Carla Vogt,[1] *Ph.D.; Andreas Becker,*[1] *B.Sc.; and Jürgen Vogt,*[2] *Ph.D.*

Investigation of Ball Point Pen Inks by Capillary Electrophoresis (CE) with UV/Vis Absorbance and Laser Induced Fluorescence Detection and Particle Induced X-Ray Emission (PIXE)

REFERENCE: Vogt C, Becker A, Vogt J. Investigation of ball point pen inks by capillary electrophoresis (CE) with UV/Vis absorbance and laser induced fluorescence detection and particle induced x-ray emission (PIXE). J Forensic Sci 1999;44(4):819–831.

ABSTRACT: In the process of examining fraudulent documents ink analysis is a small but important part of the operation of forensic laboratories. Systematic approaches to ink comparison and identification have been performed by optical methods and various chromatographic techniques.

Capillary electrophoresis (CE), a relatively new separation technique with very high resolution power, and Particle Induced X-Ray Emission (PIXE) were used for the analysis of ball point pen inks. In comparison to water soluble fountain pen inks, ball point inks are less soluble or insoluble in water and these inks contain only few components. The study focused on the optimization of the separation of ink extracts from paper material of commercially available inks with respect to resolution and analysis time. During the method development process different buffers, organic modifiers, and surfactants were tested. Good results were obtained with a 50 mM borate buffer pH 9.0 containing 50% acetonitrile. Reproducible extraction procedures as well as separations enables one to perform the quantification of the ink peaks within 1–8% standard deviation for parallel extractions of the same ink. Electropherograms of 20 inks of various origin showed patterns which were in most cases distinctly different from each other.

PIXE measurements with an external proton beam were used to determine the metal composition. The ratio of the peak areas for copper and zinc as well as differences in the elemental composition could be used to distinguished between the samples.

No coincidence was observed between samples hardly distinguishable by electrophoretic separations and by PIXE-measurements. Samples with nearly identical metal composition showed different peak pattern in the electropherograms, and nearly identical electrophoretic behavior of two or more samples was accompanied by quite different copper/zinc-ratios or supplementary metals identified by PIXE.

KEYWORDS: forensic science, ball point pen inks, capillary electrophoresis, separation, particle induced X-ray emission, UV/VIS-absorbance, fluorescence detection

In the process of examining fraudulent documents, forensic scientists are confronted with the task of scientifically examining components of the piece of evidence including the carrier material paper, any writing or marks made with materials as diverse as pencil traces, typewriter ribbon inks, photocopy toner, ball point pen ink or fountain pen ink as well as correcting materials, eraser residues or adhesives. Investigations or forgery cases usually focus on additions or alterations to documents that frequently have profound financial implications such as insurance claims, tax returns, checks, contracts, and wills.

The composition of the ink formulation can be quite complicated in order to meet various usage requirements. Among the dyes used as coloring material are azoic compounds, acid and basic dyes and organic or inorganic color pigments. In addition, surfactants, antioxidants, viscosity adjusters, resins, glycol and glycerol could be part of the mixture, all of them in varying amounts.

Systematic approaches to ink comparison and identification have been described by Brunelle, Pro, Reed, and Cantu (1–4). In particular, chromatographic methods, including paper chromatography (5), thin layer chromatography (6,7) and high performance liquid chromatography (8–10), have been extensively applied to the analysis of inks during the last decades. The application of capillary electrophoretic (CE) techniques has also been investigated during the last decade (11–15). These 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.

Recently we reported on the CE separation of fountain pen inks (13–15). These inks are highly soluble in water or buffer mixtures and electropherograms contain many colored and non-colored components, so that these inks could be distinguished easily. In comparison, ball point inks are less soluble or insoluble in water and these inks contain only few components. Therefore, differentiation or identification is much more difficult. Ball point inks consist of mostly hydrophobic dyes, which are often composed of substances (e.g., isomers) or derivatives with similar structure and nearly identical chemical behavior. Besides neutral and charged or chargeable dyes, metal containing dyes are also applied (Fig. 1) (16,17). The composition of ball point inks is completed by polymers and resins (16,18) and about 50% solvents (Table 1). The character of the writing line on the paper material is also influenced by the chemical composition of the paper itself as well as of the ball point pen tip, which could add further traces of metals to the sample.

On this basis, the present work was aimed to test the applicability of capillary electrophoresis (19,20) to the separation of blue and black ball point pen inks from writing lines on paper materials. After the optimization of the separation conditions, diode array

[1] University of Leipzig, Institute of Analytical Chemistry, Linnéstr. 3.
[2] University of Leipzig, Institute for Experimental Physics II, Linnéstr. 5, D-04103 Leipzig, Germany.

Received 7 July 1998; and in revised form 22 Sept. 1998; accepted 7 Oct. 1998.

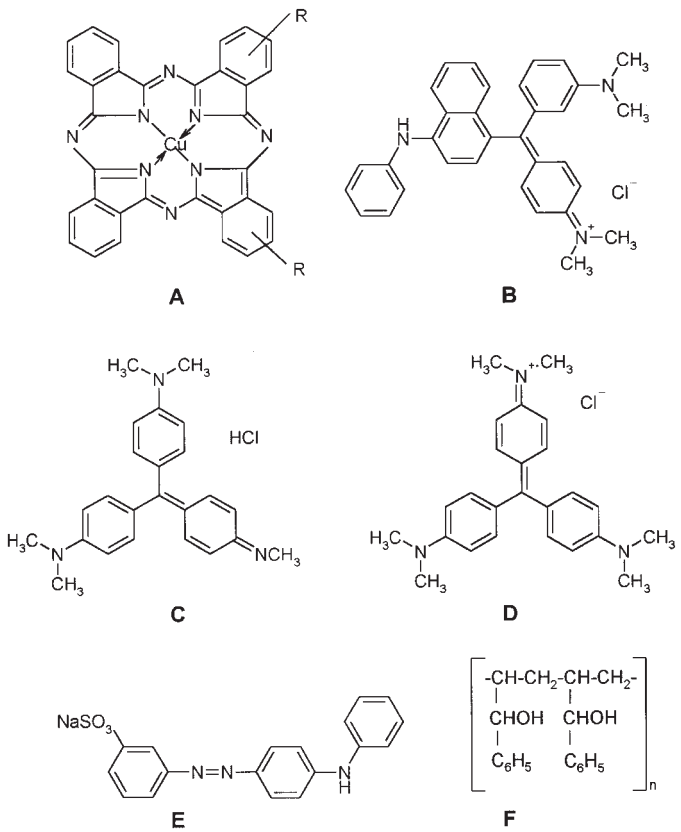

FIG. 1—*Components of ball point pen inks.* (A) *basic structure of Pigment Blue 15 (Astra Blue 6GLL),* (B) *Basic Blue 26,* (C) *Methyl Violet,* (D) *Crystal Violet,* (E) *Acid Yellow 36,* (F) *Synthetic resin SK. Compound C represents a mixture of tetra-, penta- und hexamethylated isomers; compound D contains only the hexamethylated form.*

TABLE 1—*Composition of ball point pen inks.*

Dyes and pigments	~ 25%
Resins and polymers	~ 25%
Solvents	~ 50%
Further additives	≤ 2%

UV/VIS detection and laser induced fluorescence (LIF) detection with an excitation wavelength at 320 nm have been applied to collect as much information as possible from the electropherograms. In addition, nondestructive proton-induced X-ray emission (PIXE) (14,21–23) spectrometry was used to characterize the elemental composition of dried inks on substrate materials. Finally, all the information from CE and PIXE analyses of the inks has been integrated and used for a reliable comparison of the ball point pen inks investigated.

Experimental

Instrumentation

Electrophoresis—For the separation, capillary electrophoresis instruments by Beckman (Palo Alto, CA), models P/ACE 2100 and 5510 and Perkin Elmer-Applied Biosystems (Weiterstadt, Germany), model 270A-HAT, were available. All instruments provide voltages of up to 30 kV. The Beckman instruments are equipped with a diode array capable of scanning between 190 and 600 nm and a laser induced fluorescence (LIF) detector. For the excitation of the fluorescence a He-Cd Laser Omnichrom Series 74 from Omnichrom (Chino, USA) with an excitation wavelength of 320 nm and 27 mW laser power was used. Detection of the UV/Vis absorbance was executed at 205 nm, the spectra were scanned from 200 to 600 nm. Fluorescence was detected at 405 nm. The apertures of the cartridges for LIF and UV/Vis experiments were 200 μm and 800 μm, respectively.

Fused silica capillaries with an inner diameter of 50 μm were purchased from Chromatographie Service (Langerwehe, Germany). Capillaries with a total length of 77 cm (70 cm to the detector window) were cut and fitted into the capillary cartridges of the Beckman apparatus; for the PE-apparatus fused silica capillaries with an inner diameter of 50 μm and a total length of 70 cm (50 cm to the detector window) were used.

