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ORIGINAL PAPER

Microextraction, capillary electrophoresis, and mass spectrometry for forensic analysis of azo and methine basic dyes from acrylic fibers

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Abstract Designed experiments based on a simplex mixture design were employed to explore the effects of three solvent components (water, formic acid, and aqueous acetic acid), extraction time, and extraction temperature for the automated microextraction of basic (cationic) dyes from acrylic fibers. Extractions were conducted by an automated liquid handling system, and dye extraction was evaluated using a UV/visible microplate reader. Highest extraction efficiency for two subclasses of basic dyes (methine and azo) from acrylic fibers was achieved with an extraction solvent containing 88% formic acid/12% water. Cationic dyes were analyzed by capillary electrophoresis using a 45 mM ammonium acetate buffer in acetonitrile–water at pH4.7. The utility of microextraction combined with capillary electrophoresis–mass spectrometry for analysis of extracts from trace fibers was demonstrated by the detection and characterization of three basic dyes extracted from a 2-mm length of single acrylic fiber.

Keywords Forensic analysis · Acrylic fibers · Extraction of basic dyes · Capillary electrophoresis

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Introduction

The finding of acrylic fibers as trace evidence in forensic investigations reflects partly the prevalence of acrylic in commercial products. Acrylic fibers were polymerized from acrylonitrile, patented, and marketed under the tradename Orlon® by Dupont in 1941. Acrylic apparel includes sweaters and pullovers, scarves, stocking caps and other hats, socks, and slippers. Home furnishing such as carpets and upholstery are also made with acrylic fibers, as are paint rollers and industrial pump packings. In many instances, acrylic is blended with wool, polyester, or other fibers for desired esthetic qualities or to improve performance in use. The forensic significance of acrylic fibers is also supported by presence of acrylic fibers in population and distribution studies conducted by forensic researchers [1–5].

Assessing the shape of a questioned fiber under an optical microscope can provide discriminating information quickly in the early stages of a fiber examination [6]. For example, acrylic is typically produced by dissolving in a

solvent such as dimethylacetamide and extruded into water; upon drying, the fiber collapses, taking on a shriveled appearance with crinkled cross-sections ranging from circular to dogbone shapes. Measurement of other physical characteristics such as birefringence and sign of elongation can also be employed to rapidly identify generic fiber type [6, 7]. Synthetic fibers are often crimped to make them hold together better when made into yarn and to impart bulkiness. The number of crimps per inch might itself be a distinctive signature of such fibers from their manufacturing process.

Acrylic fibers are copolymers of acrylonitrile with other monomers such as methyl methacrylate, vinyl pyridine, vinyl chloride, vinyl acetate, and/or vinylidine chloride. Polymerization is typically initiated via redox with bisulfate, sulfonate, or sulfate free radicals, and termination is effected via free radical termination with sulfate or sulfonate-free radicals. Both initiation and termination of the acrylonitrile copolymers introduce sulfate or sulfonate anionic end groups into the polymer structure that serve as dye sites. There are two types of acrylic textiles, depending on the amount of monomers incorporated into the polyacrylonitrile polymer. Acrylic fibers, which are the subject of this paper, are polymerized from a monomer mixture containing at least 85% acrylonitrile and 15% other comonomers. Modacrylic fibers, which are not included in this study, are polymerized from mixtures containing at least 65% acrylonitrile monomers mixed with other comonomers [8, 9].

Polyacrylonitrile itself tends to resist dyeing because of its hydrophobic nature. By taking advantage of strong intermolecular forces and the presence of comonomers, dyers can facilitate penetration of dye into the polymer. Basic dyes have been so-called historically in the textile industry because of their substantivity for acidic dye sites (e.g., in acrylic); basic dyes are also categorized chemically as cationic dyes because that is their ionized state in aqueous solution [9, 10]. Basic dyes attach to acrylic fibers via salt linkages (ionic interactions) between the cationic dye and the anionic sites in the acrylic polymer.

