

TECHNICAL NOTE**QUESTIONED DOCUMENTS**

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The Use of Filtered Light for the Evaluation of Writing Inks Analyzed Using Thin Layer Chromatography

ABSTRACT: Thin layer chromatography (TLC) is a scientific methodology that can be used to compare and characterize ink formulations. Occasionally, when evaluating chromatographic profiles on a TLC plate with ambient light, different ink formulations, or the same inks from different batches, may appear indistinguishable. The use of filtered light can be very effective to illuminate characteristics that are not readily apparent with ambient light. There are a diverse number of components commonly found in writing inks, and it may be that some of them respond to particular wavelengths of energy that are not visible to the unaided eye (i.e., colorless). There has been very little information published that addresses the use of filtered light for evaluating TLC plates. Twenty-nine ballpoint writing ink samples were selected for TLC analysis. Further evaluation using an alternate light source, coupled with the appropriate filter, proved to be an effective means for definitive discrimination in some cases.

KEYWORDS: forensic science, questioned documents, forensic document examination, ink analysis, writing inks, thin layer chromatography, ink dating, batch variation, infrared luminescence, filtered light, alternate light source

In their basic form, writing inks are composed primarily of a colorant(s) that is suspended in a vehicle (i.e., solvents and resins). Depending on the vehicle and its interaction with the colorant, two types of colorants can be used: dyes or pigments. Dyes are considered to be soluble compounds with highly conjugated resonance structures, while pigments consist of fine particles of insoluble material that are suspended in a vehicle. The molecular composition of the colorants dictates how certain wavelengths of light are absorbed and reflected, resulting in the observed color. Additives, including antioxidants, preservatives, and trace elements, may also be present in inks but typically account for a small percentage of the overall formulation. Nevertheless, their importance should not be discounted because it is possible that these components allow otherwise similar inks to be discriminated.

Thin layer chromatography (TLC) has been used for decades to analyze and characterize writing ink formulations and is covered extensively in the published literature (1–8). TLC can be very effective at resolving mixtures of dyes and some pigments found in writing inks using solvent systems detailed in the American Society for Testing and Materials (ASTM) International Standard Guide E 1422-05 (9). Commonly used solvent systems may include ethyl acetate:ethanol:water (70:35:30) or butanol:ethanol:water (50:10:15). Other types of pigments may not chromatograph, but they can sometimes be discriminated from mixtures and characterized as spots at the origin or colored streaks on the TLC plate. It must be emphasized that TLC is only one portion of an analytical scheme, and the “profile” of an ink is only achieved using the

results from a series of physical, optical, and chemical examinations as outlined in ASTM International Standard Guides E 1422-05 and 1789-04 (9,10).

TLC is sometimes perceived as a simplistic procedure. The comparative interpretation, however, can be over-simplified in some circumstances. For example, even when the same inks are compared on the same TLC plate, differences can still arise in the retardation factor (R_f) from inconsistent spotting technique, defects in the silica coating, or the use of different concentrations. Furthermore, colorants that chromatograph on a TLC plate are commonly visualized with ambient light, but the colorant profiles of some inks appear very similar and therefore difficult to discern. These are all factors that must be considered in order to reach accurate conclusions.

The composition of colorants and other materials will directly affect the manner in which an ink absorbs, reflects, and transmits light (i.e., the optical properties of the ink). When illuminating a sample, there are two ways the sample can generate light. The first involves light from the illuminant being reflected to a detector. The second way to detect light from a sample is through luminescence. Infrared luminescence (IRL) is the absorption of light at one wavelength and the re-radiation of that light at another, typically longer, wavelength. While luminescence is often broken down into fluorescence for other analyses, it is generally referred to as luminescence because no distinctions are made for this type of analysis. The greatest advantage in examining the luminescence of a sample is the ability to filter out the incident energy. When the incident energy is filtered out, only the energy that has been re-radiated at a longer wavelength can be observed with the appropriate equipment. An alternate light source (ALS), such as the Crimescope (Spex, Edison, NJ), can be utilized to illuminate samples under various wavelengths. Additional instrumentation, such as the Video Spectral Comparator (VSC; Foster and Freeman, Ltd., Evesham, Worcestershire, UK), may be used to observe and record the IRL properties of writing inks.

