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ORIGINAL PAPER

# Forensic analysis of anthraquinone, azo, and metal complex acid dyes from nylon fibers by micro-extraction and capillary electrophoresis

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**Abstract** The extraction and separation of dyes present on textile fibers offers the possibility of enhanced discrimination between forensic trace fiber evidence. An automated liquid sample handling workstation was programmed to deliver varying solvent combinations to acid-dyed nylon samples, and the resulting extracts were analyzed by an ultraviolet/visible microplate reader to evaluate extraction efficiencies at different experimental conditions. Combinatorial experiments using three-component mixture designs varied three solvents (water, pyridine, and aqueous ammonia) and were employed at different extraction temperatures for various extraction durations. The extraction efficiency as a function of the three solvents (pyridine/ammonia/water) was modeled and used to define optimum conditions for the extraction of three subclasses of acid dyes (anthraquinone, azo, and metal complex) from nylon fibers. The capillary electrophoresis analysis of acid dye extracts is demonstrated using an electrolyte solution of 15 mM ammonium acetate in acetonitrile/water (40:60, *v*/*v*) at pH 9.3. Excellent separations and discriminating diode array spectra are obtained even for dyes of similar color.

**Keywords** Forensic analysis · Nylon fibers · Extraction of acid dyes · Capillary electrophoresis

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## Introduction

Forensic fiber examinations involve comparison of transferred fibers with one or more known fibers to determine possible associations between victims, suspects, and crime scenes [1]. Once the fibers have been collected, "questioned" and "known" fibers are compared using microscopic techniques to determine whether or not the characteristics of the suspect fibers are consistent with those of the known fibers. Discriminating cross-sectional shapes of nylon fibers can be easily seen using optical microscopy. For example, nylon for carpets is extruded from a melt spinning process to create fibers with characteristic cross-sections (often trilobal, triangular, or y-shaped), the purpose of which is to increase surface area and translucence, as well as hide dirt. Comparison microscopy and measurement of birefringence,

sign of elongation, and refractive index using polarized light microscopy are among the initial steps used to classify fibers as natural or synthetic and to determine the generic fiber polymer type [2]. Additional comparison techniques may include fluorescence microscopy, UV/visible microspectrophotometry, and infrared spectroscopy for polymer structure characterization. Fast nondestructive methods such as these are preferred, but these techniques do not identify dyes. Two textile fibers dyed with mixtures of several, possibly different, dyes might be formulated by different manufacturers to achieve a particular (common) color. These fibers could be visually indistinguishable and exhibit very similar UV/visible spectra. Analysis of dyes extracted from evidence fibers offers further discrimination of dyed fibers by comparison of UV/visible or mass spectra or by matching of retention or migration times from chromatography or capillary electrophoresis.

Although there are many types of textile fibers to which dyes are applied, four fiber types are more common in forensic fiber examinations: cotton, nylon, polyester, and acrylics. These four fiber types are found in more than 80% of criminal cases involving textile fibers as forensic evidence [1–4]. Dyes are conjugated molecules, generally consisting of aromatic and/or unsaturated compounds that are either derived from natural sources or are made synthetically. Dyes are often classified according to their application method (e.g., reactive, disperse, and vat) and their chemical constitution (e.g., azo, anthraquinone, and metal complex azo) [5]. Knowledge of the chemistry of both fibers and dyes is relevant to the extraction of dyes from fibers.

Two chemical types of nylon dominate the marketplace for use in apparel, home furnishings, and industrial fabrics. Nylon 6 is a polymer formed through ring-opening polymerization of caprolactam, while nylon 6,6 is a condensation polymer of hexamethylene diamine and adipic acid. These nylons are very similar in both polymer structure and chemical/physical properties; thus, we refer to them generically as nylon textile fibers, and their behavior with respect to dyeing and dye extraction is practically identical. Anthraquinone and azo acid dyes are characterized by the presence of anionic carboxylic or sulfonic acid groups. The anionic groups also provide varying aqueous solubility depending on the number of groups and the size of the dye molecule. The dyes are first dissolved in aqueous dye liquor adjusted to pH 8–10 with ammonia or NaOH. This step insures that sulfonic acid groups on the dye are deprotonated, thereby increasing dye solubility and penetration into the fiber. During the dyeing process, as the bath is slowly acidified with acetic acid to protonate the terminal amine groups on the nylon, the negatively charged sulfonic acid groups on the dye attach to the positively charged amine groups on the fibers via salt linkages [5, 6].

A solvent for dye extractions must have the capability of solvating the dye molecule, reducing its substantivity for the fiber (affinity via intermolecular interactions), and transporting the dye out of the fiber into the extraction medium. Extraction assistants such as acids or bases, which titrate the ionization state of the fiber dye sites, are often employed to increase extraction efficiency. Macrae and Smalldon [3] developed a qualitative solubility test of acid dye presence using 57% pyridine/43% water (4:3 volume ratio) on single nylon fibers. These conditions have also been used by other researchers for thin-layer chromatography (TLC), liquid chromatography, or direct mass spectrometry (MS) analysis [7–12] and were also published in FBI guidelines [13]. Resua [14] extracted acid dyes from nylon prior to TLC with a slightly different solvent of 53% pyridine/47% water. In extracting black acid dyes from wool, Xu et al. [15] recommended pyridine/water or ammonia/water, both in 4:3 volume ratios.

