ELSEVIER

Journal of Chromatography A, 781 (1997) 391–405

JOURNAL OF CHROMATOGRAPHY A

Separation, comparison and identification of fountain pen inks by capillary electrophoresis with UV–visible and fluorescence detection and by proton-induced X-ray emission

C. Vogt[a,*], J. Vogt[b], A. Becker[a], E. Rohde[c]

[a]*Faculty of Chemistry & Mineralogy, Institute of Analytical Chemistry, University of Leipzig, Linnéstrasse 3, 04103 Leipzig, Germany*
[b]*Faculty of Physics and Geosciences, Institute of Nuclear Solid State Physics, University of Leipzig, Linnéstrasse 5, 04103 Leipzig, Germany*
[c]*Department of Chemistry, University of Cincinnati, Mail Location 172, Cincinnati, OH 45221, USA*

Abstract

The analysis of inks as a part of the detection of fraudulent documents is a small but important aspect of forensic science. The present study was focused on the separation of components of commercially available original fountain pen inks and on different paper substrates by capillary electrophoresis and proton-induced X-ray emission (PIXE). Electropherograms of inks from various manufacturers and countries separated in borate buffers with various methanol contents showed patterns which were in most cases distinctly different from one another. UV–Vis scans and fluorescence detection at different excitation and emission wavelengths have been used to compare similar mixtures and ink extracts from paper with the original ink composition, to identify concurrences or differences in the spectra of main and trace components and to improve the discrimination power of the technique in this way. Extraction conditions, like solvent composition, extraction time, paper carrier material, age of the ink and ink concentration have been examined with respect to signal intensity and the number of detectable components. PIXE spectra of ink samples on different paper substrates have been recorded to determine the elemental composition of the samples and to obtain additional information about the chemical composition of the dyes.

Keywords: Ink; Detection, electrophoresis; Forensic analysis; Proton-induced X-ray emission; Dyes

1. Introduction

The analysis of inks for the investigation of fraudulent documents is a small but important area of forensic science. Subtle alterations to documents such as insurance claims, wills and tax returns can have significant financial implications. The detection of alterations or additions to a document and the determination of time, when the document was written are a prime concern of document examiners and ink chemists.

Inks are complex mixtures of different chemical compounds, such as acid or basic dyes, organic or inorganic color pigments, surfactants, antioxidants, viscosity adjusters, resins, glycol and glycerol, all of them in varying amounts. The comparison of two or more inks involves chemical and physical analysis. Systematic approaches to ink comparison and identification have been described by Brunelle and coworkers [1,2]. In particular, chromatographic methods, including paper chromatography [3,4], thin-

*Corresponding author.

0021-9673/97/$17.00
PII S0021-9673(97)00621-3

layer chromatography [5,6] and high-performance liquid chromatography [7–11], have been extensively applied to the analysis of inks during the last decades. In comparison, electrophoretic techniques have been applied only rarely [12,13]. Nevertheless, modern electrophoretic techniques are characterized by high resolution power, and inherently are suitable to separate charged compounds, like most of the acid and basic dye components of inks.

On this basis, the present work was aimed at testing the applicability of capillary electrophoresis (CE) [14,15] to the separation of blue and black fountain pen inks in the original liquid form and dried on different substrate materials. After the optimization of the separation conditions diode array UV–Vis detection and laser-induced fluorescence (LIF) detection with two excitation wavelengths have been applied to collect as much information as possible from the electropherograms, in order to compare the ink extracts with the original inks. In addition, nondestructive proton-induced X-ray emission (PIXE) [16,17] spectrometry was used to characterize the elemental composition of dried inks on different substrate materials. Finally, all the information content from CE and PIXE analyses of original and dried inks has been integrated and used for the reliable identification of fountain pen inks.

2. Experimental

2.1. Chemicals

Methanol, ethanol and isopropanol of HPLC grade were from Roth (Karlsruhe, Germany). NaH_2PO_4 and H_3BO_3 were from Merck (Darmstadt, Germany). To adjust the pH of the buffers, concentrated solutions (1 or 0.1 *M*) of NaOH were utilized. For the preparation of all buffers only triply distilled water was used. Only fountain pen inks, purchased from office supply stores as refill cartridges have been investigated (Table 1). Original samples were stored in darkness at 4°C. Diluted samples were prepared fresh using 5 m*M* borate buffer, pH 8.25. Five different substrate materials (Table 2) purchased from office supply stores were investigated.

2.2. Capillary electrophoresis

Electrophoretic separations were performed on P/ACE 2050 and 5510 systems from Beckman (Palo Alto, USA) controlled by an IBM PC using GOLD 8.1 and P/ACE 3.0 software (Beckman). UV–Vis detection was performed with a diode array detector, recording spectra from 198 to 600 nm. A cartridge with an aperture of 800×100 μm was used. For laser-induced fluorescence (LIF), an He–Cd-laser Series 74 from Omnichrom (Chino, CA, USA) with an excitation wavelength of 320 nm and 26 mW power and an argon ion laser (Beckman Instruments) with an excitation wavelength of 488 nm and 3 mW power were used. Emission radiation was recorded at 436 or 520 nm, respectively. The aperture on the capillary was 200×100 μm.

Uncoated fused-silica capillary material (Laser 2000, Wessling, Germany) of 57 length (50 cm to the detection window) and 50 μm I.D. was used. Separation was carried out at 25 kV. Injection was performed in the pressure mode with 3.5 kPa and injection time was set to 10 or 15 s.

2.3. Preparation of the ink extracts

From a medium writing line, one to 10 microplugs are cut out by a hollow metal syringe of 1.5 mm I.D. After transferring the material into a tapered plastic tube (500 μl total volume), 25 μl of a solvent (methanol, ethanol, isopropanol, water) and 25 μl of a 5 m*M* borate buffer, pH 8.25, were added. The tubes were sealed and ultrasonicated for 5, 15, 30 or 60 min. Afterwards the solution was either pipetted to a clean plastic tube for storage in the refrigerator at 4°C or injected directly into the electrophoretic separation unit.

2.4. Proton-induced X-ray emission (PIXE)

The PIXE experiments were performed on the 2 MeV van de Graaff accelerator at the University of Leipzig. The proton beam with an energy of 1700 keV was collimated to a diameter of 400 μm using a set of three diaphragms. After directing the ion beam onto the writing on the substrate material the proton-induced X-rays were detected by a Si(Li)-detector with 135 eV energy resolution. The precise beam

position was controlled by a CCD-camera. A movable sample holder was used to correct the position for the measurements. Both sample holder and sample were fixed in an evacuated measuring chamber.

For each measurement, the X-ray signal was recorded until a charge of 2 μC was accumulated. After that, the calculated net areas of the K_α- or L_α-line of the characteristic X-rays were figured as 'counts'. Because of time-dependent inhomogeneities of the ion beam only the detection of the charge makes a comparison of different spectra possible. During the measuring process the proton current of 1 nA was kept as constant as possible.

The writing line with each ink was measured three times, as was as the spectrum of the substrate material adjacent to the writing. The average spectrum of the substrate was then subtracted from the average spectrum of ink plus paper. The resulting difference spectrum is characteristic for the dried ink on the substrate material.

3. Results and discussion

3.1. Optimization of the separation conditions

In most cases the dye components of the fountain pen inks (Table 1) are chargeable substances, frequently with sulfonic or carboxylic, phenolic and less frequently amino functional groups. Under strong acidic or alkaline conditions these compounds are charged, and therefore they should migrate in the electric field of an electrophoretic separation system. To compare different inks, resolution of the main components should be as large and reproducible as possible.

