although incomplete resolution of all compounds is observed, rapid assessment of contaminant plumes or remediation progress could be obtained from these determinations. Only partial resolution of all target analytes is also observed with most field-screening methods that involve cGC. As an example, a rapid separation of PNAs by cGC is illustrated in Fig. 7 using a high-temperature column and operating conditions. All PNAs elute in less than 11 min; compare this result to runs of 44 min in normal work and 18 min in field-screening applications literature [27]. Incomplete separation of analytes is common in field-screening methods.

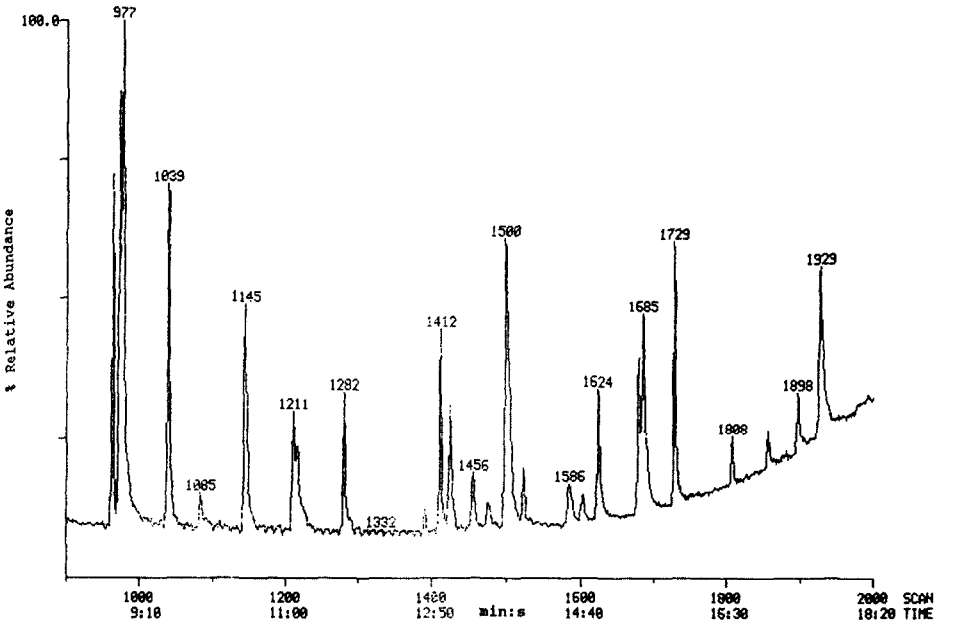

Fig. 5. cGC separation of anilines (40-m DB-5MS, GC–electron impact MS). Acridine, scan 1729; toluidines and N-methylaniline, scans 964, 975 and 977; *o*-chloroaniline, scan 1039; 3,5-dimethylaniline, scan 1085; chloromethylanilines, scans 1145 and 1211; 2,6-dichloro-3-methylaniline, scan 1282; methylnitroanilines, scans 1412 and 1425; 2,4,5-trichloroaniline, scan 1456.

Table 3
Corrected MEKC migration times for anilines (10% acetone–cholate–borate buffer)

Compound	t_m (min)
o-Toluidine	6.93
m-Toluidine	7.06
N-Methylaniline	7.13
p-Toluidine	7.16
o-Chloroaniline	7.87
3,5-Dimethylaniline	7.89
3,4-Dimethylaniline	8.03
4-Methyl-3-nitroaniline	8.72
2-Methyl-4-nitroaniline	9.32
2-Methyl-6-nitroaniline	9.41
2-Chloro-4-methylaniline	9.54
4-Methyl-2-nitroaniline	9.87
5-Chloro-2-methylaniline	9.90
3-Chloro-2-methylaniline	10.25
2,6-Dichloro-3-methylaniline	13.08
2,4,5-Trichloroaniline	14.43
Acridine (IS)	11.70

