TECHNICAL NOTE

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Thin-Layer Chromatography of Writing Inks—Quality Control Considerations

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ABSTRACT: Quality control procedures for thin-layer chromotrography (TLC) are offered to increase reproducibility and dependability of results. During preparation of a TLC ink standard library, several types of inconsistencies were observed. Observations of more than 100 chromatograms of writing inks indicated the need for the institution of quality control procedures. Procedures were developed to ensure proper solvent selection, TLC plate selection, and proper photographic recording of TLC plates. Implementing quality control procedures in TLC enables the document examiner and forensic chemist to achieve these results.

KEYWORDS: forensic science, questioned documents, thin-layer chromatography, ink chemistry, quality control

Authenticity, as well as fraud, may be detected through analysis of inks. Thin-layer chromatography (TLC) is often used to compare two ink entries on a questioned document for the purpose of determining whether the two inks are the same or different. A difference may indicate alteration of the document. TLC is a simple, useful technique to separate mixtures of dyes and other organic materials in inks. Several authors have described TLC techniques used with microquantities of inks (1–3). Kelly and Cantu (4) went on to propose standardization of TLC techniques between laboratories. This paper illustrates inconsistencies found when TLC procedures were performed on inks and offers some practical remedies involving quality control procedures.

Methods

Document examiners in the Wisconsin State Crime Laboratory System (WSCLS) ran numerous TLC plates in preliminary work to establish a TLC standard ink library. The examiners compared chromatograms to gage reproducibility of results. A number of deviations from the expected occurred. Because of these deviations in TLC results, quality control procedures were developed. Quality control procedures ensure reproducible results through the following: (1) proper solvent selection and use, (2) TLC plate selection, (3) ideal ink concentration, (4) consistent origin positions, (5) use of standard inks, (6) elimination of equipment contamination, (7) storage and preservation of plates, and (8) proper photographic recording of TLC plates.

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Solvent Selection

Selection of the proper extraction solvent (solvent used to remove ink from a substrate—usually paper) and eluent (solvent used to separate dyes on a thin-layer plate) will affect the resulting TLC. In a recent case, portions of questioned handwriting from a wall were lost because acetone was selected as an extraction solvent. Acetone evaporated rapidly and dispersed the marker ink on the wall, leaving only a thin film in one particular area of writing to analyze. The dry film of marker material was difficult to extract with a pipette. Fortunately, additional writing remained on the wall. Pyridine was then used successfully to extract the solvent-based marker material from the wall. Pyridine solubilized the marker on the wall, forming a droplet of solution. The marker ink was then easily extracted from the droplet with a 1 μL pipette for spotting on a TLC plate.

Pyridine is the primary extraction solvent used with ballpoint pen inks in the WSCLS. Certain aqueous inks, however, will not extract with pyridine (2). In such instances an alternate extraction solvent such as ethanol/H_2O (1:1) may used.

N-Butanol/ethanol/distilled H_2O (2:1:1)(HPLC grade) is the eluent of choice in the WSCLS. Although it is effective in separating most dye mixtures, certain formulas of inks will separately poorly. Should this occur, other solvent systems must be used. Ethyl acetate/ethanol/distilled H_2O (70:35:30)(HPLC grade) is a good second choice. Other eluents such as cyclohexane/chlorobenzene/ethanol (10:2:1) should be tried if neither of the previously described systems are effective (2).

Microsampling techniques in TLC allow flexibility in selection of extraction solvent. One common technique involved punching three microdots of ink from a written line and placing them in a vial (4). One drop of extraction solvent was added to the three dots to determine solubility of the ink. If the extraction solvent failed to solubilize the ink in the vial, three more dots must then be punched from the ink line and a second extraction solvent dropped in to another vial. Kuranz (5) described a technique that is used in the WSCLS. One microdot is punched out of an ink line with a No. 16 hypodermic syringe (point cut off) and placed at the origin on a TLC silica gel plate. The extraction solvent of

choice in a 1 μL pipette (Microcaps, Drummond Scientific Co.) is then allowed to flow directly through the microdot on to the plate. Should the extraction solvent fail to solubilize the dye mixture, a second microliter of alternate solvent may be used on the same microdot. Use of this direct microdot sampling technique ensures proper extraction solvent selection with a limited sample size.

Solvent Use

Improper chamber saturation[2] may result in "edge effects." Stahl (6) noted further migration of spots closer to the edge of the plate than those in the center due to evaporation of solvent at the edges of the plate. This unwelcome effect in TLC may be eliminated by filling the developing tank with the appropriate amount of eluent, covering the tank, and allowing it to equilibrate for 15 min before developing a plate. The addition of filter paper along one side of the developing tank will further assist in saturation of tank vapors.

Origin Positions

Erratic placement of ink origin points on TLC plates produces inconsistent *Rf* values (*Rf* is the ratio of the distance traveled by the dye bands to the distance traveled by the solvent front) (7). Haphazard placement of the ink application point on a TLC plate undermines reproducible *Rf* values.

