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Electrochromatography–electrospray mass spectrometry of textile dyes

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Abstract

Electrochromatography with its high chromatographic performance has been coupled with electrospray mass spectrometry (MS) for the analysis of non-ionic disperse textile dyes. Electrochromatography offers an alternative to micellar electrokinetic capillary chromatography (MECC) for the analysis of uncharged compounds in conjunction with MS, since MECC generally relies on MS incompatible compounds for micelle formation. Overall, the technique of electrochromatography–MS should find application in many areas.

1. Introduction

Electrochromatography relies on the phenomenon of electroosmosis to effect solvent flow through packed fused-silica capillaries, with the advantage that a flat flow profile is produced in contrast to the parabolic Poisseuille flow of pressure driven systems, resulting in enhanced chromatographic performance. A further advantage is that flow is independent of particle diameter and it is possible to use very small particles, with no theoretical restriction on column length. Pretorius et al. [1] first demonstrated electrochromatography in 1974, and the technique was revived in 1981 by Jorgenson and Lukacs [2], but was not developed further until 1987 by Knox and Grant [3], which led to current interest in the technique. A recent report developed an idea of Knox and Grant on the use of a pressurised electrochromatography system to suppress bubble formation in the capillary, induced by Joule heating [4].

Electrochromatography capillaries are typically 25–100 cm long with 25–100 μm I.D., filled with reversed-phase packing material. Selectivity in electrochromatography is identical to that in conventional HPLC for neutral analytes but electrophoresis contributes for ionised species. The technique has particular advantages in coupling to mass spectrometry (MS), since narrower chromatographic peaks produce a higher mass flux, and in addition offers an alternative to micellar electrokinetic capillary chromatography (MECC) for the separation of neutral analytes. MECC generally relies on MS-incompatible

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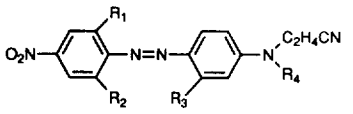
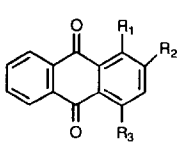


SERILENE YELLOW BROWN R-LS	R_1, R_2 = Cl R_3 = H $R_4 = C_2H_4OOCMe$
SERILENE ORANGE 2RL	R_1, R_2, R_3 = H $R_4 = C_2H_5$
SERILENE YELLOW BROWN G-LS	R_1, R_2 = Cl R_3 = H $R_4 = C_2H_4OOCPh$
SERILENE DARK RED FL	R_1 = Cl R_2 = H R_3 = Me $R_4 = C_2H_5$

Fig. 1. Structure of Serilene azo dyes.

quantities of compounds, typically anionic surfactants, for micelle formation. Initial work on the combination of electrochromatography with MS has been reported [5]. Combinations of both pressure driven and electroosmotically driven chromatography have been described, initially by Tsuda [6], who used pressure-driven flow in addition to electroosmosis, to suppress bubble formation. Later work by Verheij et al. [7] used the term pseudo-electrochromatography to distinguish the technique from pure electrically driven electrochromatography. This report was also concerned with the coupling of pseudo-electrochromatography to MS, and further work by the same group has recently been reported [8]. The flow profile in pseudo-electrochromatography approaches that in a pressure-driven system, resulting in a level of efficiency lying between electrochromatography and conventional HPLC, but biased towards the latter. However, since selectivity is based on both electrophoresis and partition, pseudo-electrochromatography does have particular advantages in coupling to MS for the separation of ionic compounds, owing to the lack of need for ion-pairing agents and buffer gradients, most of which are MS incompatible.

We report in this paper on the use of electrochromatography coupled with MS for the analysis of some non-ionic, water-insoluble, disperse textile dyes (azo and anthraquinone based with



DISPERSOL BLUE BN	$R_1 = NHCH_3$ R_2 = H $R_3 = NHCH_2CH_2OH$
DISPERSOL RED B3B	$R_1, R_3 = NH_2$ $R_2 = OCH_3$
TERASIL BLUE 2R	$R_1 = NH_2$ R_2 = H R_3 = NHPh

Fig. 2. Structure of anthraquinone-based dyes.