To extract a dye from a fiber, the solvent must reduce, or compete with, the substantivity of the dye for the fiber, then solvate and transport dye molecules from the fiber into the bulk solvent. To extract basic dyes from fibers, solvents are employed that can provide a pH low enough to protonate the anionic groups on the acrylic, which in turn will break the salt linkage that binds the dye to the acrylic fiber [10–12]. The pH of the extraction solution must be optimized for complete removal of the dye from the fiber. Beattie et al. [13, 14] found pyridine–water or pyridine–formic acid–water solvents to extract basic dyes from polyacrylonitrile. Petrick et al. [15] reported extraction of basic dyes with formic acid/water solutions. Home and Dudley [16] reported the extraction of 23 basic blue dyes from polyacrylonitrile using aqueous pyridine, formic acid, and formamide, but report structural changes upon extracting with aqueous pyridine.

The target size for forensically relevant fibers derives in part from fiber examinations and population studies reporting that recovered fibers are often as small as 2 mm in length, depending on the degree of dyeing [17–19]. Clearly, methods for the analysis of dyes extracted from fibers require high sensitivity for applicability to forensic casework. Previous studies have reported that UV–visible detection of dyes provides neither sufficient sensitivity for analysis of trace fiber extracts nor adequate discrimination for similar structurally related dyes [15, 20–24]. Analysis of dye extracted from single fibers of 2–10 mm in length has been achieved by Xu et al. [21] by sample-induced isotachophoresis with micellar elecktrokinetic capillary chromatography by Tuinman et al. [22] who infused dyes directly into electrospray mass spectrometry (MS) and by other researchers using high-performance liquid chromatography coupled to MS [15, 20, 23, 24].

The objective of the present work was to optimize and validate conditions for the automated microscale extraction of basic dyes for acrylic fibers. Complete removal of the dye from a fiber is desirable to insure detectability and reproducibility in subsequent chromatographic and/or spectroscopic analyses for limited size forensic casework samples. Combinatorial experimental designs were performed using an automated liquid sample handling workstation to program sequences of microscale extraction processing steps. The second objective was to demonstrate capillary electrophoresis (CE) conditions for separation of diverse basic dyes, as well as the trace analysis of basic dye extracts from millimeter-sized single acrylic fibers using CE–MS. The representative basic dyes selected for these studies (Fig. 1) include azo and methine dyes, which constitute 43% and 17%, respectively, of the usage on acrylic fibers [10].

Experimental

All dyed fabrics and matching dyes standards were current production samples donated by dyestuff manufacturers in the southeastern United States. All fibers were dyed at levels consistent with commercial use (2–4% by weight). For the extraction optimization experiments, basic dyed acrylic fibers (one cm lengths of thread, each containing about 30–50 fibers) were loaded into 20 500-μL glass inserts in a 96-well plate system purchased from Biotech Solutions (Mt. Laurel, NJ, USA). The 96-well plate was then placed on a BioMek 2000 automated liquid sample handling workstation (Beckman-Coulter, Fullerton, CA,