Automation of the sample-handling aspects involved in extraction of dyes from forensic fibers offers increased analysis speed and sample throughput compared to manual extraction. The most relevant aspect of an automated extraction system for the present work is the ability to program combinatorial experiments to determine the optimal solvent extraction mixtures for a given fiber–dye combination. Forensic applications of laboratory automation workstations have included analysis of biological specimens [16, 17], analysis of cocaine, benzoylecgonine [16], morphine [17], codeine [17] in urine, and DNA extraction from forensic casework samples [18].

While applications of capillary electrophoresis (CE) analysis of dyes in the textile industry have been published for several years, these studies are not based on extraction of dyes from fibers. For example, Croft and Hinks [19] reported CE separations of acid dyes using a potassium dihydrogen phosphate buffer at pH 9 with UV/visible detection at 254 nm. Robertson [20] reviewed the potential of capillary electrophoresis for forensic analysis of fiber dyes and suggested that CE compared favorably to high-performance liquid chromatography. Robertson et al. [21] found acid dyes to be extractable from wool only by organic solvents but had only marginal success with CE analyses. Sirén and Sulkava [22] analyzed acid dyes extracted from wool cloth patches and threads with ammonia, also with 254 nm detection. Extracts were evaporated, 200 μL methanol/water (1:1) was added, and the solution was diluted (1:10) with water prior to CE analysis using 3-(cyclohexylamino)-1-propanesulfonic acid electrolyte at basic pH. Poiger et al. [23] performed CE-MS separations of anionic metal complex azo dye standards using a buffer of 40% acetonitrile/60% 5 mM ammonium acetate at pH 9. Additional separations of acid dyes have also been achieved by liquid chromatography using diode

array or MS detection [10, 11, 24–27] and by infusion into electrospray MS [28], with several of these studies extracting dyes from fibers [10, 11, 24, 25].

Recovered fibers between 2 and 10 mm in length and containing between 2 and 200 ng of dye are of greatest concern in forensic fiber examinations [3]. Because forensic casework samples are size limited, efficient extraction techniques are necessary for complete removal of the dye (s) from the fiber for subsequent separation, quantitation, and identification. For that reason, the primary objective of the research described here was to optimize extraction as a function of solvent composition (pyridine/ammonia/water) and time/temperature conditions in automated micro-scale extractions of acid-dyed nylon. A secondary objective was to demonstrate capillary electrophoresis analysis of extracted acid dyes. Chemical classes of acid dyes used to dye nylon 6,6 include azo (48% of usage), metal complex azo (31%), and anthraquinone (10%) [6]. The structures of three representative dyes from these classes that were selected for the optimization of solvent extraction conditions in this work are shown in Fig. 1.

## Experimental

A laboratory robotic system was programmed for combinatorial experiments to determine the solvent composition producing the most efficient extraction conditions for a given fiber–dye interaction. For the purpose of optimizing extraction conditions, 1-cm threads of fiber (single yarns consisting of a bundle of twisted fibers) facilitated determination of the extraction completeness with high signal-to-noise ratio using a microplate reader.

Acid-dyed nylon 6,6 samples were donated by a dyestuff manufacturer in the southeastern USA, along with standards of the dyes with which each fabric was treated; the dyes selected are in common commercial use. For each extraction experiment, 1-cm lengths of thread, containing about 50 fibers, were loaded into 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, 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. To avoid loss of volatile components during extraction, especially at higher temperatures, 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. Subsequently, extracted dyes were analyzed using a SpectraMax M5 UV–Visible microplate spectrophotometer (Molecular Devices, Sunnyvale, CA, USA). All chemicals used were of analytical reagent grade.

Experimental design was applied to the extraction of acid dyes from nylon using a three-component simplex mixture design [28] that varied the composition of a ternary extraction solvent consisting of pyridine (Mallinckrodt, Phillipsburg, MO, USA), aqueous ammonium hydroxide (12 M, Fisher Scientific, Hampton, NH, USA), and water (18 MΩ, deionized). Ten design points were employed as shown in Table 1. Experiments were carried out in random order, and each design point was replicated, for a total of 20 experiments. This experimental design was performed at three different temperature/time conditions (e.g., 60 °C for 60 min; 90 °C for 60 min; and 100 °C for 15 min) and for each representative dye from the three acid dye classes (Fig. 1; azo, anthraquinone, and metal complex azo). The workstation was programmed to add amounts of liquid designated by the experimental design to each of 20 glass

