Journal of Chromatography, 636 (1993) 153–169
Elsevier Science Publishers B.V., Amsterdam

CHROM. 24 739

Role of capillary electrophoresis in specialty chemical research

Kenneth P. Evans* and Gillian L. Beaumont

Specialties Research Centre, Zeneca Specialties, P.O. Box 42, Blackley, Manchester M9 3DA (UK)

ABSTRACT

The use of capillary electrophoresis (CE) for the analysis of a range of specialty chemicals including small ions, agrochemicals, biocides and dyestuffs is reported. Quantitative aspects of the technique are also considered. In general CE is found to be complementary to ion chromatography and high-performance liquid chromatography (HPLC) but offers distinct advantages in terms of speed, resolution, ease of method development and the ability to analyse dyestuffs that are difficult by HPLC.

INTRODUCTION

The potential scope of CE techniques is far reaching and CE techniques appropriate to specialty chemicals have been reported [1,2] covering the following diverse range of compound types: (i) inorganic/small ions → macromolecules → submicron particles; (ii) highly polar molecules → neutral molecules; (iii) chiral compounds (resolution of enantiomers).

In this paper we report a comparison of CE with conventional chromatographic techniques for assays developed in our laboratory involving the determination of small ions, agrochemicals, industrial biocides and dyestuffs. Whilst the CE methodologies employed in this study have been extensively reported, there is little published information on their practical application in specialty chemicals research. In particular the analysis of anionic and cationic dyestuffs by CE has received little attention [3].

EXPERIMENTAL

Instrumentation

The capillary electrophoresis instrument used was a Quanta 4000 (Waters Chromatography, Division of Millipore, Milford, MA, USA) with a Perkin-Elmer CLAS data system (Perkin-Elmer Corporation, Norwalk, CT, USA). The separation capillaries used were 60 cm × 75 μm I.D. fused-silica from Polymicro Technologies (Phoenix, AZ, USA). Ion chromatography (IC) was performed using a Dionex (Sunnyvale, CA, USA) 2100I chromatograph. The IC column system used consisted of a 25 cm × 4.5 mm I.D. Dionex IonPac AS4A anion-exchange column fitted with a 5 cm × 4.5 mm I.D. AS4A guard column. A 5 cm × 4.5 mm I.D. ODS Hypersil precolumn was connected to the anion-exchange column system to minimise sample fouling. The HPLC instrument used consisted of a Hewlett-Packard (Waldbronn, Germany) 1090L and HPLC columns were 25 cm × 4.5 mm I.D. ODS Hypersil (5 μm) (Shandon Scientific, Runcorn, UK) or Spherisorb ODS-1 (5 μm) (Phase Separation, Deeside, UK).

Chemicals, reagents and solvents

Purified (18 MΩ) water using a Millipore (Bedford, MA, USA) Milli Q water purification system was used for all solutions. Sodium chromate tetrahydrate, concentrated sulphuric acid, lithium hydroxide, alkyl sulphonates, sodium tetraborate decahydrate, sodium carbonate, sodium bicarbonate

* Corresponding author.

and tetrapropyl ammonium hydroxide were obtained from Aldrich (Gillingham, UK). *p*-Hydroxybenzoic acid was obtained from Sigma (Poole, UK). The anion and cation standards were obtained from BDH (Poole, UK). Sodium dodecyl sulphate (SDS) and dicyclohexylamine were supplied by Fluka (Glossop, UK) and orthophosphoric acid from May and Baker (Eccles, UK). HPLC solvents were obtained from Rathburn (Walkerburn, UK). The electroosmotic flow modifiers OFM Anion-BT and UVCat-1 were obtained from Waters.

RESULTS AND DISCUSSIONS

Capillary ion electrophoresis versus ion chromatography

Capillary ion electrophoresis (CIE) is a technique introduced by Waters Chromatography Division of Millipore in 1991 (under the trade name Capillary Ion Analysis, CIA) for the CE determination of small ions using buffer systems containing a number of patented electroosmotic flow modifiers. The range of ions currently amenable to CIE is some 130 comprising inorganic anions, organic acids, small chain anionic surfactants and inorganic cations [4–10].

Figs. 1 and 2 show the CIE and IC separation of a standard solution of inorganic anions commonly determined. Fig. 3 is the CIE separation of chloride and sulphate in a boiler water sample taken from one of our manufacturing sites. The main advantages of CIE for this analysis is the increased speed. We have also used these CIE conditions to determine chloride and sulphate in the anionic dye shown in Fig. 4. This dye is used for ink-jet printing and the level of chloride and sulphate must be carefully controlled. Fig. 5 is the CIE separation of a dye sample containing 1.7% (w/w) chloride and 800 ppm (w/w)

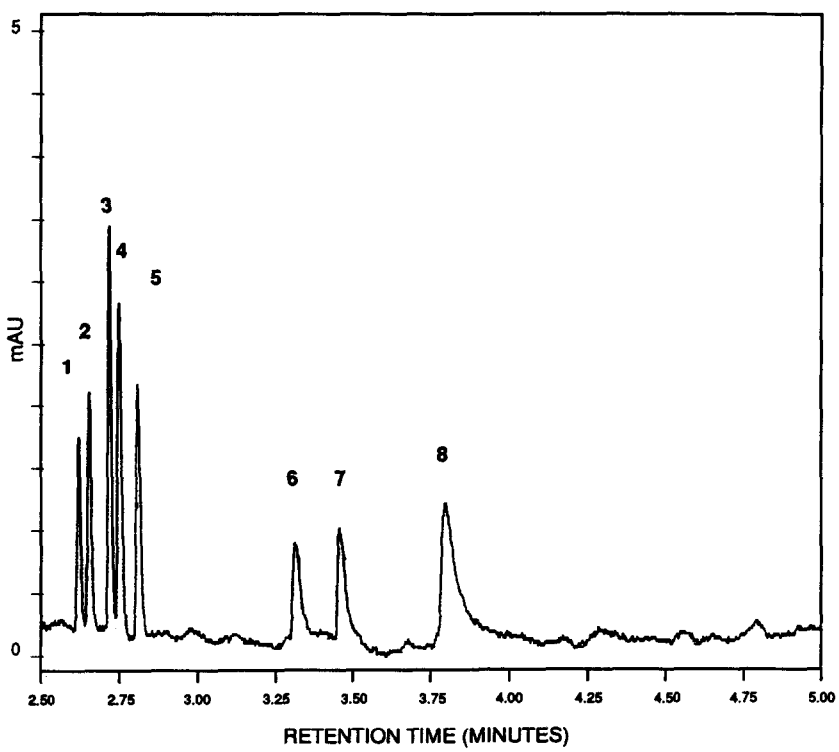


Fig. 1. Electropherogram of standard anions. Conditions: capillary, 60 cm × 75 μm I.D. fused silica; power supply, negative; electrolyte, 5 m*M* chromate with Nice-Pak OFM Anion BT at pH 8.0; injection, hydrostatic for 30 s; detection, indirect UV at 254 nm. Solutes: 1 = bromide (4 ppm); 2 = chloride (2 ppm); 3 = sulphate (4 ppm); 4 = nitrite (4 ppm); 5 = nitrate (4 ppm); 6 = fluoride (1 ppm); 7 = phosphate (4 ppm); 8 = carbonate (4 ppm).

