Journal of Chromatography, 640 (1993) 413–417
Elsevier Science Publishers B.V., Amsterdam

CHROMSYMP. 2740

Capillary zone electrophoresis in the analysis of dyes and other compounds employed in the dye-manufacturing and dye-using industries

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ABSTRACT

High-resolution separation of several dyes and related intermediates, as well as other compounds employed in the dye-manufacturing and dye-using industries, has been achieved using capillary zone electrophoresis (CZE).

The analysis of anionic dyes and some non-coloured anionic intermediates has been achieved using 10 m*M* $Na_2B_4O_7$–40 m*M* sodium dodecyl sulphate (SDS) buffer; high-resolution separations of water soluble anionic, neutral and cationic intermediates were also achieved using this micellar buffer. Micellar electrokinetic capillary chromatography (MECC) has also been developed for the analysis of aqueous insoluble, electrically neutral compounds by incorporating a co-solvent, acetonitrile, into a micellar buffer. In addition, MECC has been used successfully for following all the major steps involved in the synthesis of a disperse dye.

INTRODUCTION

Previous work on the analysis of dyes by capillary electrophoresis (CE) has largely involved separations using conventional aqueous buffers such as phosphate, borate or citrate [1–3]. Although, in general, efficient separations have been achieved, the range of compounds which could be successfully analysed have been limited to charged simple anionic and cationic dyes and some related intermediates. The analysis of most aqueous soluble neutral analytes and all aqueous insoluble compounds is beyond the scope of conventional capillary zone electrophoresis (CZE). Terabe's introduction of micellar electrokinetic capillary chromatography (MECC) [4] enabled aqueous soluble, electrically neutral species to be separated. Only recently has MECC been used for the analysis of dyes [5] and their intermediates.

This paper demonstrates the range of compounds which can be separated using MECC with only two micellar buffer systems. A conventional MECC buffer has been used for the separation of anionic, cationic and neutral species, and also an MECC buffer containing acetonitrile as co-solvent has been used for the separation of aqueous insoluble, neutral compounds.

EXPERIMENTAL

Materials

p-Phenylenediaminesulphonic acid, *m*-phenylenediaminesulphonic acid, orthanilic acid, *m*-nitrobenzenesulphonic acid and *p*-sulphinic acid-phenylacetanilide were supplied by ICI Specialties (Blackley, UK). *m*-Trimethylammoniumaniline and 2-(aminoethyl)pyridinium chloride were prepared by the method described by Evans *et al.* [7]. HPLC-grade acetonitrile was supplied by BDH Chemicals (Poole, UK). All other reagents were analytical grade supplied by BDH Chemicals. All samples were filtered and degassed.

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Analysis

A Dionex capillary electrophoresis system CES1 (Dionex, Sunnyvale, CA, USA) was used for all analyses. Uncoated silica capillaries were used of internal diameter 75 μm and 60 cm length; the capillary was activated by pressure injection of 0.5 *M* sodium hydroxide solution for 10 min followed by rinsing with deionised water. Sample injection was by gravity: the samples were elevated by 100 mm for 12 s. A voltage of 25 kV was applied for each run with the detector positioned at the cathode and detection was by on-line UV absorbance at 254 nm. Deionised water was used throughout.

RESULTS

Use of MECC in following dye synthesis reactions involving aqueous insoluble compounds

It is important for the dye synthetic chemist to be able to accurately follow how a particular reaction is proceeding. This is often achieved using either high-performance liquid chromatography (HPLC) or thin-layer chromatography (TLC). HPLC is perhaps most commonly used for the separation of charged dyes and intermediates whilst TLC is suited to the analysis of water insoluble dyes such as disperse dyes; TLC, however, generally only provides qualitative information.

CE has been shown to offer higher resolution separations, compared to HPLC, for some dyes and intermediates [2,3], but so far it has only been achieved with charged samples. MECC enables efficient separations of water soluble neutral compounds to be achieved but water insoluble compounds cannot be analysed by conventional MECC. However, by incorporating a co-solvent into the buffer system aqueous insoluble, neutral species have been separated thus enabling the analysis of uncharged water insoluble (disperse) dyes.