Table 1
Fountain Pen Inks investigated

No.	Manufacturer	Color
1	Cross (USA)	blue
2	Cross (USA)	black
3	Pelikan (Germany)	royal-blue
4	Pelikan (Germany)	brilliant-black
5	Pilot (Japan)	blue
6	Pilot (Japan)	black
7	Lamy (Germany)	black
8	Lamy (Germany)	blue
9	Geha (Germany)	royal blue
10	Geha (Germany)	brilliant-black
11	Waterman (France)	black
12	Mont Blanc (Germany)	royal blue

If capillary electrophoresis (CE) is performed in uncoated fused-silica capillaries, separation of the inks is possible in strong acidic medium (pH<4) at reversed polarity (no osmotic flow) or at normal polarity in alkaline buffers (high osmotic flow). Separation in acidic medium (pH 2.5–5.0) did provide resolution of only a few negatively charged components and most dye components remained unresolved. On the contrary, in alkaline buffers (pH 8.0–10.0) good resolution was achieved. Positively charged species could be detected only seldom, while anionic dyes were more frequently found in the investigated inks. Basic buffers assured good analyte ionization and strong electroosmotic flow allowing simultaneous detection of both anionic and cationic species. Different buffering substances and additives, like organic solvents and micelle-forming substances have been tested to optimize the separation, but only with addition of simple borate buffers with methanol between 10 and 30% was sufficient resolution achieved with both detection modes, UV–Vis and fluorescence. The pH was kept as low as possible (pH 8.0) to reduce the electroosmotic flow in the system, to lower the difference between the electrophoretic migration of the dyes and the osmotic velocities, and to increase the resolution of similar compounds. At pH 8, a tremendous impact on the resolution of the ionic strength of the buffer was observed. Fig. 1 demonstrates the separation of 100-fold diluted original ink 2 (Table 1) in the same buffer with concentrations of 20 (A), 100 (B) and 250 (C) m*M* borate. The increase of the ionic strength leads to a drastic reduction of the osmotic flow and therefore better resolution. On the other hand, for some signals, peak depression (lower peak intensities and peak areas) was observed and prolongation of the separation time took place. Especially for the separation of ink extracts, the sensitivity should not be neglected in order to get a maximum number of ink components detected. Therefore, the separation in Fig. 1B (100 m*M* borate buffer) presents a good compromise between maximum resolution, short separation time and signal intensity. A content of 20% methanol was

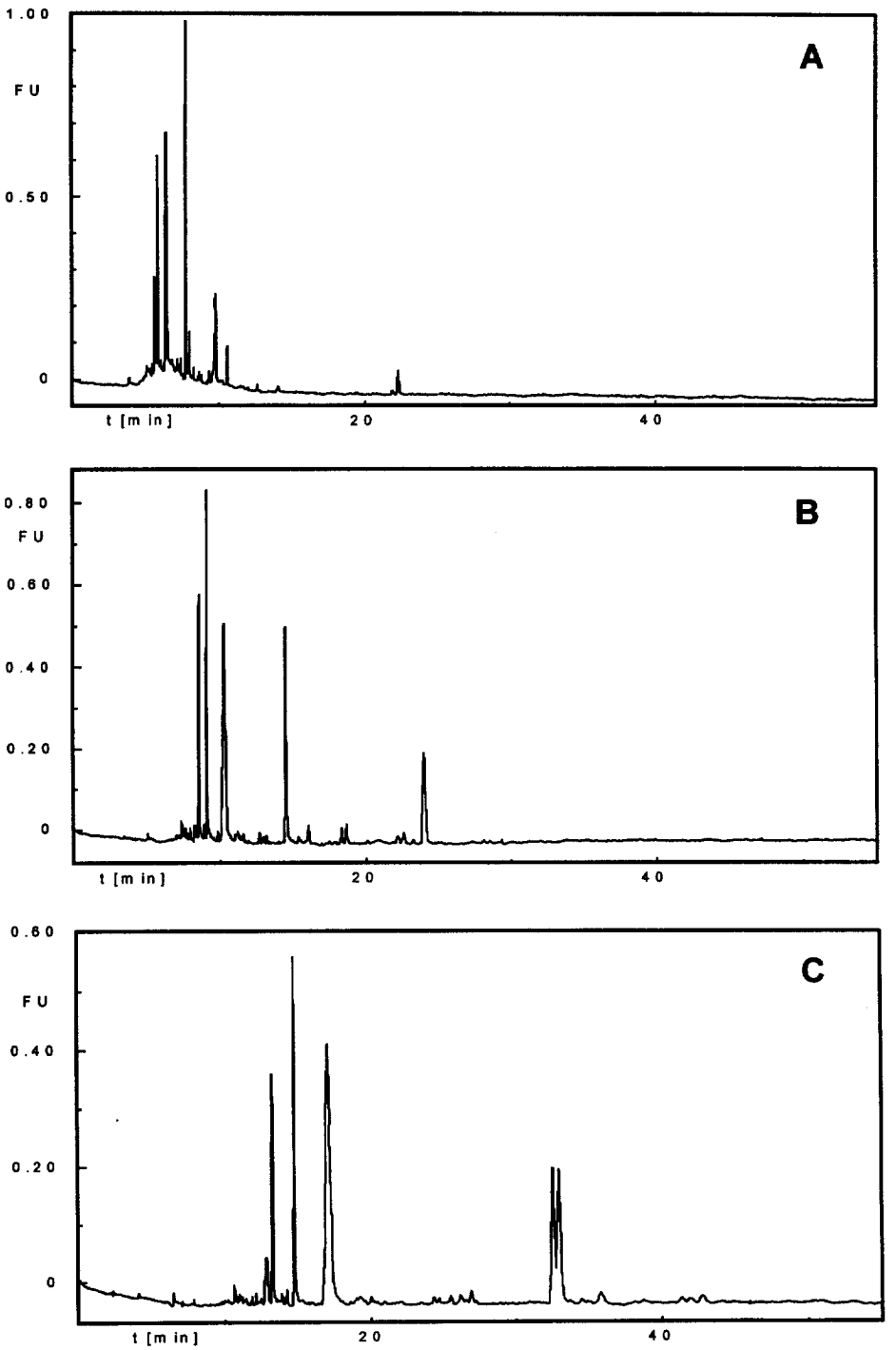

Fig. 1. Separation of ink 2 (Table 1) in buffers of different ionic strength: (A) 20 m*M* borate, pH 8.00, 20% MeOH; (B) 100 m*M* borate, pH 8.00, 20% MeOH; (C) 250 m*M* borate, pH 8.00, 20% MeOH; 10 s pressure injection of an original ink sample diluted 1:100; 25 kV; 22°C; detection, LIF He–Cd, $\lambda_{ex/em}$ 320/436 nm.

observed to provide best resolution and helped to minimize cleaning procedures of the capillary material at the end of each separation due to minimized adsorption effects. Good resolution was also achieved in buffers without methanol but an ionic strength above 150 m*M* borate, however under these conditions cleaning procedures after each run took much more time because, to a great extent, adsorption of the dye components onto the capillary wall took place. Rinsing the capillary with SDS containing borate buffer, pH 9, followed by methanol and 1.0 *M* NaOH, standard deviation of the migration times of the dye components could be reduced below 2%. Therefore, all following separations have been performed in buffers with 100 m*M* borate, pH 8.00, and 20% methanol.