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In 1973, Kevern (11) discovered that components represented on a thin layer chromatogram, when exposed to IRL, can be further characterized as luminescent, infrared absorbing, or transparent (i.e., infrared radiation is reflected off of the underlying surface). Kevern used IRL photography to capture the previously described effects and concluded that “infrared luminescence photography offers a very sensitive physical method of spot detection on thin layer chromatograms of inks” (11, p. 27). Additionally, Blackledge and Iwan (12) reported on the use of IRL photography for the visualization of TLC plates in 1983. Since that time, however, visualization techniques using infrared reflectance, IRL, or an ALS have not been discussed in the literature. The technique of visualizing TLC plates using an ALS or VSC has been employed to further characterize inks for over two decades, despite the limited number of articles that have been published on the topic.

The objective of this research was to examine groups of ballpoint writing inks that were known to have similar colorant profiles and determine whether further evaluation of the chromatograms with filtered light could provide additional discrimination. The authors also examined different batches of the same ink formulation to determine if batch variations can be detected using this methodology.

Materials and Methods

Collection

Based on known TLC profiles and the difficulty in discerning inks within certain groups, 29 ink samples comprising 21 distinct ink formulations were selected for analysis. A summary of the ink samples and the respective manufacturers is outlined in Table 1. The 21 different ballpoint ink formulations (five black and 16 blue) were produced by seven different manufacturers. In addition, four groups of ink samples, each containing three inks of the same formulation produced in different batches throughout a given year were analyzed to determine whether variations in batches might be detectable. The Sample Numbers 1–21 refer to the different ink formulations studied, and the lowercase letters (i.e., a, b, and c) correspond to different ballpoint ink batches from the same ink formulation and manufacturer. For example, the inks designated Samples 1a, 1b, and 1c refer to three batches of the same ink formulation, with Sample 1b referring to the specific batch that was manufactured on June 28, 1999. A sample of each ink was placed onto Whatman® #2 filter paper (Catalog Number 1002-917; Whatman International, Ltd., Maidstone, Kent, UK) and allowed to dry for 2 days. Whatman® #2 filter paper was used because it contains no additives, coatings, or brighteners. However, in casework, it is necessary to run a paper blank alongside samples on a TLC plate to ensure that the paper does not contain any additives (e.g., fluorescent brighteners) that could interfere with the interpretation of the results, especially when visualized with filtered light.

Extraction

Three to five micro punches (*c.* 1.0 mm in diameter), or ink plugs, were removed from each writing sample contained on the Whatman® #2 filter paper using a hypodermic needle-like apparatus (Catalog Number 69034-10, Harris™ micro-punch; Electron Microscopy Sciences, Hatfield, PA). The ballpoint ink samples were extracted in glass vials with *c.* 5 µL of pyridine (Catalog Number PX2014-6; EM Science, Gibbstown, NJ) and then manually agitated for *c.* 30 sec to ensure sufficient extraction of the ink components.

TABLE 1—Detailed list of the ballpoint ink samples studied.

Sample Number	Manufacturer	Color	Batch	Date Manufactured
1a	Formulabs	Black	a	1/15/1999
1b	Formulabs	Black	b	6/28/1999
1c	Formulabs	Black	c	10/26/1999
2a	Formulabs	Blue	a	1/31/1984
2b	Formulabs	Blue	b	6/25/1984
2c	Formulabs	Blue	c	12/13/1984
3	Hartley	Blue	–	–
4	Hartley	Blue	–	–
5	Bic	Black	–	–
6	Papermate	Black	–	–
7a	Formulabs	Black	a	2/20/1997
7b	Formulabs	Black	b	7/15/1997
7c	Formulabs	Black	c	10/15/1997
8a	Formulabs	Black	a	2/19/1986
8b	Formulabs	Black	b	4/7/1986
8c	Formulabs	Black	c	11/5/1986
9	Anja	Blue	–	1/30/1974
10	Anja	Blue	–	1/9/1968
11	David Kahn	Blue	–	3/2/1972
12	Anja	Blue	–	1/9/1968
13	Anja	Blue	–	10/26/1971
14	Anja	Blue	–	2/22/1971
15	Anja	Blue	–	10/31/1975
16	Fisher	Blue	–	4/1/1970
17	Fisher	Blue	–	7/21/1970
18	Fisher	Blue	–	07/65
19	Fisher	Blue	–	06/67
20	Fisher	Blue	–	06/68
21	Fisher	Blue	–	7/21/1970