The operation voltage was 15 or 25 kV and hydrodynamic injections were performed for 5, 8 or 10 s. The instrument specific softwares P/ACE 3.0, System Gold version 7.1 and Turbochrom 4 were used for data acquisition and management.

Particle Induced X-Ray Emission—The PIXE measurements were performed on the 2-MeV van de Graaf accelerator at the University of Leipzig. The proton beam with an energy of 1700 keV was collimated to a diameter of 200 μm using a set of 3 diaphragms. After directing the ion beam onto the writing on the substrate material the proton induced X-rays were detected by a HP-Ge-detector with 175 eV energy resolution. This high efficient detector provides measurements with good statistics and low detection limits. The beam was extracted from the beam line of the vacuum system into the air by a small slit of 150 μm diameter and differential pumping. The precise beam position was controlled by a CCD-camera. A movable sample holder was used to correct the position for the measurements. For the measurements under normal pressure sample holder and sample both were fixed in a distance of 6 mm to the slit.

For each measurement, the X-ray signal was recorded until a charge of 10 μC was accumulated. 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 allows to compare different spectra. During the measuring process, the proton current of 10 nA was kept as constant as possible.

The written line of each ink was measured three times as well as the spectrum of the substrate material right next to the writing. The average spectrum of the substrate material 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.

Chemicals

The chemicals used for the buffer solutions were obtained from Merck (Darmstadt, Germany) and Fluka (Buchs, Switzerland). H_3BO_3 was used to prepare borate buffers between pH 8.0 and 10.0, H_3PO_4 and $NaH_2PO_4 \cdot 2H_2O$ for phosphate buffers with pH between 2.8 and 10.4. To adjust the pH of the buffers, concentrated solutions of NaOH or HCl were utilized.

Organic solvents were used as buffer additives and for the extraction of inks from writing traces. Acetonitrile, acetone and methanol, all of HPLC grade, were from Merck (Darmstadt, Germany). 2-Propanol was from Sigma-Aldrich (Daisenhofen, Ger-

many), glacial acetic acid, ethylene glycol, toluene, pyridine, 1-hexanol and 2-methyl-2-butanol were purchased from Fluka (Buchs, Switzerland).

The studies of the separation of ink components by micellar electrokinetic chromatography (MEKC) or solvophobic association were performed with sodium dodecyl sulfate (SDS, $C_{12}H_{25}NaO_4S$) from Riedel-de Haën (Seelze, Germany), 1-decane sulfonic acid ($C_{10}H_{21}O_3SNa$) from Fluka (Buchs, Switzerland) and 1-hexane sulfonic acid ($C_6H_{13}NaO_3S \cdot H_2O$) from Fluka. Hexadecyltrimethylammonium bromide (HTABr, $C_{19}H_{42}BrN$) and tetrabutylammonium bromide (TBABr, $C_{16}H_{36}BrN$) from Merck have been used to modify the electroosmotic flow for some of the separations. All solutions were prepared with distilled water from a Christ ModuPure Plus system.

The ball point pens investigated are numbered and listed in Table 2. They were supplied by the Bundeskriminalamt (Wiesbaden, Germany) (ink 1–11) or purchased in office supply stores (number 12–20).

Pigment Blue 15 (Astra Blue 6GLL), Basic Blue 26, Methyl Violet, Crystal Violet, Acid Yellow 36, synthetic resin SK and Phthelopal LR 8525, all components of ball point pen inks 1 or 2, were also supplied by the Bundeskriminalant (Wiesbaden, Germany).

Results and Discussion

Methods Development for the Separation of the Dyes

For the method development pure dyes have been mixed to simulate mixtures representative for ball point pen inks. Therefore, Pigment Blue 15 (Astra Blue 6GLL), Basic Blue 26, Methyl Violet, Crystal Violet and Acid Yellow 36 have been dissolved in methanol:water 50:50 to blue and black solutions and the saturated supernatants were used for further investigations. Ball point pen inks are only little soluble in aqueous solution. To provide a high extraction quantity for inks from paper material and to avoid precipitation and/or adsorption of the ink components inside the separation unit, extraction solvents and separation buffers have to contain high amounts of organic solvents. The separation of these mixtures was optimized according to an optimum resolution of the ink components.

In a buffer study, a number of different buffers were tested for their suitability to separate these mixtures. In addition to the pH of borate buffers (pH 8.0–10.0) and phosphate buffers (pH 2.8–10.4) also type and amount of organic solvents (methanol, acetonitrile and 2-propanol) and the influence of surfactants (SDS, 1-decane sulfonic acid and 1-hexane sulfonic acid), added to the buffer, have been investigated. To allow for the assignment of the inks to the separated peaks spectra of the diode array were accumulated from 200 to 600 nm over the time of the separation.

TABLE 2—*Inks under investigation.*

Ink	Color	Supplier
1	blue	BKA*
2	black	BKA*
3	black	BKA*
4	black	BKA*
5	black	BKA*
6	black	BKA*
7	black	BKA*
8	black	BKA*
9	black	BKA*
10	black	BKA*
11	black	BKA*
12	blue	Schneider 75 M, Germany
13	blue	Senator, Groβraummine, Germany
14	blue	Hauser, Gigant 211, Germany
15	blue	Bullograph, Groβraummine, Sweden
16	black	SM 0757M
17	black	Profi-Mine, Germany
18	black	S 12
19	green	Garantie-Mine, Germany
20	red	Schneider Büro 575M, Germany

*Samples were supplied by the Bundeskriminalamt Wiesbaden, Germany. Names of the manufacturers are subject to imposed secrecy.

Buffer Concentration—Generally, at higher buffer concentrations the separation efficiency should be improved. The increased ionic strength of the buffer is responsible for reduced adsorption effects onto the capillary wall as well as a decreased electroosmotic flow. Under such conditions slight differences in the mobilities of similar analytes gain more importance because the components need a longer time to reach the detector. Best separation was obtained with 50 mM borate or phosphate buffers. At higher concentrations the ink mixture was partly precipitated which gave rise to current problems during the separation and irreproducible separation results.

pH—In a 50 mM phosphate buffer with 10% methanol and pH 2.8 only one ink signal could be detected for a black ink mixture (Crystal Violet, Methyl Violet and Acid Yellow 36) which was attributed to a violet ink (Methyl Violet and/or Crystal Violet) by the UV-VIS-scan. At this pH Acid Yellow 36 is not charged and therefore will not migrate electrophoretically at all. The electroosmotic flow (EOF) in the system is still very low, and so the yellow ink does not reach the detector. The simple buffer composition does not allow to separate both violet inks with similar structures.

At a buffer pH of 6.5, a second ink signal for the yellow component was observed. The EOF is now high enough to carry the yellow compound to the detector, although Acid Yellow 36 is negatively charged under these conditions due to increasing dissociation. With increasing pH (at/above pH 8), the dissociation of the yellow component is increased too while EOF remains almost unchanged. This leads to longer migration times due to a higher electrophoretic mobility and a reduced net mobility towards the detector.

Generally, for all ink mixtures optimum separation according to the number of components separated, the separation time and the peak shape was obtained at pH of 8–9.

Organic Solvents—To 50 mM phosphate buffers pH 3.0 50% of the organic solvents methanol, acetonitrile and 2-propanole were added. With methanol and acetonitrile as buffer additives a higher number of separated sample components could be detected in comparison to buffers with 2-propanol. In addition, buffers with acetonitrile provided much faster separation. High amounts of organic solvents in aqueous buffers have great influence on the dielectricity constant and the viscosity of the buffer and could change the solubility of the analytes as well as the interaction of the solutes with buffer components. The ratio of the dielectricity constant and the viscosity of an organic solvent will provide first information about the expected separation (Table 3). A ratio close to that for water will provide fast separation under conditions comparable with the pure aqueous medium but better solubility for analytes

which are not completely soluble in aqueous medium (e.g., acetonitrile). Lower ratios indicate a drastic reduction of the current transport in the electrophoretic separation system which leads to very long migration times (e.g., 2-propanol). For our purpose acetonitrile provides the best conditions for the separation of the ink components.

Surfactant Additives to the Buffer—In micellar electrokinetic chromatography (MEKC) micelle-forming agents, usually sodium dodecyl sulfate, offer the possibility for additional interactions of poorly resolved analytes with these additives in aqueous buffers. Nevertheless, hydrophobic substances with similar structures are difficult to resolve in MEKC due to their low solubility in water and high partition coefficients into the micellar phase. With the addition of organic modifiers in concentrations higher than 20% to electrophoretic buffers containing charged micelles, the micelle formation will be inhibited, but the surfactants and the analytes can still interact. The interaction between the nonionic parts of the analytes and the surfactants is called solvophobic association. In addition to the influence of the electrophoretic migration of the analytes, separation is now based on differences in the strength of analyte-surfactant association complexes, which results in differences in effective electrophoretic mobilities.