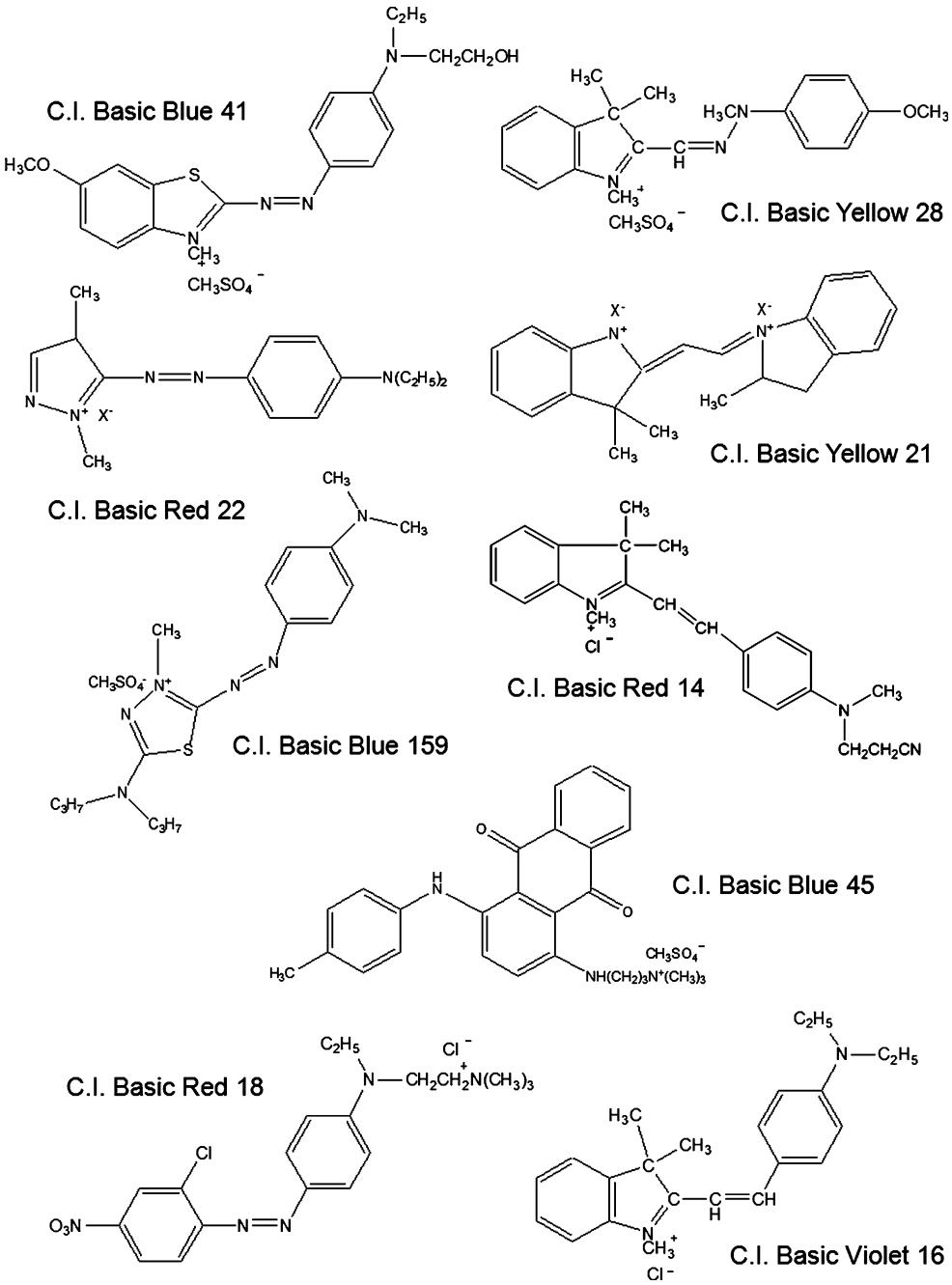

Fig. 1 Chemical structures of representative basic dyes

USA) in accordance with extraction programs written for specific fiber–dye combinations. Solvents were added to the threads in the 96-well plate under program control.

A Teflon liner was placed between the glass inserts and a plastic lid to minimize solvent evaporation during high-temperature extractions. Additionally, to avoid solvent evaporation during extraction, aluminum plates were placed above and below the 96-well plate system and clamped tight with metal clips to form an impervious seal between the Teflon liner and the glass inserts. The 96-well plate was then placed in a laboratory oven at specified temperatures and for prescribed times for extraction of dyes from the fibers. Extracted dyes were analyzed using a SpectraMax M5 UV–visible microplate spectrophotometer (Molecular Devices Corp., Sunnyvale, CA), USA.

A ternary component simplex mixture design [25, 26] consisting of water (distilled), formic acid (88%, ACS Certified, Fisher Scientific, Hampton, NH), and acetic acid (glacial, HPLC Grade, Fisher) was applied to the extraction of basic dyes from acrylic. Ten design points were employed as shown in Table 1. The experiments were carried out in random order to avoid systematic errors, and each design point was replicated twice, for a total of 20 experiments. A quadratic Scheffé three-component mixture

Table 1 Experimental design used in the combinatorial extraction of basic dyes from acrylic fibers

Design point	Water (%)	Formic acid (%)	Acetic acid (%)
1	100	0	0
2	0	100	0
3	0	0	100
4	0	50	50
5	50	0	50
6	50	50	0
7	66	16	16
8	16	66	16
9	16	16	66
10	33	33	33

model (first order effects for each component and two-factor interactions) was fitted to the extraction response data for each dye. Design of experiments, modeling, and graphics were carried out using *Design Expert*, v. 7 (StatEase Corp., Inc., Minneapolis, MN, USA).