3.2. Optimization of the extraction parameters

Each plug cut out from the substrate material represents a unique sample, because the amount of ink and the degree of binding of the ink components to the paper is different at each position of the writing line. Therefore, the recovery for ink components during repeated extraction procedure will inevitably be very poor.

Therefore, extraction parameters have been optimized in view of preparation time, number of signals and signal intensity. For this purpose, influence of extraction time, solvent composition and number of plugs were investigated. To confirm the results each extraction was performed twice.

To five plugs cut out from an ink line (ink 2) of medium thickness, 50 μl of a solvent mixture were added: first, 25 μl ethanol, methanol, isopropanol or water, followed by 25 μl 5 m*M* borate buffer, pH 8.25. All samples were sonicated under identical conditions before the supernatant liquid was transferred to a new vessel. The electropherograms obtained from extracts obtained with different extraction solvents showed only few differences. Best resolution was obtained with methanol as organic solvent. But water or ethanol also provided good resolution for most of the peaks. Only with isopropanol in the extraction mixture was a larger amount of unresolved peaks observed. Since separation was performed in buffers with 20% methanol, all further extraction procedures were also performed with methanol.

Concerning extraction time, already after 5 min a remarkable amount of signals could be detected. On the other hand, no significant signal increase could be observed at extraction times above 30 min. Since the signal intensities for a 15-min extraction time were equal or higher than 90% of those obtained after 30 min extraction, all further extractions were performed for 15 min.

The number of plugs cut out plays an important role in the number of detectable peaks on the electropherograms. In some cases, only one plug (1.5 mm writing line) was sufficient to get most of the components of the dried ink detected. In other cases, even 10 plugs (about 20 mm ink line) gave only two or three peaks in the electropherogram.

The number and the intensity of the obtained signals depend not only on the number of the components in the original ink, but also on the chemical behavior of these compounds and on the paper material and its ability to bind the dyes irreversibly. To allow a comparison of all selected inks (Table 1) each extraction was performed with five plugs cut out from a medium writing line.

Independently of the ink and the extraction solvents, a large number of additional peaks compared to the signals of the original ink have been observed using LIF detection. These components are either degradation products of the ink, formed during the ultrasonication step of the extraction procedure, or dyes extracted from the paper substrate material.

3.3. UV–Vis detection

Signal intensities of ink extracts and diluted original inks of the same sample have been compared. Based on this comparison a dilution factor of 1:100 for original inks was found to be best, in most cases, to obtain comparable peak intensities with electropherograms from the extracts. In Fig. 2, electropherograms of four different black inks are compared. Despite being of quite different origin, the electropherograms in Fig. 2A,B show many concurrences. The main components, 1 and 2, are characterized by nearly identical UV–Vis spectra with maxima at 412 nm for peak 1 and 585 nm for peak 2 (violet dye). Unfortunately, both peak pairs do not

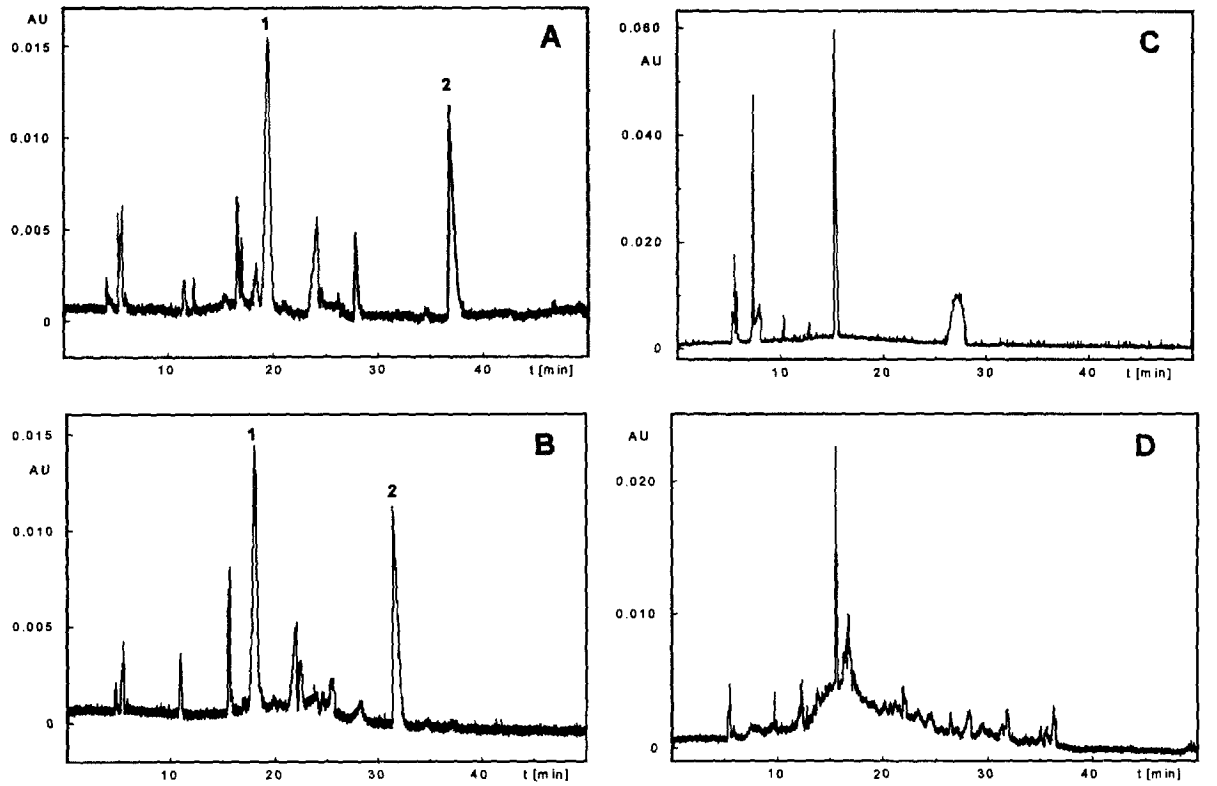

Fig. 2. Comparison of four different original black inks (see Table 1) diluted 100-fold (in 5 m*M* borate, pH 8.25) after separation. Detection at 200 nm; 25 kV; 22°C; 15 s pressure injection. (A) Ink 2, (B) ink 4, (C) ink 6 and (D) ink 11.

possess the same migration times. To compensate for adsorption effects during the separation and for influences from additional ink components (which are not dyes), normalization of the peak migration times to the migration time of the electroosmotic flow is helpful. Doing so, the peak pair 1 in Fig. 2A,B possesses the same migration time. For peak pair 2, identical migration time was not obtained. For many other samples similar behavior was observed for the longer migrating components with migration times above 25 min (highly negatively charged components). By all known odds these effects are caused by adsorption effects during the separation, which could not be influenced by cleaning steps prior to or after the run. Working with internal standards offers additional possibilities to improve the reproducibility for the results obtained. Unfortunately, for the separation of unknown samples, the application of internal standards often leads to peak overlapping with sample components important for the pattern or the identification of the sample.

Despite identical migration times and spectra for most of the components in both inks, few peaks are unique representatives in each sample. Therefore, both inks in Fig. 2A,B could be distinguished easily.

