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# POLICE SCIENCE

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## PAPER ELECTROPHORESIS IN THE IDENTIFICATION OF WRITING INKS

### Comparison with Horizontal Paper Chromatography\*

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Professor Paul L. Kirk, School of Criminology, University of California, has been a frequent contributor to this Journal during the past years. This paper is his second dealing with advanced methods in the identification of writing inks. His early paper on this subject appeared in the September-October issue and dealt with the identification of ball point pen inks.—EDITOR.

Identification of liquid writing inks by paper chromatography (1, 2, 3, 4) marks the most important step in the field of ink identification in recent times. With relatively simple means, most inks can be differentiated and much can be ascertained rapidly regarding their composition. The dyes become visible individually, and with ultraviolet light and chemical testing, invisible constituents can be located on the paper.

Chromatography, even by the horizontal paper method (5, 6, 7) leaves something to be desired in practical document examination. The requirement usually imposed, that the document must not be significantly defaced, limits severely the quantity of ink that can be removed from original ink lines. When the amount of ink is very small, the diffusion that occurs during chromatography may spread it so much as to make it invisible or nearly so. The more rapid the technique of separation, the less this effect will be, and for this the horizontal paper method has proven most effective of the chromatographic techniques tested in this laboratory. Description of this procedure has been given elsewhere (7), and the results of testing it with fluid writing inks is included in this discussion.

Chromatography depends primarily though not entirely on slight solubility differences and resulting partition ratios between different solvents. Paper electrophoresis depends on an entirely different principle (8, 9, 10, 11). Materials which are ionic or can be rendered ionic by adjustment of the environment will move in an electric field toward the electrode of opposite sign. The velocity of movement will be determined by the size of the ionic particle and its charge. When the material is placed on a piece of paper and subjected to an electric field the paper serves only as a support, and one which minimizes convection currents. Diffusion will still occur as in chromatography. A number of ionic species will migrate in a particular ionic field at different rates, thereby being separated from each other and eventually establish-

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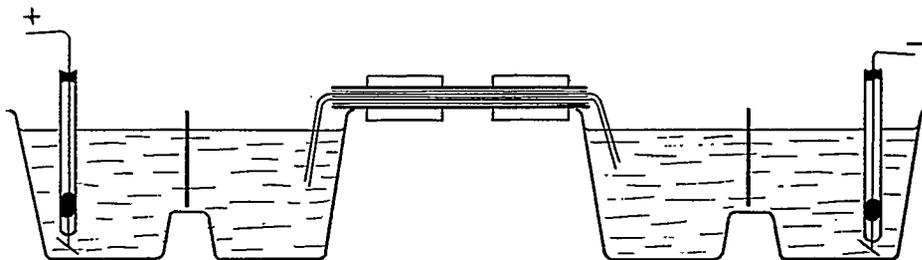


Figure 1

## Simplified Electrophoretic Apparatus for Ink Study

ing a separate zone for every ionic species that has a unique mobility. Since the dyes and other materials present in ink are either ionic, or may become ionic under proper pH adjustment, it is possible to separate them very rapidly and cleanly by subjecting them to electrophoresis on paper. Most constituents of liquid writing inks migrate in an electrical field and are adaptable to identification and possibly other studies by the electrophoretic approach. It has also proven applicable to ball point inks but seems less satisfactory for these than paper chromatography (7).

## EXPERIMENTAL

*Electrophoretic Equipment*<sup>1</sup>. A number of large paper electrophoresis outfits are available commercially. While any of these are applicable to ink study, such large differences exist in the mobility of the constituents of ink, that a very small and inexpensive unit was found to be eminently satisfactory and far cheaper than the conventional units that are manufactured primarily for protein separations. The design of unit is shown in Figure 1. It consists of two end vessels with a partial partition in the middle of each. Double ice-cube molds of polyethylene plastic were found suitable. The partition was made from a sheet of Vinylite plastic cut to fit snugly in the molds leaving a slit open at the bottom for electrical pathway. Platinum wire electrodes were introduced in each end vessel. The vessels were placed in a rack which separated the adjacent edges  $2\frac{1}{4}$  inches. These edges were used to support two glass plates three inches square between which were sandwiched the papers carrying the samples.

Power for the electrophoresis was supplied by a small direct current power supply capable of furnishing up to 130 volts and supplying currents up to 50 ma. While the voltages obtainable were lower than is common in paper electrophoresis, the short length of paper more than compensated for this fact, and the voltage drop per cm. of paper length was actually higher than is normally used, thus leading to very rapid separations. Such short distances of movement are entirely feasible with ink electrophoresis because of the very great differences in mobility of the constituents. It would not be suitable for many of the other applications of paper electrophoresis in which the spread in mobility values is often very narrow.