The automated workstation was programmed to add the designated amounts of liquid to each of 20 glass inserts in a 96-well sample plate for a total volume per well of 200 µL. Identical length acrylic yarns dyed with the basic dye were extracted in each well. After addition of the prescribed extraction mixture, the samples were sealed and placed in an oven at three time/temperature conditions (90 °C for 60 min, 100 °C for 60 min, and 100 °C for 15 min). Samples were allowed to cool to room temperature, and 100 µL of each extract was transferred into a clean glass insert using automated liquid sample transfer. This process isolated the extracted basic dye from the fiber and quenched further extraction so that optimum solvent ratios for that particular oven temperature and time might be determined. The plate holding the resulting extracts was returned to the oven, and the solvent was allowed to evaporate to dryness at 50 °C. Once dry, the basic dye residues were reconstituted in 100 µL methanol. Analysis of dye extraction completeness was performed using the plate reader by measuring the absorbance at the wavelength maximum (for C.I. Basic Blue 41 and C.I. Basic Yellow 28, 590 and 430 nm, respectively).

For CE analysis, the robot was programmed to remove the extraction solvent and, after drying, to reconstitute the extract in 190 µL water. Dyes were analyzed using a PACE-MDQ CE system (Beckman-Coulter, Fullerton, CA, USA) equipped with a diode array detector that monitored absorbance from 190 to 600 nm. Solutions of cationic dye standards were prepared in deionized water at concentrations of 1.0 mg/mL. A fused silica capillary with an internal diameter of 50 µm and length of 50 cm (40 cm effective length) from Polymicro Technologies (Phoenix, AZ, USA) was used for CE. Capillary ends were burned prior to use to prevent degradation of polyimide coating. The capillary was conditioned by rinsing with 0.5 M NH_4OH for 12 min, water for 10 min, and then electrophoresis medium for 12 min. Between injections, the capillary was rinsed with electrophoresis buffer for 3 min. Cationic dyes were separated using a buffer consisting of 45 mM ammonium acetate in acetonitrile–water (60:40, *v*/*v*), adjusted to pH4.7 with acetic acid. The CE electrolyte was replaced after every five runs to minimize solvent evaporation effects. Injections were completed in hydrodynamic mode at 1 psi for 5 s. Separations were performed at 25 °C with an applied voltage of 20 kV.

For extraction prior to CE–MS analysis, 10 µL of aqueous formic acid (88%) was added to a single tri-dyed acrylic fiber and heated at 100 °C for 60 min in a sealed vial. After evaporation to dryness, the extract was reconstituted in 5 µL of water. Hydrodynamic CE injections were performed at 1 psi for 5 s with the electrospray ionization (ESI) voltage off. A Micromass Q-Tof Micro with an ESI source was connected to the CE instrument via a coaxial sheath flow interface (Waters Corporation, Milford, MA, USA). For positive MS, the sheath liquid was 50/50 methanol/water with 1% formic acid at 1.7 µL/min. The nebulization gas was set at 8 psi, and the ESI voltage and cone voltage were 3.72 kV and 17 V, respectively.

Results and discussion

For the purpose of optimizing extraction conditions, 1-cm threads of fiber were extracted in the present work. This amount of sample facilitates the determination of the extraction completeness with high signal-to-noise ratio for absorbance measurements using a microplate reader. Based on the previous literature, the performance of a ternary solvent system consisting of water, acetic acid, and formic acid was investigated [12–14]. The use of pyridine was avoided due to the structural changes and associated spectral changes previously reported [14]. We have also found that certain reactive dyes may change color on dissolution in pyridine.

