ELSEVIER

Journal of Chromatography A, 827 (1998) 1–12

JOURNAL OF CHROMATOGRAPHY A

Review

Analysis of carboxylic and related acids in the environment by capillary electrophoretic techniques

D.H. Craston, M. Saeed*

LGC (Teddington) LTD., Queens Road, Teddington, Middlesex TW11 OLY, UK

Received 21 July 1998; received in revised form 4 September 1998; accepted 14 September 1998

Abstract

This review article describes the application of electroseparation techniques to the analysis of carboxylic and related acids in environmental samples. The material covered includes discussion of sample preparation and detection issues, advantages and limitations, and future trends and developments.

Keywords: Carboxylic acids

Contents

1. Introduction

Over the past decade, capillary electroseparation techniques [1–4] have emerged as powerful tools for the analysis of solutions, and now provide a plausible option for obtaining complementary information to, or replacing, measurement by high-performance liquid chromatography (HPLC). CE is fast, with a

*Corresponding author.

0021-9673/98/$ – see front matter
PII: S0021-9673(98)00750-X

typical analysis time of less than 10 min; cheap, requiring very low volumes of reagents; simple, the instrumentation requires few couplings or connectors and it allows for rapid method development. Also, the resolution of separation by CE is much better than can be achieved by HPLC and, because of this, the technique is well suited to handling complex solutions containing many discrete chemical components. Given the separation mechanism, CE is particularly suited for analysing charged species such as metal ions, small anions, acids and bases.

The many benefits of CE were overshadowed in the early days of its development by problems in achieving the reproducibility and sensitivity required for many measurement applications. Improvements in available instrumentation have subsequently significantly enhanced the quality of data; however, our experience of discussions with non-users is that the stigma of poor reliability remains. In this review article, we set out to dispel some of the popular myths about CE by considering its application to measurements of acids in the environment.

Environmental samples, particularly from land or waste streams, provide a rugged test for analytical techniques because of their chemical complexity. Currently, there are a number of acid compounds that are believed to exert negative effects within ecosystems. Concerns vary from the individual toxicity of components, to their ability to associate with and assist the redistribution of other toxic reagents, such as heavy metals. This review describes CE analysis of humic and fulvic acids, phenoxy acid herbicides and other low-molecular-mass organic acids in air, land and water. For each application, the various modes of CE [5–11] are considered, and emphasis is given to work that covers the analysis of real samples, rather than just standard solutions. For this reason, this article also describes the procedure used for preparing samples prior to measurement (extraction and clean-up) and the enrichment [12–16] process required for trace analysis.

2. Analysis of acids in the environment

2.1. Humic and fulvic acids

Humic substances (HSs), that is humic and fulvic acids, are formed by the decomposition of plants and animal tissues and are widespread in soils. The humic acid (HA) matrix consists of a skeleton of alkyl/aromatic units that are crosslinked mainly by oxygen and nitrogen groups, with the major functional groups being carboxylic, phenolic and alcoholic hydroxyls, ketone and quinine groups. Fulvic acids (FAs) are more aliphatic and are richer in carboxylic, phenolic and ketone groups, which increase their water solubility [17–20]. Both HA and FA can act as ligands for radionuclides and toxic metals and, consequently, are thought to participate in the migration and mobilisation of these elements at contaminated sites [19,20]. The characterisation of HS could therefore provide valuable information in understanding the environmental fate of metal contamination. Spectroscopic, chromatographic and slab gel electrophoresis methods have all been used for this purpose, but results have generally been poor due to the low resolving power of these techniques [17,18].

2.1.1. Sample preparation

The extraction and isolation of HA and FA in soil and water is normally performed according to the procedures outlined by the International Humic Substances Society [21–23]. For soils, the procedures suggest solid/liquid extraction using solutions of 0.1 *M* sodium hydroxide [24] or sodium pyrophosphate [25]. Aqueous, sodium hydroxide provides the highest recovery, but at the price of altering the chemical composition through oxidation or condensation reactions. Most of the text detailed below is concerned with CE separations that were performed directly on the extracts, however, a few workers [26] have recommended some processing of the extracts, involving fractionation by either gel electrophoresis or gel permeation chromatography.

2.1.2. Capillary electrophoresis

HSs are large (molecular mass of between 1000–17 000 g mol^{-1}) [18–20] charged molecules that are amenable to separation by capillary isotachophoresis (CITP), capillary gel electrophoresis (CGE) and capillary zone electrophoresis (CZE). All of these techniques have been studied but most reports describe separation by CZE. Both HA and FA contain an abundance of UV-absorbing chromophores and,

hence, this has been the normal mechanism for end detections. Due to the complexity of HA and FA compounds in a typical soil sample, resolution of components is not feasible; instead, analysis has strived to provide characterisation profiles, the environmental relevance of which can then be interpreted.

The one study of CITP [26] used both UV and conductivity detection to analyse commercial and natural preparations of HA and FA (Fig. 1). Conductivity was found to be unreliable, requiring regular cleaning of the electrodes. Good run-to-run reproducibility was obtained by adding a polymer (PVP, polyvinylpyrrolidone; M_r 360 000) to the leading electrolyte, which served to reduce the electroosmotic flow and act as a form of sieving buffer. While the CITP approach was successful, the procedure adopted cannot be readily transferred to current commercial CE instrumentation, due to the requirement for abnormally high sample injection volumes (15–30 μl).

A single report describes analysis by CGE [27] using a polymer-sieving media and a coated capillary (e.g. DB-Wax) to reduce the electroosmotic flow (EOF). HA and FA were profiled on the basis of molecular size. A variety of sieving buffers were investigated, but methyl cellulose (MC) [27] at low

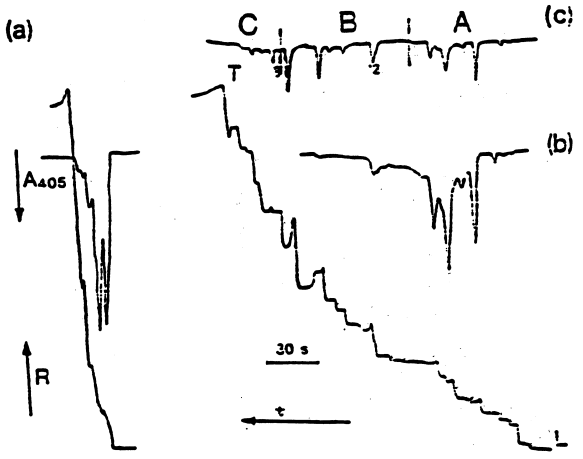

Fig. 1. Isotachopherogram of the separation of humic acid (HA); molecular mass range, 600–1000. (a) Conductivity and UV detection for a 5-μl injection volume employing an electrophoresis buffer without PVP; (b) as for (a) except that a 30-μl volume of spacers was injected; (c) as for (b) but employing a 10-μl sample volume containing 0.025% PVP in the leading electrolyte.

concentration was found to be the best at providing structure in the electropherogram within a short analysis time (less than 5 min).