To study the influence of the surfactant structure in 50 mM borate buffers pH 9.0 with 50% acetonitrile, a 30 mM concentration of different surfactants (1-hexane sulfonic acid, 1-decane sulfonic acid and sodium dodecyl sulfonate, SDS) was adjusted. The surfactant with the longest alkyl chain (SDS) provided the best resolution of the analytes. A longer alkyl chain of the surfactant leads to a more intensive interaction with the hydrophobic parts of the analytes and a stronger retardation of these components due to the migration of the formed negatively charged association complex towards the anode of the separation system (detection at the cathodic end). At the end, the concentration of SDS was varied between 0 and 60 mM (Fig. 2). The mixture of blue and vi-

TABLE 3—*Properties of organic solvents and water.*

Solvent	Viscosity η (mPa·s) at 20°C	Dielectricity Constant ε	ε/η
Water	1.00	80.4	80.4
Acetonitrile	0.37	37.5	101.4
Methanol	0.60	33.6	56.0
2-Propanol	2.39	18.3	7.7

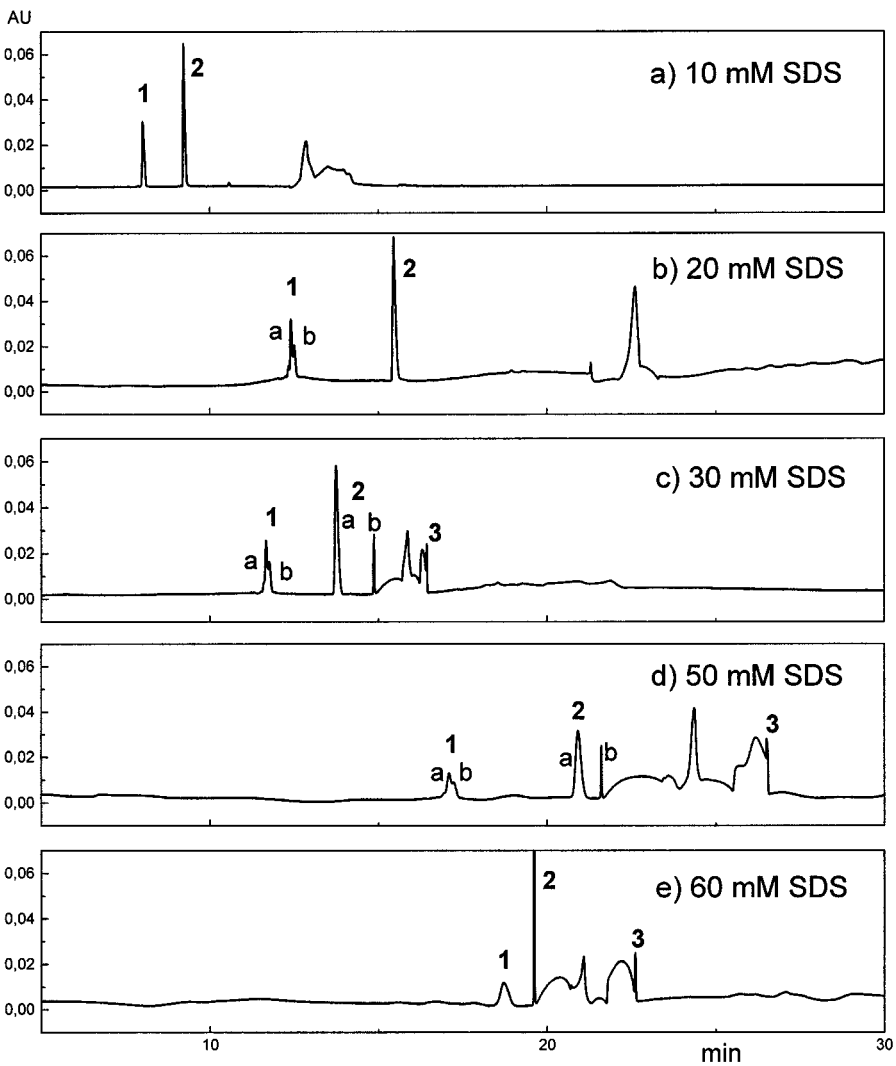

FIG. 2—*Separation of a dye mixture (Basic Blue 26 and Basic Violet 1, the last is a mixture of Methyl Violet and Crystal Violet) in 50 mM borate buffer pH 9.0, 50% acetonitrile and varying amounts of SDS. Only dye components are numbered; (1) Basic Violet 1, (2) Basic Blue 26, (3) unknown.*

olet dyes was separated into two components at 0 and 10 mM SDS concentrations in the buffer. From 20 mM SDS concentration onwards better resolution was observed, which was best at a 30 mM concentration. Here, a separation of both, the violet and the blue dye, into two components (isomers) was obtained. A higher surfactant concentration of 50 and 60 mM leads to a strong interaction of all analytes with the surfactant monomers which blurs the differences between the dye components and aggravates the separation efficiency.

From the optimization of all buffer parameters, a 50 mM borate buffer pH 9.0 with 50% acetonitrile and 30 mM SDS was proved to allow the best separation of the dye components. A very fast analysis is possible without the addition of SDS to this buffer. Therefore, all further analyses of ink extracts from paper material have been performed with these two buffer systems (buffer 1: 50 mM borate pH 9, 50% acetonitrile; buffer 2: buffer 1 with 30 mM SDS).

Methods Development for the Separation of the Resins

The separation of the resins in the ball point pen ink mixture will provide additional information for the comparison and identification of the ink. Unfortunately, most of the methods applied for ink analysis, are unable to separate the resin components. In comparison, capillary electrophoresis is able to resolve mixtures of molecules with high molecular weights in fused silica capillaries when using sieving gel medium. In buffers without a sieving medium resolution for analytes with molecular weights above 1000 gets worse. Nevertheless, a complete or baseline resolution of the polymers is not necessary when characteristic peak pattern enables to compare different polymer or resin types.

Therefore, the buffer previously optimized for the dye separation was modified according to the requirements of the resin separation. For the separation, a 50 mM borate buffer pH 9.0 with 50% acetonitrile was modified with 1 mM HTABr. The resins phthalopal and SK were dissolved in pure acetonitrile (about 10 mg/mL) and injected. Figure 3 shows the separation of both resins in two different electrophoretic buffers (with and without HTABr). Phthalopal, an anionic resin with free carboxylic groups, migrates electrophoretically towards the anode while detection is performed at the cathode. The multiple charged components possess a high electrophoretic mobility. Due to the electroosmotic flow, which is high enough to overcompensate the electrophoretic migration, the analytes are transported to the detection end. Because the differences in electrophoretic and electroosmotic velocity are low under these conditions, the migration times for most of the analytes are very high due to a very low resulting migration towards the detector (Fig. 3A). The addition of the cationic surfactant HTABr provides

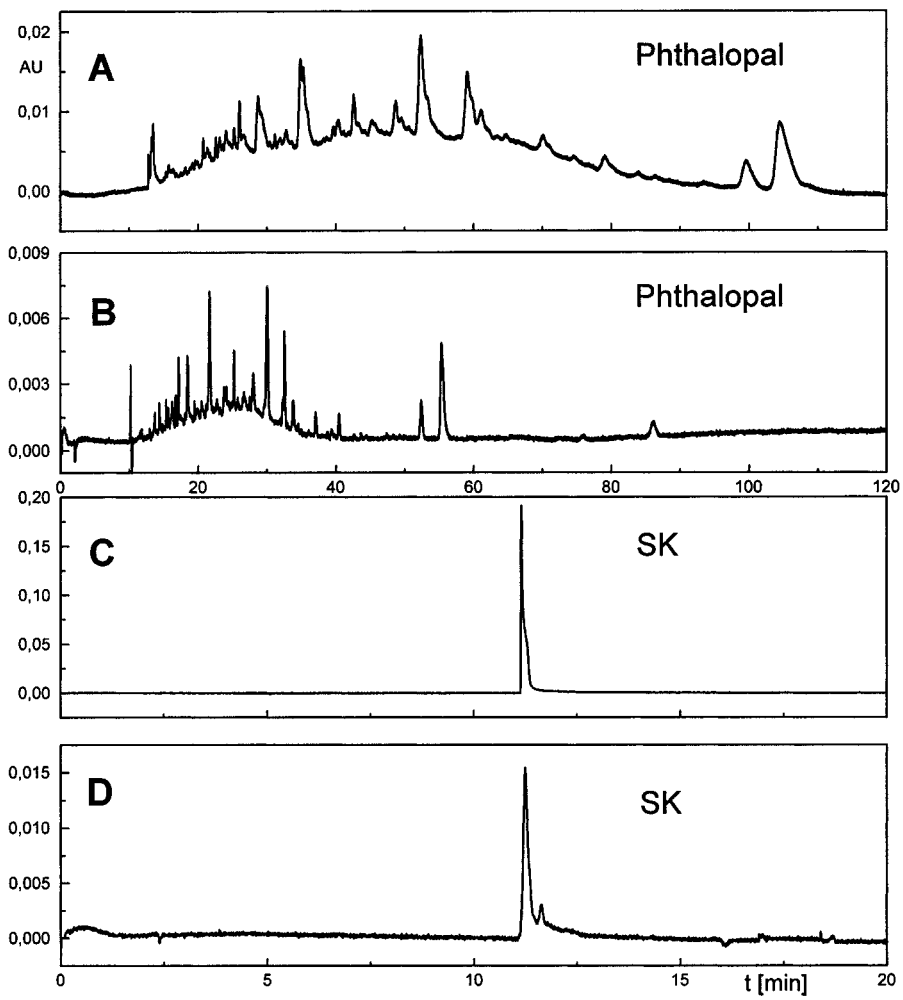

FIG. 3—*Separation of synthetic resins commonly used as ball point ink components. Separation in 50 mM borate buffer pH 9.0, 50% acetonitrile and no HTABr (*A, C*) or 1 mM HTABr (*B, D*).*

best conditions for ion pairing or the formation of association complexes in the buffer which leads to reduced negative charges of the resin components. The resulting velocity (the difference of electroosmotic and electrophoretic migrations) is increased and the migration times of all components are drastically reduced (Fig. 3B) while resolution is kept constant.