Figure 2 summarizes graphically the results of extraction mixture experiments, conducted at 90 °C for 60 min, for acrylic fibers dyed with the azo basic dye, C. I. Basic Blue 41. An iso-response contour plot and a three-dimensional perspective view of the fitted absorbance response surface are shown in Fig. 2. The model fits the data adequately, with a coefficient of determination (R^2) of 0.9192, and lack of fit of the model is not significant [25, 26]. Relative to the highest measured absorbance, the pooled percent relative standard deviation of the replicates was 9.12%. Extraction

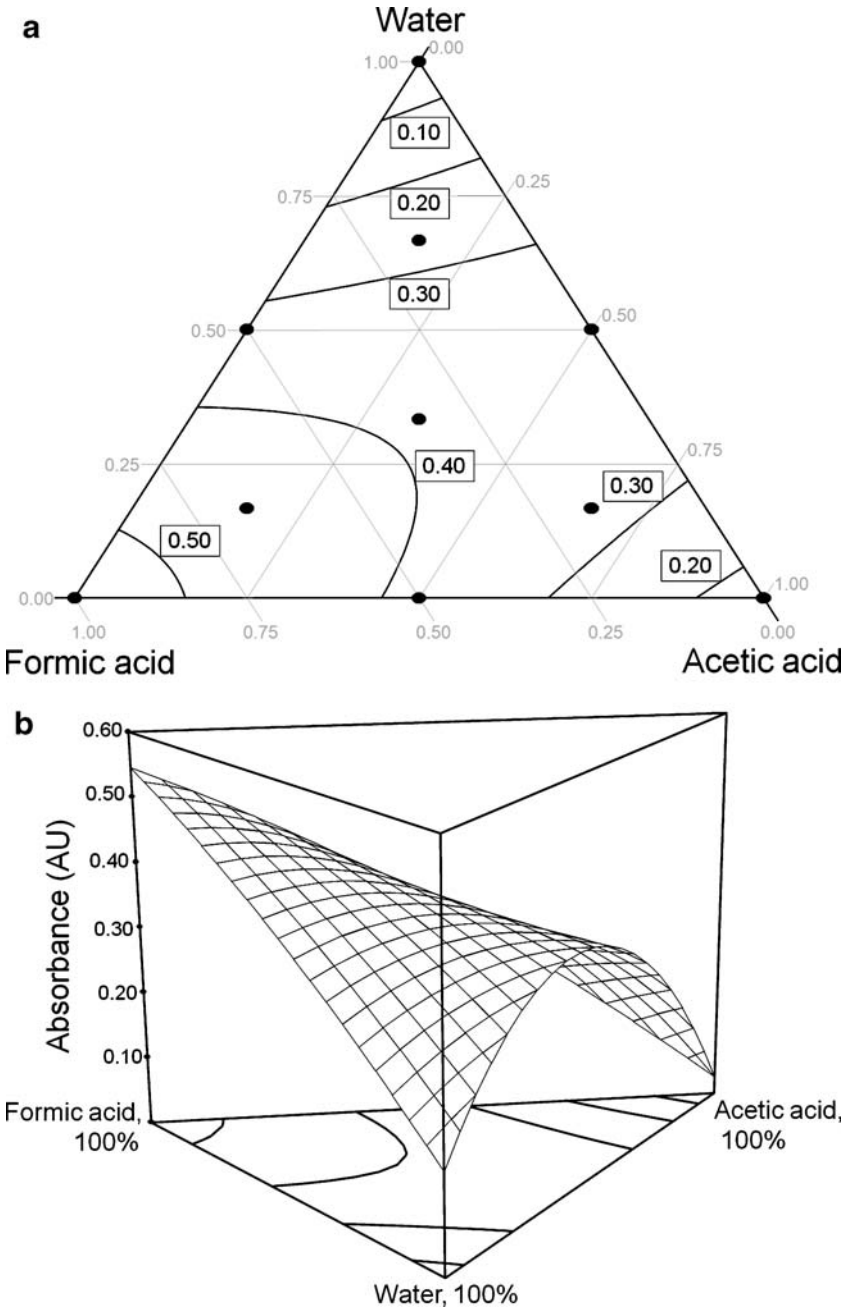

Fig. 2 **a** Two-dimensional contour plot of the fitted absorbance response surface for the extraction of C.I. Basic Blue 41 from acrylic using water, formic acid, and acetic acid (90° C for 60 min); *dots* represent design points at which two replicate experiments were performed. **b** Three-dimensional view of the same response surface