structures shown in Figs. 1 and 2, respectively), particularly as an alternative to MECC. The combination of extremely efficient chromatography with the unrivalled specificity of MS results in a very powerful analytical technique. There are several areas where an efficient method for the analysis of dyes and related compounds is required, these include both the production of dyes and their use in textile dyeing, but also wider applicability such as environmental and forensic fields. In dye synthesis, side-reaction products are usually formed which may be economically wasteful and/or may be actively involved in the dyeing process. Degradation of dye when present on the textile is a further area of interest to the textile industry, and relates to a current interest of the authors, namely the analysis of dyes from textiles of archaeological significance [9]. The analysis of dyes and related compounds has been typically carried out by TLC, HPLC or GC, each technique having particular drawbacks, such as difficulties in quantitation with TLC, use of relatively large volumes of solvent in conventional HPLC and requirement for sample volatility in GC. More recently CE has been applied to dyestuff analysis following the pioneering work of Lee et al. in 1989 [10], who also coupled the technique to MS. Further work on CE–MS of dyestuffs by another group has also been recently reported [11]. These reports and others [12–14] concerned with CE alone, mostly involved the separation of charged species. However, following the work of Terabe et al. [15] on MECC, neutral species could also be analysed by CE techniques, as well as improving separation efficiency of some charged compounds. This technique has recently been applied to the analysis of both charged and neutral dyestuffs [14,16], including some disperse dyes, when acetonitrile was used as a co-solvent [16].

2. Experimental

2.1. Materials

All solvents used were of HPLC grade obtained from Rathburn, Walkerburn, UK, and

were filtered before use with the aid of vacuum through a 0.2-μm PTFE filter (Alltech Associates Applied Science, Carnforth, UK). Dyes were used as received: Serilene azo dyes were a gift from Yorkshire Chemicals, Leeds, UK; Dispersol red B3B, Dispersol blue BN and Terasil blue 2R were obtained from Zeneca, Manchester, UK. All other reagents from BDH, Poole, UK.

2.2. Preparation of capillary columns

The electrochromatography column consisted of uncoated fused-silica capillary (Polymicro Technologies, AZ, U.S.A.), 100 cm × 75 μm I.D. × 375 μm O.D., slurry packed with acetonitrile to a depth of 25 cm with 3-μm ODS-Hypersil (Shandon Scientific, Runcorn, UK), as described in detail elsewhere [5]. The progress of packing was observed through the capillary wall with the aid of a microscope, and when completed, the column was equilibrated with the mobile phase to be used for electrochromatography separations [acetonitrile–4 m*M* sodium tetraborate (80:20, v/v) adjusted to pH 8 with dilute sodium hydroxide solution].

The outlet end of the packed section of capillary was not fitted with a retaining frit, since when voltage was applied the negatively charged packing material would be pulled toward the inlet frit against the electroosmotic flow. Thus there is an absence of dead space, which is advantageous compared with conventional pressure-driven systems when a dead space often develops at the head of the column, and leads to a decline in chromatographic performance.

2.3. Chromatography

Electrochromatographic separations were performed using an Isco model 3140 electropherograph (Isco, Lincoln, NE, USA), which is designed primarily as a capillary electrophoresis instrument, but is equally suitable for electrochromatography.

Samples were dissolved in the eluent, and were applied to the column electrodynamically. Detection was by UV absorbance (210 nm) through a window in the capillary wall 30 cm from the inlet. A voltage of 30 kV was applied to the anode buffer solution into which the inlet end of the capillary was immersed. The outlet end of the capillary was linked to the mass spectrometer as described below.

2.4. Mass spectrometry

MS was carried out on VG Quattro quadrupole mass spectrometer fitted with an electrospray source (VG Biotech, Altrincham, U.K.), to which the electrochromatography capillary was linked via a co-axial interface-probe (VG Biotech) and is shown diagrammatically in Fig. 3. The interface comprises two concentric stainless-steel capillaries surrounding the inner electrochromatography capillary contained within a probe which is inserted into the instrument, and each is fitted into a separate tee-piece, as shown in the diagram. Methanol–water (1:1, v/v) containing 1% (v/v) acetic acid flowing at 10 μl/min was incorporated via the capillary at the first tee-piece, delivered by a Brownlee Microgradient syringe pump (Brownlee Labs., Santa Clara, CA, U.S.A.), to provide a "make-up" flow of liquid to compensate for the low flow-rate of electrochromatography. This was necessary for efficient operation of the electrospray process. The outer capillary supplied nitrogen gas via the second tee-piece to nebulize the liquid flow and