The synthesis of a disperse dye (Fig. 1) was followed using MECC; the buffer used was 10 m*M* $Na_2B_4O_7$–50 m*M* H_3BO_3–20 m*M* SDS and 50% (v/v) acetonitrile as co-solvent. All the main reagents used in the reactions were detected (Fig. 2).

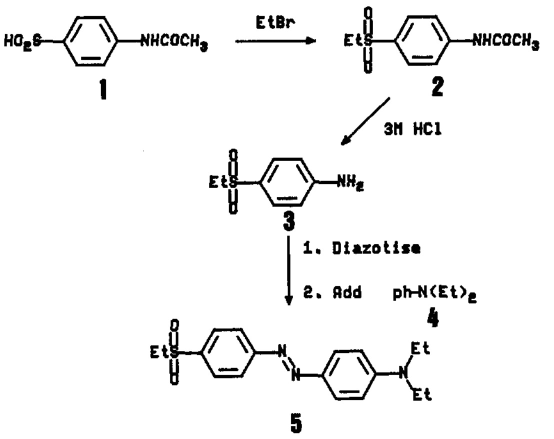

Fig. 1. The synthesis of a disperse dye (**5**).

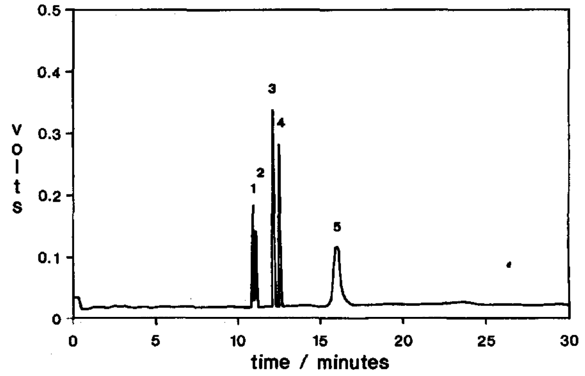

Fig. 2. Separation of the major reagents involved in the synthesis of **5** (shown in Fig. 1) using the following conditions. Buffer: 10 m*M* $Na_2B_4O_7 \cdot 10H_2O$–50 m*M* H_3BO_3–20 m*M* SDS (pH 10) and 50% (v/v) acetonitrile; sample concentration: 10–40 mg dm^{-3}; sample injection: gravity (100 mm for 12 s); voltage: 25 kV; detection (at the cathode): 254 nm; capillary: glass silica 60 cm × 75 μm I.D.

This MECC buffer system was unsuitable for the separation of anionically charged dyes as the electro-osmotic flow of buffer was insufficient, due to the presence of acetonitrile, to force the dyes to elute at the cathode. Also, due to the alkaline pH of the buffer (pH 10), the analysis of cationic dyes using this system was also found to be unsuccessful. The separation of cationic dyes usually requires acidic buffer conditions in order to prevent the dyes interacting with the capillary wall [3]; at high pH values the capillary wall is highly dissociated and positively charged dyes appear to have affinity for the negatively charged silanol groups on the interior surface of the capillary wall. Consequently, cationic dyes often exhibit low electrophoretic mobility and poorly resolved separations under alkaline buffer conditions and, due to the alkaline nature of this

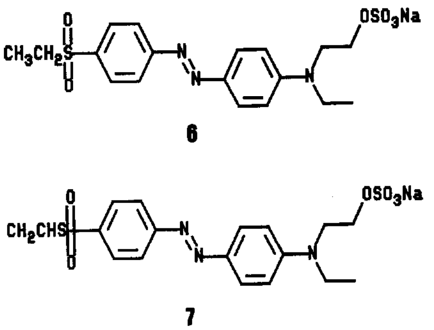

Fig. 3. Molecular structure of two acid dyes.

buffer, no satisfactory separations of cationic dyes were achieved using this system.