Ideal reproducibility is obtained using 13 mm by volume of solvent from the base of the plate in the developing tank. Ink origin positions are spotted exactly 19 mm from the base of the plate. The origin position on a plate is critical to reproducibility of *Rf* values of dye bands.

Figure 1 shows the same ink spotted and developed on a TLC plate from three positions: A is the ideal position (19 mm from the base of the plate), B is too high, and C is too low.

Visually, the band positions (not the *Rf* values) in Fig. 1 are similar. This occurs because the individual dyes in the sample possess characteristic affinities for the components of the eluent as it moves up the plate (8). Each dye is only carried so far by the eluent before it is separated. Visually, the band positions of the ink spotted are similar, even though the origin positions are not consistent. If, however, *Rf* values are measured, care must be taken to spot uniform origin positions.

Origin positions, as well as solvent flow rates and the changing composition of the solvent mixture as it travels up the plate, account for the differences observed in Fig. 1. Three different origin positions will produce three sets of *Rf* values for the same ink. Solvent flow rates also contribute to changes in separations of dyes from a single ink. The eluent front travels faster at the ink application point than it does further up the plate. This phenomenon is likely due to the strength of capillary forces at the starting point on the plate versus the force of gravity further up the plate pulling downward (personal communication with Jerry P. Kelly, former Chief Document Examiner; Wisconsin State Crime Lab-Madison, Nov. 1990). The favored eluent contains three molecules: *N*-butanol, ethanol, and distilled H_2O. These molecules have distinct polarity. Composition of the solvent mixture changes as it moves up the plate. Dyes from one ink may also separate differently due to changes in solvent system composition at various positions on the chromatogram.

Therefore, to obtain consistent dye separations, all application points must encounter the solvent front moving up the plate at the same time and position. Use of a 19-mm template for marking application points on TLC plates or careful measuring and marking with a ruler will ensure exact placement of ink application positions.

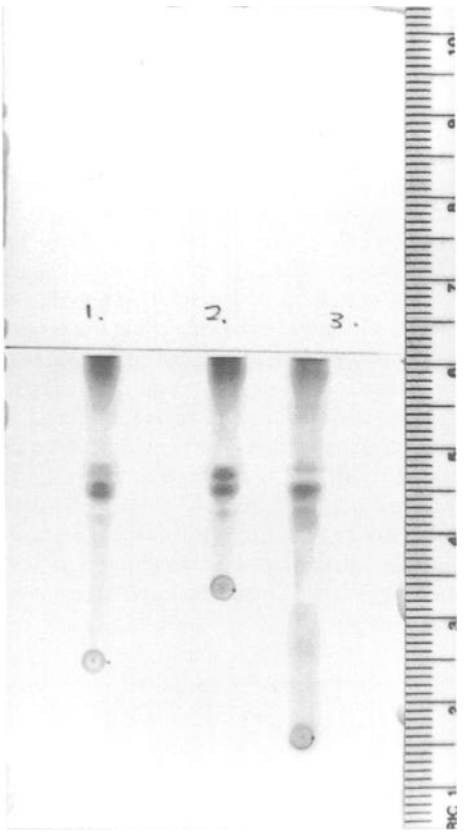

FIG. 1—*Photograph of ink origin positions depicting erratic placement of ink origin points: (1) 19 mm from base of plate is ideal position, (2) placement is too high, (3) placement is too low (dyes dissolve into solution). Ink: Formulab blue ballpoint pen ink (blue 373 1989); eluent: N-butanol:ethanol:H_2O (2:1:1); development time:25 min.*

TLC Plate Selection

Plate selection may affect the resolution of TLC dye bands and ease of handling. One type of TLC plate may develop five dye bands, whereas another only develops three dye bands for the same ink. Both silica gel 60 5 by 20 cm glass plates from E. Merck[3] and silica gel polyester TLC plates manufactured by Whatman Paper Ltd.[4] are used routinely. The glass plates provide better quality of resolution and sturdiness. The polyester-backed plates have certain disadvantages. The polyester-backed plates are difficult to write on with a pencil, which could be desirable for handling and identification purposes. Also, silica gel flake off along the edges. The Merck plates flake less, are easy to mark on, and can be adjusted to size. One advantage of the polyester-backed plates is that they are easier to cut, using ordinary scissors, than glass plates, which require glass cutters for adjustments. Silica gel glass plates generally provide better resolution of dye bands than the

[2]Developing chambers may be insufficiently saturated as a result of the mixture of solvents of varying densities used as eluents in TLC (6).

[3]Em Science, PO Box 70, 480 Democrat Rd., Gibbstown, NJ 08027-1296.

[4]Whatman Ltd., Maidstown, Kent, England. Made in West Germany.

polyester-backed plates. Although both types of plates are used in the WSCLS, the glass-backed plate is preferred for standard ink library work.

