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EXAMINATION OF MICRO-QUANTITY OF BALL POINT INKS FROM DOCUMENTS BY THIN-LAYER CHROMATOGRAPHY

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For the document examiner, the crux in any method for the examination and identification of ink from a written line has been the limited amount of sample which could be obtained without defacement and damage to the document. Document examiners have sought a simple method that would afford the best possible resolution of components for identification, high sensitivity, accuracy, speed, and convenience.

A number of techniques for removing ink from writings and separating dye components have heretofore been described. Paper chromatographic methods (1, 2, 3, 4) have generally been used for separating dyes. Although satisfactory results have been obtained from paper chromatography, some preliminary results obtained from thin-layer chromatography (TLC) has revealed that the resolution pattern of dye components are definitely superior. It was apparent at the outset in experimenting with TLC, that this method had an inherent ability to produce dye spots which were more compact and their separation more discrete than those yielded by paper chromatography (Fig. 1).

Since the amount of ink which is removed from a writing is extremely small, TLC presented at once an opportunity for characterizing such a microquantity of sample. Its possible application in ink analysis was described by Doud (5) in 1958 and more recently by Witte (6). A solvent was sought in this Laboratory which would enable a quick separation of dye components on a microscope slide layered with silica gel preparation. While no all-purpose solvent mixture could be formulated for all ball pen inks, it was decided to standardize only one solvent mixture to simplify the procedure and to eliminate haphazard guesses as to which formula to use on a small amount of unknown ink available for examination. Since the amount of ink sample removed from a writing was limited, the chromatographic run necessarily had to be short with the best possible resolution of dyes at lower R_f values.

A number of techniques were tested for collecting ink samples from writing including those which entailed scraping (6), bleeding (3, 5), and punching (7) for application onto a chromatogram. One must be reconciled to some damage to the document upon removal of ink sample, however the latter technique of removing a minute disc from a written line with a hypodermic needle left a clean line without unsightly smears and tears along the sampling source. The methods for comparing inks as to their chromatographic characteristics and for spot testing the component dyes are described in the following paragraphs.

METHOD

Chromatoslides. Chromatography plates are prepared from 1″ x 3″ glass microscope slides (8). A large number of plates can be layered simultaneously using a commercial spreading apparatus and then stored in a desiccator for future use. However, if only a few slides are required in a single test, chromatoplates can be made rapidly

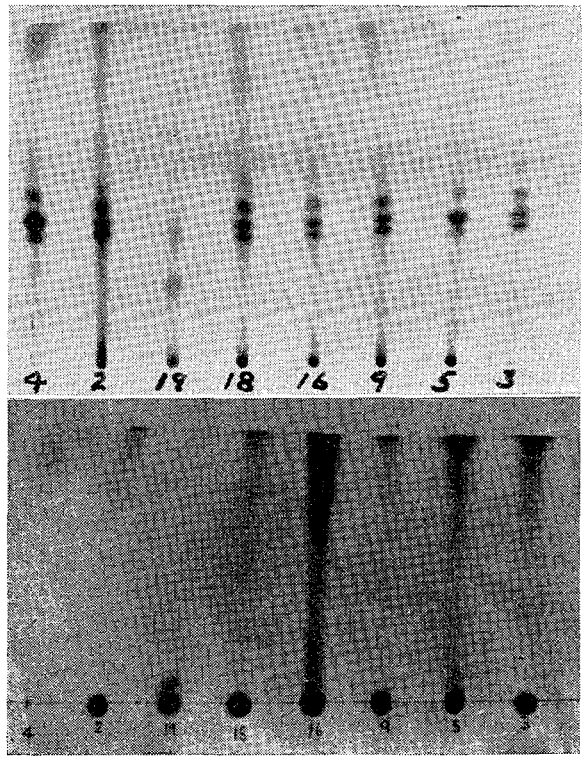

FIGURE 1

Comparison of a thin-layer chromatoplate (top) and a paper chromatogram (bottom) using the same solvent mixture (1-butanol:50, ethanol:10, water:15) on the same group of blue ball pen inks.

Other solvent systems described in (1, 2, 3, 4) improved the resolution of dyes by paper chromatography but they did not afford nearly the separation exhibited by TLC as in Figure 1b. Legend: (4) Zebra. (2) Tombow Crown. (19) Eversharp KEC. (18) Wearever. (16) Eversharp. (9) Script. (5) Ballograf. (3) Ballcon.

by dipping slides into a small jar containing 10 grams of "Wakogel B-5" (Silica gel powder containing 5% calcium sulfate as binder, Wako Pure Chemical Industries, Osaka, Japan) suspended in 38 ml of chloroform. Layer thickness can be measured with a micrometer ordinarily used for measuring the thickness of papers. Optimum thickness for TLC of ink in this method was found to be approximately 250 microns. Typical "microchromatoplates" showing separation of ball point ink dyes are presented in Figure 2.