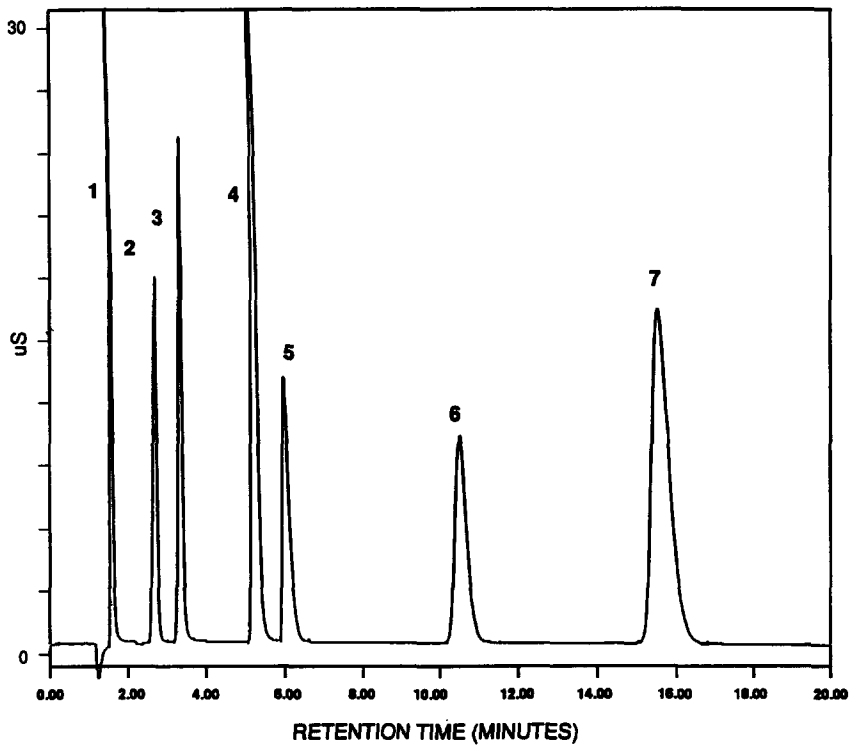


Fig. 2. Ion chromatogram of standard anions. Conditions: column system, Dionex IonPac AS4A (25 cm × 4.5 mm I.D.) fitted with AS4A guard column (5 cm × 4.5 mm I.D.) and ODS Hypersil pre-column (5 cm × 4.5 mm I.D.); mobile phase, 1.8 m*M* sodium carbonate–1.7 m*M* sodium hydrogencarbonate in 0.5 m*M* tetrapropyl ammonium hydroxide at a flow-rate of 2 ml/min; injection, 25 μl; detection, conductivity. Solutes: 1 = fluoride (3 ppm); 2 = chloride (4 ppm); 3 = nitrite (10 ppm); 4 = bromide (25 ppm); 5 = nitrate (10 ppm); 6 = phosphate (25 ppm); 7 = sulphate (25 ppm).

sulphate. IC is currently used for this application but a common problem encountered is fouling of the expensive anion-exchange column by the dye, however, using the CIE procedure the dye is simply removed from the capillary during a purge cycle with the running electrolyte.

A further illustration of the benefits of CIE is shown in Figs. 6–9. Figs. 6 and 7 show the CIE and IC separation of a standard solution of C_1–C_5 alkyl sulphonic acids. In this case the benefit of CIE includes enhanced resolution as well as increased speed. A practical example of this CIE method is the determination of ethane sulphonic acid in waste brine effluents. Fig. 8 is the CIE separation of an effluent sample containing 70 ppm (w/w) ethane sulphonic acid. In contrast the IC separation of this sample showing fewer resolved components in a longer analysis time is given in Fig. 9. A problem common to both CIE and IC in this case was the inability to directly inject the percent levels of chloride present, which had to be removed prior to injecting using a Dionex On Guard-Ag cartridge.

Disadvantages of CIE compared to IC are poorer detection levels and quantitative precision. Area reproducibilities by CIE for 30 injections of the ppm common anions shown in Fig. 1 varied between 3–5% R.S.D. compared to < 1% R.S.D. by IC. CIE detection levels of 0.1–4 ppm are some 100–200 times higher than in IC. The relevance of these limitations of CIE are dependent on the particular analytical assay involved. The majority of our applications involve the determination of small ions present in specialty chemicals at < 2% (w/w) and the poorer quantitative precision of CIE is acceptable. Similarly the higher detection levels of CIE are only a problem for trace analysis or if the sample matrix

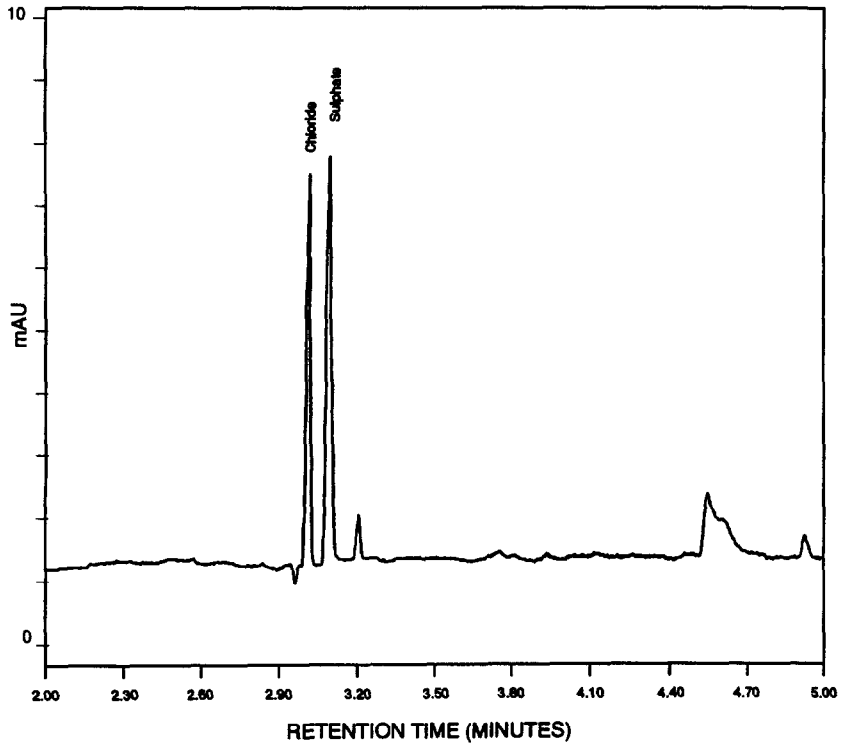
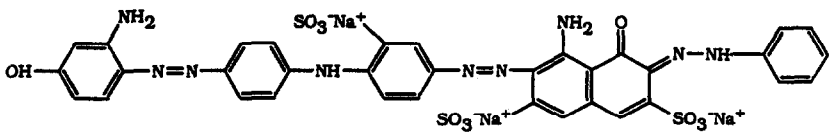
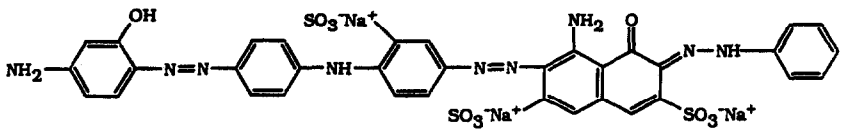


Fig. 3. Electropherogram of boiler water sample. Conditions as for Fig. 1.



ISOMER 1



ISOMER 2

Fig. 4. Structure of ink-jet dye.

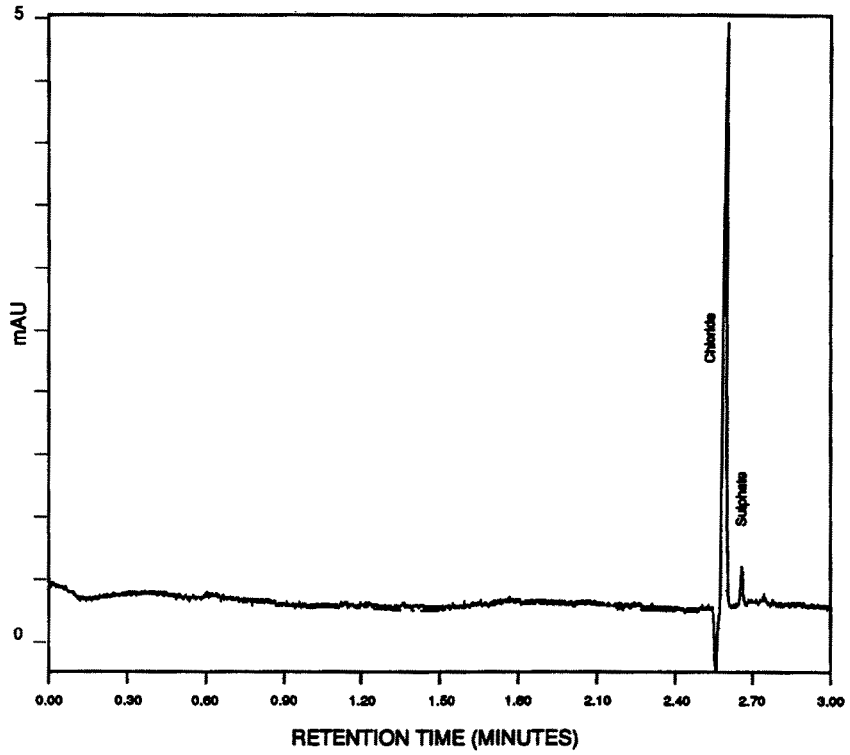


Fig. 5. Electropherogram of 0.1% (w/v) anionic ink-jet dye in water. Conditions as for Fig. 1.