In contrast, the neutral synthetic resin SK shows only one (Fig. 3C) or two signals (Fig. 3D) when separated under the same conditions. The uncharged resin components coelute all under the same peak (Fig. 3C), because the buffer composition used did not allow the separation of uncharged analytes. The addition of HTABr to the buffer slightly improves the separation (Fig. 3D), but interpretation of the electropherogram still remains uncertain. The main peak could represent a vast number of analytes with different molecular weights but the same electrophoretic mobility or only one or few representatives with similar electrophoretic mobilities. Unfortunately, higher concentrations of the cationic surfactant in the buffer as well as the addition of the anionic surfactant SDS in varying amounts did not provide an improvement of the separation efficiency. Therefore, for the analysis of resins in ink extracts from paper material a 50 mM borate buffer pH 9.0 with 50% acetonitrile and 1 mM HTAB was used.

Separation of Ink Extracts by Capillary Electrophoresis

Optimization of the Extraction Procedure—Previous studies about the extraction of fountain pen inks from paper (13,14) showed that 10–20 min extraction time under ultrasonic conditions will suffice for an almost complete dissolution of the ink components. Although the dye components from ball point pen inks are bound more tightly to the paper, an extraction time of 10 min was used for all experiments. The conditions of the extraction procedure have been optimized with regard to the number of plugs cut out from the writing line for each analysis and to the extraction solvent.

1 to 20 plugs with a diameter of 1.5 mm have been cut out from a writing line of ink 6 (see Table 2). The plugs were transferred to a microvial and 25 μL buffer (50 mM borate pH 9.0, 50% acetonitrile, 30 mM SDS) as well as 25 μL acetonitrile were added. After 10 min ultrasonication the supernatant of the extract (usually 30–40 μL) was pipetted to a fresh microvial and injected directly into the separation unit. Extracts obtained from 20 plugs were scanned for the three dye peaks with the highest peak area. These peaks were used as markers for the assessment of the extracts obtained from less than 20 plugs. Even one plug of the writing line was sufficient to generate all three dye peaks with areas high above the detection limit, so that quantification was still possible. Nevertheless, only with 10 and more extracted plugs (1.5 to 2.0 cm writing line) electropherograms have been obtained which showed other components in addition to the dyes.

Prior to an extraction of the plugs, cut out from the paper, systematic experiments have been performed to investigate the elution strength of different solvents (methanol, ethanol, 2-propanol, 1-hexanol, glacial acetic acid, ethylene glycol, toluene, pyridine, and 2-methyl-2-butanol) for the inks 1, 2, 17, 19, and 20 on paper ma-

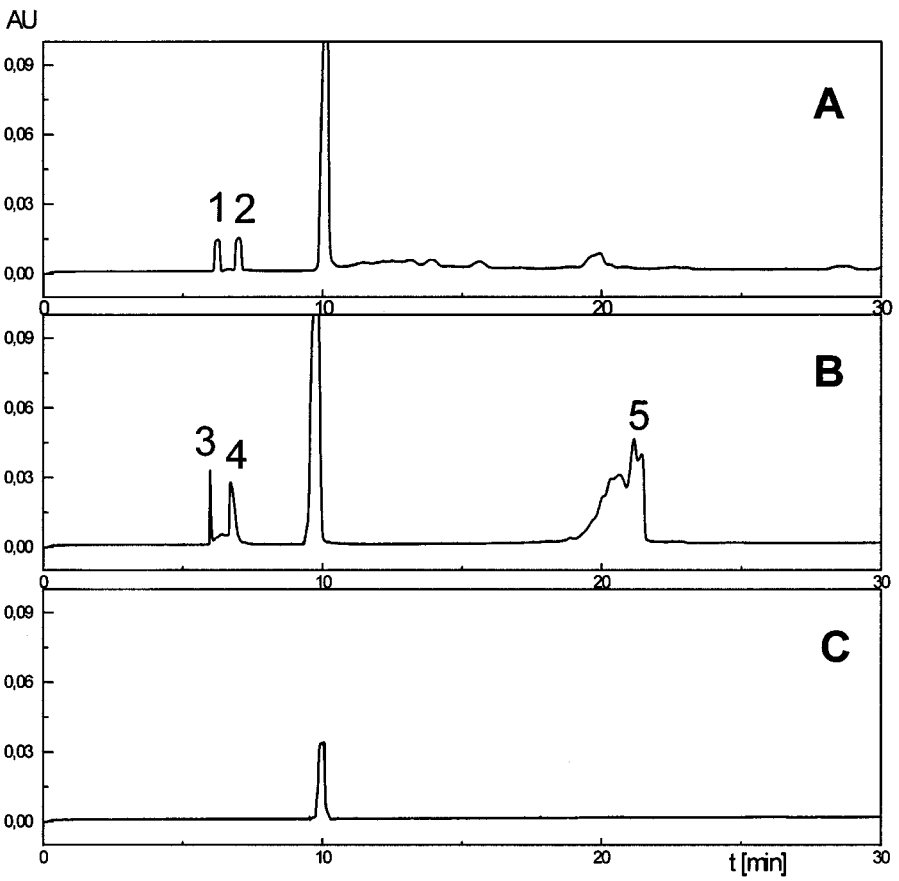

FIG. 4—*Separation of ball point pen inks extracted from paper material. Separation buffer: 50 mM borate pH 9.0, 50% acetonitrile; Detection at 205 nm. (A) ink 1 (blue), (B) ink 2 (black) (see Table 2), (C) extracted paper. For extraction conditions see text.*

terial. For this purpose one drop of the solvent was allowed to interact with the writing line of the corresponding ink and the efficiency of dye extraction was observed visually. In addition, plugs were cut out from one line, each of the plugs was extracted with one of the solvents and the intensities of the dye remained on the paper after ultrasonication were compared. With acetonitrile and the alcohols an efficient extraction was achieved for the inks 1 and 2 both with high concentrations of dyes. The red ink 20 was dissolved only partly, whereas the black and green inks 17 and 19 have hardly been dissolved. With pyridine and glacial acetic acid all inks investigated could be dissolved to a greater extend. So the blue ink 2 was extracted from the paper completely with glacial acetic acid, because the Pigment Blue 15 (Astra Blue 6GLL) (see Fig. 1), responsible for the coloring effect of this ink, is highly soluble in this acid. Unfortunately, the application of these acidic sample solutions with high ionic strength and low pH in alkaline buffer medium will cause problems in capillary electrophoretic separations. Due to local depletion of the buffer capacity and thermal effects baseline stability, peak profiles and reproducibility of the measurements are aggravated. Therefore, all further experiments were performed with acetonitrile, because the amount of ink extracted from all blue and black inks was sufficiently high for electrophoretic separations and the identical solvent was used in the previously optimized separation buffers.

A mixture of 50% electrophoretic buffer (50 mM borate pH 9.0, 50% acetonitrile, 30 mM SDS) and 50% acetonitrile provided the best results for the extraction compared to buffer or acetonitrile only. This mixture with a conductivity between that of the buffer and the acetonitrile leads to zone sharpening effects during the electrophoretic separation and provides smaller peaks and better resolution.

Separation of Ink Extracts—In Fig. 4, the separation of ink 1 and 2 after extraction from paper are shown. Ten plugs of a medium writing line have been extracted with 50 μL of the optimized mixture of 50% buffer and 50% acetonitrile. For the blue ink (Fig. 4A) two dye components (1 Crystal or Methyl Violet, 2 Basic Blue 26) and for the black ink (Fig. 4B) three dyes (3 and 4 violet dyes, 5 Acid Yellow 36) could be separated and identified in a buffer to which no surfactants have been added. The blank extract of ten plugs of the paper material (Fig. 4C) shows only one main component, which does not belong to the blue, violet or yellow dyes of the inks. As expected, no difficulties have been observed for the differentiation between blue and black inks. However, a comparison within the group of blue inks and black inks, respectively, is complicated by the similarity of most of the obtained electropherograms.