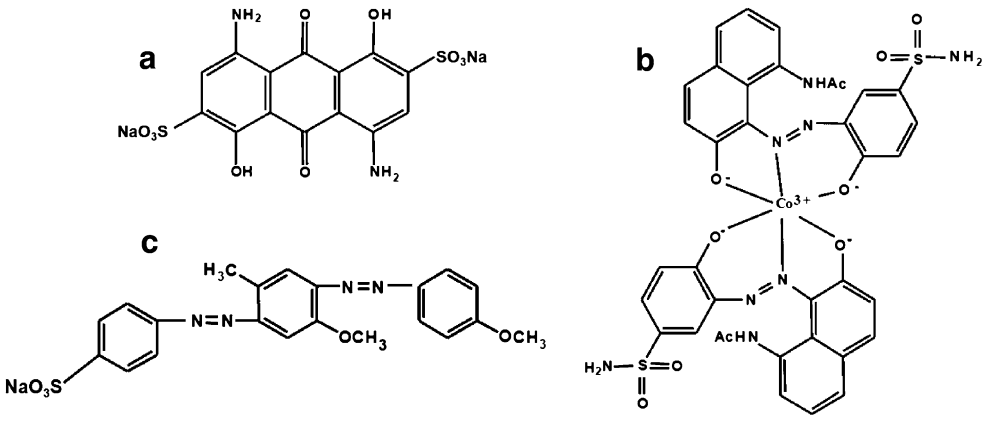


**Fig. 1** Representative acid dyes and their chemical structures: **a** C.I. Acid Blue 45 (an anthraquinone dye); **b** C.I. Acid Blue 171 (a metal complex azo dye); **c** C.I. azo Orange 156 (an azo dye)

**Table 1** Simplex mixture experimental design for optimization of extraction of acid-dyed nylon fibers using mixtures of pyridine, ammonium hydroxide, and water

| Design point | Pyridine (%) | Ammonia (%) | Water (%) |
|---|---|---|---|
| 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 |

Compositions are expressed as volume %

inserts in a 96-well sample plate for a total volume per well of 400 μL. *Design Expert*, v. 7 (Stat-Ease, Minneapolis, MN, USA) was employed for design of experiments, regression modeling, and graphics.

After addition of the prescribed extraction mixture, the samples were sealed and placed in an oven for the durations and temperatures as described. The samples were subsequently allowed to cool to room temperature, and 200 μL of each extract was transferred into a clean glass insert using automated liquid sample transfer. This process isolated the extracted acid dye from the nylon and served to quench further extraction. The plate holding the resulting extracts was returned to the oven, and the solvent was allowed to evaporate to dryness at 50 °C. Dye residues were reconstituted in 100 μL deionized water. Evaluation of dye extraction completeness was performed using the plate reader by measuring the absorbance at the wavelength maximum (for C.I. Acid Blue 145, C.I. Orange 156, and C. I. Blue 171 at 595 nm, 380, and 650 nm, respectively).

Prior to CE analysis, the robot was programmed for extract removal and addition of 190 μL water to the dried extract. Dyes were analyzed using a P/ACE-MDQ CE system (Beckman-Coulter) equipped with a diode array detector that monitored absorbance from 190 to 600 nm. A fused silica capillary with an internal diameter of 50 or 75 μm and length of 50 cm (40 cm effective length) from Polymicro Technologies (Phoenix, AZ, USA) was used for CE. Before use, capillary ends were burned to prevent degradation of polyimide coating. The capillary was then 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. Similar to the buffer employed by Poiger et al. [23], the electrolyte solution consisted of 15 mM ammonium acetate in acetonitrile–water (40:60, *v*/*v*), pH 9.3. Dye standards were prepared in deionized water at concentrations of 1.0 mg/mL. Injections were completed in hydrodynamic mode at 0.2 psi for 2 s. Separations were performed at 25 °C with an applied voltage of 30 kV. The electrolyte was replaced after every five runs to minimize solvent evaporation effects that might cause irreproducible electro-osmotic flow and migration times.

## Results and discussion

A three-component Scheffé mixture model (including first-order effects for each component and all two-factor interactions) [28, 29] was fitted to the absorbance data from the extraction of C.I. Acid Blue 45, an anthraquinone dye. Figure 2a shows contours of the predicted extraction response as a function of solvent composition. For the extraction carried out at 60 °C for 60 min, the model fits the data with a coefficient of determination ($R^2$) of 0.9480, and lack of fit of the model was not significant at the 95% level of confidence [29, 30]. Using the replicate experiments at the ten design points, an estimate of the experimental variability of the extraction process was obtained by calculating a pooled standard deviation relative to the highest extraction response. The pooled relative standard deviation for the replicate absorbance measurements was 4.9% (based on ten degrees of freedom)—an acceptably low level of experimental uncertainty.