A completely different peak pattern is observed in the electropherograms in Fig. 2C,D. Ink 6 (Fig. 2C, Table 1) from Japan consists of only few compounds which absorb in the UV–Vis region. In comparison, besides a main compound, ink 11 (Fig. 2D) contains many other UV–Vis-absorbing substances which also leads to a unique peak pattern.

The comparison of extracts from dried inks with electropherograms from diluted original inks is more important. Fig. 3 presents extracts of a blue and a black dried ink compared with the original inks diluted 1:100-fold by borate buffer. All dried inks were extracted from paper 2 (Table 2). Fig. 3A,B

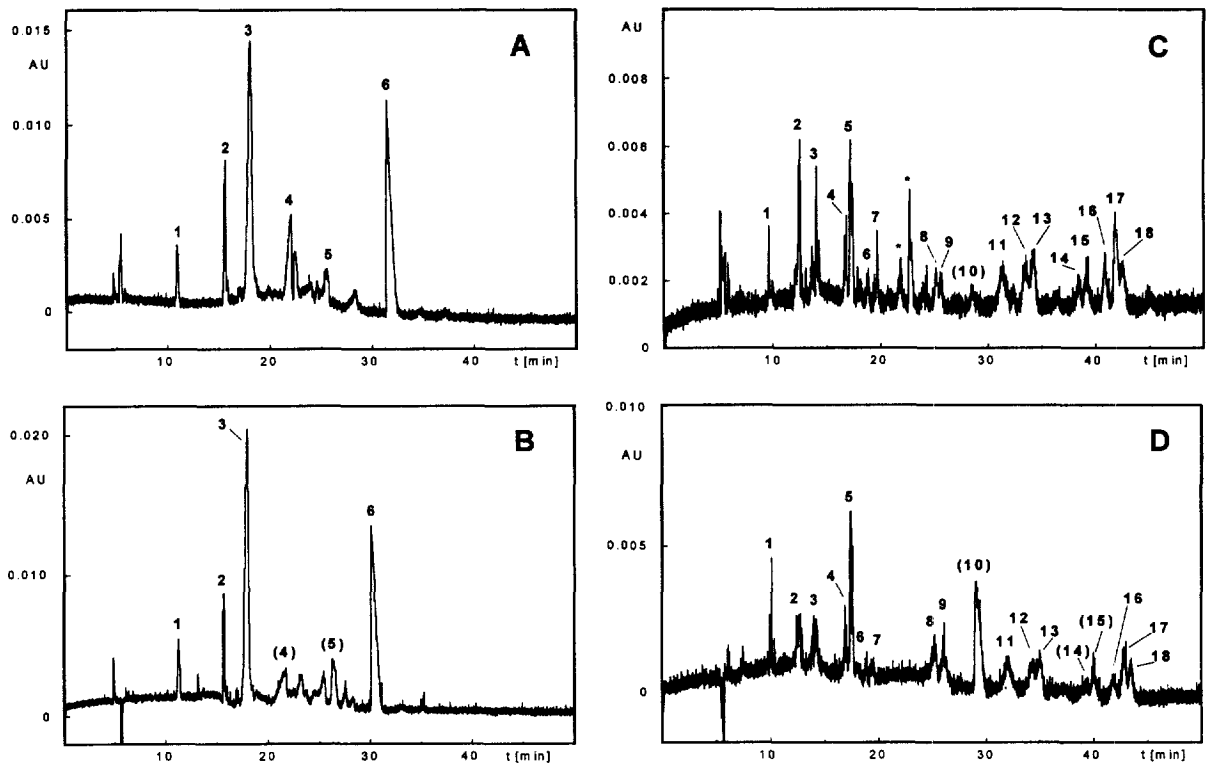

Fig. 3. Comparison of black and blue inks (see Table 1) with their extracts from substrate paper 2 (see Table 2). Original inks diluted 1:100 in 5 m*M* borate buffer, pH 8.25; 25 kV; 22°C; 15 s pressure injection; detection at 200 nm. (A) Ink 4 original ink, (B) ink 4 extract, (C) ink 9 original ink, (D) ink 9 extract.

show the peak pattern of the original black ink and the extract of this ink. Both electropherograms show nearly the same peak pattern. Using migration times and UV–Vis scans (only for main components, peaks 3 and 6) four peaks (peak numbers 1, 2, 3 and 6) could be identified as being the same in both electropherograms (for peak 6 only the scans were identical, migration time is influenced by adsorption effects again). Although this result seemed encouraging, there were other black inks with nearly the same or identical peak patterns (inks 2, 7, 10 and 11). This leads to the conclusion, that most of the black inks under investigation contain dyes with a similar structure. Only for ink 6 from Japan were quite different peak patterns and UV–Vis scans for the main components observed.

Table 2
Substrate materials (papers) investigated

No.	Substrate material	Color
1	Fine cardboard	white
2	Copy paper	white
3	Writing paper (eco material)	grey
4	Eurocheck	multicolored
5	Copy paper	faintly yellow

In Fig. 3C,D an extract and the original blue ink are compared. Again, both electropherograms show nearly identical patterns. Due to the low intensity of the peaks, only migration times have been used to compare single signals in both electropherograms. Nevertheless, more than 15 compounds could be assigned in both electropherograms. Only one group of signals, marked with an asterisk in Fig. 3C, could not be found in the electropherogram of the ink extract. Probably these compounds are tightly bound to the paper material and could not be extracted under the described conditions. Also, destruction during the ultrasonication process has to be taken

into account. For blue inks under investigation in this work, the original electropherograms differ much more than for black inks. In some cases a good correlation between the electropherograms of the extract and the original sample could be achieved (inks 9 and 5), in other cases no dye signal could be found in the extract (inks 1, 3 and 8) or only few of many compounds could be assigned (ink 12).

The results confirm that UV–Vis detection is applicable to the characterization of inks extracted from substrate materials although some problems with longer migrating components and signal intensities may occur. Fortunately, no components extracted from substrate material interfere with the extracted ink components. Because not all inks produce sufficiently high signals, UV–Vis scans could only be applied to few main compounds. However, specially designed on-line preconcentration steps (stacking) could help to overcome this disadvantage of the UV–Vis detection mode. The extension of the scan range up to 800 nm will allow better identification of all dye components. Using a scan range to 600 nm (as in this work) only violet, purple, yellow and red dyes (absorption maxima at 550–580, 500–530, 400–450 and 450–480, nm respectively) could be identified. Blue and green dyes possess absorption maxima beyond the accessible range.