The many studies of CZE have investigated different buffer components, such as borate, phosphate and citrate [24,28–30,33], at a range of pH values. For species of high mass–charge ratio, best results (Fig. 2) have been obtained when operating at high pH where the carboxyl groups are fully ionised. One report described the use of a background electrolyte composed of 100 m*M* boric acid at a pH of 3.15, and observed that three fractions were separated (Fig. 3) [34]. This phenomena was thought to be caused by the formation of borate complexes containing two or more HA molecules. A further reports suggests that fingerprinting of HSs can be improved by the addition of organic solvents (e.g. acetonitrile, acetone, 2-propanol and tetrahydrofuran) to the buffer system [31]. This improvement is believed to originate from a reduction in the EOF and an increase in the solubility limits of the individual HS components.

CE is believed to allow rough quantification of HSs because of the approximate linear relationship between signal and concentration [35]. Also, by including certain metals within the buffer system, it has been possible to relate acid profiles with the ability to form complexes [32,34], thereby providing meaningful information for environmental studies. However, overall, it is our view that CE offers little benefit over HPLC and related techniques for this application. The improved resolution of CE is still insufficient to resolve specific components and, hence, as with the other methods, chemometric approaches will be required to make sense of the data produced.

2.2. Phenoxy acid herbicides

Phenoxy acid herbicides are used routinely to protect arable land from weed infestation. Their widespread use has led to increased levels of native compounds and metabolites in water and soil [36,37]. Some acid herbicides are administered to the environment as racemic mixtures, with only the D-isomer being active. Chiral analysis is viewed as being important as the enantiomers have different toxicities [38–40].

Acid herbicides are non-volatile and, hence, can-

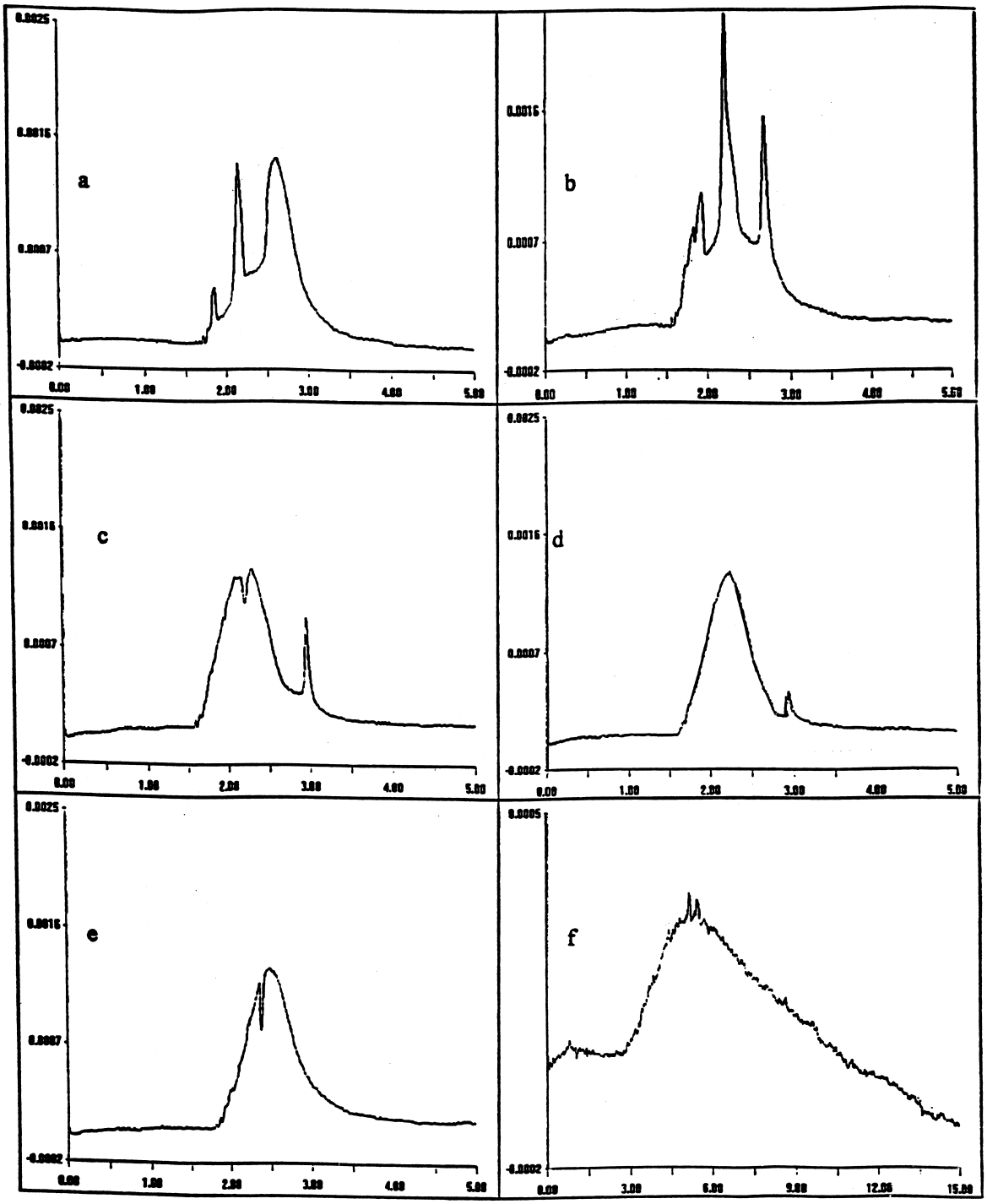

Fig. 2. Electropherograms of FA extracted with NaOH using different buffer solutions: (a) borate buffer at pH 9.7; (b) borate buffer at pH 8.9; (c) borate buffer at pH 8.15; (d) phosphate buffer at pH 7.1; (e) citrate buffer at pH 6.25 and (f) citrate buffer at pH 2.3.