Figure 2b shows a three-dimensional view of the fitted response surface. A diagonal ridge of high extraction response runs across the surface from about 50% pyridine/50% water to 50% pyridine/50% ammonia. Of the three pure solvents, pure water gives the best extraction of C.I. Acid Blue 45 dye from nylon, although the amount of dye extracted is very low. Pure pyridine does not dissolve the dye completely; the solubility of the dye in water is four times higher than that in pyridine. However, pure water is not sufficiently basic to deprotonate the nylon amine end groups and to release acid dyes completely from nylon. Likewise, although aqueous ammonia dissolves acid dyes better than does pure pyridine, aqueous ammonia lacks the organic content necessary to fully extract the organic anions of acid dyes. The diagonal ridge runs across the ternary solvent triangle at constant pyridine content of about 45–50%. For extraction of the anthraquinone C.I. Acid Blue 45 dye from nylon, the predicted optimum is at a solvent composition of 42% pyridine/58% water.

Studies on batch dyeing of nylon with acid dyes have shown increased dye uptake, probably due to increased dye penetration by diffusion, at temperatures up to 100 °C [31]. To investigate whether faster extraction might be possible at

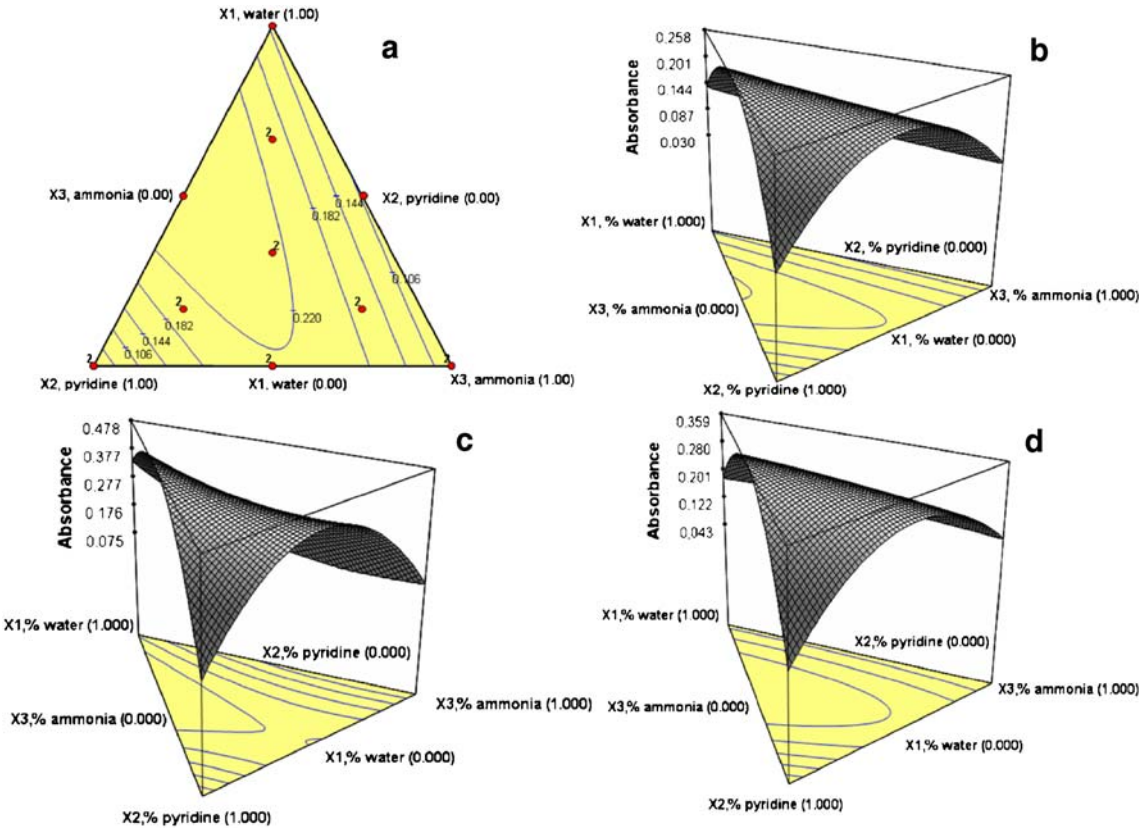


**Fig. 2** **a** Two-dimensional view of the fitted response surface for the extraction of C.I. Acid Blue 45 from nylon using pyridine, ammonia, and water (60 °C for 60 min). Two replicate experiments were performed at each of ten design points marked by *dots*. **b** Three-dimensional view of the fitted response surface (60 °C for 60 min). **c** Fitted response surface at 90 °C for 60 min. **d** Fitted response surface at 100 °C for 15 min

higher temperature, the anthraquinone dye extraction response surface was reexamined at two higher temperatures.

The 20-experiment mixture design was performed with the same extraction time (60 min), but at a higher extraction temperature of 90 °C. The resulting response surface is depicted in Fig. 2c. As might be expected, at this higher temperature, the amount of dye extracted increased, and the general appearance of the fitted model is similar to that observed in Fig. 2b, with a near-stationary ridge running diagonally across the surface. The model exhibited an ($R^2$) of 0.9477, no lack of fit, and optimum extraction of the acid dye from nylon is predicted at 37% pyridine/63% water. Slightly more dye is extracted under these conditions than the 42% pyridine/58% water extraction at 60 °C. The pooled relative standard deviation of replicate measurements, calculated as described previously, was 6.96%.

