

TECHNICAL NOTE

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Some Separations of Black and Red Water-Soluble Fiber-Tip Pen Inks by Capillary Zone Electrophoresis and Thin-Layer Chromatography

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ABSTRACT: Capillary zone electrophoresis was tried for analyzing water-soluble fiber-tip pen inks from documents. The separation process for the compounds—most of them move as anions—is usually less than 10 min. Some examples of possible applications, also combined with thin-layer chromatography, are given.

KEYWORDS: questioned documents, inks, electrophoresis, chromatographic analysis, capillary zone electrophoresis

In forensic science, ink analysis is a very important field. So, it is understandable that many analysis techniques and their optimal experimental conditions have been published by forensic document experts [1–4].

A number of papers proposing methods of paper, thin-layer, and high-performance liquid chromatography, as well as their applications in reversed-phase chromatography and also paper and gel electrophoresis, have been published to provide more information about ink composition [5–15].

Capillary zone electrophoresis (see Ref 16)—with its great resolution power, high efficiency, short analysis time, and other advantages—is a relatively new technique used for the separation of compounds with different mobility by using a buffer-filled capillary cartridge and applying a relatively high electric field. Capillary zone electrophoresis was introduced by Hjerten [17], Jorgenson and Lukacs [18], and Mikkers et al. [19] and was used for dyestuff separations [20], but, as far as we know, until now it has not been applied to the analysis of inks, and we wish to report here some successful separations.

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Experimental Procedure

Apparatus

The experiments were performed with a Bio-Rad HPE 100 apparatus (Richmond, California) equipped with an ultraviolet (UV) detector with a deuterium lamp (190 to 380 nm). The detection wavelength was set at 206 nm. The apparatus was equipped with a power supply able to deliver up to 12 kV. Sampling and electrophoresis were controlled by a microprocessor.

Separations were performed in a Bio-Rad 148-3002 HPE capillary cartridge (20 cm by 0.025 mm, coated). The capillary cartridge was filled with the background electrolyte by using a 100- μ L Hamilton microsyringe. The background electrolyte was a mixture of 0.1M ammonium acetate buffer solution (pH = 4.5) and methanol (3:1). The electric chamber near the detector was positively charged, which means that the dyes were moving as anions.

Electropherograms were recorded with an LKB 2210 line recorder at a chart speed of 10 mm/min.

Chemicals

We used double-distilled water, the methanol was a high-performance liquid chromatography (HPLC)-grade product from Carlo Erba (Milan, Italy). The acetic acid and the ammonium acetate were obtained from Carlo Erba (quality "puro").

HPTLC plates, RP-18, precoated and manufactured by E. Merck (Darmstadt, Germany), were used.

The fiber-tip pens examined were those locally available.

Results and Discussion

In the present work, in which we used ink samples from water-soluble red and black fiber-tip pens, we employed the following two basically different kinds of comparisons. The first one was to observe and, if possible, to establish that two (or more) different inks produce two (or more) different electropherograms or, in other words, that every ink has an individual pattern. The results are shown in Fig. 1.

The differences between the water-soluble fiber-tip pen inks were evident; the reproducibility of these analyses has also been tested.

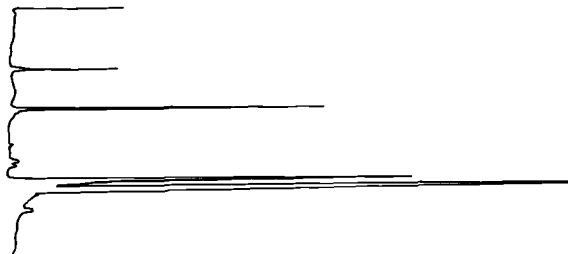
When using this first kind of comparison, one can prevent eventual chemical interaction problems by using internal standards. The following procedure is proposed: A part of one ink is separated by capillary zone electrophoresis under the described conditions. The electropherogram is recorded. Afterwards, a second ink is analyzed under the same conditions. The two electropherograms are then compared. Now, an equal part of each of the two ink samples is mixed and separated by capillary zone electrophoresis. The electropherogram will show the result of the electrophoretic separation of the mixture. In this manner, it is possible to avoid eventual problems by using internal standards.

Usually, the different peaks in the third electropherogram can be associated with one of the two original inks by means of the first two electropherograms.

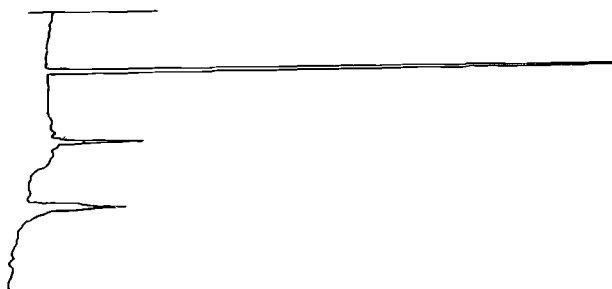
It seems important to mention that, for the moment, we are only interested in a qualitative comparative comparison of the inks.