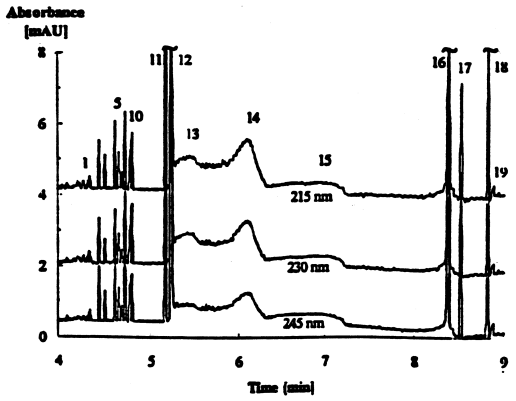

Fig. 3. Electropherograms of HAs at 215, 230 and 245 nm in 100 m*M* boric acid.

not be analysed by gas chromatography (GC) without derivatisation [41]. Analysis by HPLC is less sensitive and requires gradient elution to achieve sufficient resolution [42]. Although chiral separations can be achieved by HPLC, this is an expensive option and no single column will resolve all of the phenoxy acid herbicides [43]. The enhanced resolution of CE, coupled with its proven capacity to effect chiral analysis, make the technique ideally suited to this application.

2.2.1. Sample preparation

There are two approaches to sample preparation that are dependent on the sample matrix under investigation. Recovery from water is generally achieved by passing the sample through a solid phase (C_{18}) support after adjusting the pH or adding ion pairing reagents [41–43]. Extraction from soil can be performed by sonication or soxhlet methods (water–methanol, 25:75, v/v, can be used): simple mixing of the extraction solvent and the solid sample, followed by filtering, has proved adequate in some circumstances [43]. For trace analysis, extracts are concentrated to low volumes and reconstituted with minimum amounts of background electrolyte prior to analysis.

2.2.2. Analysis by capillary electrophoresis

Micellar electrokinetic capillary chromatography (MECC) and CZE have both been used to analyse acid herbicides [36,42]. MECC has been preferred due to the similar charge-to-mass ratios of the different acid components [42]. Studies of chiral analysis are detailed below. Where there has been no attempt to separate optical isomers, sodium dodecyl sulphate (SDS) and cholic acid have been used, with the latter providing the best separation due to its higher affinity for anionic compounds (Fig. 4) [39,41]. For CZE, acetate buffers of high concentration [43] have been preferred and these have been shown to generate reasonable efficiency separations (Fig. 5). Most acid herbicides absorb light sufficiently in the 180–220 nm range to be measured by UV detection [41].

2.2.3. Chiral selectors for CE separation

The co-addition of cyclodextrin (CD) and SDS [39] is a normal route for separating chiral components and has been described for the analysis of acid herbicides [41]. In one study, it was found that neither α-CD or β-CD (Fig. 6) on their own allowed resolution of all of the acid herbicides [42], but that a combination of the two was effective in achieving complete separation (Fig. 7). This study also indicated that the resolution was heavily influenced by the cavity size and concentration of CDs [42] and that a combination of a stacking technique and preconcentration with C_{18} membrane disks allowed measurement down to around 1 ppb in lake water [42].

Another study compared 2,6-di-*O*-methyl-β-CD with α-CD and β-CD for resolving acid herbicide impurities [40]. The work indicated that phenoxy acid herbicides can be baseline separated using CD, provided that an appropriate pH is selected. The different forms of CD gave different selectivities, and the combination of the resulting electropherograms allowed determination of a range of impurities. A tri-methyl-β-CD has also been used to study the degradation of dichloroprop enantiomers in soil [44]. The quality of the separation was found to be highly dependent upon temperature, due to its impact on micelle formation, partitioning of the analyte and electrophoretic mobility of the chiral complex. The conclusion reached, i.e. that the (−)-isomer degraded faster than the (+)-isomer, was in contrast to that