Analysis of acid dyes by MECC

Two acid dyes were analysed with similar molecular structure and relative molecular mass (Fig. 3). When a mixture of the dyes was analysed by a conventional buffer (10 m*M* KH_2PO_4, pH 9) the two dyes did not separate (Fig. 4). A micellar buffer system, however, (10 m*M* $Na_2B_4O_7$–40 m*M* SDS) separated the compounds very well (Fig. 5). This improved separation efficiency when using MECC indicates strongly that the technique has potential to be an excellent method of analysis for the dye chemist.

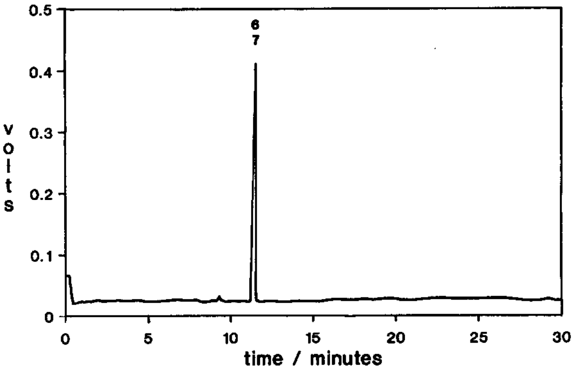

Fig. 4. Separation of two acid dyes (shown in Fig. 3) using conventional CE. The following conditions were employed. Buffer: 10 m*M* KH_2PO_4 (pH 9); sample concentration: 20–50 mg dm^{-3}; sample injection: gravity (100 mm for 12 s); voltage: 25 kV; detection (at the cathode): 254 nm; capillary: glass silica 60 cm × 75 μm I.D.

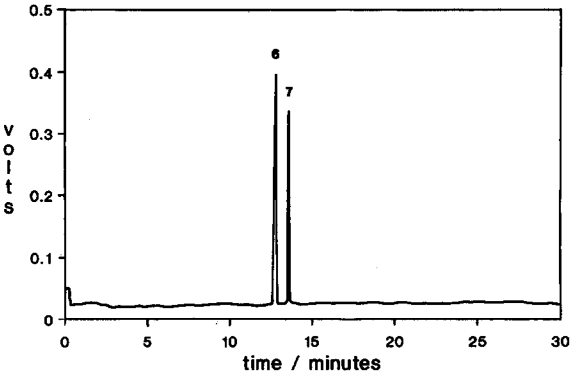

Fig. 5. Separation of two acid dyes (shown in Fig. 3) using MECC. The following conditions were employed. Buffer: 10 m*M* $Na_2B_4O_7 \cdot 10H_2O$–40 m*M* SDS; sample concentration: 30–40 mg dm^{-3}; sample injection: gravity (100 mm for 12 s); voltage: 25 kV; detection (at the cathode): 254 nm; capillary: glass silica 60 cm × 75 μm I.D.

Analysis of dye intermediates by MECC

MECC has been used for the analysis of a number of charged and uncharged, water soluble and water insoluble compounds commonly used in dye synthesis.

Anionic intermediates. Several anionic dye intermediates (Fig. 6) were analysed using 10 m*M* $Na_2B_4O_7$–40 m*M* SDS as buffer (Fig. 7). The two isomers, **8** and **10**, separated exceptionally well.

Cationic intermediates. Four fully cationic compounds (Fig. 8) were separated using 10 m*M* $Na_2B_4O_7$–40 m*M* SDS. Excellent separation was achieved (Fig. 9). The analysis of cationic *dyes*, however, was unsuccessful using this buffer due to

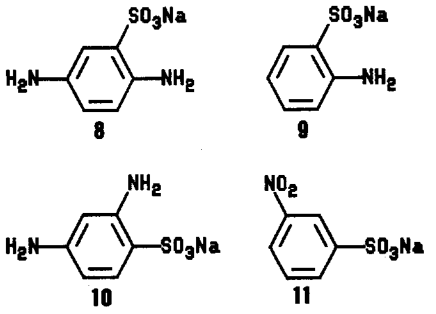

Fig. 6. Molecular structure of four sulphonated dye intermediates.