In the second kind of ink comparison, we first separated the dyes of the ink by means of chromatographic techniques—usually reversed-phase thin-layer chromatography [21]. We next compared dye spots which were not (or were hardly) discernible when the chromatographic process was used. (The optimization of the chromatographic conditions is yet to be established; in the present work, we used a mixture of acetate buffer (pH = 4.5) and methanol at a 2:8 ratio). The procedure is as follows: Samples of the

R 1: 8 kV, 9.2 μ A; Loading: 5 kV, 6 s



R 2: 8 kV, 11.2 μ A; Loading: 5 kV, 5 s



R 6: 8 kV, 9.4 μ A; Loading: 5 kV, 5 s



R 12: 8 kV, 8.6 μ A; Loading: 4 kV, 5 s



FIG. 1—The electropherograms of four red fiber-tip pen inks and of three black fiber-tip pen inks. The fiber-tip pens used were the following: R 1: Pilot—S, made in Germany; R 2: Pentel Sign Pen, made in Japan; R 6: Ball Pentel Extra Fine R 56, France; and R 12: Pentel Super Ball (EEF) BH16, Japan.

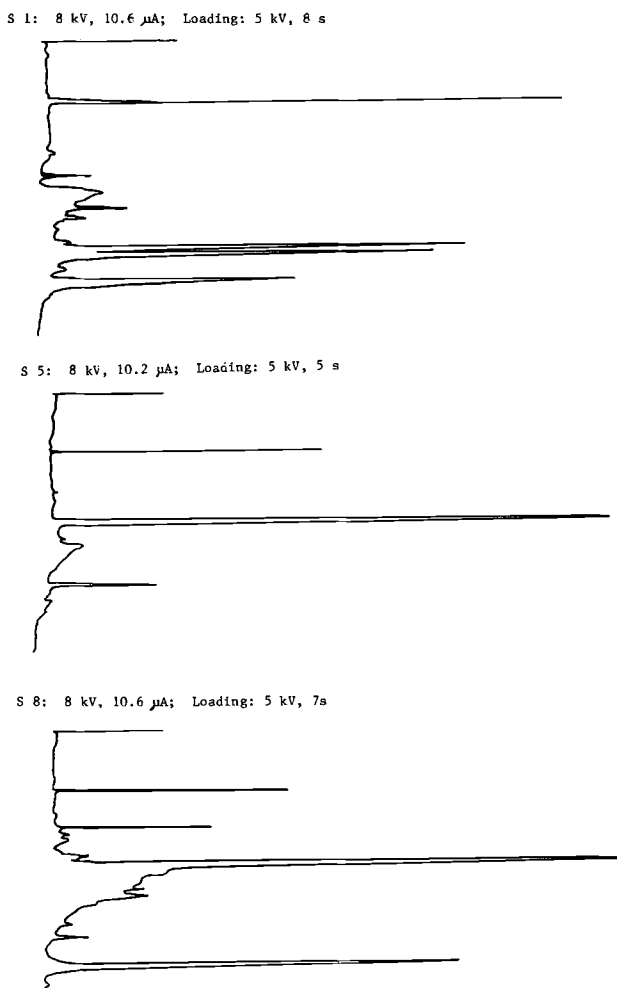


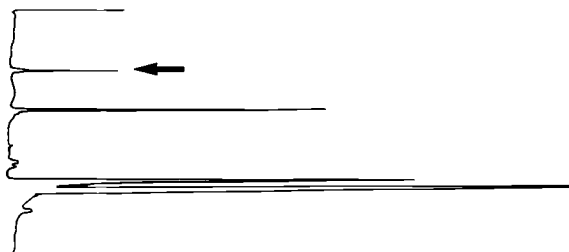
FIG. 1—Continued: S 1: Stabilo-OHPen 97 Fine, Germany; S 5: Pilot Fineliner; and S 8: Pentel Sign Pen, made in Japan.

corresponding thin-layer chromatography (TLC)-separated dye spots are extracted from the chromatographic plate and compared by capillary zone electrophoresis as in the previously described method.

This second method has been applied to our samples in the following manner. One of the ink samples (No. R 1) was chromatographed, and each of the separated spots was added to a part of the whole (not yet separated) ink sample. This was then separated by capillary zone electrophoresis. It was thus possible to see whether the TLC-extracted dye corresponded to one of the dyes in the ink mixture (by checking the number of peaks obtained) and, if so, to associate the peaks in the electropherogram with the different dyes (separated by chromatography), according to the increased concentration (see Fig. 2).

We think that the proposed techniques could be useful in the examination of documents. We have not, so far, dealt with the extraction of the ink from a document (especially problems of dye solubility or of adhesion forces between some of the dyes and the cellulose

R 1: 8 kV, 9.2 μ A; Loading: 5 kV, 6 s



R 1 + fraction of the 2nd spot: 8 kV, 9.2 μ A; Loading: 5 kV, 6 s

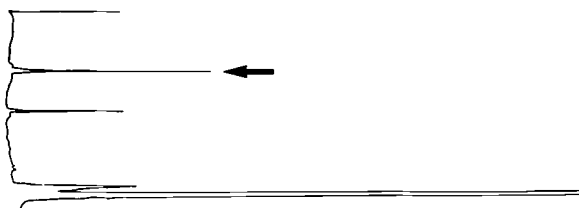


FIG. 2—Differences between the same basic ink: (top) the extracted ink and (bottom) the extracted ink with the addition of a red dye obtained by a TLC-separation of the same ink. The arrow shows the peak which increased on addition of the red dye.

fibers in the paper); also the problem of the decomposition of inks with time will be dealt with in due course.

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