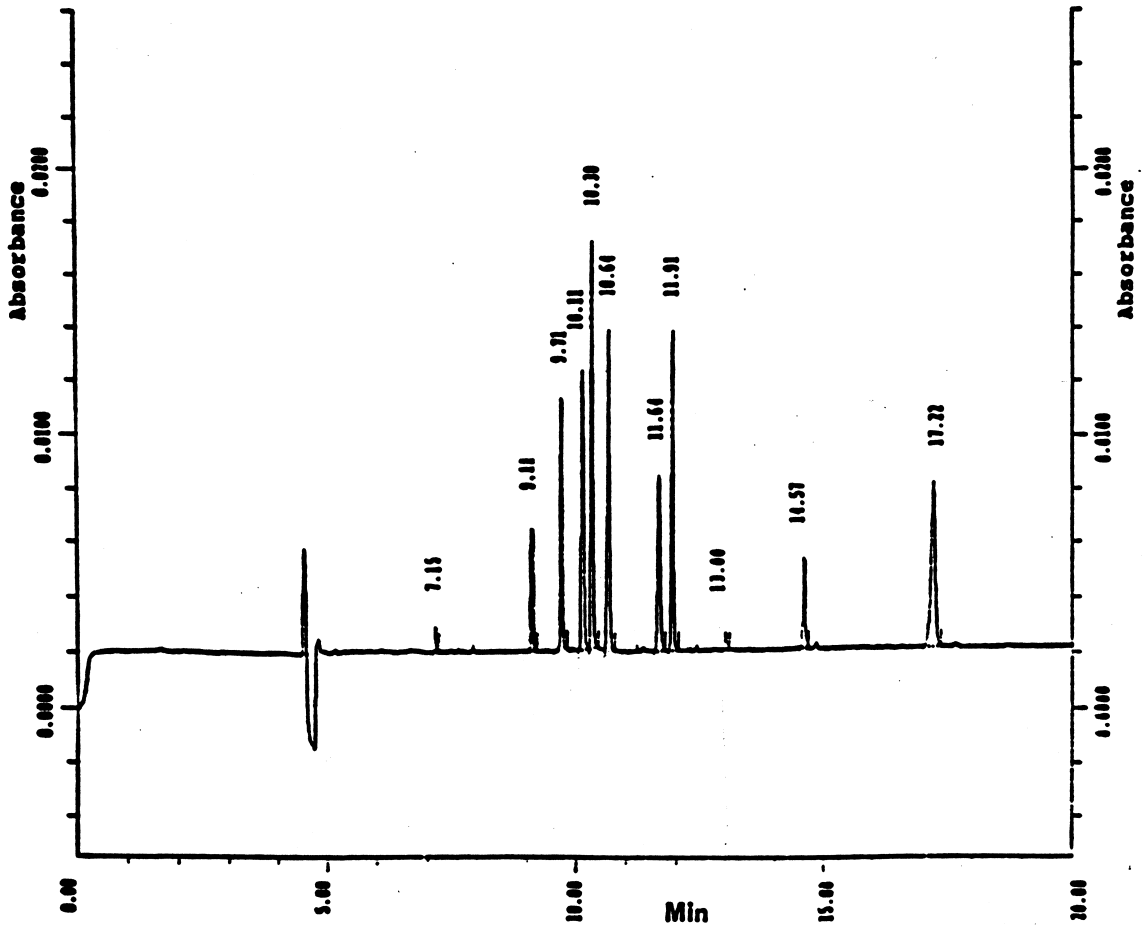

Fig. 4. Electropherogram of the seven standards under micellar conditions. (1) 2,4-DB [4-(2,4-dichlorophenoxy)butyric acid]; (2) MCPB [4-(4-chloro-2-methylphenoxy)butyric acid]; (3) 2,4-DP [4-(2,4-dichlorophenoxy)propionic acid]; (4) 2,4-D (2,4-dichlorophenoxyacetic acid); (5) MCPA (4-chloro-2-methoxyphenoxyacetic acid); (6) 2,4,5-TP [2-(2,4,5-trichlorophenoxy)propionic acid] and (7) 2,4,5-T (2,4,5-trichlorophenoxyacetic acid).

found in previously published [37] studies in water, indicating that a different pathway was responsible for environmental breakdown.

Although CD is the most widely used chiral selector, bile salts [39], (*R*,*R*)-tartaric acid [39] and *n*-nonyl- (or *n*-octyl)-β-glucopyroside [44], 1-allyltergluride [38] and vancomycin [45] have also been tested. For the first four of these, studies indicate that CD is the better choice in terms of resolution, range and efficiency of the separations obtained. Vancomycin, a chiral macrocyclic antibiotic, has been reported to give better results [45]; the separation of six racemic acid herbicides in 9 min has been reported (Fig. 8). However, this positively charged chiral selector requires the use of a polyacrylamide-coated capillary to prevent adsorption on the capillary surface and to reduce the EOF. Also, the strong UV absorption of this chiral additive means that a partial filling method needs to be employed to keep the optical detection cell free of absorbed material. Under optimised conditions, a limit of detection of $5 \cdot 10^{-7}$ *M* was achieved for each enantiomer.

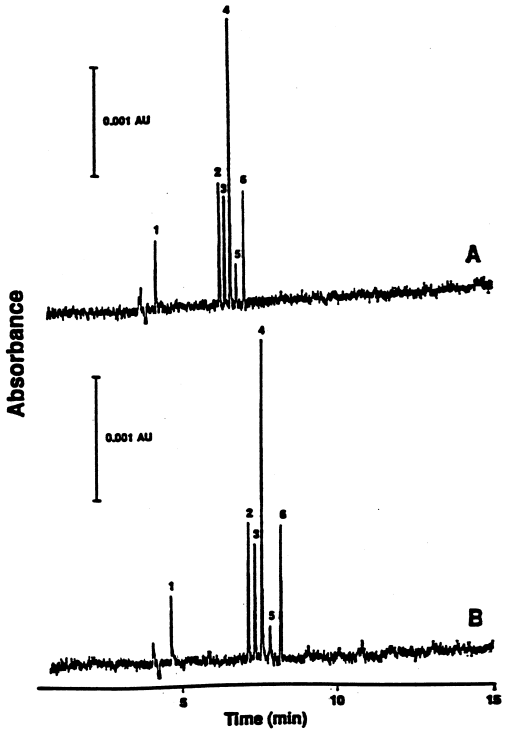

Fig. 5. Electropherograms of herbicide mixtures at different buffer concentrations at pH 4, with an applied voltage of 30 kV and a wavelength of 230 nm. Graph A represents a separation using a buffer concentration of 100 m*M* and Graph B shows the results using a buffer concentration of 150 m*M*. The peaks are as follows: 1=MCPB [4-(4-chloro-2-methylphenoxy)butyric acid]; 2=MECOPROP [2-(4-chloro-2-methylphenoxy)propionic acid]; 3=DICHLORPROP [2-(2,4-dichlorophenoxy)propionic acid]; 4=MPCA (4-chloro-2-methoxyphenoxyacetic acid); 5=2,4D (2,4-dichlorophenoxyacetic acid) and 6=DICAMBA (dichloro-2-methoxybenzoic acid).

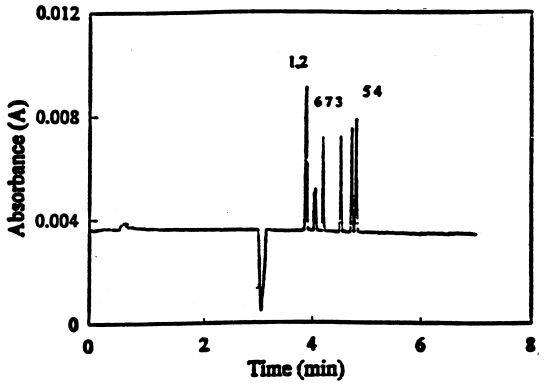

Fig. 6. Electropherogram of phenoxy acid herbicides in 2 m*M* β-cyclodextrin. (1) 2,4-DB, (2) MCPB, (3) 2,4-DP, (4) 2,4-D, (5) MCPA, (6) 2,4,5-TP and (7) 2,4,5-T.

2.3. Miscellaneous low-molecular-mass organic acids

A range of small organic acids occur in the environment that are derived largely from atmospheric [46–49] or industrial processes [50,51]. Analysis of these is important in a number of contexts, for example, in:

- examining acid reagents in rain water to study the global transport of air-borne pollutants
- determining the corrosion potential in industrial processes through acid attack on metal surfaces
- understanding geochemical factors in oil wells through the profile of acids present within underground water

In most cases, the analytical options for these acid compounds are either ion-exchange chromatography or CE. The only CE papers that have been found to date relate to the analysis of air and water samples.