Table 4
Corrected MEKC migration times for polynuclear aromatics (γ-CD–cholate–borate buffer)

Compound	t_m (min)
Acenaphthylene	7.23
Naphthalene	7.40
Fluorene	9.93
Phenanthrene	10.32
Anthracene	10.64
Pyrene	10.83
Fluoranthene	10.96
Chrysene	11.17
Benzo[*a*]pyrene	11.44
Benzo[*ghi*]perylene	11.57
Benzo[*b*]fluoranthene	11.63
Dibenz[*a*,*h*]anthracene	11.64
Indeno[1,2,3-*c*,*d*]pyrene	11.68
Acridine (IS)	8.20
Acenaphthene	N.D.
Benzo[*a*]anthracene	N.D.
Benzo[*k*]fluoranthene	N.D.

N.D. = Not determined.

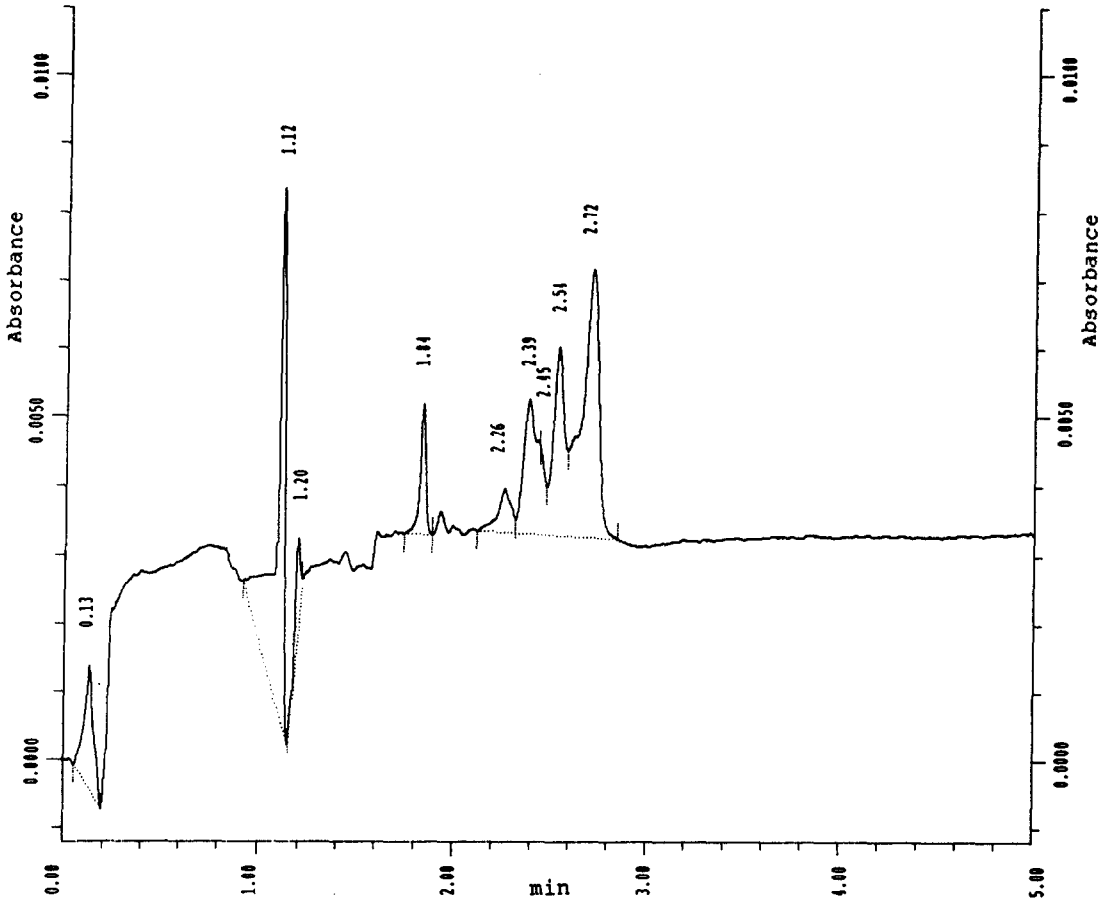

Fig. 6. Short capillary (27-cm) separation of PNAs (γ-CD–cholate–borate buffer). Acridine (IS), $t_m = 1.84$ min.