Figure 5 shows the separation of 4 different blue inks in a 50 mM borate buffer pH 9.0 and 50% acetonitrile. In all 4 samples two

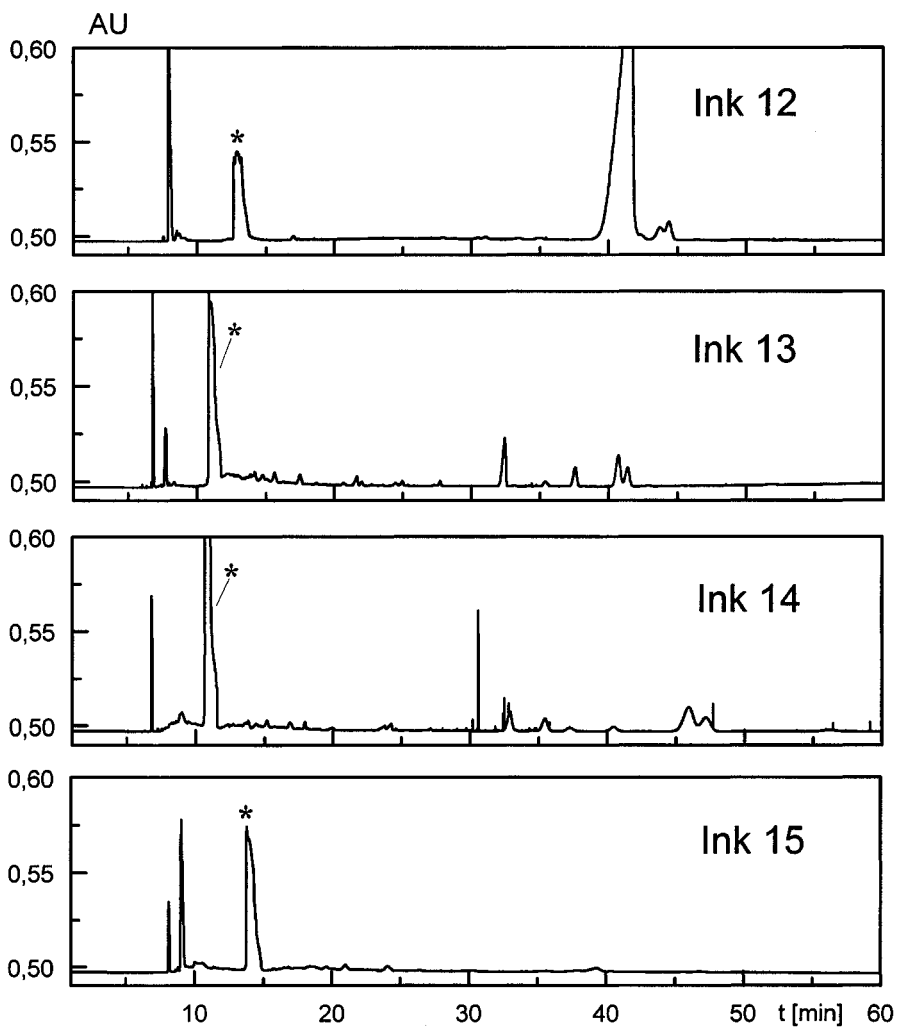

FIG. 5—*Separation of blue inks in 50 mM borate buffer pH 9.0 with 50% acetonitrile. For inks see Table 2.*

main groups of peaks were observed. To the first group of peaks in the region between 5–15 min migration time belong blue dye components (migration times below 10 min) and the compound (*) extracted from the paper material. The slight shifts in the migration time, especially observed for this component, could be caused by differences in the extract composition due to other ink components coextracted with the dyes. A second group of signals from 15–50 min migration time contains no blue or violet dyes. Because all samples have been extracted from the same sheet of paper these components represent the individual differences in the composition of the ink. Each of the five electropherograms obtained for blue ink extracts (four in Fig. 5, one in Fig. 4B) under identical conditions show a different peak pattern either in the region of the dyes (5–15 min) or in the region of ink additives (15–50 min).

A discrimination within the group of black inks is more difficult due to a much higher similarity for most of the samples. Therefore, a simple comparison of the electropherograms will not provide reliable information for the characterization of identical or different samples. In this case only quantification of the separated inks or ink isomers could help to overcome this problem.

Several ink extracts have also been investigated for the resins in the ink composition. Unfortunately, signals of resin components have not been identified unambiguously in the electropherograms obtained under the same conditions as for the separation of standards in Fig. 3. Neither longer extraction time nor higher temperatures during the extraction procedure have been useful to improve this result. Possibly the elution strength of the solvent mixture applied for the extraction procedure is insufficient to solve the resins, which could be tightly bond to the paper material. A new set of solvents have to be tested in further experiments to establish optimum extraction conditions for the resins.

Quantification of Extraction Products

Because of their stability and availability, some dyes, e.g., Methyl Violet, are becoming popular with ink manufacturers. Therefore, for many samples with similar peak pattern only quantification of the separated dye components (subpeaks for isomers) will allow to discriminate between the inks. To characterize the influence of the sample preparation and the capillary conditions on the reproducibility of the measurements 9 different samples were obtained from the same ink line of inks 7 and 11, respectively, each containing 10 microplugs. In a 50 mM borate buffer pH 9.0 with 50% acetonitrile two main dyes could be separated (peaks 1 and 2; Fig. 6A). The same

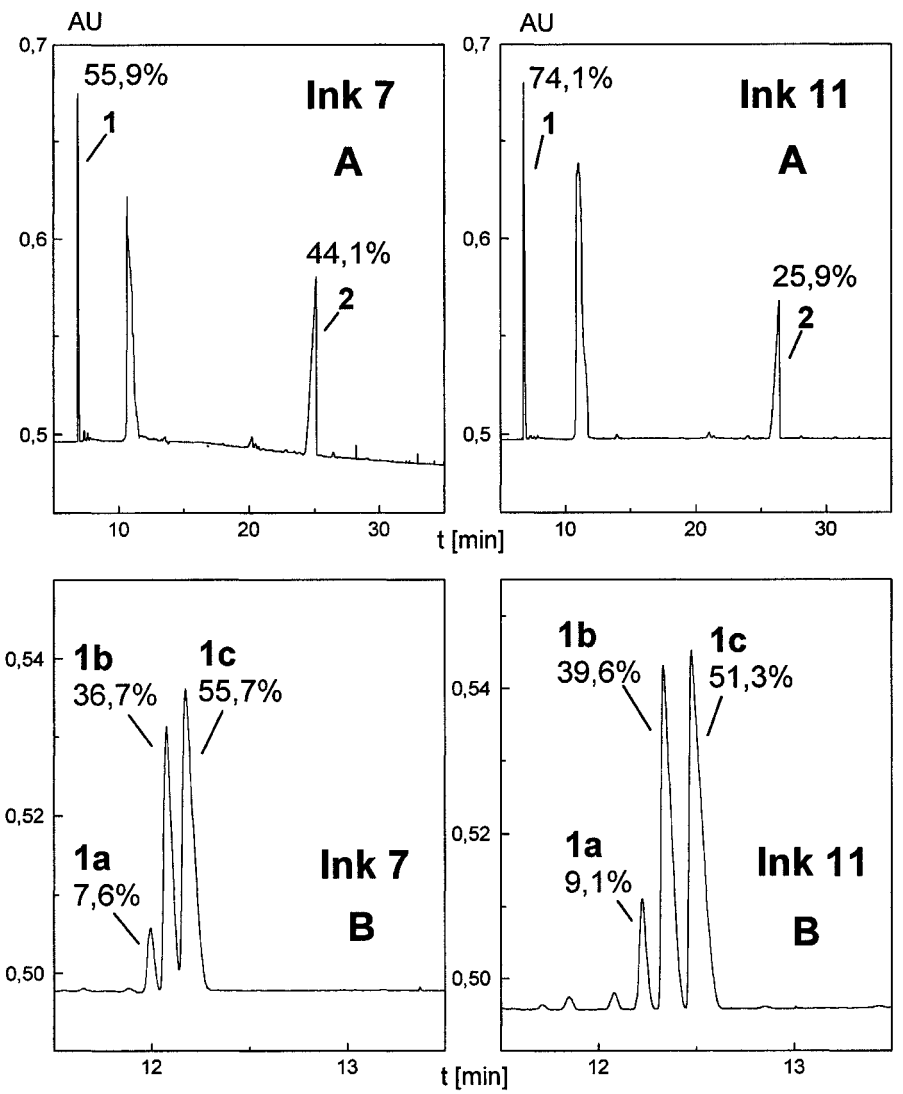

FIG. 6—*Comparison of black ball point pen inks 7 and 11. Separation in* (*A*) *50 mM borate pH 9.0 with 50% acetonitrile, and* (*B*) *50 mM borate pH 9.0 with 50% acetonitrile and 30 mM SDS.*

samples gave 3 subpeaks for dye 1 in a 50 mM borate buffer pH 9.0 with 50% acetonitrile and 30 mM SDS (peaks 1a, 1b and 1c; Fig. 6B). For the 9 different samples of inks 7 and 11, respectively, all 5 peaks have been quantified.