Highlighted cells separate the nine different groups of ballpoint inks studied that have similar colorant profiles when visualized with ambient light.

Lowercase letters correspond to different ballpoint ink batches from the same ink formulation and manufacturer.

Thin Layer Chromatography

TLC analysis was conducted in accordance with ASTM International Standard Guide E 1422-05 (9). The extracted ballpoint ink samples were applied to Silica Gel 60 precoated glass plates (Catalog Number 5721-7; EMD Chemicals, Inc., Gibbstown, NJ) with a layer thickness of 250 µm. Micro-capillary pipettes were used to transfer *c.* 2.0 µL of the extracted ballpoint ink to the designated TLC plate. The samples were also examined at increasing concentrations (0.5-, 1.0-, and 2.0-µL spots) to ensure that any observable differences were not attributable to concentration differences. The TLC plates were developed in a solvent system composed of ethyl acetate, ethanol, and water (70:35:30). The solvent front was allowed to elute 4 cm from the origin, which was located 1 cm above the bottom edge of the TLC plate. Additionally, High-performance TLC (HPTLC) Silica Gel 60 glass plates (Catalog Number 540025417; EM Separations Technology, Gibbstown, NJ) with a layer thickness of 200 µm were used with a solvent system composed of butanol, ethanol, and water (50:10:15) as a secondary approach to possibly achieve different resolutions of the colorants.

Ambient Light and Alternate Light Source Examinations

The developed thin layer chromatograms were first observed using a light box (Model Number BL182; Hall Productions, San Luis Obispo, CA) with 42 W of high-intensity ambient transmitted light. Visual differences, if any, between the groups of ballpoint inks were recorded with the Repostar 3 (Catalog Number

TABLE 2—Detailed list of the different groups of ballpoint inks studied that have similar colorant profiles when visualized with ambient light. The ballpoint ink samples that were differentiated when visualized with ambient and/or filtered light are also listed.

Group Number	Sample Numbers	Differentiated with Ambient Light/Sample #	Differentiated with Filtered Light/Sample #	ALS Wavelength Setting and Filter (Goggle) Color
I	1a, 1b, 1c	NO	YES/1c	515 nm; Red
II	2a, 2b, 2c	NO	YES/2c	515 nm; Red
III	3, 4	NO	YES	515 nm; Red
IV	5, 6	NO	YES	495 nm; Orange
V	7a, 7b, 7c	YES/7c	NO	515 nm; Red
VI	8a, 8b, 8c	YES/8c	YES/8a	515 nm; Red
VII	9, 10, 11	NO	YES/9	495 nm; Orange
VIII	12, 13, 14, 15	NO	YES/15	495 nm; Orange
IX	16, 17, 18, 19, 20, 21	NO	YES/17 and 21	535 nm; Red

ALS, alternate light source.

022.9611; Camag Scientific Inc., Wilmington, NC) using transmitted light. The chromatograms were then observed with an ALS (Crimescope CS-16-400; Spex) using multiple wavelength illumination in combination with red and orange goggles (583 and 549 nm, respectively). Any differences observed between the groups of ballpoint inks were appropriately documented and recorded with the Digital Capture System (DCS)-3 (Foster and Freeman, Ltd.). Photographs were taken with the DCS-3 using blue-green (460–510 nm) and/or green (500–550 nm) illumination and a 570-nm long-pass camera filter.