3.4. Fluorescence detection

Two different laser systems have been tested for ink analysis. A He–Cd laser with an excitation wavelength at 320 nm and an argon ion laser with an excitation wavelength at 488 nm have been applied. In general, better results were expected for the He–Cd laser, because of a higher power and lower excitation wavelength. In Fig. 4, representative blue and black inks, detected with both laser systems, are

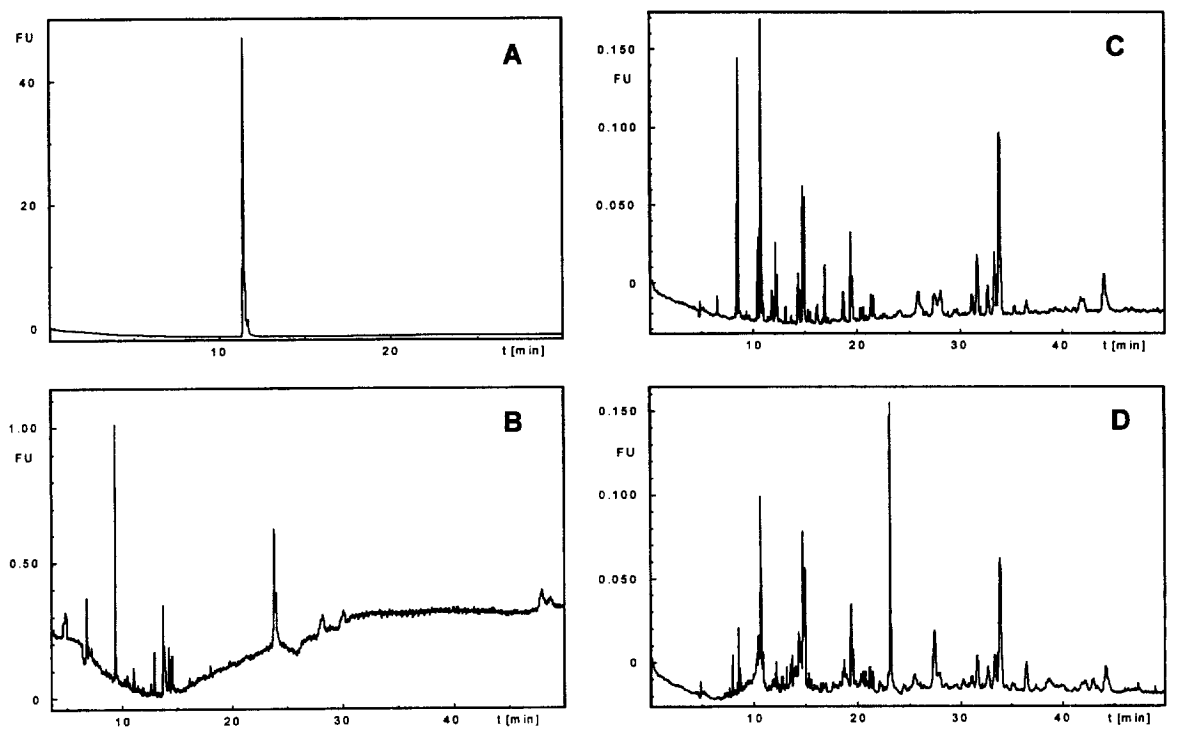

Fig. 4. Detection of black and blue inks diluted 100-fold (see Table 1) with different LIF systems. (A) Ink 5, (B) ink 6, (C) ink 9, (D) ink 11; 25 kV, 22°C, 15 and 10 s pressure injection for samples A, B, C and D, respectively; LIF detection at $\lambda_{ex/em}$ 488/520 nm (A and B) and $\lambda_{ex/em}$ 320/436 nm (C and D).

compared. For the detection shown in Fig. 4A,B the Ar laser with the higher excitation wavelength was applied, for Fig. 4C,D the He–Cd laser was used. Generally, electropherograms detected with the argon ion laser possess less components than those detected with the He–Cd laser. Also, in most cases, signal intensities using the argon laser are smaller. Two reasons could justify this behavior. First, the excitation wavelength of the argon laser is higher than the optimum wavelength for maximum excitation of the dye components. Second, the He–Cd laser is much more powerful, and more components are excited, even substances which are not dyes. However, independently of the laser applied, every ink (diluted 1:100-fold with borate buffer) gave a unique electropherogram.

From the five similar black inks (inks 2, 4, 7, 10 and 11), which gave nearly the same UV–Vis plot, only inks 4 and 10 gave identical fluorescence patterns (using the He–Cd laser). The same fluorescent components were detected in both electropherograms, on the basis of the migration times and peak areas. Therefore, we concluded that the two inks are identical. Ink 7 was very similar to inks 4 and 10, since each component of inks 4 and 10 was also detected in the electropherogram of ink 7, but there were distinct differences in the peak areas of the main fluorescent components.

With the He–Cd laser, for all original inks under investigation, good sensitivity was achieved for 1:100-fold diluted samples. In some cases, an even higher dilution was necessary for complete resolution of all detectable compounds. Black inks gave better fluorescence signals than blue inks, which could be ascribed to the high content of violet dyes in black inks, and their lower excitation wavelength in comparison to blue dyes. In this case the excitation wavelength of the laser is closer to the optimum excitation wavelength of the dyes.

Using the argon ion laser, only for the original inks 2, 5 and 6 (diluted 1:100-fold) could fluorescence signals be recorded which are comparable with those obtained with the He–Cd laser. Good results were obtained also for inks 3, 9, 10, 11 and 12, with samples diluted only 1:10. For inks 1, 4, 7 and 8, in every case only a few very small sample signals could be detected. Therefore, the argon ion laser proved unsuitable for a comparison of ink extracts and original inks, but in some cases additional information could be obtained.

In Fig. 5, a comparison of the two fluorescence patterns for ink 2 is shown. This ink is one of the few samples for which both laser systems gave electropherograms with a large number of peaks. The peak pattern of both electropherograms is quite different. Using normalized migration times, only four peaks (1–4) could be assigned in both electropherograms. Compounds 1 and 3, especially, are not excited to any great extent with the argon ion laser (Fig. 5A), but rank among the main components after excitation with the He–Cd laser (Fig. 5B). Probably, both compounds belong to the dyes with fluorescence in the violet region. The compound responsible for peak 5 behaves in a similar way

During the separation of ink extracts, very large peaks with migration times different from those of the ink compounds have been observed. Most papers contain whiteners, mostly violet dyes, which have to compensate the faintly yellow appearance of the paper after the production process. These dyes could also be extracted during the treatment with organic and/or aqueous solvents. Therefore, the extraction behavior of the paper substrate material was investigated. In Fig. 6, electropherograms of four of the investigated five paper materials are shown. The electropherograms in Fig. 6A–C show two main components (at 11.2 and 12.7 min migration times) with identical migration times in each electropherogram (after normalization). Paper 2 (Fig. 6B) and paper 3 (Fig. 6C) contain very large amounts of these substances. In these two paper materials, additional compounds in lower amounts, which migrate close to the position of ink dyes, could cause deterioration of the identification of signals from extracted inks. In Fig. 6D, the electropherogram of only one plug cut out from a white paper material with a multicolored check is shown. Smaller signal intensities and only a few compounds make the identification of co-extracted inks much easier. Red-, blue- and black-colored regions of the check have also been extracted. The electropherograms obtained from plugs cut out from white and blue regions of the check show nearly identical peak patterns; in those from black and red regions the peaks possess different migration times and much lower signal intensities. For the investigation of inks on multico-