In Table 4 the standard deviation for the normalized peak areas are summarized for ink 11. To obtain normalized peak areas the peak area was divided by the migration time of the component. This compensates for shifts in migration times from run to run. For the smallest peak (1a) the highest standard deviation of the normalized peak area (8%) was observed. In some cases very low standard deviation (1% for peak 1) was obtained which was not expected from the inhomogeneity of the sample material.

TABLE 4—*RSD for peak areas obtained for separate extractions from ink lines of ink 11.*

Peak	Number of Separate Extractions	RSD of Peak Area (%)
1	9	1.0
2	9	4.0
1a	8	8.0
1b	8	7.0
1c	8	4.0

For a further decision on the identity of similar black ink samples we used an 8% deviation for the normalized peak area of the same ink peak in two different samples. Samples with normalized peak areas within these 8% were considered to be identical; normalized peak areas with more than 8% deviation were considered to indicate different ink compositions. Even if 4 of the 5 peaks show lower standard deviation than 8% this procedure will take into consideration that differences in the peak areas of the dyes could be caused by additional parameters, e.g., different extraction rates for a dye on different papers, aging processes and batch-to-batch differences during the manufacturing process of the inks.

In Fig. 7 normalized peak areas are compared which were obtained for the 5 peaks from the separation of 9 black inks with the bo-

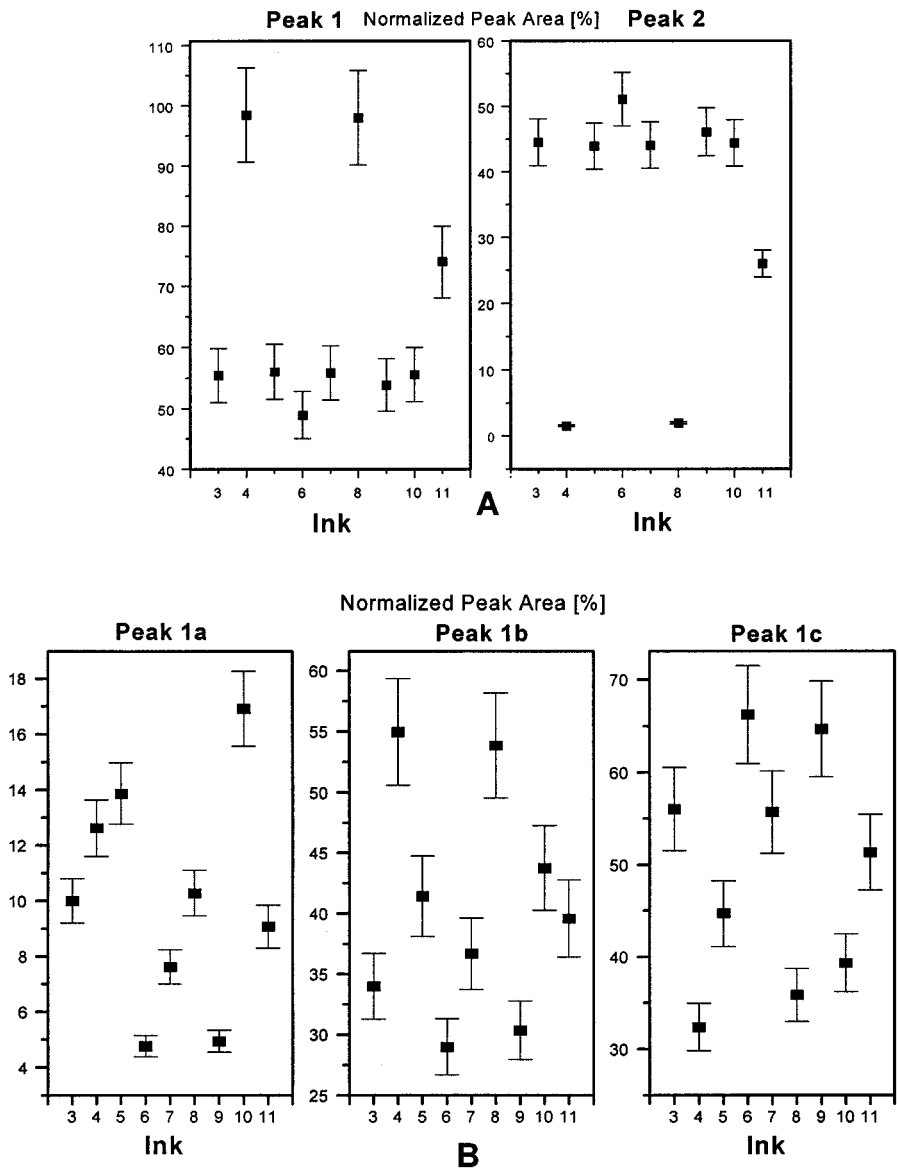

FIG. 7—*Comparison of black ball point pen inks 3–11 (see Table 2). Normalized peak areas of the inks are shown with error bars. Separation in* (A) *50 mM borate pH 9.0 with 50% acetonitrile, and* (B) *50 mM borate pH 9.0 with 50% acetonitrile and 30 mM SDS.*

rate buffers with and without SDS. Results obtained for the borate buffer with acetonitrile and no SDS were insufficient to distinguish be-tween all samples (Fig. 7A). From the normalized peak areas of dye 1 (peak 1) and dye 2 (peak 2) 3 groups of similar inks could be distinguished (group 1 - ink 4 and 8, group 2 - ink 11, group 3 - ink 3, 5, 6, 7, 9, and 10). Using the subpeaks of dye 1 (peaks 1a, 1b, and 1c) separated in the borate buffer with acetonitrile and SDS a much better differentiation is possible. Only one pair of inks (inks 4 and 8) remains which possesses similar sets of normalized peak areas in both buffer systems. For all other inks the separation in both buffers as well as a comparison of the normalized peak areas for the dye peaks allows to consider the inks to be different.

In case of identical results from quantification of the electropherograms the application of other detection methods for the separation or the comparison with the results of another analytical method could provide the information for the unambiguous characterization of the sample. Since fluorescence detection bases on a different measuring principle in comparison to UV-Vis detection and some of the ink components (dyes and other additives) do fluoresce this detection principle could be considered a suitable supplementation for the process of ink identification. In Fig. 8 UV-Vis and fluorescence detection are compared for the inks 4 and 8, which have not been distinguished by UV detection. The normalized peak areas of the subpeaks of both inks in Figs. 8A and 8B differ only by 2–3%, which does not suffice to assign the inks to different origins. By contrast, the fluorescence signals (Figs. 8C and 8D) show significant differences for both samples between 30 and 40 min migration time and therefore an identical origin of both samples is unlikely.

In comparison to thin-layer chromatography which could be considered to be the classical method for ink discrimination (7), capillary electrophoresis offers the advantages of higher resolution, flexibility in the selection of detection methods, simplicity of quantification, and the ability to perform separations under completely different separation conditions consecutively in the same capillary system. By contrast, TLC allows to separate sets of different samples simultaneously, which means often time saving for inks which could be easily distinguished. In the end, capillary electrophoresis will belong to the group of selective methods (like HPLC or GC-MS) which are able to distinguish samples not sorted by the classical methods but require more time and higher investment.

PIXE-Analysis of Writing Traces

The set of only 20 ball point pen inks investigated in this work could not be considered representative for the wide variety of ball