Artificial Aging of the Ink Samples

To determine whether common environmental factors, such as heat and light, could have an effect on the colorant profiles (e.g., chemical byproducts), Samples 1 and 2 (all batches) were collected from their respective ink cartridges and exposed to heat or high-intensity light. The composition of cartridges typically used in ballpoint pens includes plastic (e.g., polyvinylchloride, polyethylene, and polypropylene) and metal (e.g., brass and stainless steel). The authors were unsure if the use of different cartridges might have an effect on the chromatographic profiles. Depending on the composition of the cartridge and the type of ink, the use of a certain solvent(s) may not be warranted because of potential detrimental effects in the flow or composition of the ink as a result of the solvent-cartridge interaction. Therefore, all batches of Sample 2 (2a, 2b, and 2c) were further separated into two categories: ink contained within metal cartridges and ink contained within plastic cartridges. One set of Sample 1 and 2 (all batches) was placed into an oven at 100°C for 24 h, while the other set was exposed to high-intensity 515-nm light from an ALS for 15 min every hour for 8 h. Both the samples exposed to heat and the samples exposed to high-intensity light were then subjected to TLC analysis to observe any differences in the colorant profiles.

Results and Discussion

A total of 29 ballpoint writing inks were analyzed using TLC. Following an examination using ambient transmitted light to evaluate the chromatographic profiles, the inks were divided into nine discernible groups. Within each group, it was determined that some of the chromatographic profiles were similar and a more detailed examination would be necessary before reaching a categorical conclusion. The 29 inks and their respective groups are listed in Table 2. Groups I, II, V, and VI were composed of four different ink formulations manufactured by Formulabs. Each of the aforementioned groups contains the same ink formulation from three different batches that

were produced within the same year. The inks designated Sample 1c and 2c were further discriminated from their respective groups based on the appearance of different bands when examined with filtered light. One ink sample from each of Groups V and VI had noticeable differences when visualized with ambient light; however, the remaining two inks in Group VI were further differentiated when visualized using an ALS with the appropriate filter.

Each of Groups III and IV contained two inks that could not be differentiated with ambient light, but were easily discernible when visualized with an ALS. Group VII contained three blue ballpoint inks from two manufacturers, designated Samples 9, 10, and 11. Distinct differences were observed between Sample 9 (produced by Anja in 1974) and Sample 10 (produced by Anja in 1968) when compared using an ALS. In contrast, Sample 10 had a slight difference in the appearance of the origin spot when compared to Sample 11 (ink provided by David Kahn in 1972), but the authors could not conclusively discriminate the samples. The interpretation of the chromatographic profiles was hindered because of significant differences in the dye concentrations. The authors established that other analytical techniques would be warranted if the same situation were encountered in casework. Although not employed for this study because it was beyond the scope of the research objectives, the use of gas chromatography/mass spectrometry, direct analysis in real-time (DART™; JEOL USA, Inc., Peabody, MA) mass spectrometry, and/or Fourier transform infrared spectrometry would be warranted to determine whether Samples 10 and 11 are truly different. It is possible that David Kahn and Anja used the same formulation of ink at one time, as it is feasible for different pen manufacturers to use the same ink.

Group VIII was composed of four blue ballpoint writing inks that were manufactured by Anja; one of the inks could be discriminated from the others following an examination with an ALS. The ink designated Sample 15, manufactured in 1975, was discernible from the other inks designated Samples 12, 13, and 14, which were manufactured in 1968, 1971 (October), and 1971 (February), respectively. Finally, Group IX included six blue ballpoint inks manufactured by Fisher. Samples 17 and 21, both manufactured at the same time, were differentiated from the remaining four inks when the TLC plate was visualized with an ALS.