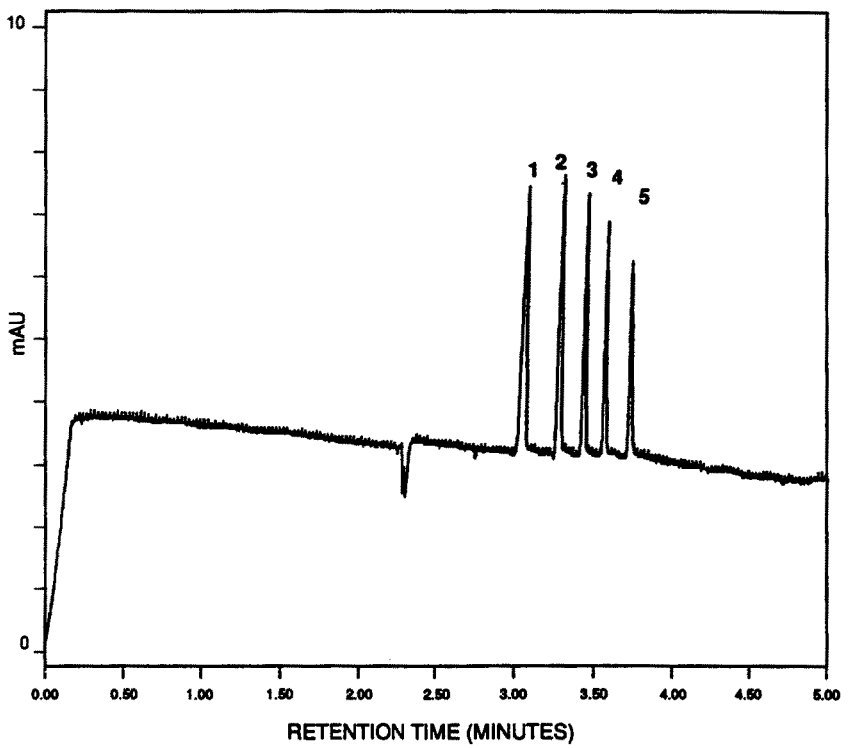


Fig. 6. Electropherogram of C_1–C_5 alkyl sulphonates. Conditions as for Fig. 1, except electrolyte: 5 m*M* *p*-hydroxybenzoic acid + OFM Anion BT at pH 6.0. Solutes (5 ppm): 1 = methanesulphonate; 2 = ethanesulphonate; 3 = propanesulphonate; 4 = butanesulphonate; 5 = pentanesulphate.

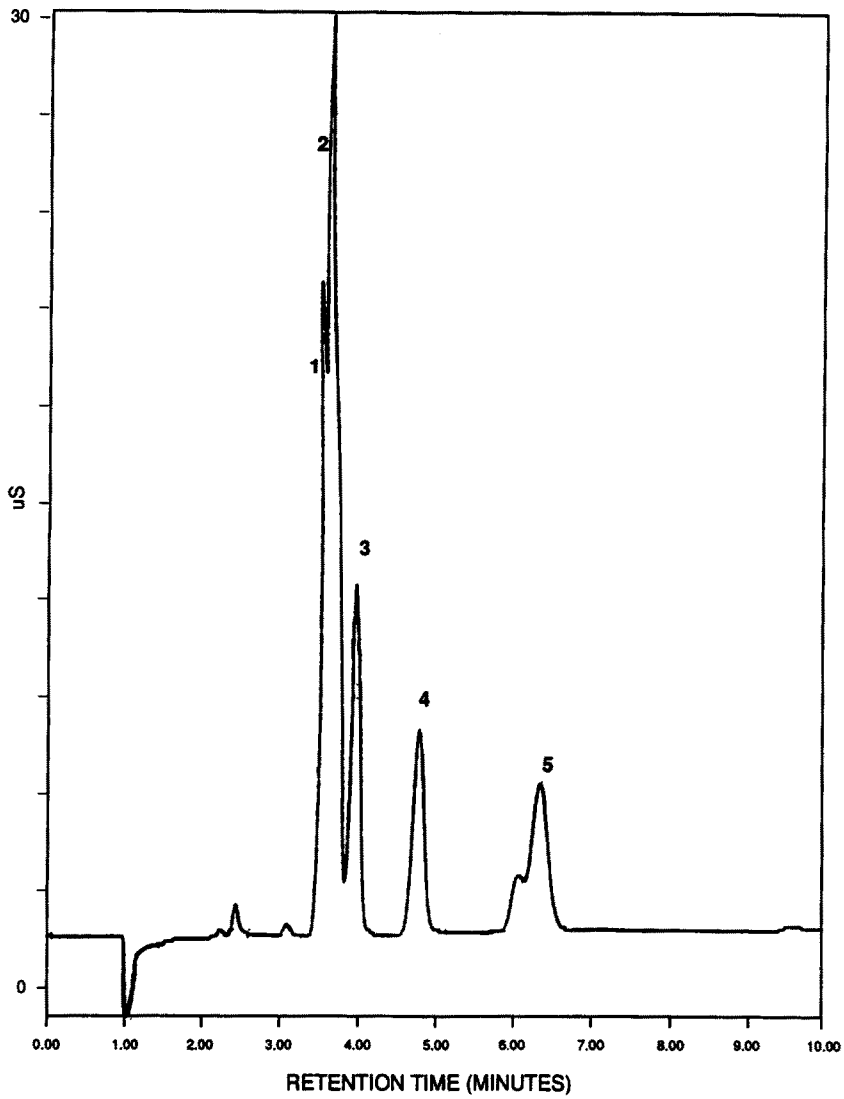


Fig. 7. Ion chromatogram of C_1–C_5 alkylsulphonates. Conditions as for Fig. 2, except mobile phase: 5 m*M* sodium tetraborate at 2 ml/min. Solutes (5 ppm): 1 = methanesulphonate; 2 = ethanesulphonate; 3 = propanesulphonate; 4 = butanesulphonate; 5 = pentanesulphonate.

limits the amount of sample that can be injected. In the case of the anionic dye described above the maximum dye concentration that could be used was 0.1% (w/w) resulting in detection levels for chloride and sulphate of 320 ppm and 270 ppm (w/w) in the sample, which was adequate for our purposes. Whilst lower detection levels in CIE have been reported using electromigrative sample injection [7], preliminary work using this approach with the anionic dye has not been successful due to poor reproducibility and linearity.

We have also used CIE to our advantage for the determination of cations. This is illustrated in Fig. 10 for the determination of cations in boiler water. However, attempts to determine the ppm levels of iron and calcium present in the ink-jet dye by CIE

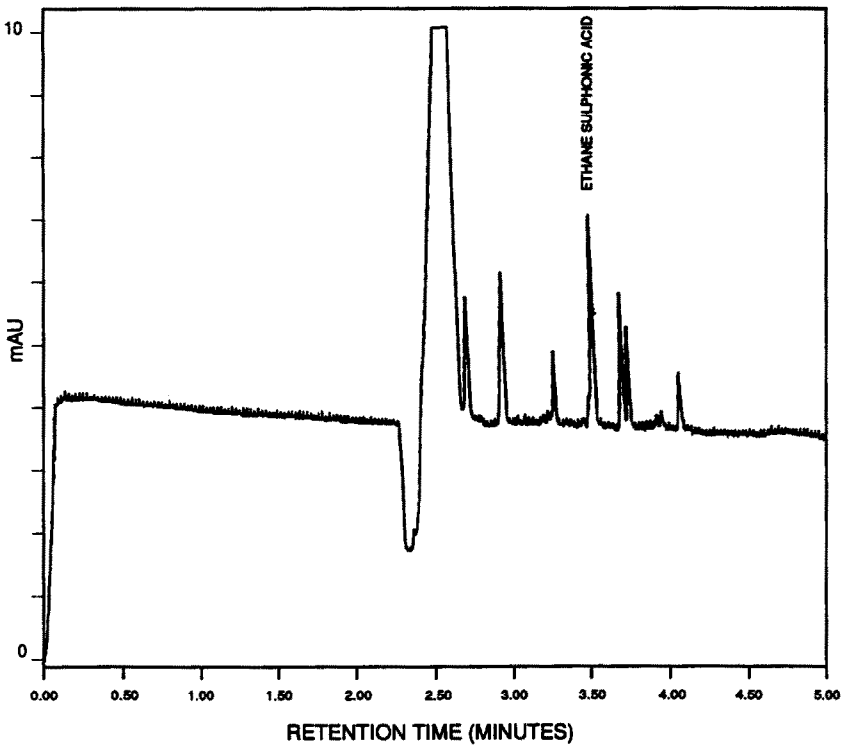


Fig. 8. Electropherogram of waste brine effluent. Conditions as for Fig. 6.

