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Analysis of Reactive Dyes and Related Derivatives Using High-Performance Capillary Electrophoresis

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ABSTRACT

Analysis of mixtures of reactive dyes has traditionally been carried out using TLC (thin-layer chromatography). More recently, HPLC (high-performance liquid chromatography) has been used. This paper describes the analysis by both HPLC and the relatively new technique of HPCE (high-performance capillary electrophoresis) of a series of reactive dyes based on substituted triazine azo derivatives of H-Acid and non-reactive derivatives of these dyes. Triazine substituents include chloro, pyridinium and 3-carboxypyridinium in the case of the reactive forms of the dye; non-reactive forms consisted of amino- and hydroxy-triazines.

1 INTRODUCTION

Reactive dyes have been the subject of considerable interest in recent years, with many innovations being introduced to the market; such products include Nippon Kayaku's range of neutral-fixing reactive dyes.[1] These dyes are very polar water-soluble substances and are not readily amenable to gas-chromatographic and mass-spectrometric analysis. Consequently, until recently, TLC and UV–visible spectrophotometry have formed the basis for analysis of this type of dye.[2] With the advent of HPLC (high-performance liquid chromatography) analysis has become considerably more efficient. However, the relatively high running costs of HPLC, and the continued need to improve separation efficiencies, have led us to investigate HPCE (high-performance capillary electrophoresis) as a possible analytical method for reactive dyes.

Recent studies[3,4] have compared HPCE very favourably with HPLC, both techniques showing comparable linearity and analysis times. Peak shape and efficiency were found to be superior with HPCE, however: HPCE efficiencies were in the range $1{\cdot}2 \times 10^5$–$6{\cdot}7 \times 10^4$ theoretical plates compared with HPLC efficiencies of $1{\cdot}8 \times 10^4$–$2{\cdot}6 \times 10^3$ theoretical plates.

Electrophoresis in general is a well-established technique for the separation and analysis of charged substances. The technique normally involves electrophoretic separation at high voltage through a stationary phase, then subsequent detection with an appropriate staining procedure. Traditional electrophoresis, therefore, has always been fairly labour-intensive, with little possibility of automation. These factors inhibited the usage of electrophoresis as a standard laboratory desk-top instrument. These problems have been overcome with the development of HPCE, where electrophoresis takes place in free solution in an open capillary filled with electrolyte, the separated components passing an on-line detector (Fig. 1).

There are several different techniques which can be applied to electrophoresis in capillaries. Capillary zone electrophoresis (CZE) is the technique most commonly employed and the one covered in this paper. The silica capillary contains immobile negatively charged silanol groups along the inner capillary wall. Protons and electrolyte cations which are free in solution are attracted to the capillary wall to form an electrical double layer. When a voltage is applied, the cations arrayed at the wall are attracted towards the cathode and pull mobile phase along with them. This is known as electroendosmotic flow (Fig. 2). Significant flow rates of several centimetres per minute can be achieved using this technique. After a potential has been applied for a time, different species will have migrated different distances, thus achieving resolution of the moieties.

Electroendosmotic flow is much more rapid than simple electrophoretic mobility, so all analytes, whatever their charge, eventually elute past the on-

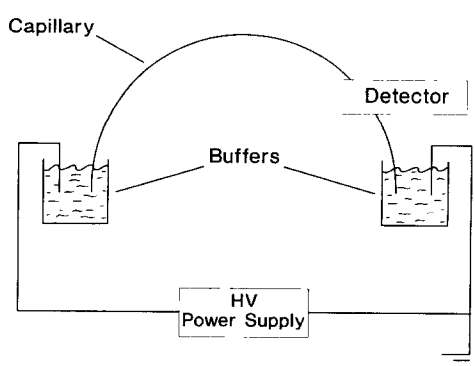


Fig. 1. Capillary electrophoresis—basic schematic.