We envision a combined SFE–SPE–HPCE instrument that could be automated with an average analysis time per sample of 6 min. This represents a novel application of MEKC to field-screening approaches and would represent at least a 10-fold reduction in analysis time over the usual methodology. Efforts at improving sensitivity and selectivity of detection under MEKC such as by using laser-induced fluorescence (LIF) detection are important to the ultimate application of this potential. Improved sensitivity lowers detection limits and allows injection of more dilute samples. Improved selectivity reduces the amount of cleanup required and increases ruggedness in the determination when a variety of matrices are encountered.

4. Conclusions

MEKC exhibits selectivity that is comparable to that of cGC for polar analytes, but PNAs are best separated by cGC under the conditions used in this work. With regard to rapid determinations, partial separations can be accomplished in about 2–3 min and this compares very favorably with cGC. Applications of this rapid separation capability are envisioned in combination with

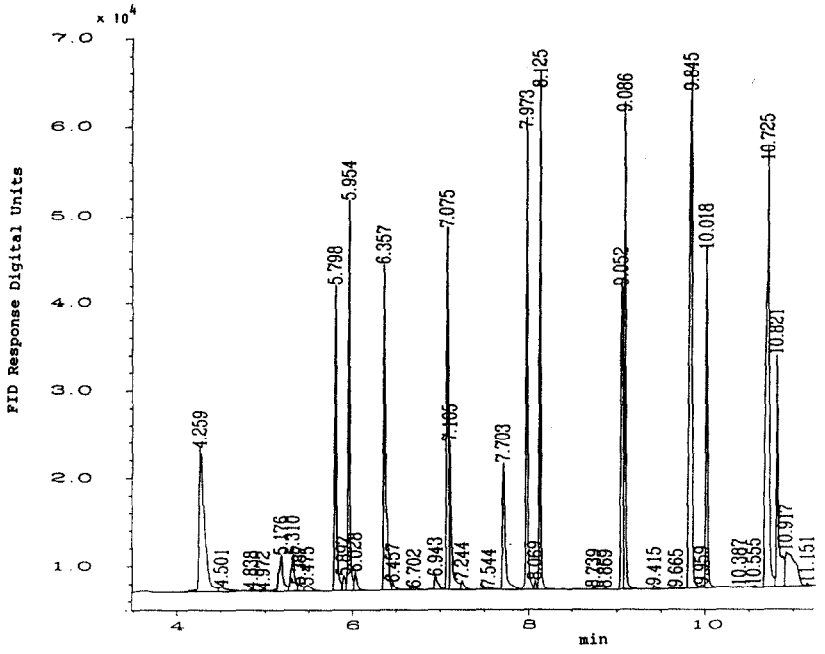

Fig. 7. Rapid separation of PNAs by use of high-temperature column and conditions [15-m DB-5HT, flame ionization detection FID)]. Naphthalene, $t_R = 4.259$ min; benzo[*ghi*]perylene, $t_R = 10.821$ min.

rapid extraction (e.g., SFE) and cleanup techniques (e.g., SPE sorbents). The approach would be particularly useful as an SFE–SPE–MEKC unified instrument with selective and sensitive detection by LIF.

Notice

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References

[1] J.W. Eichelberger, E.H. Kerns, P. Olynyk and W.L. Budde, *Anal. Chem.*, 55 (1983) 1471–1479.

[2] *Test Methods for Evaluating Solid Waste (SW-846)*, Vol. IB, US Environmental Protection Agency, Washington, DC, 3rd ed., November 1986; Proposed Update II, Rev. 2, November 1992.