The experimental design was repeated a third time with extractions carried out at 100 °C for 15 min. Figure 2d shows the extraction response surface. The ($R^2$) value was 0.9616 with no lack of fit. The pooled relative standard deviation for these results was 7.43%. The highest absorbance (and thus, extraction) is predicted at 43% pyridine/57% water. Once again, the diagonal ridge is present, along which nearly equivalent extraction efficiency is predicted. Viewing the three experimentally determined extraction surfaces in Fig. 2 together shows that a range of experimental conditions along the diagonal ridge line are near optimal for extraction of this anthraquinone acid dye.

Anthraquinone acid dyes account for only 10% of the dyes in the acid dye class used with nylon fibers; azo and metal complex acid dyes account for 48% and 31%, respectively, of dyes applied to nylon. Two additional experimental designs were conducted at 60 °C for 90 min using C.I. Acid Orange 156 and C.I. Acid Blue 171 (Fig. 1) as representative azo and metal complex acid dyes. The results of these investigations are illustrated by the fitted response surfaces shown in Fig. 3. For the extraction of the azo dye, C.I. Acid Orange 156, optimum extraction is predicted at 45% pyridine/55% ammonia. The coefficient of determination ($R^2$) is 0.9235, and the pooled relative standard deviation for replicate measurements is 7.04%. The extraction response surface for this azo dye (Fig. 3a) shows a diagonal ridge similar to that seen for the extraction of the anthraquinone dye (Fig. 2), except the optimum is tilted toward the pyridine/ammonia mixture (Fig. 3). The fitted extraction response surface for the metal

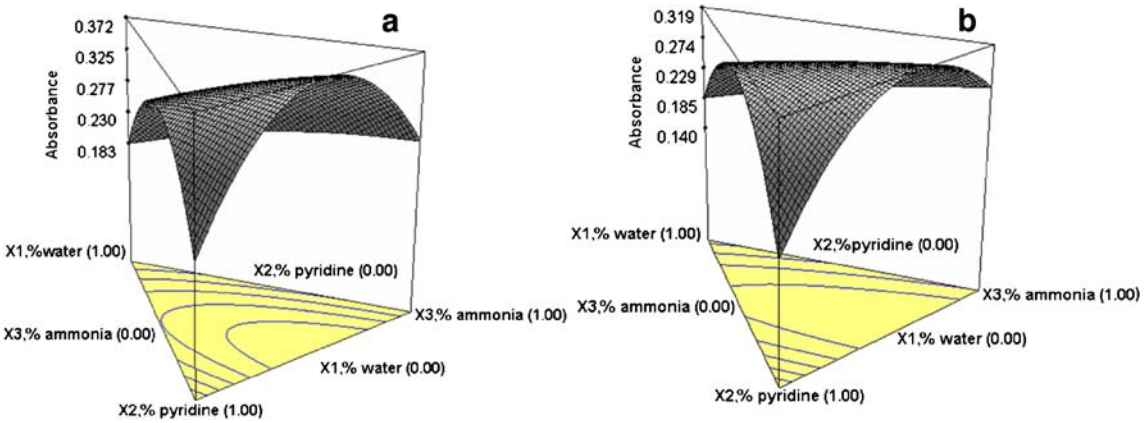


**Fig. 3** Fitted response surfaces for extraction from nylon using pyridine, ammonia, and water: **a** C.I. azo Orange 156 (an azo dye); **b** C.I. Acid Blue 171 (a metal complex azo dye). Both extractions were performed at 60 °C for 90 min

complex azo dye, C.I. Acid Blue 171 (Fig. 1b), is shown in Fig. 3b, with a predicted optimum at 33% pyridine/67% ammonia. The coefficient of determination ($R^2$) is 0.9197, and the pooled relative standard deviation for replicate measurements is 5.71%.

Examination of the appearance of Figs. 2 and 3 is informative in deciding upon a single set of extraction conditions that offer a compromise for adequate extraction of an acid dye of unknown chemical subclass from nylon fibers. All these response surfaces exhibit similar, slightly rising or falling, diagonal ridges running from a combination of pyridine/water to a combination of pyridine/ammonia. Anywhere along these diagonal ridge lines, a range of solvent conditions can be found at which optimal extraction can be achieved. For C.I. Acid Blue 45, the surfaces at different temperatures and times in Fig. 2 exhibit the same shape. For the extraction surface at 60 °C and 60 min (Fig. 2a, b), the response (0.258) measured at predicted optimum (42% pyridine/58% water) is identical to the highest measured response (0.258) which was found at the center of the design (at equal amounts of all solvent components). Extraction at 50% pyridine/50% water decreases the extraction response by only 13% compared to the highest extraction efficiency. For the other two anthraquinone surfaces at different temperatures and times (Fig. 2c, d), the predicted optimum extraction conditions range from 37% to 43% pyridine combined with 63–57% water. Comparing to the response achieved at 50% pyridine/50% water, extracting for 90 min instead of 60 min (at 60 °C) increases the absorbance by a factor of two. Running the extraction at 100 °C for only 15 min (with 50% pyridine/50% water) increases the absorbance response by a factor of 1.7 compared to the 60 °C and 60 min conditions. Not surprisingly, higher temperatures permit shorter extraction times to be used. For anthraquinone dyes, ease of use might favor the two-component mixture of 50% pyridine/50% water, which provides complete extraction when run at sufficiently high temperature (say 90–100 °C for 30–60 min).