Ideal Ink Concentration

Proper loading or concentration of inks at the origin on a TLC plate is necessary to achieve accurate results. Cantu (9) explained how overloaded TLC plates in the Howard Hughes "Mormon Will" case initially made two distinct formulas of ink appear consistent.

Different concentrations of the same ink in TLC may produce different *Rf* values. Figure 2 shows a photograph of three applications of blue ballpoint pen ink spotted on the same TLC plate at three concentrations. The ideal concentration shows eight bands. The weak concentration failed to reveal one band visible in the more concentrated application. The heaviest concentration is overloaded, making it difficult to distinguish between bands.

Six to ten microdots of ink from areas where two ink lines intersect generally provides an ideal ink concentration for TLC. Experimentation with various concentrations of inks under supervision of an ink chemist will enable the forensic scientist to recognize ideal concentrations.

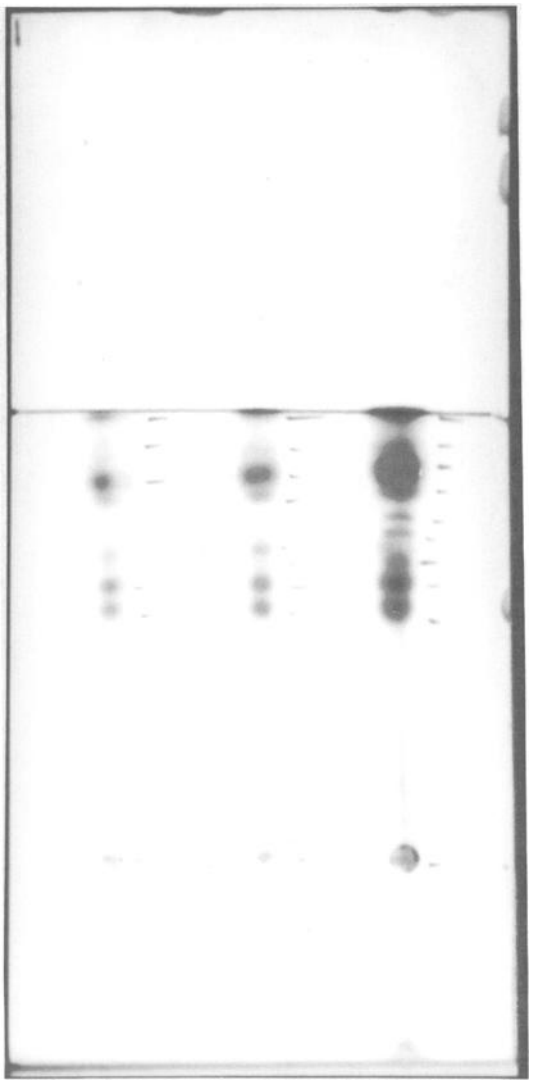

FIG. 2—*Various concentrations of the blue ballpoint pen ink used as a standard.*

Standard preparation for the TLC ink library will include three concentrations of each ink: light, medium, and heavy. The three concentrations of a single ink will be spotted and developed on the same TLC plate.

Use of Standard Inks

The performance of an eluent selected for TLC development can be ensured by running a standard ink on each plate. Should a solvent system become contaminated, the standard ink run on each plate provides an important quality control measure.

Elimination of Equipment Contamination

Equipment used in TLC may be contaminated by a previous ink. Microdot punches (cut off and sharpened hypodermic needles, 16 gage), if reused, can be cleaned by dipping in a vial, containing the appropriate extraction solvent between uses. If not, residues of inks or entire microdots of ink may lodge inside microdot punches and contaminate subsequent TLC runs.

Storage and Preservation of TLC Plates

Storage of developed TLC plates in a film box in the freezer prevents fading of the dye bands. TLC dyes exposed to light degrade rapidly. A clear acrylic spray may also preserve TLC plates (5).

Temperature Control of TLC

Few studies exist that explore the influence of temperature of TLC. However, one study found temperature reduction from 20 to 4°C showed negligible effects on time of run or *Rf* values in TLC (6). Stahl (6) recommended room temperature (approximately 20°C) as the ideal environment for running TLC plates. Good laboratory practice should include daily recording of working temperature for TLC runs.

Photographic Recording of TLC Plates

Color photographic slides (35 mm) are used to record TLC plates. Optimal color reproduction of the dye bands is achieved through conditions described in Table 1.

Conclusion

Those scientists familiar with TLC recognize that results can be capricious. As the saying goes, "Experience is a wonderful thing, for it enables you to recognize a mistake when you make it again." Recognition of inconsistencies in TLC will allow the

TABLE 1—*TLC photographic specifications.*

Film	Light Source	Exposure	Aperture
Ektachrome 160 T	3200 K	"gray card"	f 8
	Floods	$\frac{1}{4}$ s	
Ektachrome 200	Electronic	$\frac{1}{4}$ power	f 22.5
	Flash	$\frac{1}{60}$ s	

forensic scientist to avoid such pitfalls in future TLC work. Quality control procedures in TLC of writing inks will assist the forensic scientist in achieving reproducible results.

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