*Reagents—Barbital buffer.* A satisfactory buffer is made by dissolving 10.3 g. sodium diethylbarbiturate and 1.84 g. diethylbarbituric acid to make 1 liter of solution.

<sup>1</sup> Equipment is obtainable from the Microchemical Specialties Co., 1834 University Ave., Berkeley, California.

While this approximates the buffer used in electrophoresis of proteins, the ionic strength and exact adjustment of pH so necessary with proteins is not significant in this application.

*Acetate buffer.* A satisfactory acetate buffer of low ionic strength was made from 8 g. sodium acetate and 1 ml. acetic acid dissolved to make 1 liter.

*Sample Preparation.* Whatman #1 filter paper was cut into pieces 5 inches in length and  $1\frac{1}{2}$  inches in width. A light pencil line was drawn across the paper about 1 inch from the center. Samples to be studied were placed along this line at intervals in as small spots as possible, preferably by the technique previously described (7). Ordinarily not more than three samples were placed on a strip. After the spots were dry, the paper was wet with buffer solution. This was accomplished without disturbing the sample spots by dipping the ends successively in a separate bath of the buffer until the liquid had run nearly to the line of samples. The paper was laid on a dry filter paper, and the excess buffer removed by blotting with another filter paper. With a small paint brush, buffer was placed with care around the sample spots so as to wet them last of all. As soon as the last of the paper was wet, it was ready to be placed in the sandwich.

The sandwich was constructed as follows: First a 3 by 3 inch sheet of glass was made the bottom layer. On this was placed a sheet of thin polyethylene the same size as the glass. Next was placed the wet paper followed by another sheet of polyethylene. A top glass was then added if only one paper was to be used. Additional papers could be added by inserting them in order with a sheet of polyethylene between each. As many as 8 papers could be run simultaneously though 6 was a suitable number for the power supply. This allowed at least 18 samples to be studied simultaneously. The upper and lower glass sheets were finally squeezed together and clamped with four spring paper clamps. The sandwich was then placed on the end vessels with the ends of the papers dipping into buffer in the two vessels as shown in Fig. 1.

*Procedure.* Samples of original ink were placed on papers as described above using a pen or a small glass rod. Ink taken from pen lines required preliminary solution from the line, and with as little damage to the document as possible. While water can be used for this purpose as described in several publications, and with care will not produce any significant damage to a document, it also does not remove the permanent inks very well, and does not dissolve even the dye inks as rapidly as may be desired. Dyes at least are removed with greater facility by a mixture of acetone and water in equal proportions, and this was the mixture adopted. It has the disadvantage of spreading on the paper quite rapidly. To minimize this effect, the droplet was placed with a fine pipet equipped with a syringe control (12), and the liquid was immediately taken up by capillary action with a finely drawn glass capillary. When the droplet was small enough, little spreading of the ink occurred, and solution was virtually instantaneous.

The ink samples, when mounted and placed on the electrophoresis apparatus as described above was immediately subjected to current passage at about 100 volts. The current at this voltage was 5-10 ma. per paper in the sandwich. An electrolysis period of about 5 minutes was found sufficient to obtain satisfactory separation of

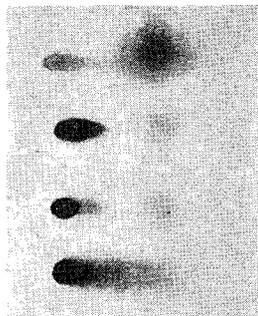


Figure 2

Electrophoretic Pattern of Four Typical Black Inks

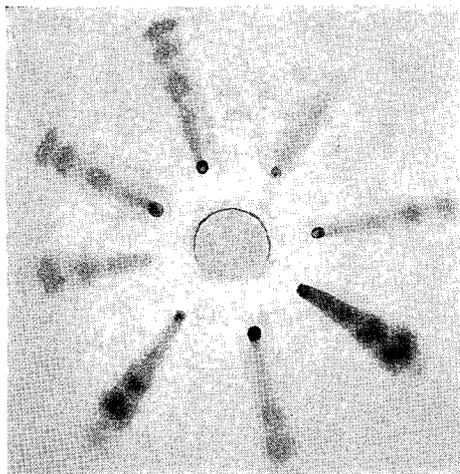


Figure 3

Typical Horizontal Chromatograms of Blue and Blue-Black Inks

the ink constituents. If it was continued for very much longer, certain ultraviolet visible materials were moved to the end of the paper and into the buffer while the dyes were still close to each other, though separated.