Two of the four Formulabs ink formulations, designated Samples 1 and 2, had indiscernible colorant profiles within their respective groups when visualized with ambient light, but had significant differences when examined with filtered light. The differences were attributed to batch variations, as the inks were identified by the manufacturer with the same ink formulation number but produced at different times within the same year. Figure 1 depicts TLC plates with similar colorant profiles under ambient light for all batches of

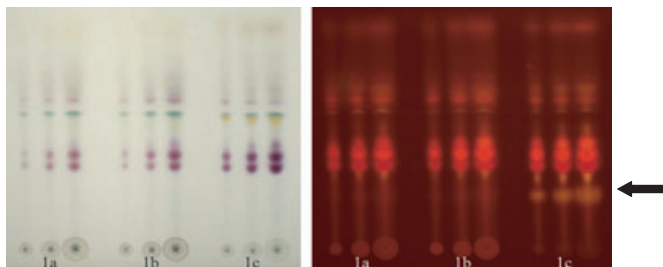


FIG. 1—(Left) Sample 1 (all batches) image captured using transmitted light (increasing spot concentrations shown: 0.5, 1.0, and 2.0 μL ; HPTLC plate). (Right) Sample 1 (all batches) image captured using filtered light (570-nm long-pass filter; increasing spot concentrations shown: 0.5, 1.0, and 2.0 μL ; HPTLC plate). Sample 1c has a distinct fluorescent band at $R_f \sim 0.35$. All inks shown were eluted with a solvent system composed of ethyl acetate, ethanol, and water (70:35:30).

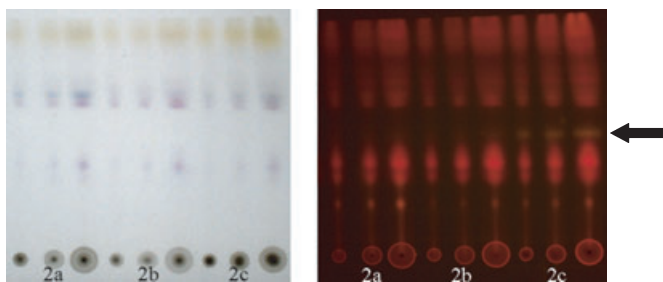


FIG. 2—(Left) Sample 2 (all batches) image captured using transmitted light (increasing spot concentrations shown: 0.5, 1.0, and 2.0 μL ; glass TLC plate). (Right) Sample 2 (all batches) image captured using filtered light (570-nm long-pass filter; increasing spot concentrations shown: 0.5, 1.0, and 2.0 μL ; glass TLC plate). Sample 2c has a distinct fluorescent band at $R_f \sim 0.45$. All inks shown were eluted with a solvent system composed of ethyl acetate, ethanol, and water (70:35:30).

Sample 1. Sample 1c clearly contains a distinct fluorescent band at $R_f \sim 0.35$, which is not present in Samples 1a or 1b. Figure 2 demonstrates that Sample 2c contains a distinct fluorescent band at $R_f \sim 0.45$. In addition, batch variations were observed in two other Formulabs ink formulations, designated Samples 7 and 8, when visualized with ambient and/or filtered light.

Comparisons were performed with artificially aged and fresh ink samples, as well as the same ink formulation from both metal and plastic cartridges. Sample 1 contained ink within plastic cartridges, and Sample 2 contained ink within both metal and plastic cartridges. When visualized with ambient light, the artificially aged inks in Sample 1 had darker purple bands at $R_f \sim 0.92$ on the chromatogram, while the fresh inks had yellow bands instead. Figure 3 illustrates the differences which are readily apparent with color photography. The differences observed may be because of a break-down product formed in response to the exposure to light or heat. When visualized with ambient light, all Sample 2 inks (i.e., fresh, exposed to heat, or exposed to high intensity light) contained within metal cartridges appeared to have more intense color bands than the inks from plastic cartridges. Additionally, ink samples contained within metal cartridges had purple/blue bands at $R_f \sim 0.97$ on the chromatogram, but the inks from plastic cartridges had yellow bands instead. These differences can be seen in Fig. 4; however, the yellow bands do not appear as dark as the purple bands when shown in black and white photography. Figure 4 also illustrates that the bands at $R_f \sim 0.97$ on the chromatogram appear