[3] W.C. Brumley, C.M. Brownrigg and G.M. Brilis, *J. Chromatogr.*, 558 (1991) 223–233.

[4] W.C. Brumley, C.M. Brownrigg and A.H. Grange, *J. Chromatogr.*, 633 (1993) 177–183.

[5] W.C. Brumley and C.M. Brownrigg, *J. Chromatogr.*, 646 (1993) 377–389.

[6] W.C. Brumley and C.M. Brownrigg, *J. Chromatogr. Sci.*, 32 (1994) 69–75.

[7] *EPA Report 600/R-92/129, Methods for the Determination of Organic Compounds in Drinking Water*, Supplement II, August 1992, Method 553, Rev. 1.1.

[8] W.C. Brumley, *J. Chromatogr.*, 603 (1992) 267–272.

[9] E.C. Huang and J.D. Henion, *Anal. Chem.*, 63 (1991) 732–739.

[10] M.A. Mosely, L.J. Deterding, K.B. Tomer and J.W. Jorgenson, *Anal. Chem.*, 63 (1991) 1467–1473.

[11] R.M. Caprioli, W.T. Moore, M. Martin, B.B. DaGue, K. Wilson and S. Moring, *J. Chromatogr.*, 480 (1989) 247–257.

[12] R.D. Smith, J.A. Olivares, N.T. Nguyen and R.H. Udseth, *Anal. Chem.*, 60 (1988) 436–441.

[13] T. Nohmi and J.B. Fenn, *J. Am. Chem. Soc.*, 114 (1992) 3241–3246.

[14] *Field Methods Compendium (FMC), OERR 9285.2-11*, Hazardous Site Evaluation Division, Analytical Operations Branch, US Environmental Protection Agency, Washington, DC, July 1993.

[15] C.A. Monnig and J.W. Jorgenson, *Anal. Chem.*, 63 (1991) 802–807.

[16] *Proceedings of the 1st International Symposium, Field Screening Methods for Hazardous Waste Site Investigation, US Environmental Protection Agency, Las Vegas, NV, October 11–13, 1988*, U.S. EPA, Environmental Monitoring Systems Laboratory, Las Vegas, NV.
[17] P. Jankik, W.R. Jones, A. Weston and P.R. Brown, *LC·GC*, 9 (1991) 634–645.
[18] K. Utasuka, S. Terabe and T. Ando, *J. Chromatogr.*, 348 (1985) 39–47.
[19] S. Terabe, Y. Miyashita, O. Shibata, E.R. Barnhart, L.R. Alexander, D.J. Patterson, B.L. Karger, K. Hosoya and N. Tanaka, *J. Chromatogr.*, 516 (1990) 23–31.
[20] C.P. Ong, H.K. Lee and S.F.Y. Li, *J. Chromatogr.*, 542 (1991) 473–481.
[21] F. Foret, V. Sustacek and P. Boček, *Electrophoresis*, 11 (1990) 85–97.
[22] S. Terabe, K. Otsuka, K. Ichikawa and A. Tsuchiya, *Anal. Chem.*, 56 (1984) 111–113.
[23] S. Terabe, K. Otsuka and T. Ando, *Anal. Chem.*, 57 (1989) 834–841.
[24] W.G. Kuhr and C.A. Monnig, *Anal. Chem.*, 64 (1992) 389R–407R.
[25] H. Nishi, T. Fakuyama, M. Matsuo and S. Terabe, *J. Chromatogr.*, 513 (1990) 279–295.
[26] S. Terabe, *Micellar Electrokinetic Chromatography*, Beckman Instruments, Fullerton, CA, 1992.
[27] *Products Catalog and Reference Guide 1992–1993*, J & W Scientific, Folsom, CA, 1992.
[28] P. Camilleri, *Capillary Electrophoresis: Theory and Practice*. CRC Press, Boca Raton, FL, 1993, p. 57.