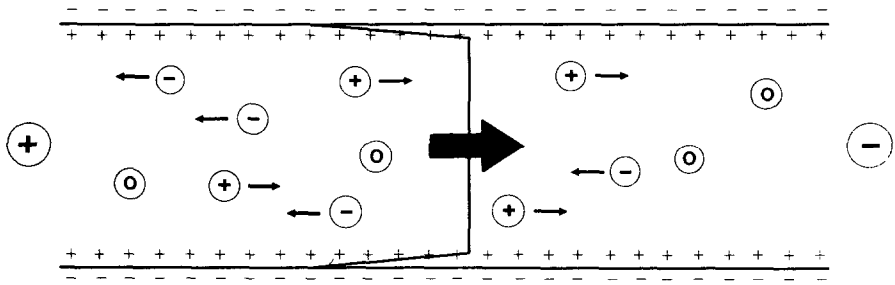
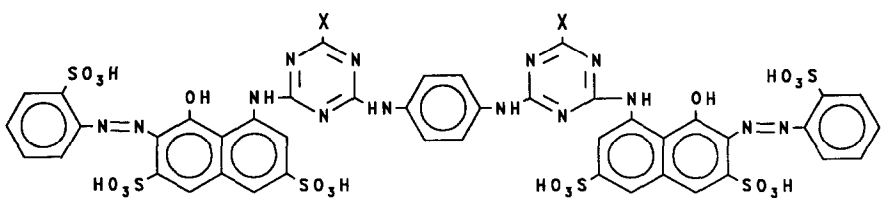


Fig. 2. Electroendosmotic flow.

line absorbance detector mounted in front of the cathode. The ability to separate samples on the basis of positive, negative or neutral charge, in one analysis, makes CZE a powerful analytical technique. HPCE has now been developed into fully automated laboratory instruments which are simple to use. Separation and detection require no moving parts, giving low maintenance requirements, and the reusable capillaries are inexpensive. Separations of water-soluble reactive dyes are described in this paper, using CZE in borate and phosphate buffers and traditional HPLC utilising a C18 reverse-phase column.

The reactive dyes used in this study were all derived from the twin-chromophore monochloro-triazinyl (MCT) type. In some dyes, however, the reactive chlorines were replaced with various quaternary amino, primary amino and hydroxy groups. Quaternisation has been shown to render the dye readily fixable to cellulose under neutral dyeing conditions and is therefore of considerable interest. The neutral fixable Kayacelon React dyes are prepared by quaternising the MCT dyes with nicotinic acid.

The structure of the dyes used throughout this study is shown in Fig. 3.



1a: X=Cl
1b: X=nicotinic acid
1c: X=pyridine
1d: X=OH
1e: $X=NH_2$

Fig. 3. Structure of dyes.

2 EXPERIMENTAL

2.1 Materials

Distilled, deionised water was used for all HPLC eluents and for all HPCE buffer solutions. HPLC-grade acetonitrile was used in HPLC eluents.

All chemicals used were laboratory-grade reagents unless otherwise stated.

2.2 Preparation of dyes

2.2.1 Dye **1a**

Cyanuric chloride (4·62 g, 0·025 mol) was added to a stirred solution of water (40 ml) and acetone (10 ml), at a temperature of $\leq 4°C$. To this was added a neutral solution (100 ml) of 1-amino-8-naphthol-3,6-disulphonic acid (7·66 g, 0·024 mol) in water and sodium hydroxide, keeping the temperature below 4°C and the pH below 3·5. The solution was stirred at this temperature for 2 h and then filtered. The filtrate was stirred with a diazo solution prepared from orthanilic acid in the following way. Orthanilic acid (4·57 g, 0·023 mol) was dissolved in sodium carbonate solution (100 ml) to give a solution of the sodium salt of the acid. The pH of the solution was adjusted to 2·5 with additions of dilute hydrochloric acid; sodium nitrite (1·73 g, 0·025 mol) was then added to the cooled, stirred suspension and stirring continued for 30 min. The solution was freed from excess nitrous acid by adding sulphamic acid (no coloration on starch iodide paper) and sodium carbonate was then added to raise the pH to 6·8. Stirring was continued for 1 h at $4 \pm 1°C$, by which time coupling was judged to be complete. A solution (10 ml) of *p*-phenylenediamine (1·35 g, 0·0125 mol) in acetone was then added over 15 min, the pH being maintained at 6–6·5 by additions of 2M-sodium hydroxide solution. The temperature was then raised to 30°C and held at this value for 3 h. The product thus formed was salted out using sodium chloride, filtered and dried.

2.2.2 Dye **1b**

Nicotinic acid (5·54 g, 0·045 mol) was added to a stirred solution of the previously prepared dye, **1a** (2·2 g, 1·5 mmol), in water (25 ml). The pH of the mixture was adjusted to 6 by additions of dilute acetic acid and the reaction was stirred at 90–95°C for 3 h. The product was salted out with sodium chloride, filtered and dried.