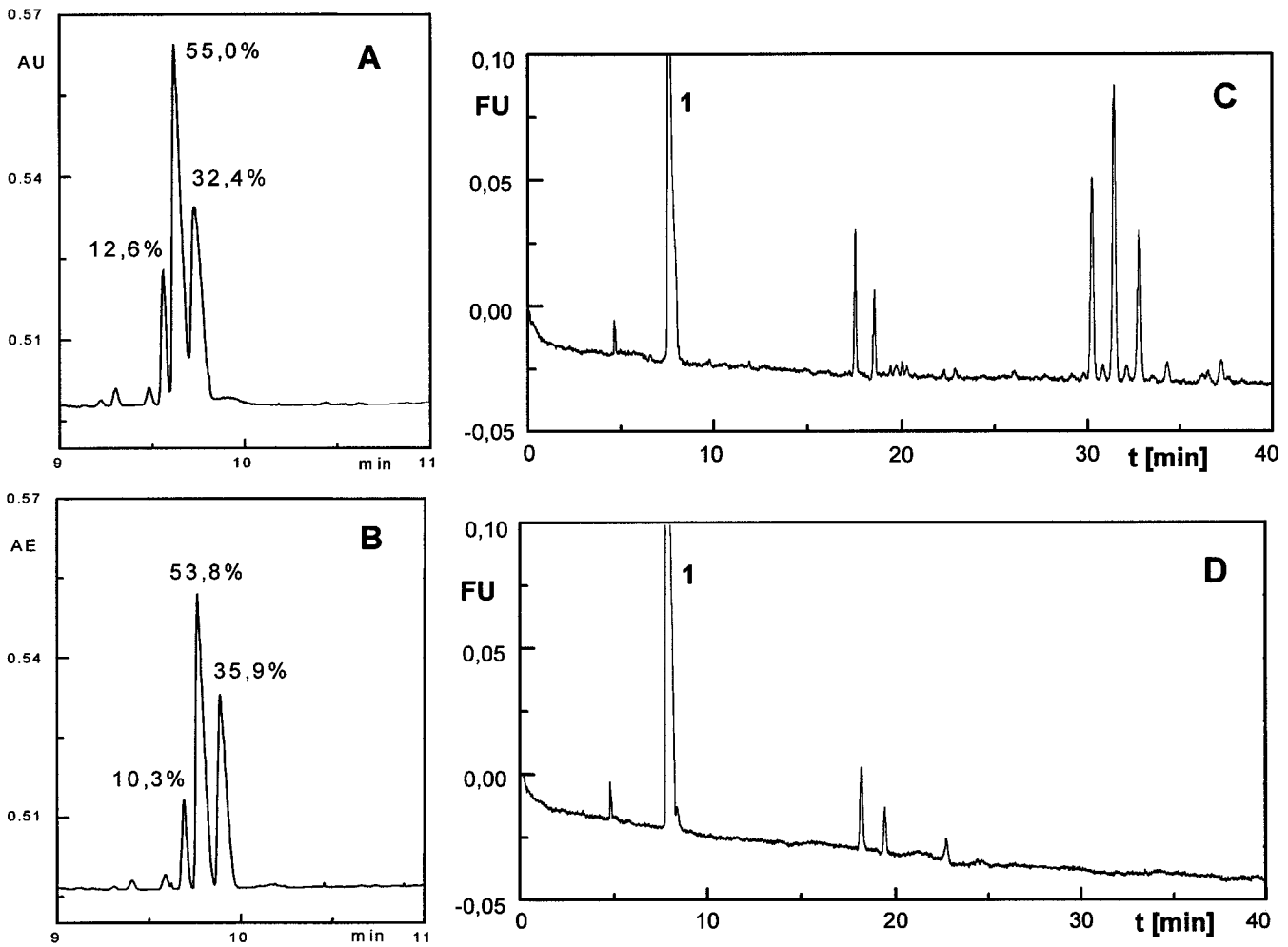

FIG. 8—*Electrophoretic separation of black inks 4 and 8 with UV- and fluorescence detection. (*A, B*): buffer: 50 mM borate, pH 9, 50% acetonitrile, 30 mM SDS, UV-detection at 205 nm; (*C, D*): buffer: 50 mM borate pH 9, 50% acetonitrile, LIF-detection:* $\lambda_{ex/em}$ *= 320/405 nm (He-Cd-laser).*

point pen inks available on the market. A higher number of samples will obviously worsen the possibility to distinguish between all inks. Therefore, the application of a second analytical technique independent of the electrophoretic method will improve the opportunity to distinguish between inks of various manufacturers unambiguously. Proton-induced X-Ray Emission (14,21–23) could be the method of choice due to several inherent advantages for ink and paper analysis.

In this work we used a measuring configuration with an external proton beam which allows to measure the sample under normal pressure conditions. The most important points favoring the use of an external beam are the efficient convectional cooling of the sample by the surrounding air during the measurement and the ability to deflect the charge on the sample through the surrounding ionized air. This way the sample damage caused by the interaction of the beam particles with the paper material could be minimized and the beam spot on the paper material was nearly invisible. In addition, samples as large as complete letters could be analyzed without any preparation or cutting. The absorption of low energetic emissions by the air was the only disadvantage of this setup, which prevents the determination of lighter elements, e.g., sulfur or chlorine.

For all 20 samples PIXE spectra have been recorded (see experimental section). The spectra in Fig. 9 are representative for the results obtained. In most of the inks only copper, nickel, zinc, and lead have been identified, sometimes also iron, titanium, chromium, and cobalt were present. Because only 4 of the elements (Cu, Ni, Zn, and Pb) are characteristic for most of the samples the identification of different manufacturers by a qualitative analysis only is complicated. A comparison of absolute amounts of copper and zinc in different samples is useless due to differences in the line width, in pressure applied during the writing process and the penetration depth of different ink formulations into the same paper material. The standardization of all these parameters is extremely difficult and not applicable to real sample analysis.

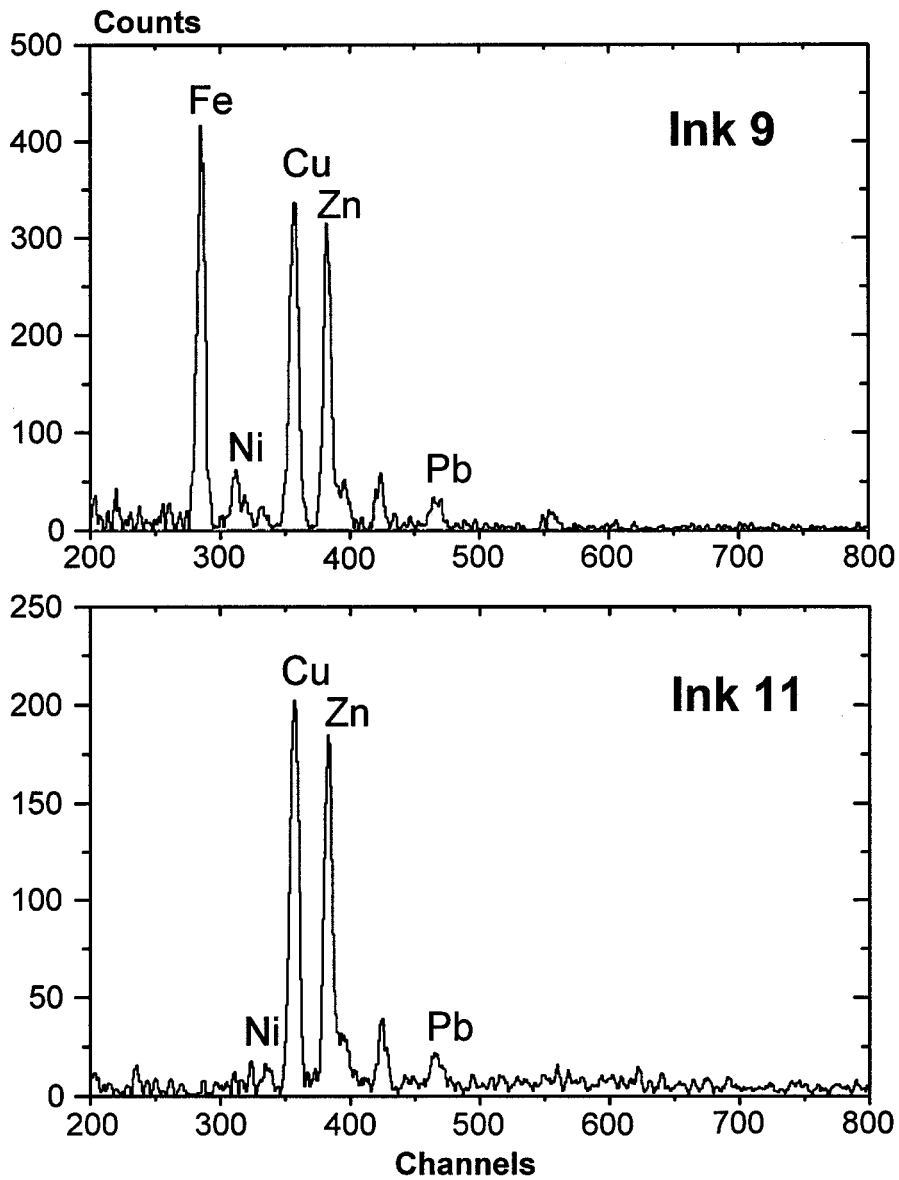

FIG. 9—*PIXE spectra of similar black inks on paper. Energy of proton beam 1550 keV; beam diameter 200 μm, measurement time 1000 s; HPGe detector, resolution 175 eV.*

However, due to little matrix influences a linear dependence of the element concentrations on the peak areas of the PIXE-signals was observed. In addition, different ratios for the signals of copper and zinc (ratios of peak areas) have been measured for most of the samples, which could be used for a differentiation between the samples. To determine the error of the determination of the copper/zinc-ratio, 10 separate measurements have been performed at different positions of the same writing line. For all samples the error was below 5%. The error obtained for additional measurements for 4 different lines of the same ink was also below 5%. Consequently, in Fig. 10 the results for all 20 samples are shown with 5% error bars for each of the measured values. Table 5 represents the numerical values and additional elements identified in the samples. From the copper/zinc-ratio the following pairs of inks could not been distinguished from each other: 4 and 8, 8 and 9, 8 and 11, 9 and 11, 4 and 17, 6 and 17, and 12 and 13. For the inks 4 and 8 (Ni), 8 and 9 (Fe), and 9 and 11 (Fe, see Fig. 9) a differentiation was possible, because nickel or iron were detected in one of both samples only. Neither the copper/zinc-ratio nor additional elements could be used to distinguish between the black inks 4 and 17, 8 and 11, and 6 and 17 and the blue inks 12 and 13.