of the dye is highest using 100% formic acid (which actually consists of 88% formic acid/12% water). Pure water extracts only 5%, and pure acetic acid only 22%, of the absorbance at the formic acid vertex. A diagonal ridge rises in response from near the 50% acetic acid/50% water edge of the ternary mixture region to the "pure" formic acid vertex. The 50% formic acid/50% water conditions used previously by Beattie et al. [13, 14] and Petrick et al. [15] are close to the predicted optimum extraction conditions.

Two additional mixture designs of 20 experiments each were conducted with C.I. Basic Blue 41 at higher temperature (100 °C) and different extraction times (15 and 60 min). The general shape of the fitted models was the same as shown in Fig. 2, with a rising ridge running diagonally across the surface and the highest extraction at the formic acid vertex (88% formic acid/12% water). Comparing measured absorbances at the optimum solvent composition, higher temperature (100 °C) and longer extraction time (60 min) produced the highest amount of dye extracted, and the resulting fiber was visually colorless. When extraction was performed at lower temperature (90 °C) but at the same 60-min extraction time, the amount extracted decreased by

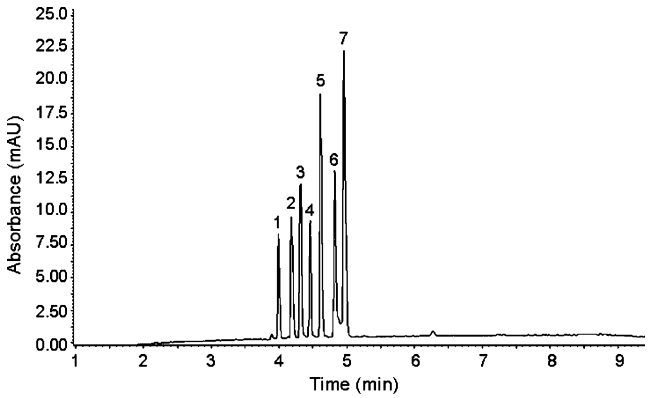

Fig. 3 Electropherogram of a mixture of seven cationic dyes. Peak identification: *1* C. I. Basic Red 22; *2* C. I. Basic Yellow 21; *3* C. I. Basic Blue 159; *4* C. I. Basic Red 14; *5* C. I. Basic Blue 41; *6* C. I. Basic Blue 45; *7* C. I. Basic Red 18. CE conditions: 45 mM ammonium acetate buffer in 60:40 acetonitrile/water at pH4.7, 50-cm effective capillary length, 2-s pressure injection at 0.2 psi, and 20 kV applied potential, detection at 214 nm

8%. At a shorter extraction time (15 min) but at the same 100 °C temperature, 21% less dye was extracted. Thus, time and temperature have significant effects on the amount extracted, and tradeoffs between speed of extraction and amount extracted could be considered. A final 20-experiment mixture design was conducted at 100 °C and 60 min for extraction from acrylic of a methine dye (C. I. Basic Yellow 28). The same diagonal ridge was present in the fitted response surface, but was flatter in response compared to that of the azo dye. The formic acid vertex of the design space again produced the highest extraction response.

Capillary electrophoresis separates the charged components of a mixture by their differing migration rates under the influence of an applied electrical field. Because basic dyes extracted from acrylic are ionic only at low pH, their separation by CE also requires a low pH electrolyte. However, a pH4.7 buffer of ammonium acetate at 15 mM causes cationic dye components to migrate too fast for adequate resolution. An ammonium acetate salt concentration of 45 mM was found to slow cation migration adequately for improved resolution. However, the high ionic strength of this electrolyte necessitated relatively high organic content (60:40 acetonitrile/water) to keep the resulting current low. To test the ability of this electrolyte to provide high resolution separations, a solution of seven basic dyes was prepared from manufacturer's standards at 0.1 mg/mL concentrations. The dyes are azo or methine dyes, except for C. I. Basic Blue 45, which is an ionic anthraquinone dye (see Fig. 1). The separation of this diverse mixture of basic dyes within 5 min using these conditions is shown in Fig. 3. Although 214 nm provides acceptable sensitivity for all seven components, not all dyes are at their maximum absorbance.