2.3.1. Sample preparation

For aqueous samples, the only sample preparation required is usually preconcentration on a solid phase [46,47,49,51–56] followed by recovery and reconstitution in either the background electrolyte or ultrapure water. In air, acid compounds are associated with air-borne particulates (solid and liquid), which are collected on filters (glass or quartz fibre filters) or on aluminium foils in an impactor for size distribution [48]. Single rain droplets are normally isolated for analysis by rapid freezing in contact with liquid nitrogen. Acids are recovered from solid phase collectors by placing them in water and agitating using an ultrasonic bath [48].

2.3.2. Capillary electrophoresis of low-molecular-mass organic acids in the environment

Analysis of organic acids has been reported by CZE using capillaries coated with a positively charged surfactant (e.g. cetyltriethyl ammonium bro-

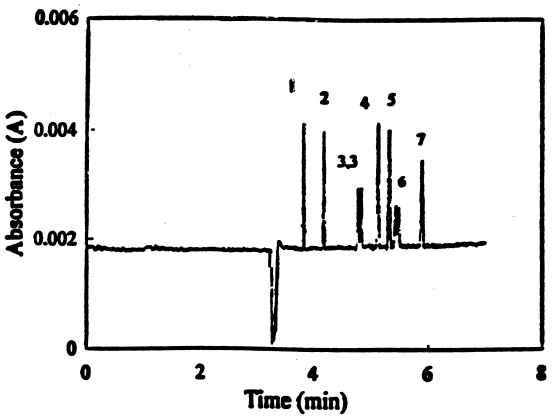

Fig. 7. Electropherogram of phenoxy acid herbicides in 4 m*M* α-cyclodextrin; 1 m*M* β-cyclodextrin. (1) 2,4-DB, (2) MCPB, (3) 2,4-DP, (4) 2,4-D, (5) MCPA, (6) 2,4,5-TP and (7) 2,4,5-T.

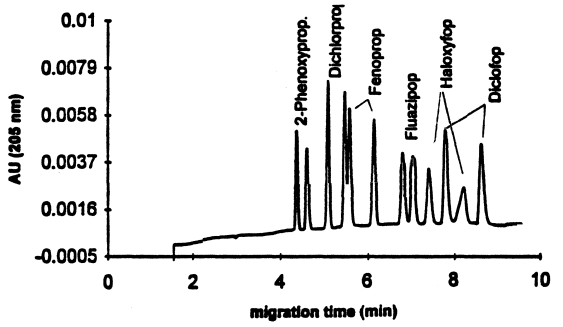

Fig. 8. Electropherogram of the enantiomeric separation of acid herbicides [(1) 2-phenoxypropionic acid, (2) dichloroprop, (3) fenoprop, (4) fluazifop, (5) haloxyfop and (6) diclofox], employing optimum conditions of 6 m*M* vancomycin at pH 5.0 with a Brittan-Robinson buffer system. The chiral selector was introduced into the capillary via a partial filling method.

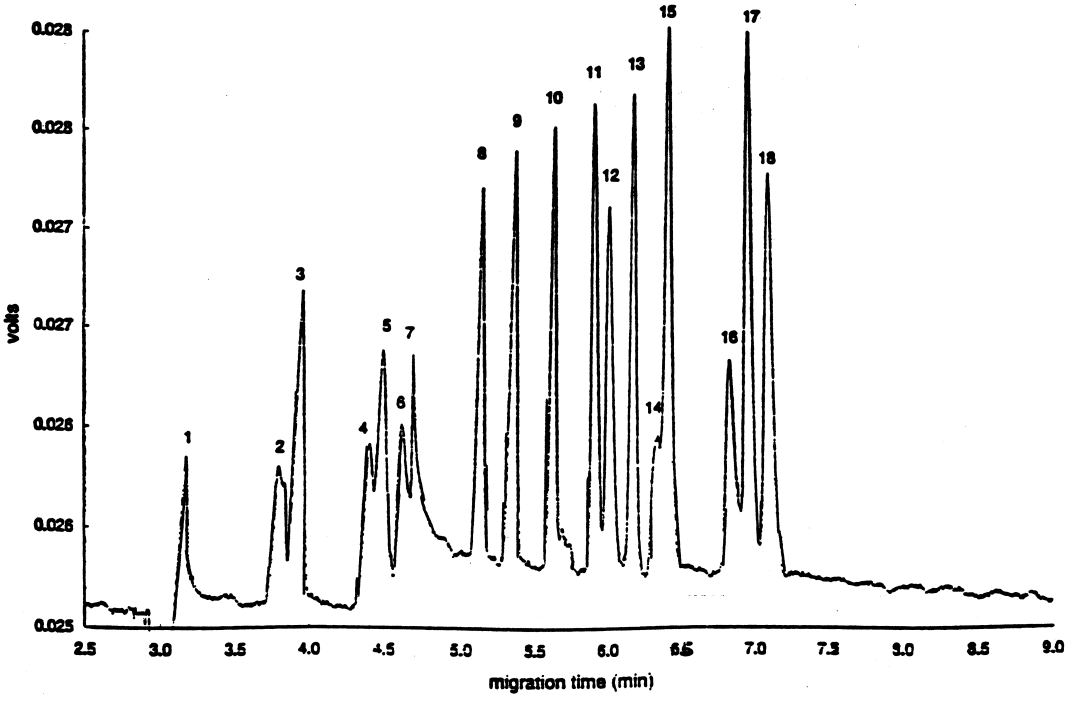

Fig. 9. Capillary electropherogram of an 18-component organic acid mixture: (1) oxalic acid, (2) formic acid, (3) fumaric acid, (4) pyruvic acid, (5) maleic acid, (6) L-malic acid, (7) citric acid, (8) D-lactic acid, (9) succinic acid, (10) DL-aspartic acid, (11) D-glucuronic acid, (12) D-gluconic acid, (13) acetic acid, (14) L-ascorbic acid, (15) shikimic acid, (16) gallic acid, (17) propionic acid and (18) *n*-butyric acid.

mide, CTAB) to reverse the EOF [49,58]. The more hydrophobic the surfactant, the more effective is the reversal of EOF and the faster the migration time of the acid analytes [53].