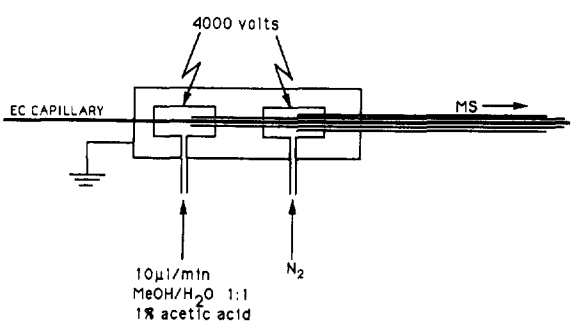


Fig. 3. Diagrammatic representation of co-axial electrochromatography (EC)–MS electrospray interface probe.

aid droplet formation. A voltage of 4 kV was applied to both stainless steel tee-pieces to provide high voltage at the probe tip for the electrospray process, and the interface is enclosed in an earthed box for operator safety. The tip of the electrospray probe at 4 kV acted as the cathode giving a net potential of 26 kV applied across the electrochromatography capillary.

MS acquisitions in positive mode were made using single ion monitoring of appropriate ions or scanning the appropriate mass range, and were initiated approximately 20 min after application of sample onto the column and commencement of elution.

3. Results and discussion

Fig. 4a is a UV chromatogram for the separation of a mixture of the four Serilene azo dyes shown in Fig. 1. Peaks 1 and 2 are just resolved, but using conventional HPLC with an analytical scale column, it was not possible to separate these compounds (data not shown), and electrochromatography-produced peaks which more closely approximated a Gaussian shape. Chromatographic performance was good, peak 4 for example, displays approximately 180 000 theoretical plates/m and a reduced plate height just below 2, although we have obtained reduced plate heights of near unity for weakly retained compounds in previous work [5]. However, a limiting factor on peak width narrowness is dictated by MS temporal requirements for full-scan data. Fig. 4b shows selected ion chromatograms of each dye, corresponding to the mass of the respective protonated molecule. Some loss of chromatographic resolution is apparent, particulary notable in the case of peaks 1 and 2, probably as a result of post detection window dispersion in the length of unpacked capillary necessary for MS coupling, and/or by a memory effect in the mass spectrometer. However, the specificity of MS obviates this problem by detection of the unique mass of each compound, and only fails when dealing with closely eluting isobaric compounds.