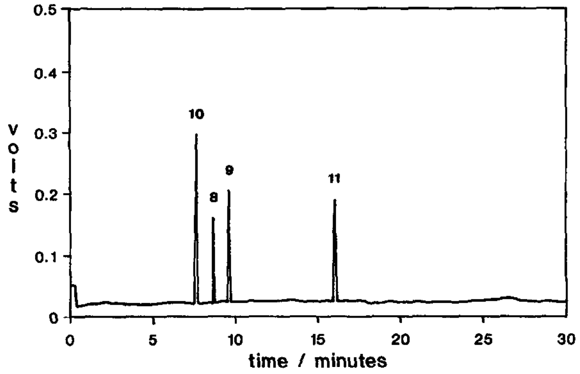

Fig. 7. Separation of anionic dye intermediates (shown in Fig. 6) using MECC. The following conditions were used. Buffer: 10 m*M* $Na_2B_4O_7 \cdot 10H_2O$–40 m*M* SDS; sample concentration: 20–40 mg dm^{-3}; sample injection: gravity (100 mm for 12 s); voltage: 25 kV; detection (at the cathode): 254 nm; capillary: glass silica 60 cm x 75 μm I.D.

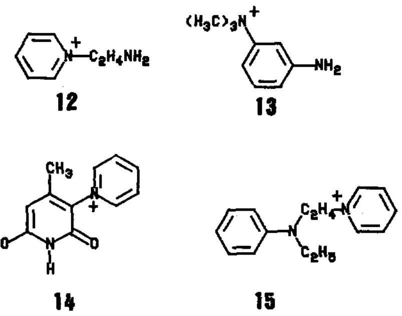

Fig. 8. Molecular structure of four cationic dye intermediates.

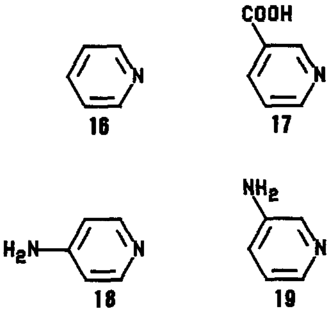

Fig. 10. Molecular structure of four pyridine based, water soluble intermediates.

the alkaline conditions employed (the reasons for this are discussed above).

Neutral, water soluble intermediates. Four pyridine based compounds with no full cationic charge (Fig. 10) were separated by MECC using an identical buffer to that above. Excellent separation was achieved (Fig. 11).

Neutral, water insoluble intermediates. Three aqueous insoluble compounds (Fig. 2) commonly used for the synthesis of disperse dyes were successfully analysed by MECC using 10 m*M* $Na_2B_4O_7 \cdot 10H_2O$–50 m*M* H_3BO_3–20 m*M* SDS and 50% (v/v) acetonitrile. Acetonitrile was required to aid dissolution of the analytes and surfactant was included in the buffer to facilitate separation of the neutral

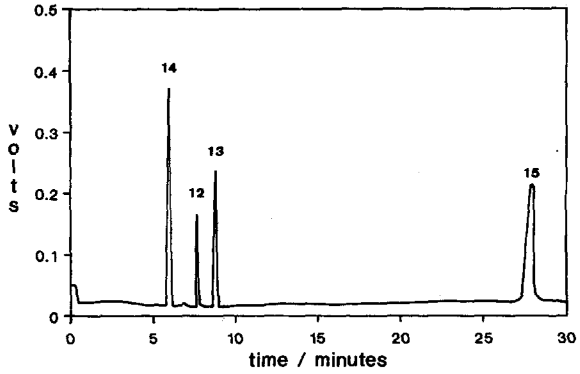

Fig. 9. Separation of cationic dye intermediates (shown in Fig. 8) using MECC. The following conditions were used. Buffer: 10 m*M* $Na_2B_4O_7 \cdot 10H_2O$–40 m*M* SDS; sample concentration: 80–100 mg dm^{-3}; sample injection: gravity (100 mm for 12 s); voltage: 25 kV; detection (at the cathode): 254 nm; capillary: glass silica 60 cm x 75 μm I.D.