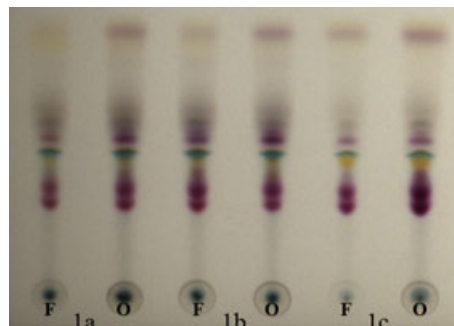


FIG. 3—Sample 1 (all batches) image captured using transmitted light (spot concentrations of c. 2.0 μL ; glass TLC plate) comparing fresh ink (“F”) to artificially aged ink through exposure to heat (“O”). The artificially aged inks have darker purple bands at $R_f \sim 0.92$ on the chromatogram, while the fresh inks have yellow bands instead. All inks shown were eluted with a solvent system composed of ethyl acetate, ethanol, and water (70:35:30).

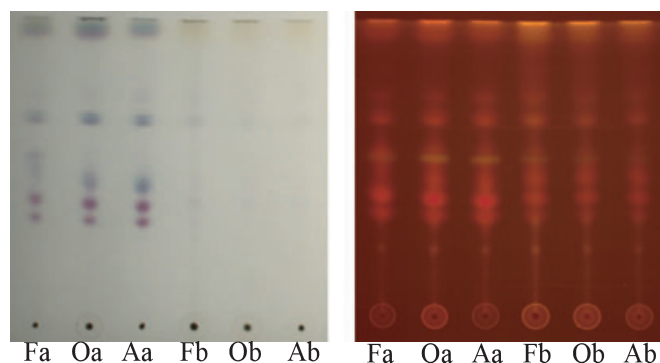


FIG. 4—(Left) Sample 2c image captured using transmitted light (spot concentrations of c. 2.0 μL ; HPTLC plate) comparing ink contained within metal cartridges (“a”) to ink contained within plastic cartridges (“b”). All ink samples [fresh (“F”), exposed to heat (“O”), or exposed to high-intensity light (“A”)] contained within metal cartridges have darker purple/blue bands at $R_f \sim 0.97$ on the chromatogram, while ink samples contained within plastic cartridges have yellow bands instead. (Right) Sample 2c image captured using filtered light (570-nm long-pass filter; spot concentrations of c. 2.0 μL ; HPTLC plate) comparing ink contained within metal cartridges (“a”) to ink contained within plastic cartridges (“b”). All ink samples [fresh (“F”), exposed to heat (“O”), or exposed to high-intensity light (“A”)] contained within metal cartridges and plastic cartridges appear similar when visualized with filtered light. All inks shown were eluted with a solvent system composed of ethyl acetate, ethanol, and water (70:35:30).

similar when visualized with filtered light. The observations noted may be because of a break-down product formed in response to the exposure to light or interference with the type of cartridge used. The authors did not identify the chemical composition of the aforementioned components.

Four groups of blue ballpoint inks and one group of black ballpoint inks appeared to have similar colorant profiles when visualized with ambient light, but had clearly distinct fluorescent bands when examined with filtered light. In Fig. 5, a distinct fluorescent band is found in Sample 3, providing discrimination between different ink formulations from the same manufacturer. In another example, Sample 5 and Sample 6 are different ink formulations from different manufacturers that appear identical under ambient light, but have distinctly different profiles when visualized with filtered light. Figure 6 displays a fluorescent band that is only present in Sample 6, enabling the ink formulation to be discriminated from all others. Typically, the distinct fluorescent bands were observed