Fig. 5 displays selected ion chromatograms

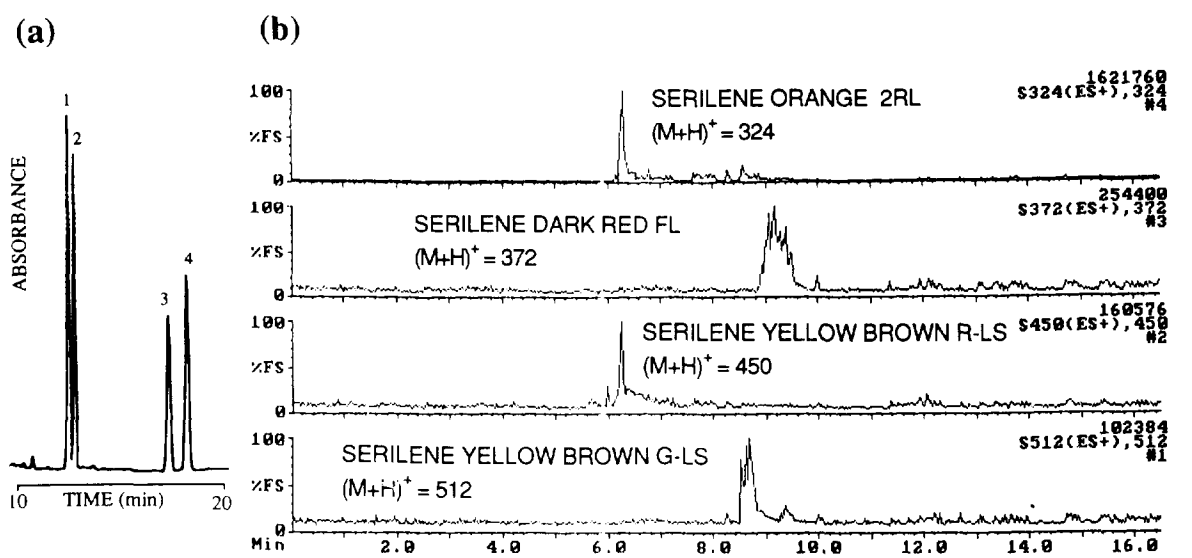


Fig. 4. On-line electrochromatography–MS separation of Serilene azo dyes, loading 100 pmol of each dye on-column. (a) UV chromatogram, peaks: 1 = Serilene yellow brown R-LS; 2 = Serilene orange 2 RL; 3 = Serilene yellow brown G-LS; 4 = Serilene dark red FL. (b) Selected ion chromatograms of $(M+H)^+$.

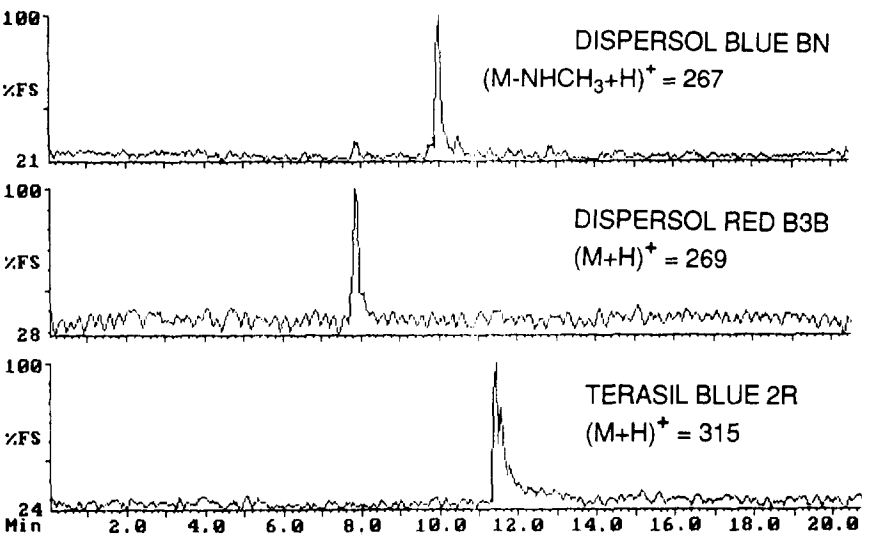


Fig. 5. Selected ion chromatograms from full-scan data for on-line electrochromatography–MS separation of anthraquinone based dyes, loading 100 pmol of each dye on-column.

from full scan data for the separation of the three anthraquinone based dyes: Dispersol blue BN, Dispersol red B3B and Terasil blue BN, demonstrating the ability to monitor fragment ions from full scan data. The lower two traces are chromatograms of intact protonated molecule ions selected from full-scan data, allowing inspection of the mass spectrum of each peak and this is further demonstrated in the next example. Fig. 6a is a UV chromatogram for the separation of one of the components in Fig. 5, Terasil blue 2R, at higher loading, in order to investigate the impurity peaks eluting before the main peak. Fig. 6b is a chromatogram of the total ion current, scanning mass range 200–600, and mirrors the UV chromatogram. Analysis of the spectra of the impurity peaks suggested that they were side reaction products arising from the synthesis of the dye. Fig. 6c shows a portion of the spectrum of the main component.

The combination of electrochromatography with MS has been shown to be a useful technique for the analysis of mixtures of neutral textile dyes, allowing identification of impurities in single dyes, and may offer wider application in the environmental and forensic areas. In addition several observations should be considered as well as the high chromatographic efficiency of the technique. These include no theoretical limitation on the use of very small particles and long column lengths, which may lead to the ability to separate previously intractable mixtures. The method offers the ability to deal with extremely small sample quantities, and there is the capability for higher loadings compared with conventional CE. Coupling of electrochromatography to MS gains advantage over the use of conventional detectors by virtue of the unrivalled specificity of mass analysis, and coupling is readily achieved owing to the compatibility of capillary flow rates with MS. Electrochromatography also obviates the major disadvantages of MECC with regard to MS, whilst retaining its virtues. Furthermore electrochromatography overcomes the difficulties encountered with open-capillary CE, where coupling to MS sources at high vacuum may result in vacuum-induced flow which degrades separation.