Therefore, a reliable differentiation between a large number of ball point ink samples by PIXE-measurements is not possible. Nevertheless, these measurements could provide useful additional information about the samples.

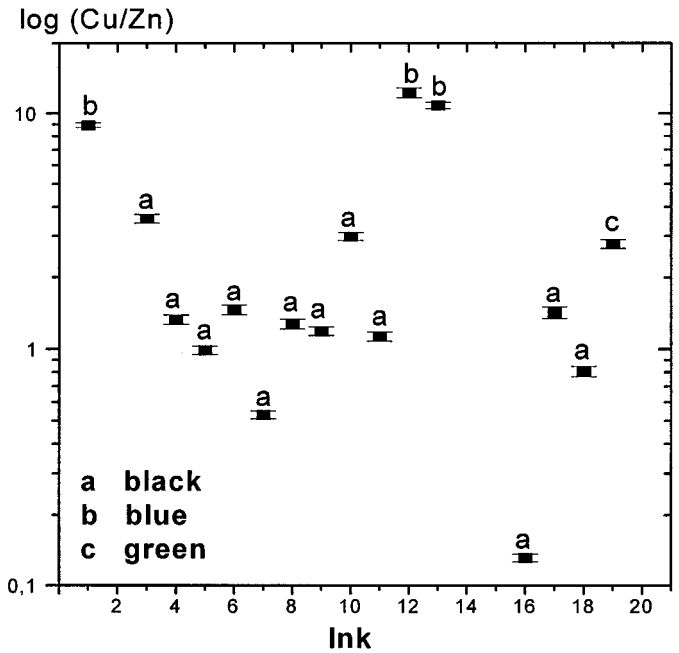

FIG. 10—*PIXE-analyses of ball point inks on paper. The Cu/Zn-ratio was determined by measurements with the external beam. The values for inks 2 and 20 are zero (no copper and zink in the sample).*

TABLE 5—*PIXE-Analyses of ball point inks on paper. Comparison of the Cu/Zn-ratios and the metal composition.*

Ink	Color	Cu/Zn-Ratio	Abs. Error	Additional Metals Detected
1	blue	8.91	0.20	Fe, Pb
2	black	0	0	
3	black	3.56	0.15	
4	black	1.33	0.06	Pb
5	black	0.99	0.04	Fe, Pb
6	black	1.462	0.07	Pb
7	black	0.53	0.02	Fe, Pb
8	black	1.274	0.06	Ni, Pb
9	black	1.19	0.05	Fe, Ni, Pb
10	black	2.99	0.12	
11	black	1.13	0.05	Ni, Pb
12	blue	12.3	0.60	
13	blue	10.84	0.35	
14	blue	58		Ni
15	blue	100		
16	black	0.131	0.005	Pb
17	black	1.423	0.08	Pb
18	black	0.808	0.04	Fe, Pb
19	green	2.78	0.12	Ti, Cr, Fe
20	red	0	0	only Co

Conclusions

For a set of 20 different samples electrophoretic separations and PIXE analyses have been performed. Many of the samples show unique peak pattern when two different buffer systems, one with borate and acetonitrile, the other with borate, acetonitrile and the surfactant sodium dodecyl sulfate, are applied for the separation. The determination of the copper/zinc-ratio by PIXE-measurements allows to distinguish between most of the ink samples. No coincidence was observed between samples hardly distinguishable by electrophoretic separations and by PIXE-measurements. Samples with nearly identical metal composition (PIXE analysis, inks 12/13, 4/17, 6/17, 8/11) show different peak pattern in the electropherograms and nearly identical electrophoretic behavior of two or more samples (inks 4/8 and 6/9) was accompanied by quite different copper/zinc-ratios or supplementary metals identified by PIXE. Nevertheless, more work is necessary to prove these results for a larger set of samples and to investigate the influence of the metal composition of the tip of the pen on the copper/zinc-ratio of the inks.

In principle, the high resolution power of capillary electrophoresis makes this method also suitable for document dating (4), since changes in dye ratios, the concentration of the dyes or the formation of metabolites of the inks could be followed much easier than by TLC (7). Nevertheless, the application of CE in the field of ink analysis is only at the beginning and definitely will focus on the dating problem in the near future. In the end, capillary electrophoresis will belong to the group of selective methods (like HPLC or GC-MS) which are able to distinguish samples not sorted by the classical methods but require more time and higher investment.

Acknowledgments

We wish to acknowledge Dr. Thomas Andermann from the Bundeskriminalamt Wiesbaden, Germany, for the constructive discussions and for providing part of the samples in this research. Furthermore, we express our gratitude to Perkin-Elmer (location Leipzig-Markkleeberg, Germany) for providing the Perkin-Elmer electrophoresis equipment.

References

1. Brunelle RL, Pro MJ. Systematic approach to ink identification. J Assoc Off Anal Chem 1972;55:823–826.
2. Brunelle RL, Reed RW. Forensic examination of ink and paper, Charles C Thomas, Springfield, IL, 1984.
3. Cantu AA. A sketch of analytical methods for document dating. Part I. The static approach: determining age independent analytical profiles. International Journal of Forensic Documents Examiners 1995;1(1):40.

4. Cantu AA. A sketch of analytical methods for document dating. Part II. The dynamic approach: determining age dependent analytical profiles. International Journal of Forensic Documents Examiners 1996;2(3):192.
5. Moon HW. Electrophoretic identification of felt tip pen inks. J Forensic Sci 1980;25:146.
6. Nakamura GR, Shimoda SC. Examination of micro quantity of ball pen inks from documents by TLC. J Crim Law Criminol Police Sci 1965; 56:113.
7. Standard Guide for Test Methods for Forensic Writing Ink Comparison. In: Annual Book of ASTM Standards, Vol 13.01, ASTM-Designation: E1422-91. West Conshohocken, 1992.
8. Colwell LF, Karger BL. Ball point pen examination by high pressure liquid chromatography. J Assoc Off Anal Chem 1977;60:613.
9. Lyter AH. Examination of ball pen ink by high pressure liquid chromatography. J Forensic Sci 1982;27:154.
10. Lyter AH. In: High performance liquid chromatography in forensic chemistry. Lurie IS, Wittwer JD, editors. New York: Marcel Dekker, 1983;399.
11. Brown C, Kirk PL. Paper electrophoresis in the identification of writing inks. J Crim Law Criminol Police Sci 1954;45:334.
12. Fanali S, Schudel M. Some separations of black and red water soluble fiber-tip pen inks by capillary zone electrophoresis and TLC. J Forensic Sci 1991;36:1192.
13. Rohde E, McManus AC, Vogt C, Heineman WR. Separation and comparison of fountain pen inks by capillary zone electrophoresis. J Forensic Sci 1997;42(6):1004–11.
14. Vogt C, Vogt J, Rohde E, Becker 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. J Chromatogr A 1997;781:391–405.
15. Rohde E, Vogt C, Heineman WR. The analysis of fountain pen inks by capillary electrophoresis with uv/vis absorbance and laser induced fluorescence detection. Electrophoresis 1998;19:31–41.
16. Stewart LF. Ballpoint ink age determination by volatile component comparison—a preliminary study. J Forensic Sci 1985;30:405–11.
17. Breedlove CH. The analysis of ball point inks for forensic purposes. J Chem Educ 1989;66:170–1.
18. Ullmann's encyclopedia of industrial chemistry, 5th edition, New York: VCH, Weinheim, 1987.
19. Kuhn R, Hoffstetter-Kuhn S. Capillary electrophoresis: principles and practice. Berlin: Springer, 1993.
20. Landers JP, editor. Handbook of Capillary Electrophoresis, 2nd Ed., Boca Raton: CRC Press, 1997.
21. Johannson SAE, Campell JL. PIXE — A novel technique for elemental analysis. New York: Wiley, 1988.
22. Lucarelli FL, Mandò PA. Recent applications to the study of ancient inks with the florence external-PIXE facility. Nucl Instr Meth Phys Res B 1996;109/110:644.
23. Cahill TA, Kusko BH. Analysis of inks and papers in historical documents through external beam PIXE techniques. Nucl Instr Methods 1981;181:205.

Additional information and reprint requests:
Carla Vogt
Faculty of Chemistry and Mineralogy
Institute of Analytical Chemistry
Linnéstr. 3
D-04103 Leipzig
Germany