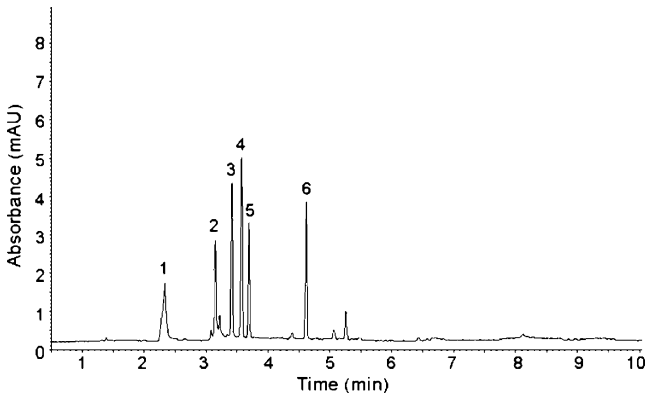


**Fig. 4** Electropherogram at 214 nm showing the separation of a mixture of five acid dyes. Peak identification: *1* neutral components, *2* C.I. Acid Blue 239, *3* C.I. azo Yellow 156, *4* C.I. Acid Blue 324, *5* C.I. Acid Red 337, *6* unidentified acid dye

The extraction response surfaces shown in Fig. 3 for an azo and a metal complex azo dye confirm the similar presence of a diagonal ridge. For example, the rising diagonal ridge in the azo extraction surface (C.I. Acid Orange 156) causes the binary 50% pyridine/50% ammonia conditions to produce 21% more absorbance than 50% pyridine/50% water conditions. For extraction of azo or metal complex azo dyes, near-optimal conditions for dye extraction are in the region of 40% pyridine/60% ammonia. However, working at equal concentrations of pyridine, ammonia, and water does not degrade extraction efficiency significantly.

As a demonstration of forensic utility, CE was used to analyze acid dye standards and extracts from fibers. Acid dyes are soluble in basic solutions and were separated using

a buffer consisting of 15 mM ammonium acetate in 40:60 acetonitrile/water at pH 9.3, which was chosen to ensure negative ionization of acid species. The electropherogram trace in Fig. 4, obtained for a mixture of five acid dye standards, shows excellent separation of all dye peaks. Theoretically, anions should elute in order of decreasing charge/mass ratio. This holds true in this separation, except C.I. azo Orange 156 migrates after C.I. Acid Blue 239. Identities of the five major peaks in the mixture were confirmed by migration times and spectra. The UV/visible spectra (not shown) of these five dyes are distinct from one another. Some minor peaks (not identified) are also seen in the electropherogram. We have observed minor peaks in separations of many extracted dye extracts and suspect that