At the end of the run, the sandwich was opened, and the papers blotted on the ends that dipped into the buffer. They could then be hung with photograph clips or clothes pins and dried for final examination or further treatment with chemical sprays.

*Chromatography.* Chromatography on paper was carried out by the technique previously described for ball point pens on horizontal, round Whatman #1 filter paper sheets. Various solvents were tested and most solvents produced a considerable degree of separation, even water (2). The solvents chosen as superior were n-butanol:acetic acid:water (52:13:35 parts by volume) and n-butanol:acetone:water (4:2:1). The former solvent revealed more components than the latter but promoted a greater spreading and loss of color intensity. When sufficient ink was present, it was superior, but with smaller amounts, the second solvent mixture gave more decisive results. As will be noted in the results, it was found desirable to use both solvents for maximum separation. The chromatographic technique did not identify inks as well as the electrophoretic technique described. (Figure 2).

## RESULTS

The electrophoretic technique was tested by applying it systematically to the identification of 36 blue and blue-black inks. It was also tested with 18 black inks which gave very striking results and easy separation. The blue and blue-black inks represent a much more difficult problem and a better test of the procedure.

The inks tested systematically, when subjected to electrophoresis in barbital buffer divided into general groups as shown. (Figure 3).

*Blue and Blue Black Inks—Barbital Buffer*

- I. Superchrome blue.
- II. Superchrome blue black.
- III. Safink.
- IV. Sheaffer permanent blue black (3 bottles of different age and designation).
- V. Quink permanent blue black with Solv-x; New Quink permanent blue black with Solv-x; Quink permanent royal blue with Solv-x; New Quink permanent royal blue with Solv-x.
- VI. Quink permanent royal blue; Graph permanent blue black; Ruxtone washable blue.
- VII. Onward blue; Penit blue black # 276M; Penit # 276M (old); Penit # 276M (new).
- VIII. Penit washable blue # 256; Penit # 925; Sanford permanent royal blue No. 16; Graph washable blue; Carter American blue.
- IX. Waterman permanent blue black (old bottle); Stafford permanent blue black; Signet blue black.
- X. Quink washable blue; Sheaffer washable blue # 42; Signet blue; Waterman washable blue (old bottle); Waterman washable blue (new bottle); Stafford washable blue.
- XI. Flo-rite washable blue.
- XII. Carter midnight blue black: Fount-O-Ink blue black # 20; Sheaffer permanent royal blue, Chem-O-Pure.
- XIII. Carter washable blue.
- XIV. Sheaffer permanent royal blue # 53.
- XV. Penit royal blue (276M—new bottle).
- XVI. Waterman permanent blue black (new bottle).

*Blue and Blue Black Ink—Acetate Buffer*

- I. Superchrome blue.
- II. Superchrome blue black.
- III. Safink.
- IV. Sheaffer permanent blue black (3 bottles of different age and designation).
- V. New Quink permanent blue black with Solv-x.
- VI. Sheaffer permanent royal blue # 53; Fount-O-Ink blue black # 20; Quink permanent blue black with Solv-x; Waterman permanent blue black (new bottle).
- VII. Penit blue black 276 (new); Penit blue black 276M (new bottle).
- VIII. Ruxtone washable blue.
- IX. Penit # 925; Graph washable blue.
- X. Waterman washable blue (old); Waterman washable blue (new); Penit royal blue # 276M (new); Penit washable blue # 256; Sanford permanent royal blue # 16 (new); Quink washable blue; Signet blue; Sheaffer washable blue # 42; Sheaffer permanent royal blue, Chem-O-Pure; Stafford washable blue.
- XI. Flo-rite washable blue.

- XII. Graph permanent blue black; Stafford permanent blue black; Carter midnight blue black; Quink royal blue.
- XIII. Signet blue black; Onward blue black.
- XIV. Carter washable blue.
- XV. Carter American blue.
- XVI. New Quink royal blue with Solv-x; Quink royal blue with Solv-x (old).

*Black Inks—Barbital Buffer*

- I. Sheaffer washable black.
- II. Signet black.
- III. Sheaffer permanent jet black.
- IV. Waterman permanent black.
- V. Quink micro-film black permanent.
- VI. Quink with Solv-x, permanent black (2 bottles of different age); New Quink with Solv-x, permanent black.
- VII. Carter midnight black, permanent; Flo-rite jet black.
- VIII. Penit jet black; Sanford royal black.
- IX. Carter raven black, washable; Graph washable black.
- X. Parker "51" India; Superchrome jet black; Higgins eternal black; Quink washable black.