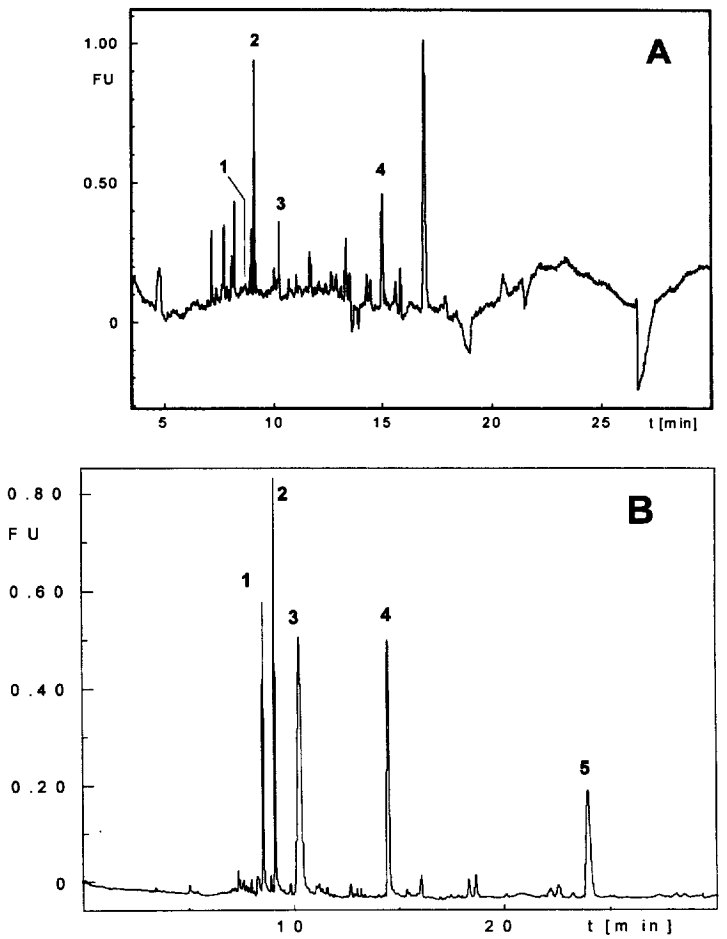

Fig. 5. Influence of excitation and emission wavelengths on electropherogramms of electrophoretically separated ink 2 (see Table 1) diluted 100-fold in 5 m*M* borate buffer, pH 8.25. (A) LIF Ar $\lambda_{ex/em}$ 488/520 nm, (B) LIF He/Cd $\lambda_{ex/em}$ 320/436 nm; 25 kV; 22°C; 15 s pressure injection.

lored substrate material, more attention has to be paid to the color of the paper sample, which has to be cut out for comparison with the extraction solution, containing dried ink and paper. Paper 5 (see Table 2) has also been investigated: the two main components observed possess nearly the same migration times to those in paper 1–3, but the intensity ratio of both peaks is different. Nevertheless, these results clearly indicate the necessity of comparing the electropherogram of the ink extract with a blank from the paper substrate material. Comparable with the results obtained for diluted original inks, no interfering signals could be observed using argon ion laser for detection. This agrees with the chemical character of the whiteners added to the paper material.

In Fig. 7, extracts from dried inks on paper 2 (see Table 2) and diluted original inks are compared. Both examples are representative of inks which are difficult to identify. Fig. 7A,B show a blue ink, which gave only few signals when measured at a 1:100-fold dilution. Most of the peaks in the extract (Fig. 7A) belong to the dyes in the paper material. From the three main compounds in the original ink (Fig. 7B), only peaks 2 and 3 could be assigned with peaks in the electropherogram of the extract using normalized migration times. A reliable identification is impossible under these conditions. Fig. 7C,D show

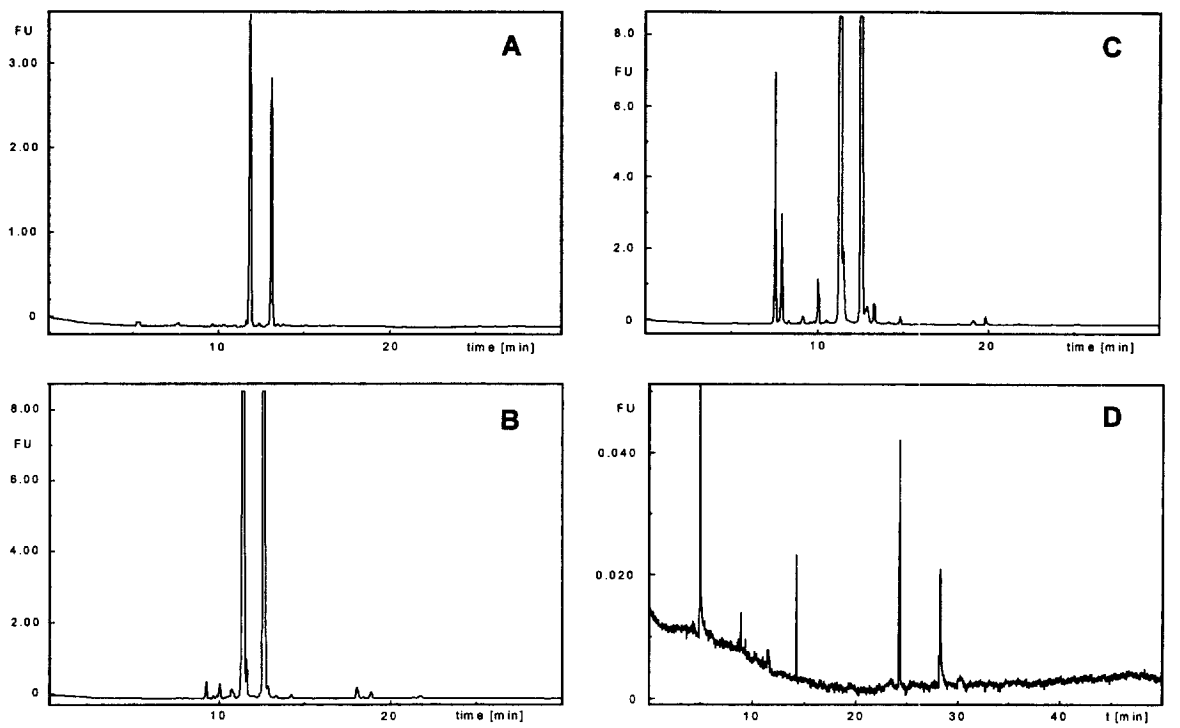

Fig. 6. Comparison of different substrate materials (see Table 2) extracted under the same conditions as dried inks (exception D). (A) Paper 1, (B) paper 2, (C) paper 3, (D) paper 4, white part (only one plug was cut out); 15 s pressure injection; 25 kV; 22°C; LIF detection with He/Cd laser, $\lambda_{ex/em}$ 320/436 nm.

a black ink. Many more compounds are detected in the diluted original sample (Fig. 7D), and the fluorescence plot of the extract is more complex than for the blue ink. Therefore, more compounds could be assigned in both electropherograms. In Fig. 7C, peak 1 represents the solvent methanol, used to perform the extraction. In Fig. 7D, under this peak, neutral compounds migrate with the velocity of the electroosmotic flow. By analogy with UV–Vis detection, only in the first part of the electropherograms was assignment (peaks 1–5) successful. At higher migration times, shifts in the migration times (due to adsorption effects) and much less effective extraction for the highly charged sample components, leads to difficulties in identification. Therefore, peaks 6–12 in the original sample (Fig. 7D) could not be reliably assigned with those in the extract

More investigation have to be done in the near future to explain or compensate the shift of peaks at longer migration times, in order to make the identification of single sample components easier and to subtract the signals of paper dyes from the electropherogram of the ink extract.

3.5. Comparison of UV–Vis and fluorescence detection

For the majority of the samples, broader peaks and worse resolution were observed for UV–Vis detection compared to fluorescence detection. This effect was caused by the wider aperture of 800 μm used for UV–Vis detection. For smaller apertures, significantly better peak resolution, but a deterioration of the detection limit, is expected. To achieve UV–Vis scans, sufficiently high signals from the sample components have to be produced. Using the wider aperture more samples are suitable for scanning UV–Vis detection, while with the smaller aperture the electropherograms, although less sensitive, are more comparable with those obtained with fluorescence detection.