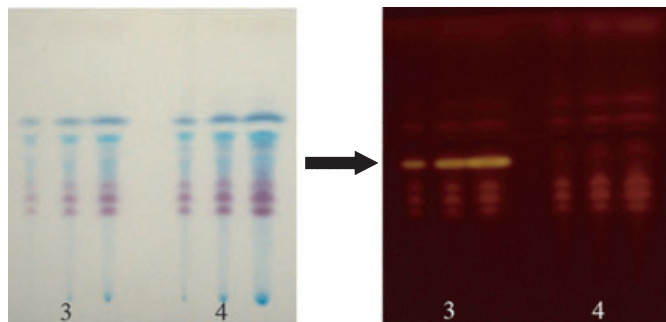


FIG. 5—(Left) Samples 3 and 4 image captured using transmitted light (increasing spot concentrations shown: 0.5, 1.0, and 2.0 μL ; HPTLC plate). (Right) Samples 3 and 4 image captured using filtered light (570-nm long-pass filter; increasing spot concentrations shown: 0.5, 1.0, and 2.0 μL ; HPTLC plate). Sample 3 has a distinct fluorescent band at $R_f \sim 0.50$. All inks shown were eluted with a solvent system composed of ethyl acetate, ethanol, and water (70:35:30).

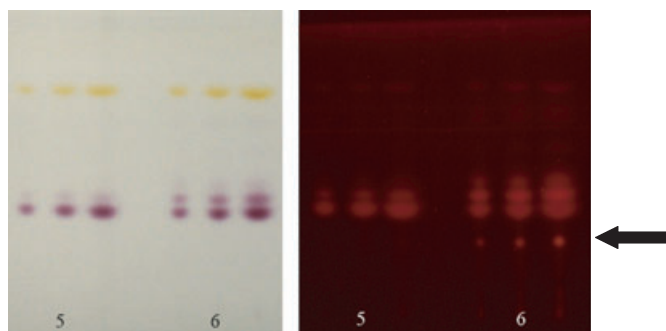


FIG. 6—(Left) Samples 5 and 6 image captured using transmitted light (increasing spot concentrations shown: 0.5, 1.0, and 2.0 μL ; HPTLC plate). (Right) Samples 5 and 6 image captured using filtered light (570-nm long-pass filter; increasing spot concentrations shown: 0.5, 1.0, and 2.0 μL ; HPTLC plate). Sample 6 has a distinct fluorescent band at $R_f \sim 0.30$. All inks shown were eluted with a solvent system composed of ethyl acetate, ethanol, and water (70:35:30).

using an ALS at a wavelength setting of 515 nm with red goggles; however, the previously mentioned distinct fluorescent band noticed in Sample 6 was observed using an ALS at a wavelength setting of 495 nm with orange goggles. Therefore, it is highly recommended to utilize a broad range of wavelength settings and various filters when utilizing this method to evaluate TLC chromatograms. Additional ballpoint ink formulations that appeared to have similar colorant profiles were studied and are listed in Tables 1 and 2, but only representative samples are displayed in the figures.

Possible explanations for the differences observed between batches of ballpoint ink, from the same formulation and manufacturer, when exposed to filtered light may be because of: (i) contamination between batches; (ii) the presence of impurities; or (iii) the presence of residual components. In addition, ink manufacturers may use a diverse number of components in the production of each ink formulation that can respond to particular wavelengths of energy in a unique way. Therefore, colorant profiles can vary between formulations as well as among ink manufacturers. Consequently, these components, in addition to the aforementioned explanations, may contribute to the differentiation observed between different ink formulations, from the same or different manufacturers, when exposed to filtered light utilizing an ALS. Further

research is needed to explain, and identify, the potential breakdown products shown on the chromatograms in the comparison of artificially aged ink to fresh ink samples, as well as ink from the same formulation contained within metal cartridges to plastic cartridges.

Conclusion

TLC is an efficient and effective method utilized to separate components and characterize ink samples. Chromatograms of inks that appear to have similar colorant profiles when visualized with ambient light can be subjected to further optical examinations in order to accurately differentiate between the inks. Twenty-nine ballpoint writing inks were subjected to TLC analysis and classified into nine groups that were considered to each contain inks with similar colorant profiles following ambient light visualization. Eleven of the inks from the nine groups that could not be conclusively discriminated were further differentiated following an examination with filtered light. The results obtained from this study clearly show that utilizing an ALS, with the appropriate filters, to evaluate TLC plates can provide additional discrimination following an examination with ambient light. Filtered light examinations provide an added dimension to identify components that are not visible to the unaided eye. Moreover, variations in batches of inks were detectable, but further research using more inks may be warranted before making definitive conclusions regarding manufacturing batch variations.

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