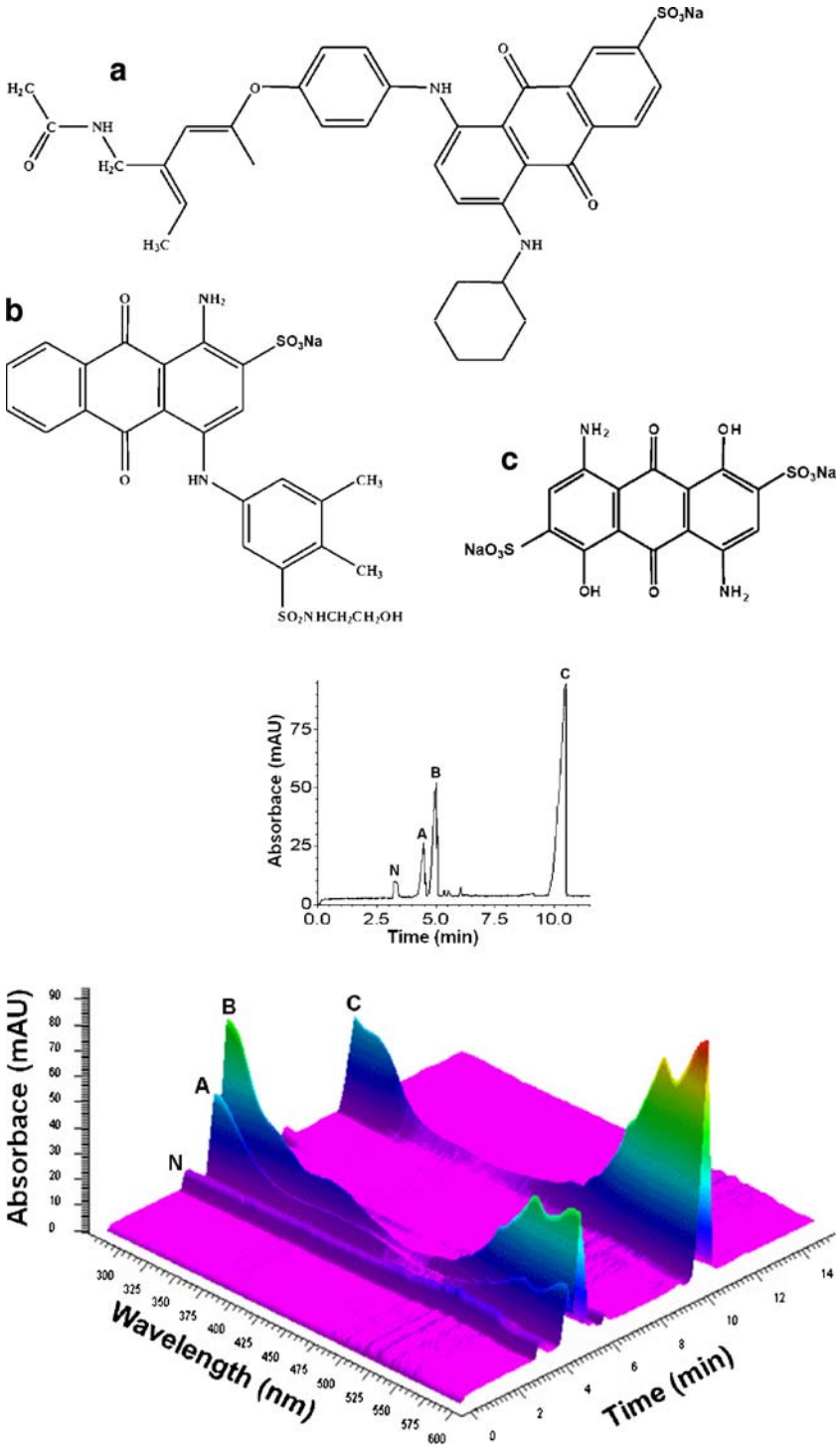


**Fig. 5** *Top* Peak identities: **a** C. I. Acid Blue 239, **b** C.I. Acid Blue 277, **c** C.I. Acid Blue 45. *Middle* Electropherogram at 600 nm of acid dyes extracted from nylon 6,6 fiber; peak *N* represents neutral components. *Bottom* Electropherogram showing UV/visible absorbance spectra

these peaks might be possible signatures of manufacturing processes or contaminants in "pure" dyestuffs. Incomplete dye synthesis can produce side products, and purified component dyes are neither required nor economically feasible on a commercial scale as long as the dyes possess the desired properties.

Figure 5 shows the CE analysis of three different anthraquinone acid dyes extracted from a 5-cm thread of nylon 6,6 using 37% pyridine/63% water at 90 °C for 60 min. The separation of the dyes is more than adequate; the three dye peaks are the major peaks in the electropherogram; and some minor components (unidentified) are seen, as before. Four sets of triplicate CE analyses of dye standards, conducted over a 4-h period, produced a pooled standard deviation of 0.65 min. Thus, migration times, at least over the short term, were reproducible to within a few seconds. Because the blue color of the three acid dyes used here are very similar, visual differentiation of separately dyed fibers is difficult and susceptible to misinterpretation at different dyeing levels. However, the CE migration times and spectra of these similarly colored dyes are distinguishable and might be matched to those of standard samples of dyes (if available).

## Conclusions

A laboratory automated workstation was employed to conduct a simple mixture experimental design to determine optimum extraction conditions for representative acid-dyed nylons from the anthraquinone, azo, and metal complex azo classes. The use of an automated workstation for micro-extractions offers programmed extraction protocols for high sample throughput and reproducibility in routine analysis.

We have employed designed experiments to explore optimum conditions for the extraction from nylon of a diverse variety of acid dyes. The three acid dye subclasses studied in this work account for 89% of acid dye types used on nylon. The solvent extraction conditions chosen for study span the range of solvents used previously for forensic analysis of fiber dyes. The solvent composition region for highest extraction efficiency of all three acid dye subclasses lies along a diagonal response ridge running from roughly 40% pyridine/60% water to 50% pyridine/50% ammonia. The comprehensive view of the extraction response surfaces affords recognition that the variety of extraction conditions previously employed for forensic analysis of acid dyes from nylons [3, 7–15] are all located close to this region of highest extraction. Anthraquinone dyes are almost equally well extracted across this range. For the azo and metal complex azo dyes examined here, extractions were complete at solvent mixtures near 40% pyridine/60% ammonia. Based on these results, 50% pyridine/50% ammonia may a reasonable compromise solvent combination for extraction of an unknown acid dye. Tradeoffs can also be made in variations of extraction time and temperature for ease of use or speed of analysis. After 100 °C for 15 to 30 min, extracted fibers are colorless, and complete extraction of dye is achieved, although shorter durations could be employed if sufficient fiber is available.