Indirect detection is normally required for the analysis since most of the components of interest (e.g. oxalate, malonate, formate, carbonate, acetate and propionate [52]) do not fluoresce or absorb in the UV region. Indirect detection is achieved by including an absorbing ion (UV-absorbing chromophore, UAC, or fluorescence absorbing chromophore, FAC) in the buffer, which provides a high background absorbance. Displacement of the absorbing ion by analytes within the sample produces a negative absorbance peak [53]. Various indirect chromophores have been used in the analysis of organic acids; these include naphthalene dicarboxylic acid (NDC) [53,54], pyridine-2-6-dicarboxylic acid (PCDA) [55] 4-hydroxybenzoate [58], sodium chromate [50] and phthalate [59]. The choice of UAC or FAC is governed by the need to obtain reasonable electrophoretic peak shapes by closely matching their charge and mobility with those of the analyte ion. In comparison with other modes of detection, UAC and FAC provide less concentration sensitivity and poorer resolution. However, the detection method

Table 1
Summary of published papers: CE of small organic acids in the environment

Compounds determined	Background electrolyte[a]	Sample	L.O.D.	Ref.
Aminobenzoate, hydroxybenzoate, toluate, benzoate	pH=4	Spiked water		[46]
Oxalate, formate, acetate, propionate, malonate, butyrate, valerate, pelargonate, azelate	Salicylic acid, hexadimethrion bromide, pH=7.5	Rain drop	50 fmol	[47]
Oxalate, malonate, formate, succinate	Sodium chromate, anion-BT pH=8	Atmospheric aerosols	100 ppb	[48]
Oxalate, formate, fumarate, pyruvate, malonate, maleate, citrate, lactate, succinate, aspartate, glucuronate, gluconate, acetate, ascorbate, shikimate, gallate, propionate, butyrate	4-Hydroxybenzoate, anion-BT, pH=4.75	Spiked water		[49]
Oxalate, formate, acetate, propionate	Chromate, CTAB, pH=8	Osmotically treated water	100–500 ppb	[50]
Formate, acetate, glycolate, propionate, lactate, butyrate, hydroxybutyrate	3,5-Dinitrobenzene, CTAB, pH=5.9	Atmospheric particulates	50–350 ppb	[51]
Oxalate, malonate, formate, carbonate, acetate, propionate, butyrate, benzoate, succinate, glutarate, adipate, permilate, suberate, acrylate, valerate, hexanoate, hydrocinnamate, ethyl hexanoate	Phthalate, CTAB, pH=7	Various industrial (e.g. process streams)	100–200 ppb	[52]
Formate, methane sulphonate, carbonate, acetate, chloroacetate, dichloroacetate, propionate, butyrate, benzoate, fumarate, glutarate, adipate, pimelate, suberate, azelate, sebacate, propionate	2,6-Naphthalene dicarboxylic acid (NDC), tetradecylammonium bromide	Air extracts (solid and liquid)	100 ppb	[53]
Formate, carbonate, acetate, oxalate	*p*-Amino benzoate, TTAB(H), pH=9.4	Rain drop		[54]
Formate, acetate, propionate, butyrate, oxalate, malonate, succinate, maleate	Pyridine-2,6-dicarboxylic acid, TTAB, pH=7.8	Waste water	500 ppb	[55]
Acetate, propionate, butyrate, valerate, caprionate	Dinitrobenzoic acid, CTAB, pH=7.8	Oil field water	500 ppb	[56]
Propionate, acetate, ascorbate, formate, malate, citrate, lactate, tartarate	8-Hydroxyquinoline–5-sulphonic acid, pH=3	Spiked water		[57]
	Chromate, HDB, pH=10.8	Waste streams from pulp processing		[58]
Succinate, levulinate	Phthalate, CTAB pH=7	Industrial process streams	500 ppb	[59]

[a]UV-absorbing chromaphore, EOF modifier and pH.

has proved reliable and is amenable to application on real samples, provided that appropriate capillary conditioning procedures are employed [59].

Table 1 summarises the range of studies that have been reported. In general, results have been encouraging, with resolution of up to 18 organic acids achievable within 7 min (Fig. 9) [49]. In addition to including EOF modifiers, separations have, in some cases, been improved by the addition of other components such as cationic complexing agents (calcium) [49]. The choice of background electrolyte pH for the separation depends on the pK_a values of the acids present. In general, a high pH is preferred, as complete ionisation of all of the compounds is ensured [50]: however, analysis at low pH has also been demonstrated (Fig. 10) [57]. Where the acids are of similar molecular mass (and, hence, mass–charge ratio), the choice of pH becomes critical [51] for achieving reasonable separations (Fig. 11).

A number of studies have directly compared CE analysis with ion chromatography and have demonstrated both separation power and time as the benefits of the former [51,52]. However, sensitivity remains an issue [51] and, although certain UV-absorbing chromophores (e.g. NDC) have proven better than others in this respect [53], the reported concentration sensitivity has typically been between 100 ppb and 1 ppm. Some further improvements in sensitivity might be achieved by moving to indirect fluorescence or by adopting alternative detection strategies (e.g. amperometry).

For a number of applications, simultaneous measurement of inorganic and organic acids is required [48,50,54,55,58]. This has been achieved (Fig. 12) [48] under a range of conditions and for a number of different sample types. CE is thus a viable alternative to IC for these applications.

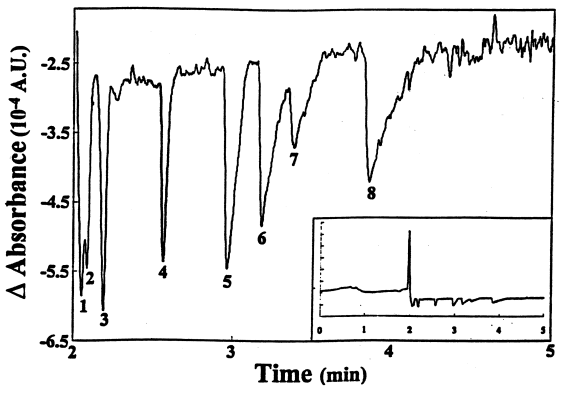

Fig. 10. Separation of eight analytes in a 5-m*M* 8-hydroxyquinoline–5-sulfonic acid (pH 3.00) buffer solution, with indirect absorption detection and dynamic control in CZE. Column: 35 cm (30 cm effective length)×75 μm I.D.×365 μm O.D. The detection wavelength was set at 290 nm. Peaks: (1) propionic acid; (2) acetic acid; (3) ascorbic acid; (4) lactic acid; (5) formic acid; (6) citric acid; (7) malic acid and (8) tartaric acid. The inset shows the whole scale.