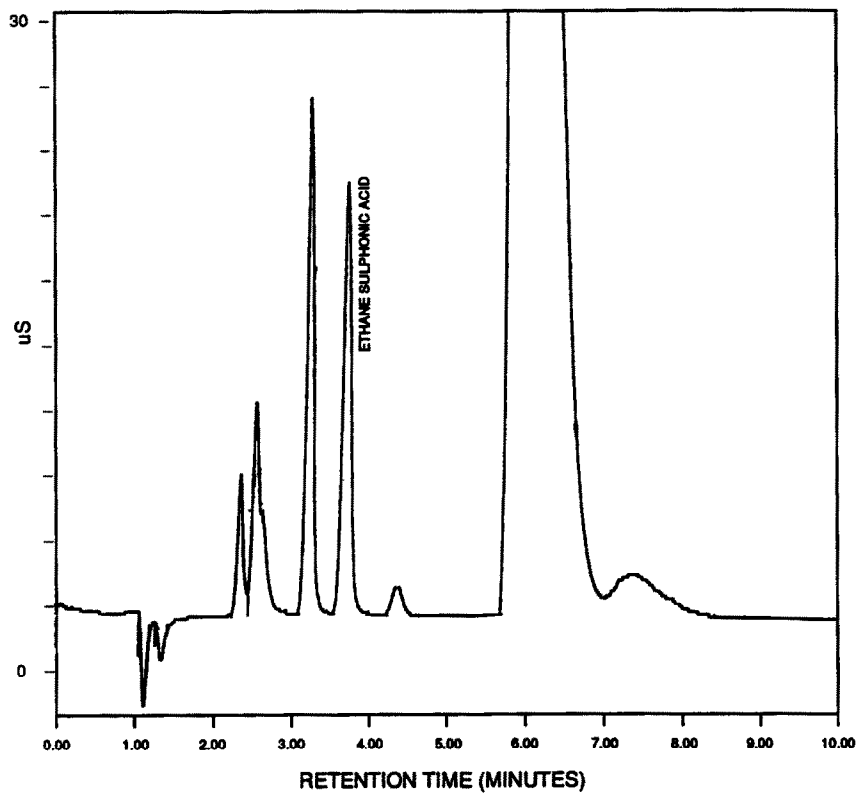


Fig. 9. Ion chromatogram of waste brine effluent. Conditions as for Fig. 7.

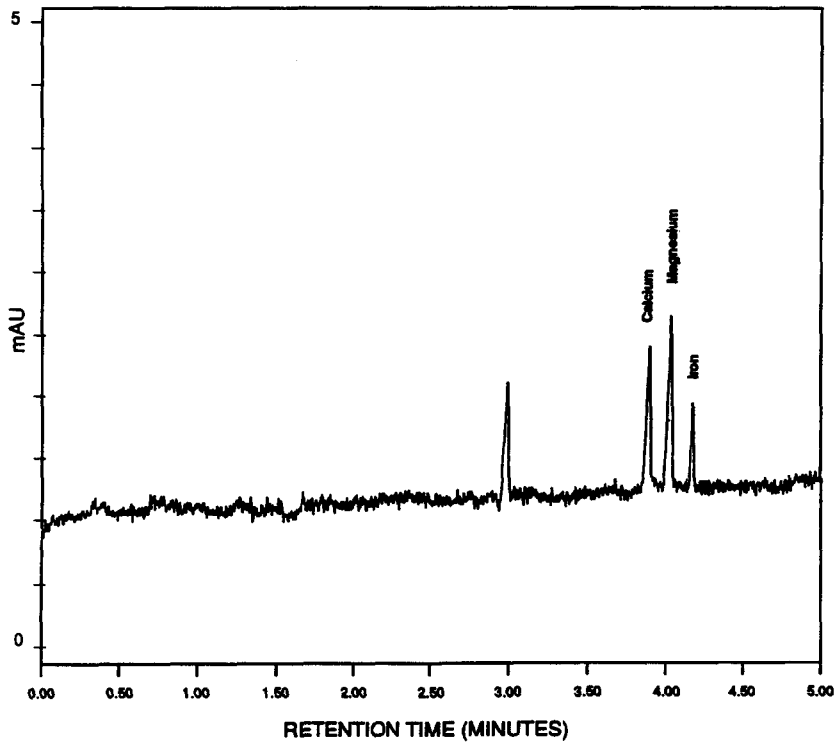


Fig. 10. Electropherogram of boiler water sample. Conditions: capillary, 60 cm × 75 μm I.D. fused-silica; electrolyte, 5 m*M* UVCat-1, 6.5 m*M* HIBA at pH 4.4; power supply, positive; detection, indirect UV at 214 nm.

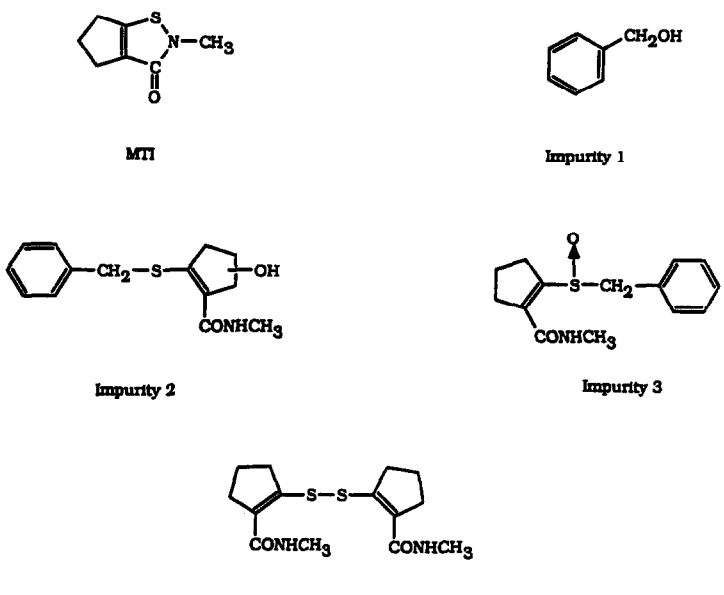


Fig. 11. Structure of industrial biocide MTI and related impurities.

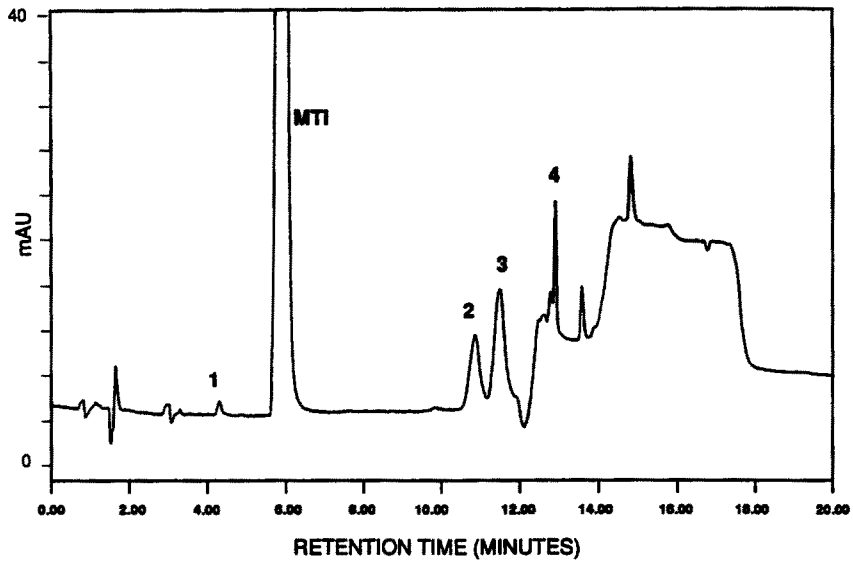


Fig. 12. HPLC separation of industrial biocide (MTI) and related impurities. Conditions: column, 25 cm × 4.5 mm I.D,. Spherisorb ODS-1; mobile phase, water–acetonitrile–phosphoric acid (79.5:20:0.5), isocratic for 10 minutes, programmed to water–acetonitrile–phosphoric acid (49.5:50:0.5) over further 10 min; flow, 2 ml/min; injection, 10 μl of 0.1% (w/v) sample dissolved in acetonitrile–water (50:50); detection, UV at 295 nm, temperature 40°C. The peak Nos. refer to impurities 1–4, respectively, in Fig. 11.