Inspection will show duplication of only about five inks between the classes in the first two lists above. This illustrates the desirability of making determinations with at least two types of buffer. Close inspection of the individual patterns when run together on the same paper will reveal differences between most of the members of a single group. It is thus doubtful if any of the inks tested cannot be distinguished from all of the others if enough care is used. The distinctions also become easier with black inks which could all be separated as to brand by color and fluorescence except those of Group IX. The fluorescent patterns were particularly striking in Group X which were clearly different. Because of the small amount of ink necessary with this technique it is not significantly more difficult to identify the ink removed from an ink line than that taken directly.

Slow fading was observed to occur after drying the papers, presumably because of the alkalinity of the buffer. The original colors could be readily restored by spraying the paper with dilute acetic acid or very dilute hydrochloric acid.

Chromatography by the horizontal paper method was also employed for comparative results, with the same 39 inks listed above. With n-butanol:acetic acid:water (52:13:35) the inks were divided into the following classes.

- I. Superchrome blue.
- II. Superchrome blue black.
- III. Safink.
- IV. Sheaffer permanent blue black (three bottles).
- V. Carter American blue; Penit #925; Graph washable blue; Carter washable blue.
- VI. Quink with Solv-x, royal blue; New Quink with Solv-x, royal blue; New

Quink with Solv-x, permanent blue black; Quink washable blue; Sheaffer washable blue # 42; Waterman washable blue (old bottle); Waterman washable blue (new bottle); Flo-rite washable blue.

- VII. Ruxtone washable blue.
- VIII. Signet blue black.
- IX. Carter midnight blue black; Onward blue black; Quink royal blue; Graph permanent blue black; Sheaffer permanent royal blue (Chem-O-Pure).
- X. Penit blue black # 276 (new bottle); Penit blue black # 276 (old bottle); Penit # 256 washable blue; Stafford permanent blue; Quink with Solv-x, permanent blue black; Fount-O-Ink # 20 blue black; Sheaffer permanent royal blue # 52; Waterman permanent blue black (new bottle).
- XI. Waterman permanent blue black, (old bottle).
- XII. Signet blue; Penit # 276 royal blue; Sanford # 16 permanent royal blue; Stafford washable blue.
- XIII. Penit # 276M (old bottle).

With a solvent of butanol:acetone:water (4:2:1), the same inks were divided into the following groups.

- I. Superchrome blue
- II. Superchrome blue black.
- III. Safink.
- IV. Sheaffer permanent blue black (three bottles).
- V. Signet blue black; Waterman permanent blue black, (old bottle).
- VI. Penit # 925; Carter American blue.
- VII. Ruxtone washable blue.
- VIII. Quink washable blue; Sheaffer washable blue # 42; Waterman washable blue, (old bottle); Waterman washable blue, (new bottle).
- IX. Carter washable blue; Graph washable blue.
- X. Flo-rite washable blue.
- XI. Stafford washable blue.
- XII. Fount-O-Ink blue black # 20; New Quink with Solv-x, permanent blue black; New Quink with Solv-x, permanent royal blue; Quink with Solv-x, royal blue; Penit # 276M royal blue; Penit # 256 washable blue; Sanford # 16 permanent royal blue.
- XIII. Sheaffer permanent royal blue, Chem-O-Pure; Sheaffer "52" permanent royal blue.
- XIV. Penit # 276M, (old bottle).

#### DISCUSSION

Paper electrophoresis and paper chromatography should be considered to be mutually supplementary to each other in the identification of inks. The indications are that electrophoresis will ultimately prove considerably more valuable than chromatography because it appears to separate and utilize constituents of the ink that do not separate well with chromatography as it has been applied up to the present. Some inks contain materials that give excellent fluorescent patterns when subjected

to electrophoresis, but which are absent on paper chromatograms. These have been found to vary with the age of the writing in some cases tested, and indicate a strong possibility that this technique may often be valuable in determining age of writing. Further experiments are in progress on this point.

Direct comparison of the two techniques indicates a preference for paper electrophoresis over paper chromatography, both in certainty of identification and in speed of operation. Added to this is the greater reproducibility of the electrophoretic technique and the less empirical conditions than in chromatography. Migration of materials by solvent action alone is determined by precise adjustment of the solvent mixture in many cases and small differences in procedure may at times produce serious alterations in results. The mobility of an ion, on the contrary, is a constant. The measured movement depends only on the existence of the ion in a constant electrical field, provided only that extraneous solvent movements are avoided or controlled.

The use of the horizontal paper technique of chromatography has a number of definite advantages over the strip methods previously proposed. It is considerably more rapid, allowing smaller samples to be used, and involves a much simpler technique. The conditions can be controlled more easily, and the separations produced are equivalent in every respect to those obtained on the strip. Identifications are probably no different in reliability as compared with strip methods.

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