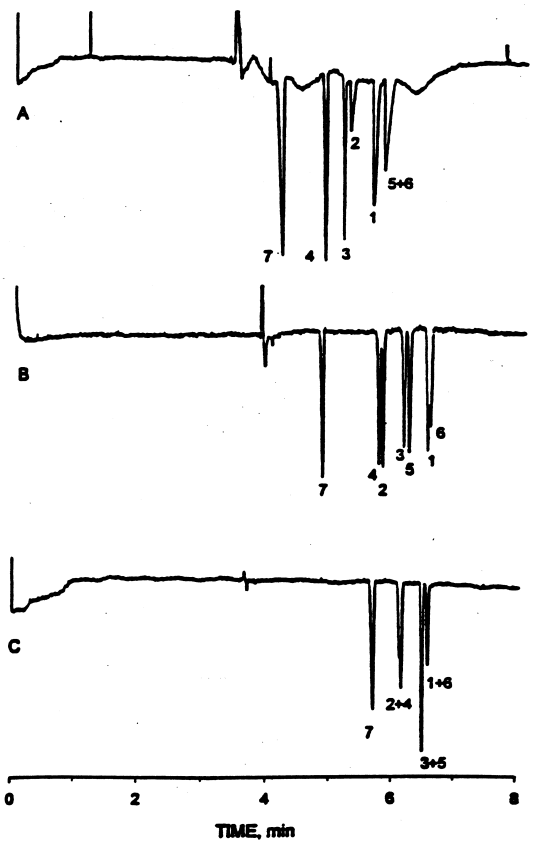

Fig. 11. Electropherograms of the standard mixture obtained in 10 m*M* 3,5-dinitrobenzoic acid containing 0.1 m*M* CTAB at pH 5.0 (A), pH 5.9 (B) and pH 6.2 (C). Peaks: (1) β-hydroxybutyric acid; (2) acetic acid; (3) lactic acid; (4) glycolic acid; (5) propionic acid; (6) butyric acid and (7) formic acid.

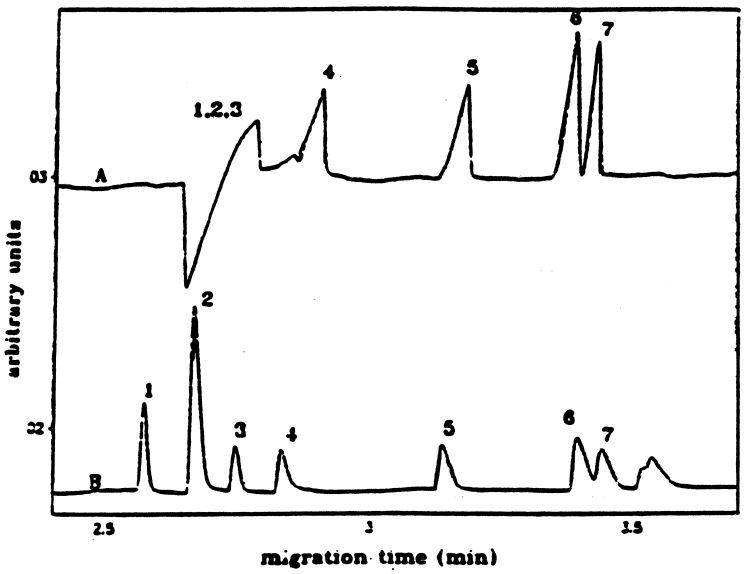

Fig. 12. Simultaneous separation of inorganic anions and organic acids in (A) 5 m*M* phthalate and (B) 6 m*M* chromate electrolyte. Both background electrolytes contained 2.5 cm^3 Anion BT in 100 cm^3 solution. Capillary: 60 cm×75 μm I.D. fused-silica. Applied voltage, 20 kV (negative). Hydrostatic injection (gravity), 10 cm for 30 s. UV detection was at 254 nm. Peaks: 1=chloride; 2=sulphate; 3=nitrate; 4=oxalate; 5=malonate; 6=formate and 7=succinate.

3. Future trends

The growing requirement for environmental monitoring increases the need for fast and cheap methods with low reagent consumption.

This should favour the uptake of CE, particularly if the available instrumentation continues to develop to allow increased sensitivity and selectivity of detection. The emergence of capillary electrochromatography (CEC) is likely to further assist acceptance of CE methods, although the full benefit of this technique will need to be realised by including the capacity to perform gradient elution [60], and by increasing the range of available stationary phases [61].

Acknowledgements

Work described in this paper was supported under contract with the Department of Trade and Industry as part of the Analytical Innovation Programme.

References

[1] M.J. Gordon, X. Huang, S.L. Pembreg Jr., R.N. Zare, Science 242 (1988) 224.
[2] A.G. Ewing, R.A. Wallingford, T.M. Olefirowicz, Anal. Chem. 61 (1989) 292.
[3] S.F. Li, Capillary Electrophoresis Principles and Applications, Elsevier, Amsterdam, 1992.
[4] R. Weinberger, Practical Capillary Electrophoresis, Academic Press, New York, 1993.
[5] C.A. Monning, R.T. Kenndy, Anal. Chem. 66 (1994) 280R.
[6] R. Khun, S.H. Khun, Chromatographia 34(10) (1992) 505.
[7] A.G. Ewing, R. Wallingford, Adv. Chromatogr. 29 (1989) 1.
[8] P. Sandra, J. Vindevogel, Introduction to Micellar Electrokinetic Chromatography, Huthig Buch Verlag, Heidelberg, 1992.
[9] E.K. Korchenaya, A.N. Ermakov, L.R. Bachkova, Anal. Chem. (USSR) 33 (1978) 635.
[10] R.S. Sahota, M.G. Khaled, Anal. Chem. 66 (1994) 1141.
[11] O.R. Cole, L.D. Hiller, A.C. Chwojdak, M.J. Sepaniak, J. Chromatogr. A 736 (1996) 239.
[12] W.C. Brumley, J. Chromatogr. Sci. 33 (1995) 670.
[13] G.M. Mclaughlin, A. Weston, K.D. Hauffe, J. Chromatogr. A 744 (1996) 123.
[14] M.W.F. Neilen, Trends Anal. Chem. 12(9) (1993) 345.
[15] M.C. Hennion, Analusis 25 (1997) M25.