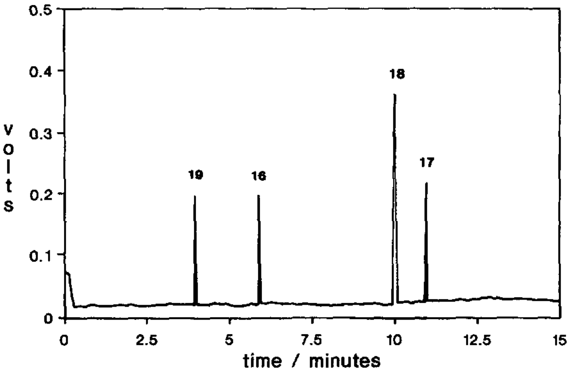

Fig. 11. Separation of four water soluble pyridine based compounds (shown in Fig. 10) using MECC. The following conditions were used. Buffer: 10 m*M* $Na_2B_4O_7 \cdot 10H_2O$–40 m*M* SDS; sample concentration: 50–100 mg dm^{-3}; sample injection: gravity (100 mm for 12 s); voltage: 25 kV; detection (at the cathode): 254 nm; capillary: glass silica 60 cm x 75 μm I.D.

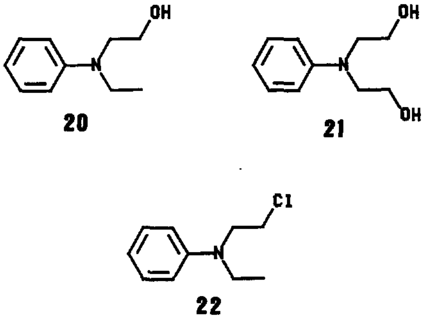

Fig. 12. Molecular structure of four neutral, water insoluble dye intermediates.

samples by micellar electrophoresis. The separation, shown in Fig. 13, strongly indicates that this technique can be used for the analysis of water insoluble anilino derivatives used for the synthesis of disperse dyes. Presently these compounds are usually analysed by conventional TLC or HPLC methods.

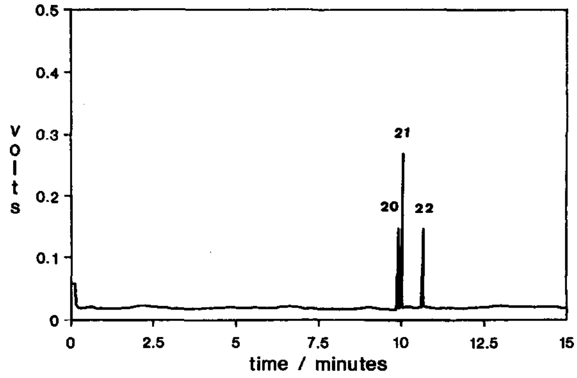

Fig. 13. Separation of three water insoluble dye intermediates (shown in Fig. 12) by MECC. The following conditions were used. Buffer: 10 m*M* $Na_2B_4O_7 \cdot 10H_2O$–50 m*M* H_3BO_3–20 m*M* SDS; sample concentration: 20–30 mg dm^{-3}; sample injection: gravity (100 mm for 12 s); voltage: 25 kV; detection (at the cathode): 254 nm; capillary: glass silica 60 cm × 75 μm I.D.

CONCLUSIONS

Micellar electrokinetic capillary electrophoresis has been used to analyse non-ionic aqueous insoluble compounds by incorporating a co-solvent into a micelle buffer. The synthesis of a non-ionic disperse dye was followed using this technique. MECC has also been used for the successful analysis of a number of charged and uncharged, water soluble and water insoluble dye intermediates. In addition, two almost identical acid dyes, which could not be separated by conventional CE methods, were successfully separated using MECC.

By utilising the various modes of capillary electrophoresis, this technique clearly has potential to be a powerful and diverse analytical method for both the dye-using and dye-manufacturing industries alike.

ACKNOWLEDGEMENTS

The authors would like to acknowledge the financial support of Zeneca Specialties and the Science and Engineering Research Council.

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