The applicability of capillary electrophoresis methods for analysis of acid dyes extracted from nylon has also been demonstrated. Combined with efficient extraction protocols, CE with diode array detection provides simple, rapid, and discriminating analysis of multiple dye components extracted from fibers. Separation and detection of individual dye components provides a qualitative and semi-quantitative fiber dye "fingerprint." Determining the number and relative amounts of dyes present and characterizing those dyes with diode array detection offers expanded capability for forensic discrimination of fibers.

Research on extraction of dyes from cotton, acrylic, and polyester fibers will be reported in subsequent publications. Detection limits and limitations to dye analysis from single fibers remains to be fully explored. Forthcoming research applies the extraction protocols used here and targets the sensitive detection and identification of dye extracts from textile fibers at forensically relevant levels by capillary electrophoresis with both diode array and electrospray mass spectrometric detection.

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## References

1. Rendle DF, Wiggins KG (1995) Rev Prog Color Relat Top 25:29–34
2. Gaudette BD (1988) In: Saferstein R (ed) Forensic science handbook, vol II. Prentice Hall, Englewood Cliffs, pp 209–272
3. Macrae R, Smalldon KW (1979) J Forensic Sci 24:109–116
4. Webb-Salter M, Wiggins KG (1999) In: Robertson J, Grieve M (eds) Forensic examination of fibres, 2nd edn. Taylor & Francis, London, pp 364–378
5. Trotman ER (1964) Dyeing and chemical technology of textile fibres. Griffin, London
6. Shore J (2002) Colorants and auxiliaries, vol 1, 2nd edn. Society of Dyers and Colourists, West Yorkshire, England
7. Beattie IB, Dudley RJ, Smalldon KW (1979) J Soc Dyers Colour 95:295–302
8. Beattie IB, Dudley RJ, Smalldon KW (1981) Forensic Sci Int 17:57–69
9. Wiggins KG, Cook R, Turner YJ (1988) J Forensic Sci 33:998–1007

10. Laing DK, Gill R, Blacklaws C, Bickley HM (1988) J Chromatogr 442:187–208
11. Tuinman AA, Lewis LA, Lewis SA (2003) Anal Chem 75:2753–2760
12. Wiggins K, Holness J-A (2005) Sci Justice 45:93–96
13. Federal Bureau of Investigation, Scientific Working Group on Materials Analysis. Forensic fiber examination guidelines (1999) Forensic Sci Commun 1(1). URL: http://www.fbi.gov/hq/lab/fsc/backissu/april1999/houcktoc.htm, accessed 24 April 2009
14. Resua R (1980) J Forensic Sci 25:168–173
15. Xu X, Leijenhorst H, Van den Hoven P, De Koeijer JA, Logtenberg H (2001) Sci Justice 41:93–105
16. Taylor RW, Le SD (1991) J Anal Toxicol 15:276–278
17. Vidal DL, Ting EJ, Perez SL, Taylor RW, Le SD (1992) J Forensic Sci 37:1283–1294
18. Greenspoon SA, Ban JD, Sykes K, Ballard EJ, Elder SS, Baisden M, Covington BL (2004) J Forensic Sci 49:1–11
19. Croft SN, Hinks D (1993) Text Chem Color 25:47–51
20. Robertson M (1999) In: Robertson J, Grieve M (eds) Forensic examination of fibres, 2nd edn. Taylor & Francis, London, pp 328–336
21. Robertson J, Wells RJ, Pailthorpe MT, David S, Aumatell A, Clark R (1993) In: Advances in forensic sciences. Proceedings of the Meeting of the International Association of Forensic Sciences, Duesseldorf, pp 247-249
22. Sirén H, Sulkava R (1995) J Chromatogr A 717:149–155
23. Poiger T, Richardson SD, Baughman GL (2000) J Chromatogr A 886:259–270
24. Yinon J, Saar J (1991) J Chromatogr 586:73–84
25. Huang M, Yinon J, Sigman M (2004) J Forensic Sci 49:1–12
26. Huang M, Russo R, Fookes BG, Sigman ME (2005) J Forensic Sci 50:1–924
27. Zhang YP, Zhang YJ, Gong WJ, Gopalan AI, Lee K-P (2005) J Chromatogr A 1098:183–187
28. Smith W (2005) Experimental design for formulation. Cambridge University Press, New York
29. Deming SN, Morgan SL (1993) Experimental design: a chemometric approach, 2nd edn. Elsevier Science, Amsterdam
30. Deming SN, Morgan SL (1979) Clin Chem 25:840–855
31. Dawson TL, Todd JC (1972) J Soc Dyers Colour 95:417–426