In our laboratory, CE with diode array detection (DAD) alone has not reliably detected extracted dyes from single fibers of lengths much below 1 cm in length. For example, CE–DAD of a basic dye (C. I. Basic Red 18) extracted from a 1-cm acrylic thread (consisting of 35–50 single fibers) generated a peak only 13 mAU in height with baseline peak-to-peak variations of 0.2 mAU. In electropherograms of extracts from smaller single fibers, peak detection becomes problematic, and the usefulness of the spectrum for component identification is severely diminished.

The electrolyte buffer used here for cationic dyes not only provides efficient separation of multiple dye components but also is sufficiently volatile to facilitate electrospray ionization. A 2-mm length of a single acrylic fiber (~15 μm diameter) dyed with C. I. Basic Yellow 28, C. I. Basic Blue 159, and C. I. Violet 16 was subjected to extraction at 100 °C for 60 min with stock aqueous formic acid (88% formic acid/12% water). Figure 4 shows reconstructed single ion electropherograms for the standard samples and for the extract sample, monitored at the appropriate ion mass for each analyte. These dye peaks were not visible in the corresponding DAD electropherogram. Figure 5 shows mass spectra of the corresponding sample and extract peaks. The expected molecular ions for each dye component and additional confirmatory ions are present, and mass spectra of the extracted dyes are relatively free from contamination. As a further indication of the applicability of CE–MS for trace analysis of extracted dyes, calibration experiments for seven basic dye standards yielded estimated limits of detection in the low picogram range for the amount of dye injected.

Conclusions

Solvent extraction conditions for the automated microextraction of basic dyes from acrylic fibers have been investigated using designed experiments. A liquid handling workstation was programmed to deliver the planned amount of solvent, and the absorbance response at different solvent extraction conditions was measured with a microplate reader. The extraction of representative basic dyes from two classes commonly applied to acrylic fiber, an azo dye and a methine dye, were tested. For both basic dyes, optimum extraction is achieved using solutions of aqueous formic acid (88%, with 12% water). Increasing temperature and extraction time (to 100 °C and 60 min, respectively) resulted in exhaustive extraction of dyes.

Capillary electrophoresis with an electrolyte buffer containing 45 mM ammonium acetate in acetonitrile–water at pH4.7 was suitable for both diode array and CE–MS analysis. Using this buffer, a mixture of seven basic dyes could be separated in 5 min. UV–visible detection