We are convinced that electrochromatography coupled to MS offers considerable advantages to many areas of interest, especially in the biological field.

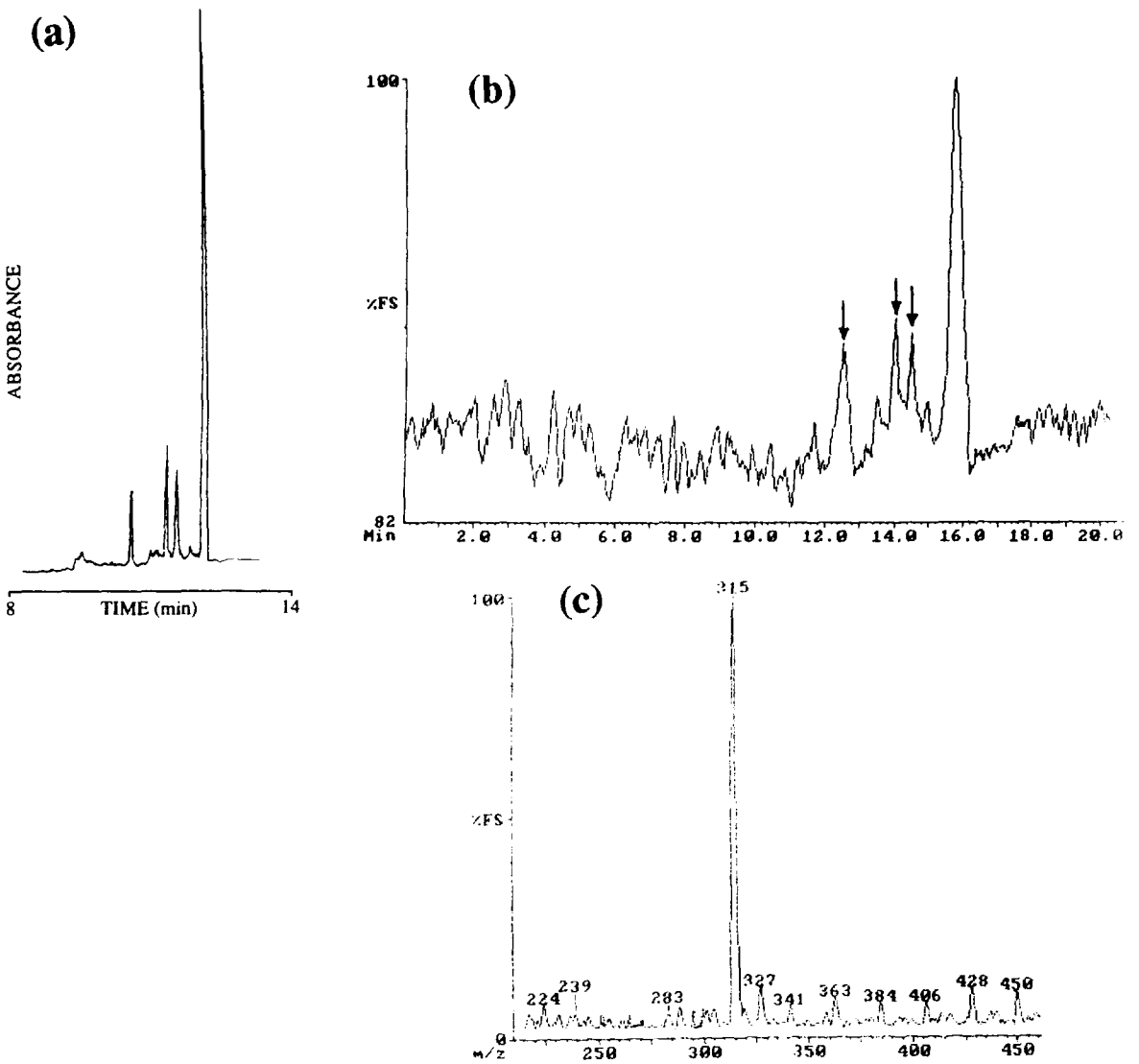


Fig. 6. On-line electrochromatography–MS separation of single dye (Terasil blue 2R) from mixture in Fig. 5, loading 1 nmol on-column, to investigate impurity peaks eluting prior to the main component as seen in (a) UV chromatogram, and arrowed in (b) full-scan total ion chromatogram. (c) Portion of mass spectrum of main component.

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