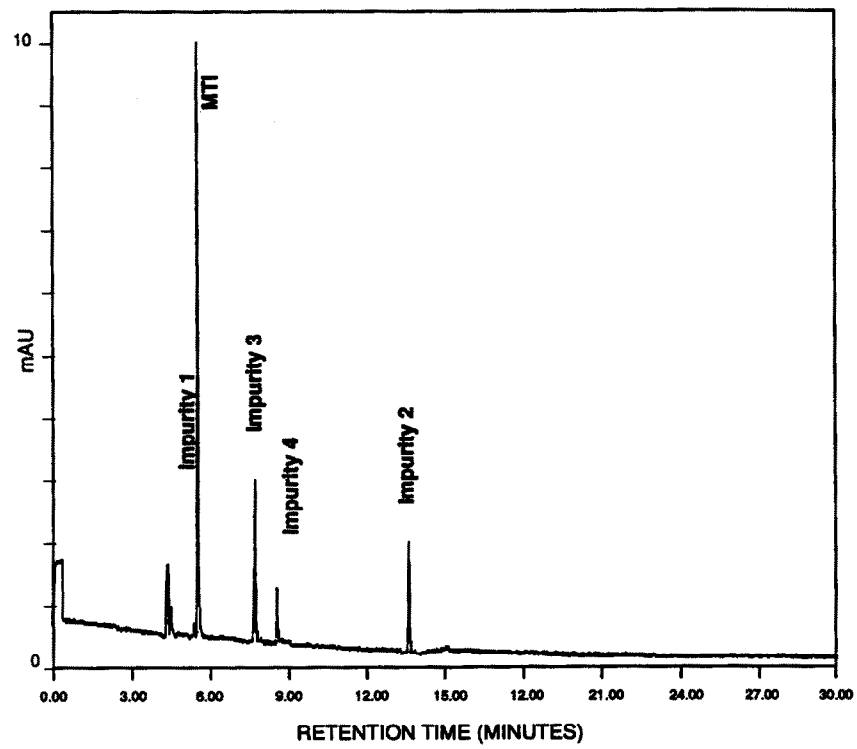
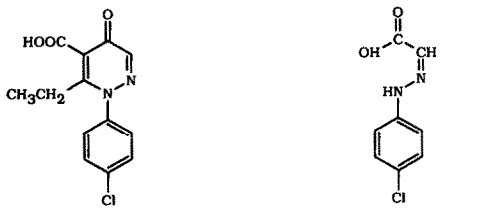


Fig. 13. MECC separation of industrial biocide (MTI) and related impurities. Conditions: capillary, 60 cm × 75 μm I.D. fused silica; power supply, positive; electrolyte, 10 m*M* sodium tetraborate containing 50 m*M* SDS and 10% (v/v) methanol at pH 9.4; injection, hydrostatic for 30 s of 0.01% w/v sample in water–methanol (90:10); detection, UV at 254 nm.



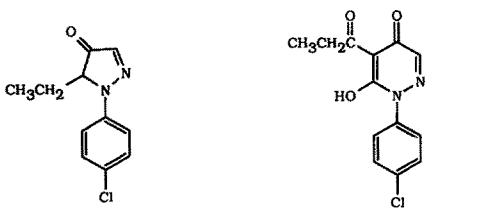


Fig. 14. Structure of agrochemical SC1158 and related impurities.

were unsuccessful due to the high level of sodium present.

Capillary electrophoresis versus HPLC

Water-soluble agrochemicals and industrial biocides. Micellar electrokinetic capillary chromatography (MECC) has proved useful for the characterisation of agrochemicals and biocides. The information provided by MECC is complementary to reversed-phase HPLC as shown by the following examples.

N-Methyltrimethyleneisothiazolin-3-one (MTI) is an industrial biocide whose structure and those of its related impurities are shown in Fig. 11.

The current method used to analyse this material is gradient reversed-phase HPLC. An HPLC separation of MTI and related impurities is shown in Fig. 12 and the MECC separation using a buffer consisting of 10 m*M* sodium tetraborate containing 50 m*M* SDS and 10% (v/v) methanol in Fig. 13. The advantage of MECC in this case is the ability to dispense with gradient HPLC and associated inherent problems of baseline shifts caused by refractive index/UV changes. We have found this ability of MECC to visualise hydrophobic compounds which

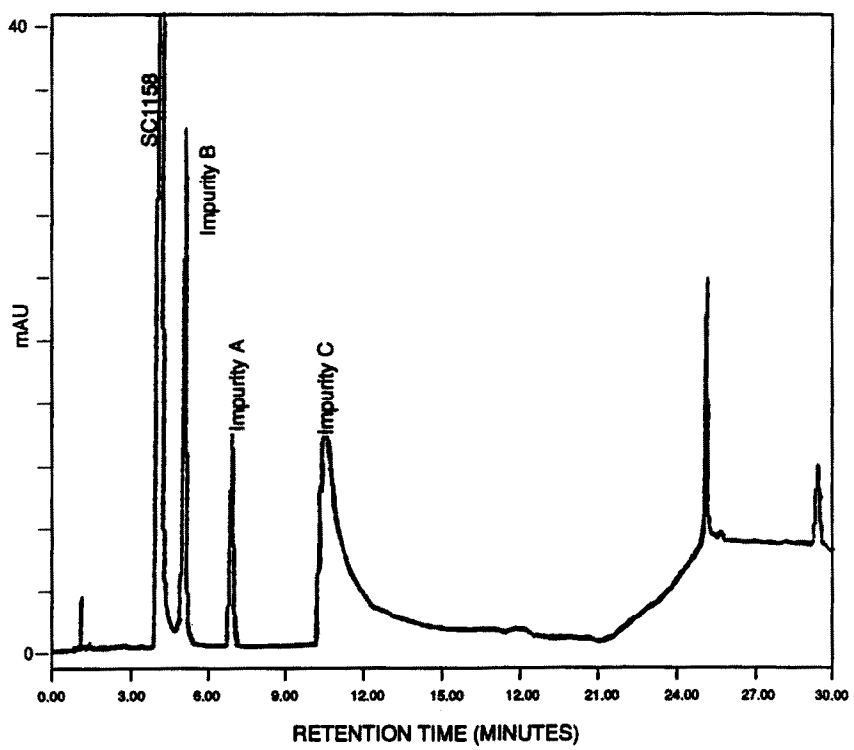


Fig. 15. HPLC separation of agrochemical SC1158 and related impurities. Conditions: column, 25 cm × 4.5 mm I.D. ODS Hypersil; mobile phase, water–methanol–tetrahydrofuran (THF) (81:13:6) containing 5.75 g/l $NH_4H_2PO_4$ and 6.6 g/l $(NH_4)_2HPO_4$, isocratic for 19 min, programmed to water–methanol–THF (62:26:12) containing 5.75 g/l $NH_4H_2PO_4$ and 6.6 g/l $(NH_4)_2HPO_4$ over 5 min; flow, 2 ml/min; injection, 10 μl of 0.1% (w/v) sample dissolved in water–methanol (90:10); detection, UV at 248 nm; temperature, 40°C.

would necessitate gradient elution by HPLC to be a useful characterisation tool. This behaviour can be attributed to the limited retention window operative in MECC [11].

A further example of MECC is the separation of the agrochemical SC1158 and its related impurities (Fig. 14). The gradient reversed-phase HPLC separation employed is shown in Fig. 15. To obtain satisfactory resolution in this case a ternary HPLC mobile phase was required which was developed using a laboratory written computer mobile phase optimisation programme. In contrast the MECC separation using a simple borate–SDS buffer is shown in Fig. 16. Advantages of MECC in this case include simpler method development and improved peak shape for the hydroxy impurity C.