The absence of interfering peaks from dye com-

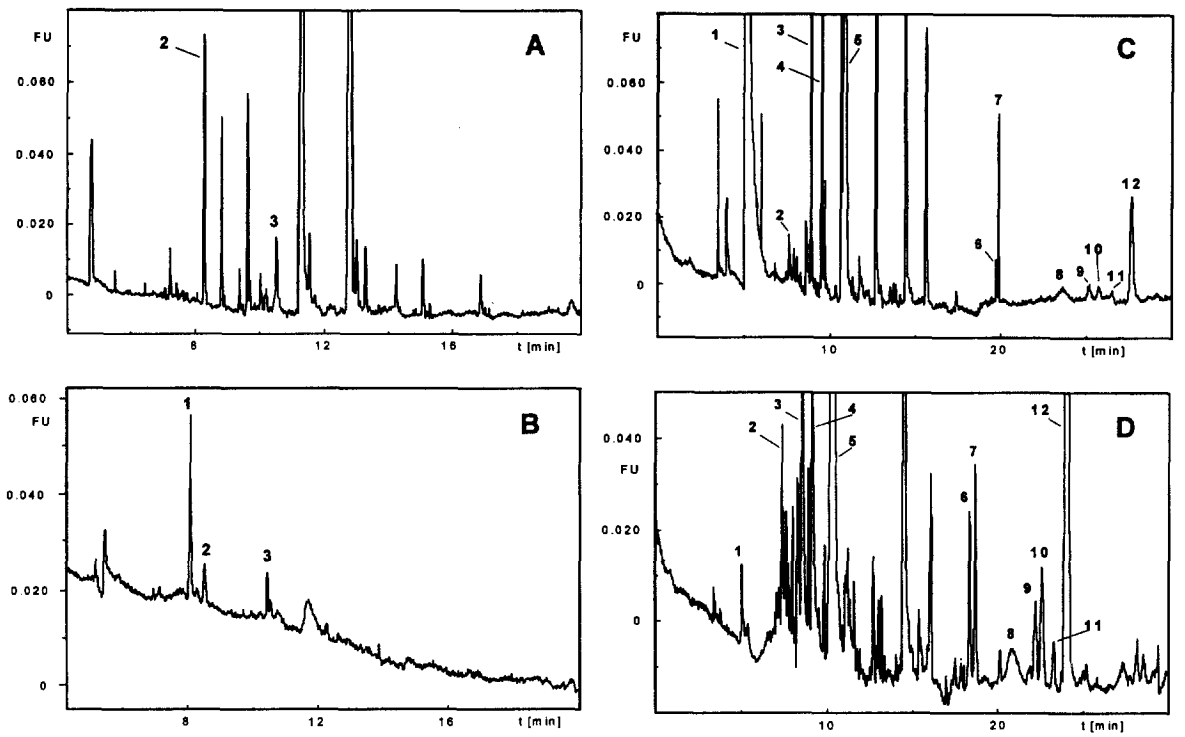

Fig. 7. Comparison of extracts from dried blue and black inks (see Table 1) with original inks diluted 100-fold in 5 m*M* borate buffer, pH 8.25; 10 s pressure injection each sample; 25 kV, 22°C; LIF detection at $\lambda_{ex/em}$ 320/436 nm. (A) Extract ink 1, (B) original ink 1 (1:100), (C) extract ink 2, (D) original ink 2 (1:100).

ponents of the paper substrate material is the main advantage of UV–Vis detection. If the concentration of dye compounds of extracted inks allows detection, the UV–Vis plots have a higher discrimination power for comparison with those of diluted original inks.

The main advantage of LIF detection is sensitivity. The highly fluorescent components of dried inks could be detected in much higher dilution compared to UV–Vis detection. Therefore, in some cases only one plug (1.5 mm diameter) from a writing line is enough to detect the main components of the ink, but more investigation is necessary to reduce the interfering influence of the dye components from paper substrate material.

3.6. PIXE measurements

PIXE measurements could provide additional information about the elemental components of the organic molecules (sulfur, halogenides) or colored complexes formed with heavy metals (Cu, Fe and others). The basics of the PIXE measurements are explained in Fig. 8 [16]. All elements with atomic

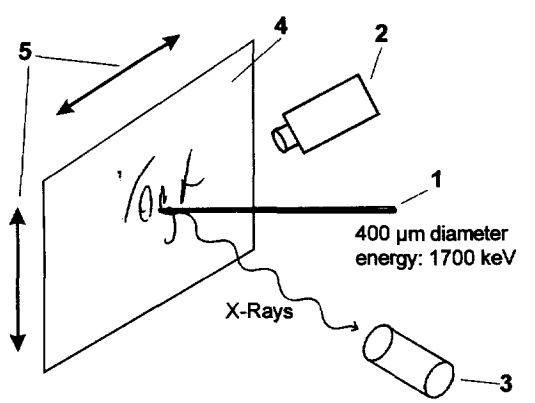

Fig. 8. Principle of PIXE measurements. (1) Collimated proton beam; (2) CCD-camera for precise definition of the beam position; (3) Si(Li)-detector with 135 eV energy resolution; (4) substrate material (e.g. paper); (5) movable sample holder.

numbers above 13 are detected simultaneously according to the energy of emitted X rays.

The writing line for each ink, as well as the spectrum of the substrate material adjacent to the writing, was measured three times. The average spectrum of the substrate material was then subtracted from the average spectrum of ink plus paper. In two cases, dried inks chosen by random selection,