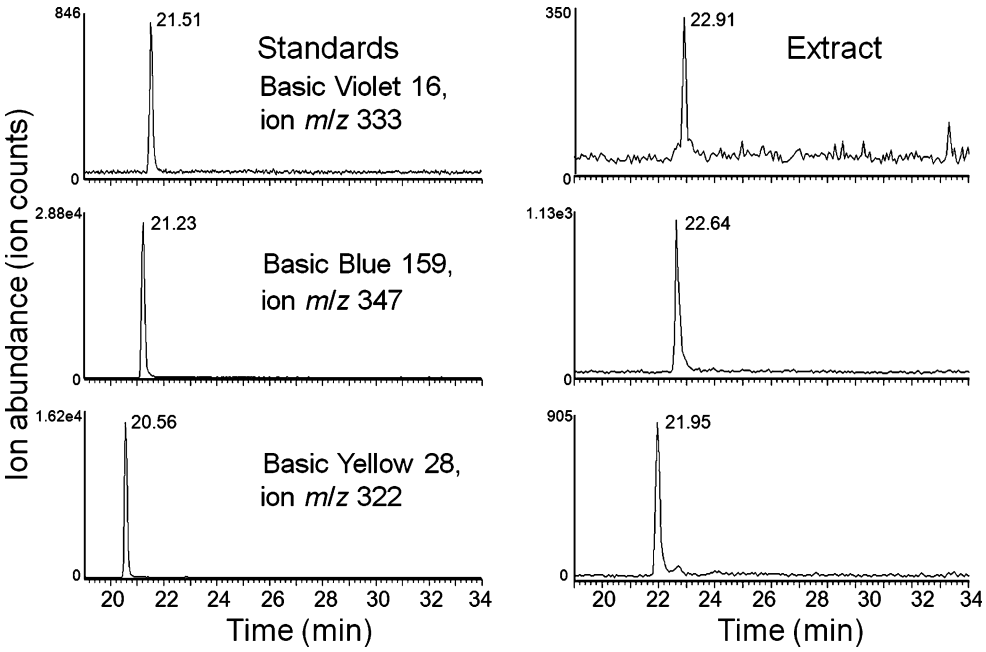

Fig. 4 Reconstructed single ion CE–MS electropherograms: *left* dye standards and *right* extracts from a single 2-mm tri-dyed acrylic fiber. Peaks are labeled with migration times (minutes)

following CE is not generally suitable for analysis of dye extracts from millimeter-length single acrylic fibers, but may provide discriminating information for dye extracts from longer threads of several fibers based on peak migration times and UV–visible spectra.

The utility of microextraction combined with CE–MS for analysis of extracts from trace fibers has been demonstrated by the detection of three basic dyes extracted from a 2-mm length of single acrylic fiber. Although the analysis is destructive to the sample, only an extremely small sample is required (~2 mm of a single 15 μ diameter fiber). If these methods can be reliably implemented at the casework level, determining the number and relative amounts of dyes present and characterizing those dyes at the molecular level offer a new level of forensic discrimination. Future research from our laboratory will address validation issues for trace dye analysis from forensically relevant fiber samples.

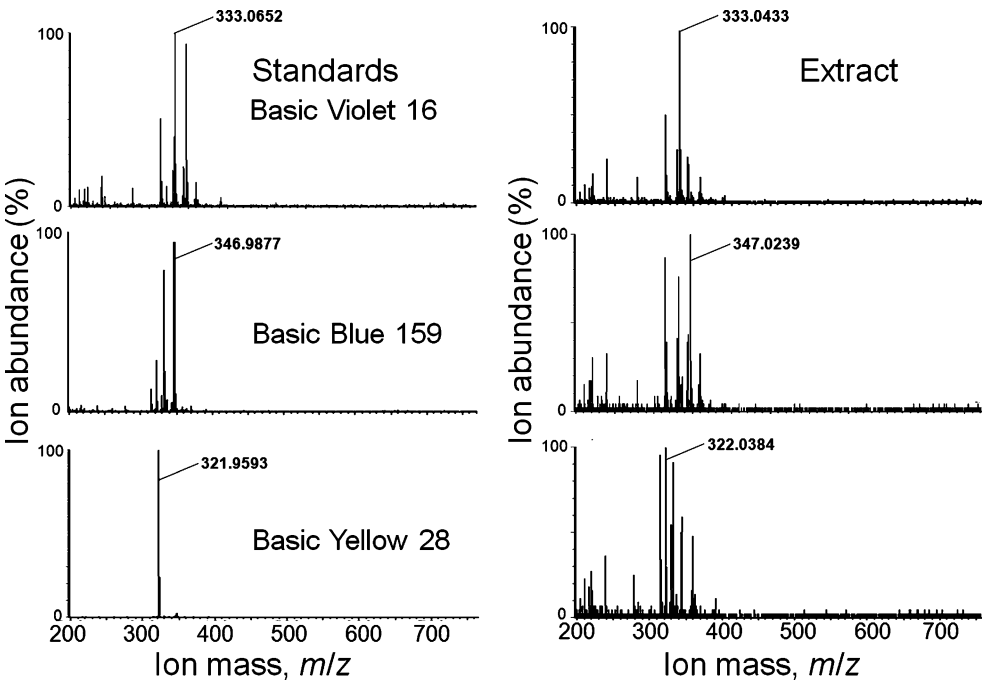

Fig. 5 Mass spectra taken at dye peak apexes in CE–MS electropherograms: *left* dye standards; *right* extract from a single 2-mm tri-dyed acrylic fiber

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