2.2.3 Dye **1c**

Pyridine (3·56 g, 0·045 mol) was added to a stirred solution of the previously prepared dye, **1a** (2·2 g, 1·5 mmol), in water (25 ml). The pH was adjusted to 7 by additions of dilute acetic acid and the reaction was stirred at 90–95°C for 3 h. The product was salted out with sodium chloride, filtered and dried.

2.2.4 Dye **1d**

Sodium hydroxide (2·0 g, 0·05 mol) was added to a stirred solution of the previously prepared dye, **1a** (2·2 g, 1·5 mmol), in water (25 ml) to bring the pH of the mixture to 10·5. The reaction was stirred at 90–95°C for 3 h. The product was salted out with sodium chloride, filtered and dried.

2.2.5 Dye **1e**

A solution of the previously prepared dye, **1a** (2·2 g, 1·5 mmol), in aqueous ammonia (25 ml, s.g. 0·88) was refluxed for 8 h. After cooling, dilute hydrochloric acid was added to bring the pH to *c.* 1. This product was filtered, and the filtrate washed with 2M-hydrochloric acid. The product was then redissolved in water (25 ml) and sodium carbonate added to raise the pH to 6·5. The product thus formed was salted out with sodium chloride, filtered and dried.

2.3 Analysis

An equimolar mixture of the five prepared dyes was analysed using both HPLC and HPCE.

2.3.1 HPLC

HPLC was carried out using the Varian Series 500 HPLC system, incorporating a C_{18} reverse-phase Apex Octadecyl 15 cm column (Jones Chromatography). The method used was as follows.

Eluent: 60% deionised water/40% acetonitrile (fixed ratio), containing 1×10^{-3} mol litre^{-1} tetrabutylammonium bromide (ion-pairing agent) and 1 ml litre^{-1} 20% v/v acetic acid + 1 ml litre^{-1} 5% w/v potassium hydroxide (buffer system)
Flow: 1 ml min^{-1}
Pressure: 50 atm
Detection: 520 nm

2.3.2 HPCE

HPCE was carried out using a Dionex capillary electrophoresis system,

incorporating a silica capillary (50 μm i.d. × 60 cm length). The methods used were as follows.

Potential: 20 kV
Injection: Gravity (sample vial raised 100 mm for 25 s)
Detection: 520 nm
Electrolyte buffer, method 1: 10 mM-$Na_2B_4O_7$, 50 mM-H_3BO_3 (pH 10·0)
Electrolyte buffer, method 2: 50 mM-Na_2HPO_4 (pH 9·0)

3 RESULTS AND DISCUSSION

An HPLC separation of the dyes (Fig. 4) shows quite a limited separation efficiency. Further method development, including gradient elution, was unable to provide increased separation efficiency, highlighting the problem of analysing mixtures of essentially similar dyes.

As can be seen from the CZE traces (Figs 5 and 6), superior separation efficiency over HPLC is achieved with both buffer systems, no organic solvents or gradient elutions being required in either case. The buffer used in CZE is chosen on the basis of pH as this controls both solute mobility and the level of electroendosmotic flow. The most commonly used buffer is an aqueous phosphate buffer (pH 7) which gives a moderate level of electroendosmotic flow.

The CZE separations of the reactive and analogous non-reactive dyes

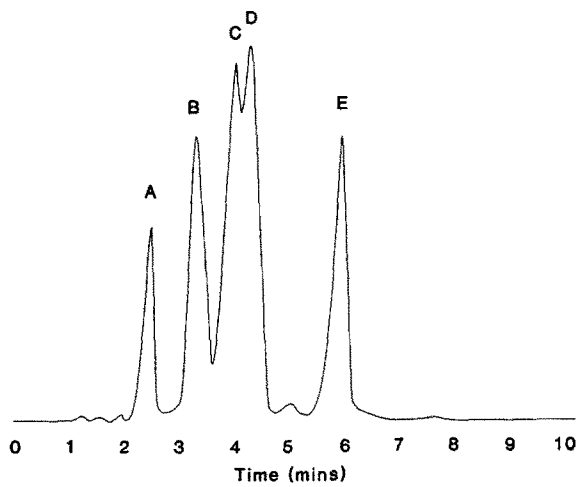


Fig. 4. Analysis of the dye mixture using HPLC. Peak A, dye **1c**; B, **1b**; C, **1e**; D, **1d**; E, **1a**.