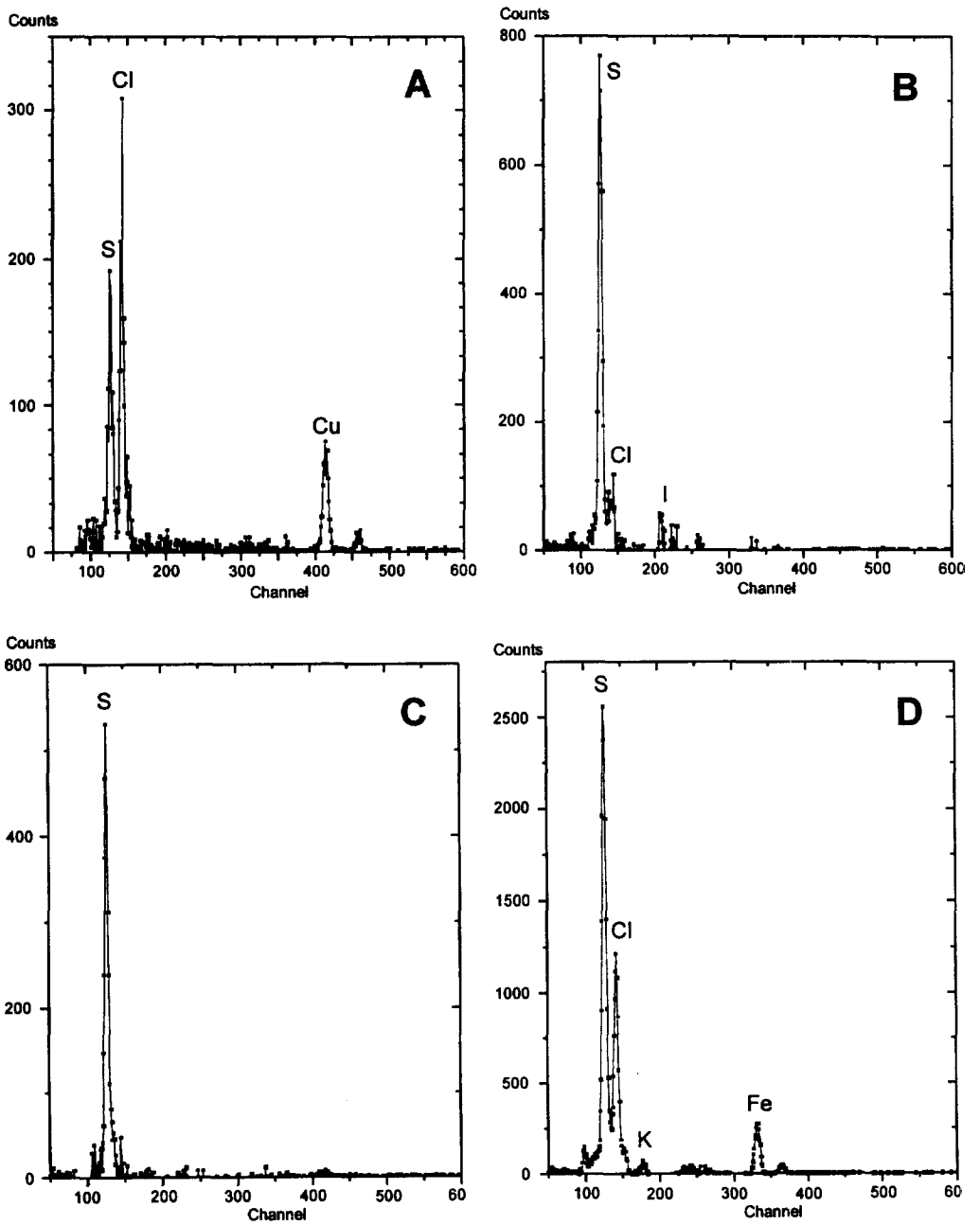

Fig. 9. Comparison of X-ray spectra of dried black and blue inks on paper 4 (check). (A) Ink 1, (B) ink 5, (C) ink 2 and (D) ink 11.

were examined three or five times by the described procedure, to control the influences of ink homogeneity, width of the writing line and the homogeneity of the substrate material. No difference in the resulting ink spectra could be observed.

Besides sulfur, bromine, chlorine and iodine, K, Ca, Cu, Fe, Cr and Zn have also been detected in the inks investigated. In Fig. 9 the spectra of two blue and two black inks are compared. A copper complex is probably used as the dye component of ink 1 (Fig. 9A). The high copper signal characterizes this element as a main component of the ink mixture. The high chlorine content is caused either by chlorinated dye substances or a high chloride content, if chloride is used as a counter ion for positively charged substances. Nearly all ink samples are characterized by a high sulfur content, which comes from sulfate or sulfonate groups in dyes or other compounds, e.g. surfactants, of the ink mixture. Ink 5 (Fig. 9B), like ink 1, also contains chlorine and sulfur. In addition, this sample contains a remarkably high iodine content, indicating that either iodinated organic molecules or an iodine complex is responsible for the intensive blue color. In Fig. 9C, the PIXE spectrum of ink 2 shows only a high sulfur signal. Furthermore, this ink is characterized by only few signals in the UV–Vis plot (with maximum absorbance of the main dye component above 600 nm) and nearly empty electropherograms using fluorescence detection. Probably the color relies on a blue organic substance containing sulfur and nitrogen in functional groups and/or aromatic ring systems, which show no fluorescence in the blue and green region. Fig. 9D shows the spectrum of ink 11. The high iron content indicates the existence of an iron complex, and the dye could be based on complexes comparable with those in iron gallate inks. Ink 3 (not shown, Table 1) contains smaller amounts of Ca, K and Fe; ink 8 is characterized by a high Ca and ink 12 by a high Ca and a low Cl content. Similar to the spectrum of ink 2, those of inks 4, 7, 9 and 10 show only sulfur signals as main components. But ink 9 also contains low amounts of Fe, and ink 7 low amounts of Br. Therefore only inks 2, 4 and 10 could not be distinguished by this method. Due to the experimental conditions, a direct comparison of these inks by a quantification of the sulfur content is impossible. For each ink, a different depth profile of ink concentration is created during the writing procedure. Since the PIXE method is a surface-sensitive method, only the ink in the first few micrometers of the paper material is used to generate the PIXE spectra. Different depth profiles will lead to different quantities of the elements recorded. Nevertheless, the method could provide useful information about the structure of the main ink components in addition to those obtained by UV–Vis and fluorescence detection after electrophoretic separation. The nondestructive character of the method, and also the ability to characterize paper substrate material, are advantages which make the application of this method to ink analysis very attractive.

4. Conclusions

Capillary electrophoresis is well suited to separate the components of fountain pen inks. UV–Vis and fluorescence detection of electrophoretically separated diluted original inks and ink extracts from substrate material provide sufficient information for comparison of different inks. For the identification of certain inks, PIXE could provide useful additional information.

Despite the demonstrated successful application of the CE technique to ink analysis, further efforts have to be directed towards stacking for the preconcentration of ink extracts and the elimination of dye signals from substrate materials.

References

[1] R.L. Brunelle, M.J. Pro, J. Assoc. Off. Anal. Chem. 55 (1972) 823.
[2] R.L. Brunelle, R.W. Reed, Forensic Examination of Ink and Paper, Charles C. Thomas, Springfield, IL, 1984.
[3] A.W. Somerford, J.L. Souder, J. Crim. Law Criminol. Police Sci. 43 (1952) 124.
[4] H.W. Moon, J. Forensic Sci. 25 (1980) 146.
[5] G.R. Nakamura, S.C. Shimoda, J. Crim. Law Criminol. Police Sci. 56 (1965) 113.
[6] R.L. Brunelle, J.F. Negri, A.A. Cantu, A.H. Lyter, J. Forensic Sci. 22 (1977) 807.
[7] L.F. Colwell, B.L. Karger, J. Assoc. Off. Anal. Chem. 60 (1977) 613.
[8] R.O. Reto, J. Forensic Sci. 29 (1984) 198.
[9] A.H. Lyter, J. Forensic Sci. 27 (1982) 154.

[10] A.H. Lyter, in: I.S. Lurie, J.D. Wittwer (Eds.), High Performance Liquid Chromatography in Forensic Chemistry, Marcel Dekker, New York, 1983, p. 399
[11] R.M.E. Griffin, T.G. Kee, R. Adams, J. Chromatogr. 445 (1988) 441.
[12] C. Brown, P.L. Kirk, J. Crim. Law Criminol. Police Sci. 45 (1954) 334.
[13] S. Fanali, M. Schudel, J. Forensic Sci. 36 (1991) 1192.
[14] R. Kuhn, S. Hoffstetter-Kuhn, Capillary Electrophoresis: Principles and Practice, Springer, Berlin, 1993.
[15] P.D. Grossman, J.C. Colburn, Capillary Electrophoresis. Theory and Practice, Academic Press, San Diego, 1992.
[16] S.A.E. Johannson, J.L. Campell, PIXE – A Novel Technique for Elemental Analysis, Wiley, New York, 1988.
[17] F.L. Lucarelli, P.A. Mandò, Nucl. Instrum. Methods Phys. Res. B 109/110 (1996) 644.