Anionic and cationic dyestuffs. Lee *et al.* [3] investigated the CE–MS of sulphonated dyes, however, little information on the use of CE for dyestuff analysis has appeared in the literature and the most

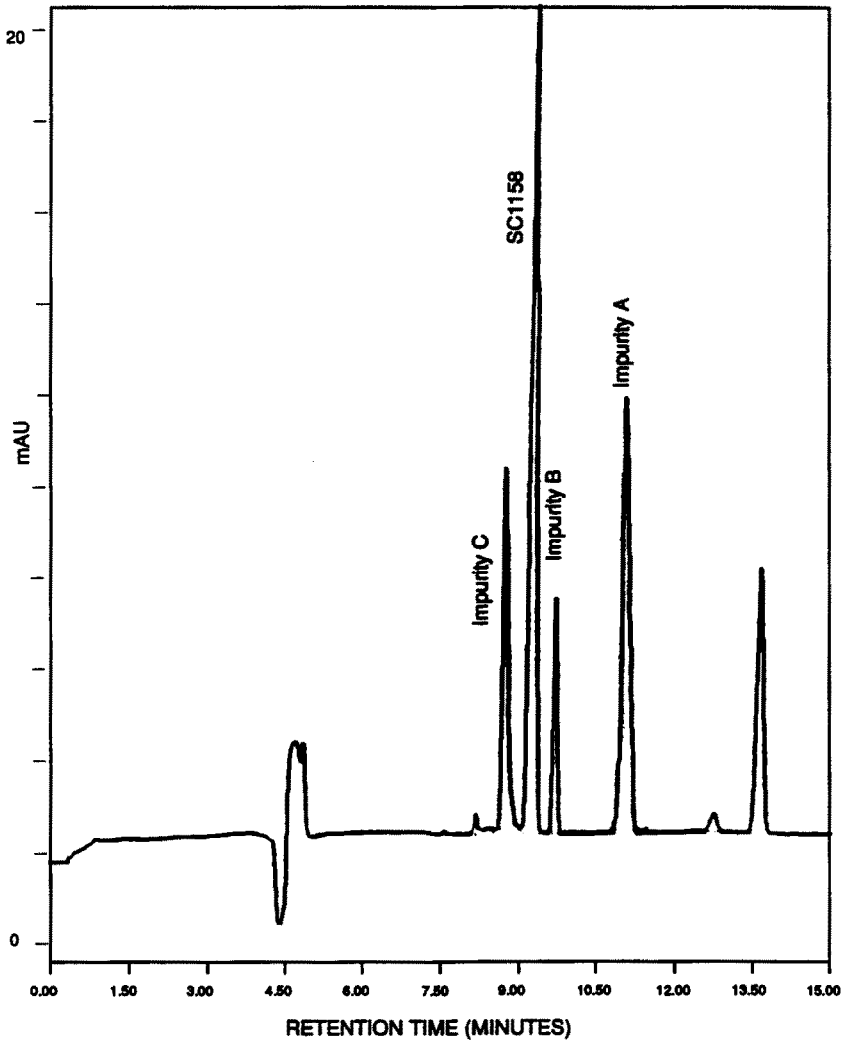
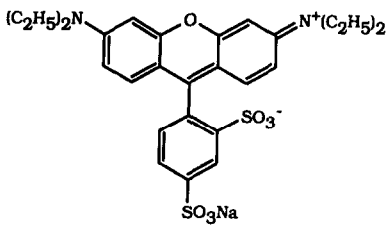
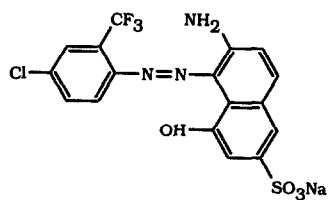


Fig. 16. MECC separation of agrochemical SC1158 and related impurities. Conditions as for Fig. 13, except electrolyte: 50 m*M* sodium tetraborate–8 m*M* boric acid containing 50 m*M* SDS at pH 10.0.

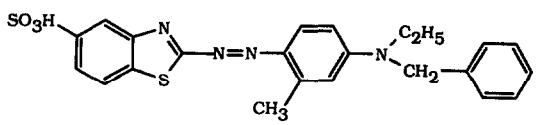
popular method for the analysis of charged dyes is HPLC, particularly ion-pairing HPLC. From our research, however, CE provides an alternative method of analysis which offers superior separation efficiency and simpler method development. In addition, although the separation of anionic dyes and intermediates by HPLC has been developed to a high standard, the analysis of cationic dyes is problematic due to the interaction of cationic groups with silanol sites on reversed-phase packing materials [12]. We have found the two most effective buffer systems for the separation of both anionic and cationic dyes to be 10 m*M* sodium tetraborate or an MECC buffer of 10 m*M* sodium tetraborate containing 20–50 m*M* SDS at a pH of 9–10. These buffer systems have been used to successfully separate the diverse range of dyes and intermediates shown in Figs. 17–20 plus numerous other proprietary dyestuffs. In cases where the free zone separation of anionic dyes was not succesful, due to lack of reso-



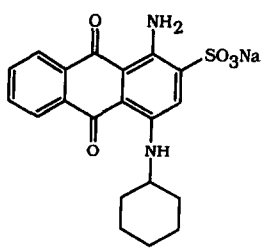
(1)



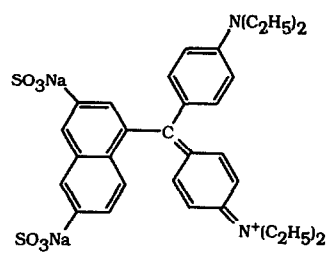
(2)



(3)

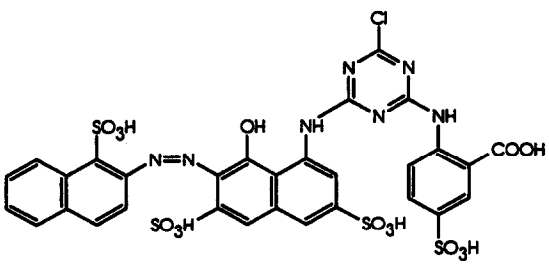


(4)

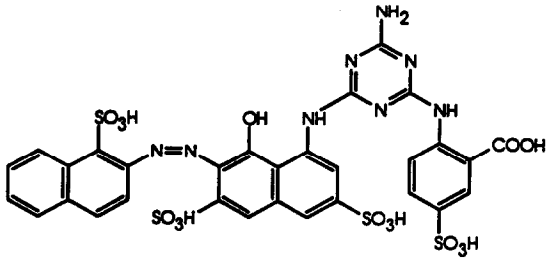


(5)

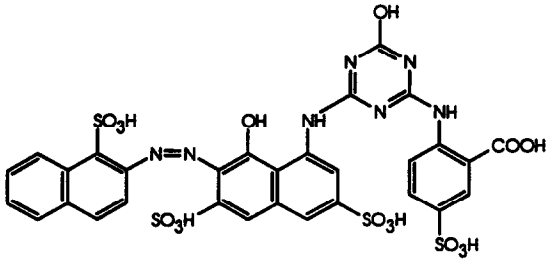
Fig. 17. Structures of acid dyes amenable to CE.



(6)

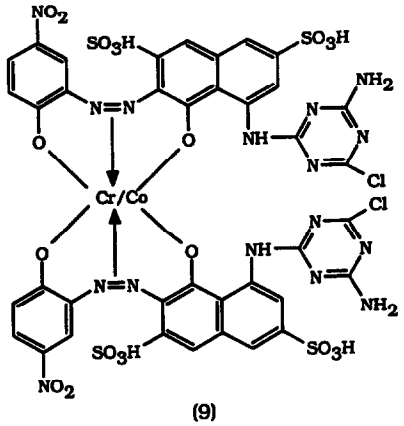


(7)

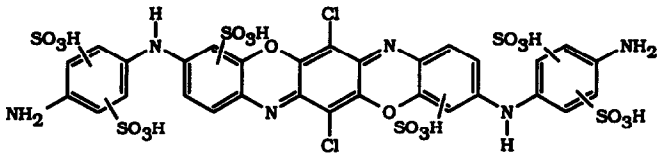


(8)

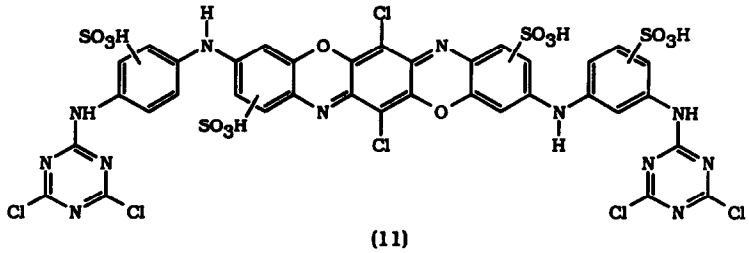
Fig. 18. Structures of reactive dye and related compounds.