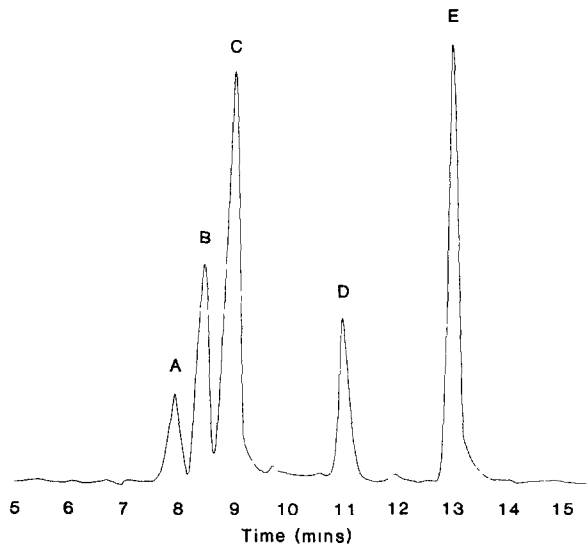


Fig. 5. Analysis of the dye mixture using CZE method 1 (borate buffer system). Peak A, dye **1c**; B, **1e**; C, **1a**; D, **1b**; E, **1d**.

illustrate the electrophoretic mobility of the molecules as a function of their charge and size. Separation is based on the charge/mass ratio of the analytes. Since these sulphonated dyes are all negatively charged in solution due to the dissociation of the cations or protons from the sulphonate groups, their electrophoretic mobilities are governed by the charge/mass ratio of the

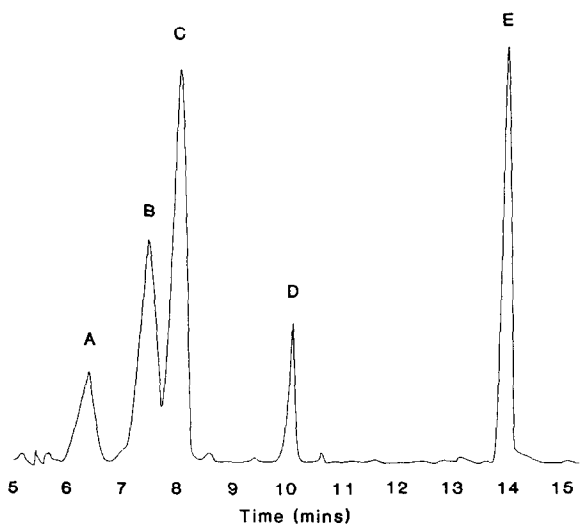


Fig. 6. Analysis of the dye mixture using CZE method 2 (phosphate buffer system). Peak A, dye **1c**; B, **1e**; C, **1a**; D, **1b**; E, **1d**.

triazine-substituted groups only. The negative charge at the silica surface of the capillary results in a high electroendosmotic flow towards the cathode end of the HPCE column, resulting in the net migration of the sulphonated dyes towards the detector. Since an increase in molecular size decreases electrophoretic mobility and an increase in negative charge increases migration away from the detector, the species with the lowest charge/mass ratio should elute first and the species with the highest charge/mass ratio should elute last. The elution order is therefore as expected, the retention time increasing with increased negative charge on the triazine groups.

4 CONCLUSIONS

The technique of capillary zone electrophoresis has proved extremely useful in the analysis of water-soluble reactive and related non-reactive triazine dyes. Short analysis times with high separation efficiencies were obtained. Method development time for CZE was also significantly lower than HPLC, partially due to the very high efficiencies.

The results from the two electrophoretic methods used show that buffer control can considerably increase the resolution achieved. Other advantages seen with the technique are low sample consumption (approximately 5-nl injections were used in these separations, compared with 10-μl injections required for HPLC), low running costs and low maintenance. Lee *et al.*[4] have shown that HPCE can readily be combined with mass spectrometry to provide an excellent technique for the determination of sulphonated azo dyes, and several other authors[5–9] have reported work with HPCE MS. From the results achieved in our study, it is our belief that capillary electrophoresis may be readily applicable to many other applications in the determination of dyestuffs, replacing existing HPLC techniques.

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