[16] B.A.P. Buscher, U.R. Tjaden, J. Van der Greef, J. Chromatogr. A 788 (1997) 165.
[17] H.R. Schulten, Fresenius' J. Anal. Chem. 351 (1995) 62.
[18] J.S. Gaffney, N.A. Marley, S.B. Clark, Humic and Fulvic Acids: Isolation, Structure and Environmental Role, ACS Symposium Series, Vol. 651, ACS, Washington, DC, 1997, pp. 43–56, 79–95, 96–107.
[19] J.H. Ephrain, H. Boen, C. Petterson, I. Arserie, B. Auard, Environ. Sci. Technol. 23(3) (1989) 356.
[20] D. Grosjean, Environ. Sci. Technol. 23(12) (1989) 1506.
[21] G. Woelki, S. Freidrich, G. Hanschmann, R. Salze, Fresenius' J. Anal. Chem. 357 (1997) 548.
[22] R.L. Malcolm, in B. Allard, H. Boren, A. Grimvah (Editors), Humic Substances in the Aquatic and Terrestrial Environment, Springer, Berlin, 1991, p. 9.
[23] R.L. Warshaw, P.J. Pinckeny, J. Res. U.S. Geol. Surv. 1 (1973) 361.
[24] A.W. Garrison, P. Schmitt, A. Kettrup, Water Res. 29 (1995) 2149.
[25] A. Rigol, J.F. Lopez-Sanchez, G. Rauret, Quim. Anal. 13 (1994) 11.
[26] P. Kopacek, D. Kaniansky, J. Hejzlar, J. Chromatogr. 545 (1991) 461.
[27] S. Wakida, S. Takeda, S. Miyazaki, Y. Yakushiji, K. Fukushi, H. Yamore, K. Higashi, Bunseki Kagaku 46(6) (1997) 483.
[28] S. Pompe, K. Heiniz-Heise, H. Nitsche, J. Chromatogr. A 723 (1996) 215.
[29] C. Ciavatta, M. Govi, C. Gessa, J. High. Resolut. Chromatogr. 20 (1997) 67.
[30] C. Ciavatta, M. Govi, L. Sitti, C. Gessa, Commun. Soil. Sci. Plant Anal. 26(19–20) (1995) 3305.
[31] D. Fetsch, J. Havel, J. Chromatogr. A 802 (1998) 189.
[32] M. Norden, E.D. Ziotorzynska, Electrophoresis 18 (1997) 292.
[33] A. Rigol, M. Vidal, G. Rauret, J. Radioanal. Nucl. Chem. 208(2) (1996) 617.
[34] M. Norden, E.D. Ziotorzynska, J. Chromatogr. A 739 (1996) 421.
[35] A. Rigol, M. Vidal, G. Rauret, J. Chromatogr. A 664 (1994) 301.
[36] G. Dinelli, A. Vicari, P. Catizone, J. Chromatogr. A 733 (1996) 337.
[37] A.W. Garrison, P. Schmitt, D. Martens, K. Kittrup, Environ. Sci. Technol. 30 (1996) 2449.
[38] B.A. Ingelse, J.C. Reijenga, M. Flieger, F.M. Everaerts, J. Chromatogr. A 791 (1997) 339.
[39] M.L. Marina, A.L. Crego, J. Liq. Chromatogr. Rel. Technol. 20(9) (1997) 1337.
[40] M.W.F. Nielen, J. Chromatogr. 637 (1993) 81.
[41] C. Spagone, C. D'Orazio, M. Rossi, D. Rotilio, J. High. Resolut. Chromatogr. 19 (1996) 647.
[42] W.C. Brumley, C.M. Brownrigg, J. Chromatogr. 646 (1993) 377.
[43] Y.Z. Hsieh, H.Y. Huang, J. Chromatogr. A 745 (1997) 217.
[44] Y. Mechref, Z.E. Rass, J. Chromatogr. A 757 (1997) 263.
[45] C. Desiderio, C.M. Polcaro, P. Padiglioni, S. Fanali, J. Chromatogr. A 781 (1997) 503.
[46] M.M. Hseieh, H.T. Chang, J. Chromatogr. A 793 (1997) 263.
[47] B. Tenberken, K. Bachmann, J. Chromatogr. A 775 (1997) 372.
[48] Z. Krivacsy, A. Molnar, E. Torjanyi, A. Galenscer, G. Kiss, J. Hlavay, J. Chromatogr. A 781 (1997) 223.
[49] J. Farre, F. Borrull, M. Calull, Chromatographia 44(5/6) (1997) 235.
[50] S.R. Souza, M.F.M. Tavares, L.R.F. De Carvalho, J. Chromatogr. A 796 (1998) 335.
[51] J. Chen, B.P. Preston, M.J. Zimmerman, J. Chromatogr. A 781 (1997) 205.
[52] O. Devevre, D.P. Putra, B. Botton, J. Garbaye, J. Chromatogr. A 679 (1997) 349.
[53] E.D. Zlotorzynska, J.F. Diocihy, J. Chromatogr. A 685 (1994) 145.
[54] B. Tenberken, P. Ebert, M. Hartmann, M. Kibbler, A. Manka, T. Prokop, A. Roder, K. Bachmann, J. Chromatogr. A 745 (1996) 209.
[55] M.P. Kallia, M. Kuitunnen, P.K.G. Manninen, Chemosphere 35(7) (1997) 1504.
[56] L. Changquing, M. Rting, F. Xiaolin, M. Bowen, Chem. J. Chromatogr. 15 (1997) 173.
[57] H.T. Chang, H.S. Chen, R. Lee, J. Chromatogr. A 800 (1998) 339.
[58] D. Volgger, A. Zemann, G. Bonn, J. High. Resolut. Chromatogr. 21 (1998) 3.
[59] M. Saeed, M. Depala, D.H. Craston, T. Lynch, Chromatographia 47(11/12) (1998) 709.
[60] C.G. Huber, G. Choudhary, C. Horvath, Anal. Chem. 69 (1997) 4429.
[61] D.L. Hugo, H. Knobel, V.T. Remcho, J. Chromatogr. A 695 (1997) 169.