(9)



(10)



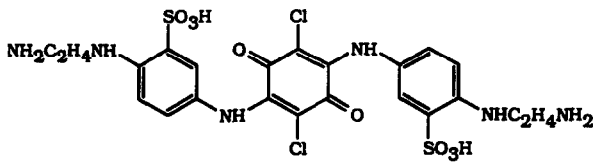
(11)

Fig. 19. Structures of miscellaneous reactive dyes amenable to CE.

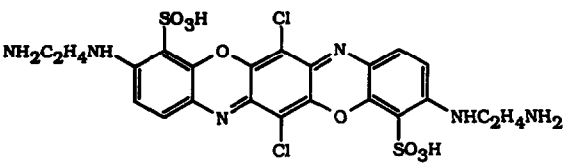
lution or badly tailing peaks, MECC using SDS was more effective than MECC using a cationic surfactant such as cetyltrimethyl ammonium bromide (CTAB). This may be due to the predominant mechanism with a cationic surfactant being strong ion-pairing or for some other reason.

Advantages of CE methodologies for the analysis of dyes is illustrated by the following examples.

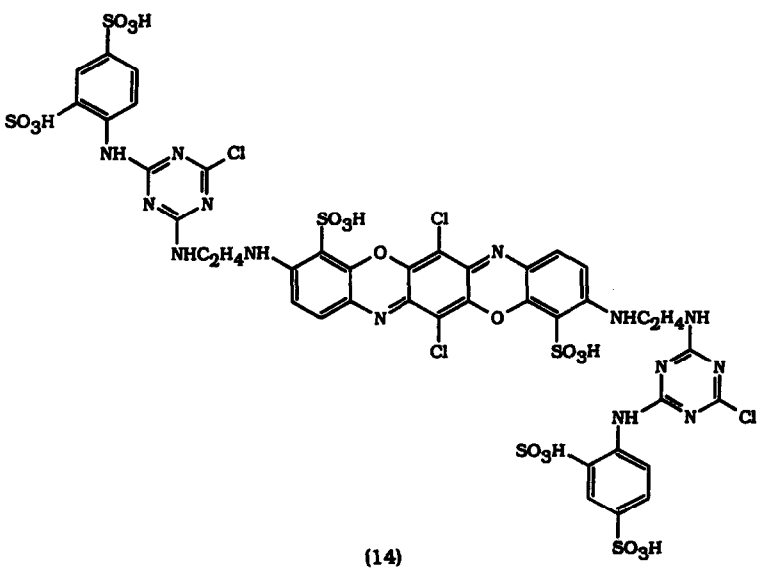
The reactive dye (structure 6, Fig. 18) is used in the printing of cotton and viscose.To investigate the degree of fixation a method was required which would allow determination of unfixed dye, amino derivate (structure 7) formed by possible reaction with urea used in the printing process and the hydrolysed species (structure 8). Whilst we were unable to separate the dye and amino compounds by



(12)



(13)



(14)

Fig. 20. Structures of miscellaneous reactive dyes and intermediates amenable to CE.

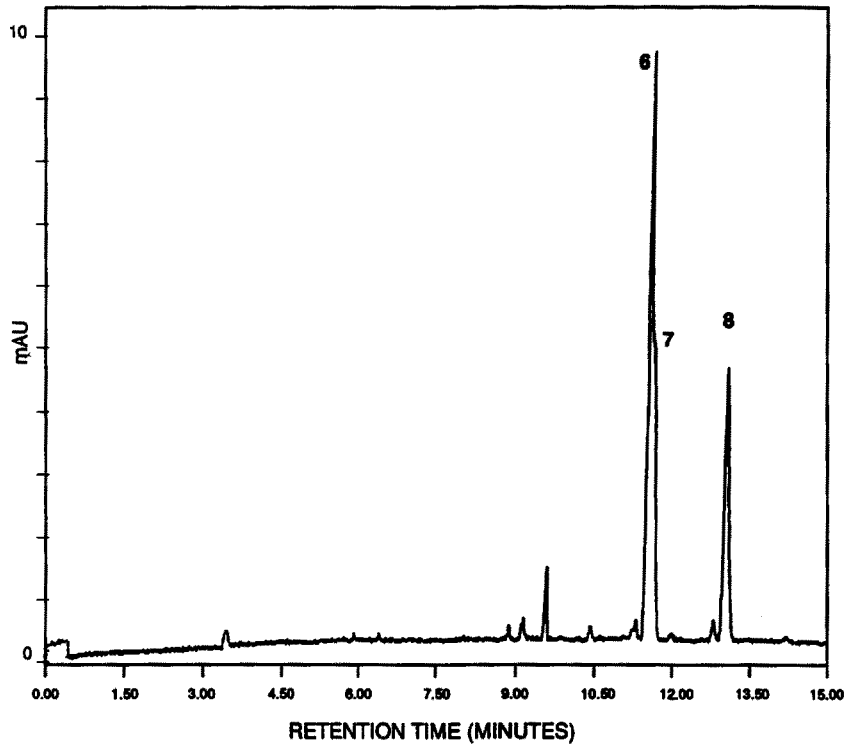


Fig. 21. CZE separation of reactive dye (structure 6) and related compounds. Conditions as for Fig. 13 except electrolyte: 10 m*M* sodium tetraborate at pH 9.4; sample: 30 ppm dye, 30 ppm amino derivative, 30 ppm hydroxyderivate dissolved in water. Peak Nos. refer to structures in Fig. 18.

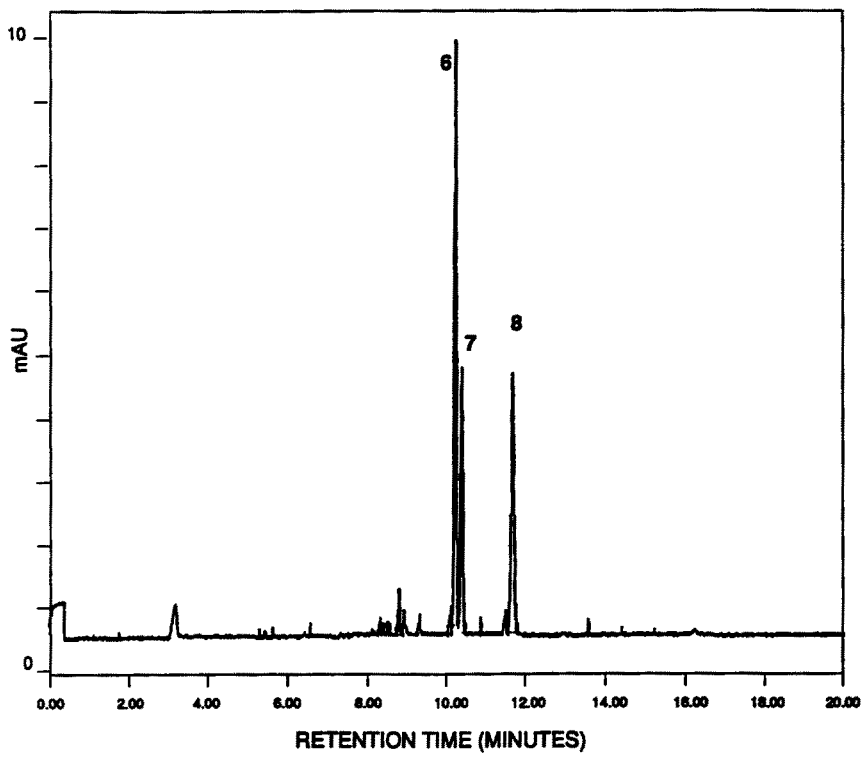


Fig. 22. MECC separation of reactive dye (structure 6) and related compounds. Conditions as for Fig. 21 except electrolyte: 10 m*M* sodium tetraborate containing 50 m*M* SDS at pH 9.4. Peak Nos. refer to structures in Fig. 18.

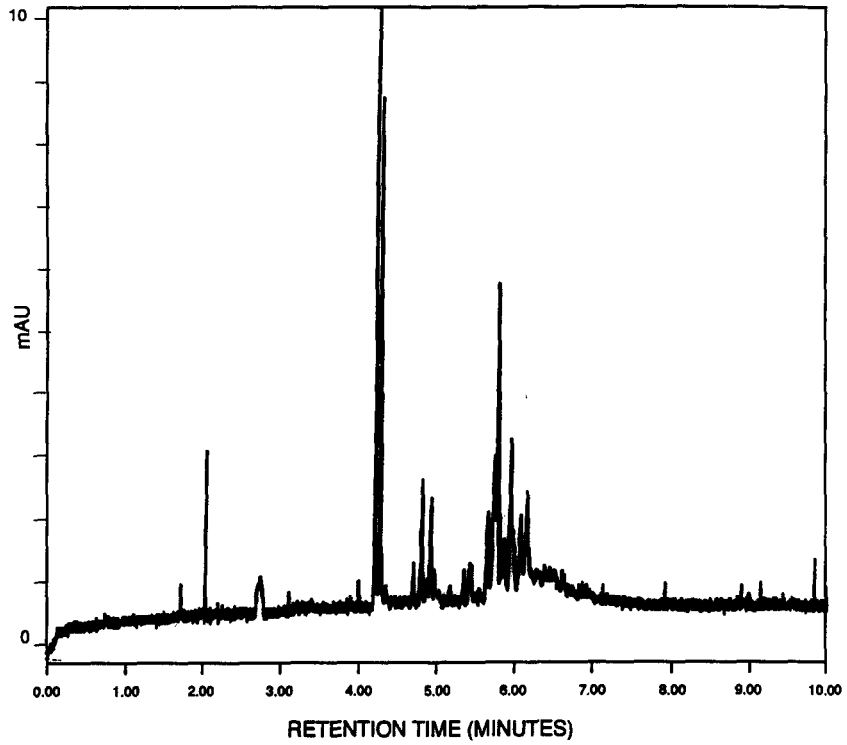


Fig. 23. CZE separation of proprietary reactive dye, Conditions as for Fig. 20 except sample 50 ppm in water.

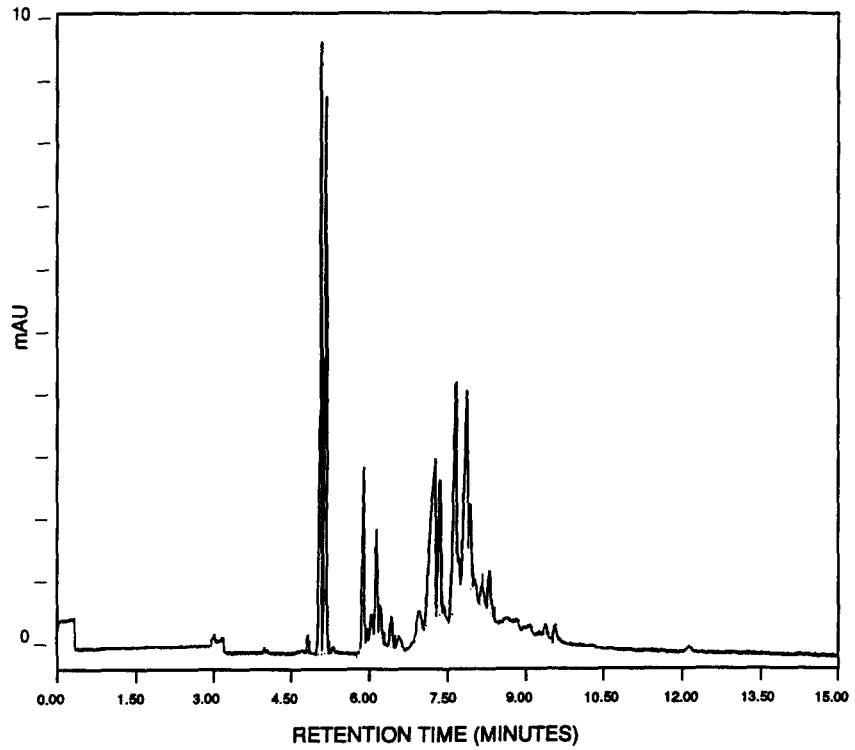


Fig. 24. MECC separation of proprietary reactive dye. Conditions as for Fig. 22 except sample 50 ppm in water.

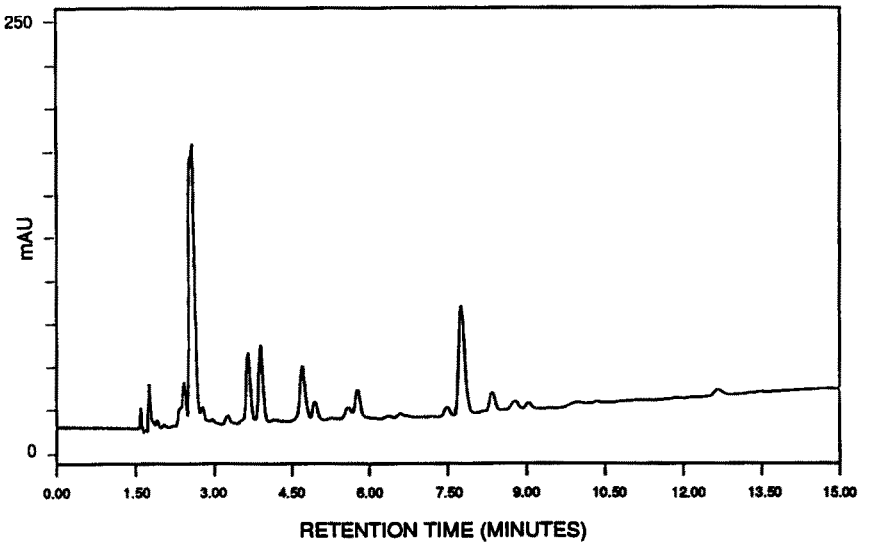


Fig. 25. HPLC separation of proprietary reactive dye. Conditions: column, 25 cm × 4.5 mm I.D. ODS Hypersil; mobile phase, water–acetonitrile–dicyclohexylamine phosphate (48:51.8:0.2) programmed to water–acetonitrile–dicyclohexylamine phosphate (34.8:65:0.2) over 20 min at 1.5 ml/min; injection, 10 μl of 0.1% (w/v) sample dissolved in water; detection, UV at 254 nm; temperature, 40°C.

ion-pairing HPLC or capillary zone electrophoresis (CZE) (Fig. 21), MECC (Fig. 22) using SDS resolves all three compounds of interest.

The last example cited is the CE separation of an impure proprietary reactive dyestuff consisting of a substituted hexasulphonated disazo compound. The CZE and MECC separations (Figs. 23 and 24) show a similar profile, in contrast the ion-pair HPCL separation (Fig. 25) fails to separate the two major components.

CONCLUSIONS

CE techniques are shown to make a significant contribution to specialty chemical research providing information which complements traditional separation methods. Whilst we have previously demonstrated that the current generation of CE instrumentation, including the instrument used in this study is capable of assay precisions of better than 2% R.S.D. by external standardisation for the CZE determination of anthraquinone sulphonic acids [13], precisions of 3–5% R.S.D. for determination of common anions was obtained in this work. This may be due to the lower signal-to-noise characteristics of CIE, however, further research on the quantitative aspects of CE is required under a variety of operating conditions to fully define the reproducibility of the technique.

ACKNOWLEDGEMENT

The authors wish to thank Waters Chromatography Division of Millipore (Europe) for the loan of the capillary electrophoresis instrument.

REFERENCES

1 W. G. Kuhr, *Anal. Chem.*, 62 (1990) 403R.
2 K. D. Altria, M. M. Rogan and G. Finlay, *Chromatography Analysis*, 12 (1990) 9.
3 E. D. Lee, W. Muck and J. D. Henion, *Biomed. Environ. Mass Spectrom.*, 18 (1989) 253.
4 W. R. Jones and P. Jandik, *Am. Lab.*, 22 (1990) 51.
5 J. Romano, P. Jandik, W. R. Jones and P. E. Jackson, *J. Chromatogr.*, 546 (1991) 411.
6 B. F. Kenney, *J. Chromatogr.*, 546 (1991) 423.
7 P. Jandik and W. R. Jones, *J. Chromatogr.*, 546 (1991) 431.
8 W. R. Jones and P. Jandik, *J. Chromatogr.*, 546 (1991) 445.
9 B. J. Wildman, P. E. Jackson, W. R. Jones and P. G. Alden, *J. Chromatogr.*, 546 (1991) 459.
10 P. Jandik, W. R. Jones, A. Weston and P. R. Brown, *LC · GC Int.*, 5 (1992) 20.
11 S. Terabe, K. Otsuka, K. Ichikawa, A. Tsuchiya and T. Ando, *Anal. Chem.*, 56 (1984) 113.
12 P. C. White and A. Harbin, *Analyst*, 114 (1989) 114.
13 S. J. Williams, D. M. Goodall and K. P. Evans, *J. Chromatogr.*, submitted for publication.