Sampling. Using a hypodermic syringe needle with its point removed and resharpened (7), sizes 17 to 20, depending on the thickness of the writing stroke, punctures are made along the written line especially at junctures showing a clot of ink. Three or four discs are removed, with the aid of a spatula or razor blade if necessary, and then transferred to a well of a spot plate. An amount of pyridine sufficient to cover the discs is introduced to extract the ink. The process can be speeded by prodding the discs with the tapered point of a drawn glass rod. The paper fragments are moved to the edge of the well, and the extract is evaporated to near-dryness. Then the entire ink extract is removed by a capillary tube having an inside diameter of about 0.5 mm and deposited onto a chromatoslide, effecting a spot about 2 mm in diameter and about 15 mm from the bottom edge of the slide. The spot should be dried to remove the pyridine prior to chromatography; a hot air blower facilitates this step.

Chromatography. The chromatoplate bearing the unknown ink spot and a standard ink spot for comparison is placed in a small jar such as that used commercially for pickles, jellies, etc. An amount of solvent mixture is placed in this jar to immerse the end of the chromatoplate without touching the applied ink spot. The solvent mixture is comprised of 50 parts of 1-butanol, 10 parts of absolute ethanol, and 15 parts of water. The chromatographic run is completed within twenty

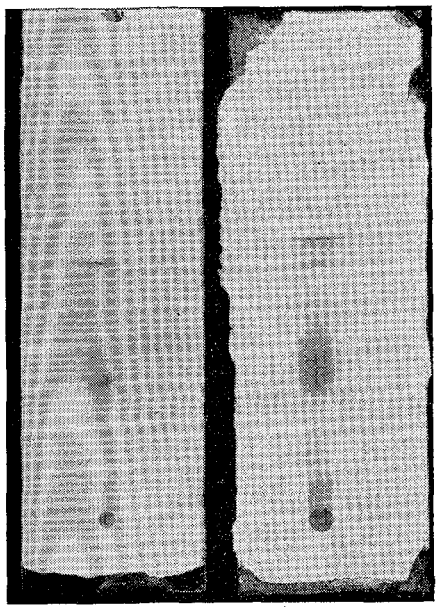

FIGURE 2

Micro-chromatoplates showing separation of two different ball pen inks. The ink was removed from a writing on a document by the punch method utilizing a hypodermic needle.

minutes. The slides are removed and air dried, and may later be photographically recorded.

Spot Tests. With the aid of a pointed instrument, four or more small portions of each separated dye is removed from the chromatoplate and transfered to wells of a spot plate. To the four wells containing particles of dye-impregnated silica gel, concentrated HCl, concentrated H_2SO_4, NH_4OH, and 10% NaOH are introduced in small drops from a capillary pipette to the respective wells. Color changes are noted, then a drop or more of a saturated sodium carbonate solution is added to the "HCl" well to observe any further change; many dyes undergo an indicator type shift back to the original color due to change in pH (2). Solubility of the separated dyes, then, are tested in water, absolute ethanol, acetone, and chloroform in four additional wells. Agitation is provided with a thin glass rod or a capillary tube having a sealed tip. Observation under a microscope set at four to ten power is an aid to these micro-spot tests.

For reference, Table I lists some of the common dyes distributed by major chemical and dye manufacturers in the United States for the manufacture of colored writing inks and their immediate reactions to these test reagents and their responses to ultraviolet and infrared lights. This list of dyestuffs is not to be construed as being complete for ball pen ink dyes and is presented only to serve as a guide. For presumptive identification, an actual comparison examination with a standard dye sample would be indispensable.

DISCUSSION

Ostensibly, it seemed forensically advantageous to identify the dye components of ink instead of characterizing ink by commercial brands, inasmuch as manufacturers change formulation frequently without notice. Some sixty or more commercially prepared dyestuffs which are used in the manufacture of colored inks were tested on our chromatoplates. While most of them were chromatographically pure compounds, some of them were shown to be mixtures containing presumably isomers and/or unreacted constituents as has already been suggested by Coldwell (1) in his discussion of dye identification. For this reason, direct spot testing of some of these dyes was valueless for our purpose and the principal spot from each separated dye preparation on our chromatoplates was tested with solvents and reagents listed in (1), (2), and (3).

While a presumptive identification can be made of some of the more common dyes used in the manufacture of ball pen ink, the primary value of spot testing must remain in the comparison of a questioned ink sample from that of a standard. The examination of blue ink samples from popular American and Japanese ball point pens selected at random (e.g.: Playmate, Parker, Script, Pilot, Mitsubishi, etc.) revealed that the dye formulation employed in the manufacture of ball pen ink is not too diverse, and the TLC method and spot testing confirmed that such dyes as methyl violet, rhodamine-B, Victoria blue, phalocyanine blue, and alkali blue are of common occurrence.

While only a few red dyes exhibited fluorescence in ultraviolet light, the marked difference particularly shown by eosin and rhodamine furnished an added criterion for their identification. Nearly all of the dyes were made invisible by infrared photography, hence differentiation of these compounds by this procedure was not of great value.

Black ink containing nigrosine or indulin type of dye, was difficult to resolve in any of the many solvent mixtures tested; the dye manifested itself on the plates as streaks and not as spots. Such dyes, therefore, were not listed in Table I. The formulation of dyes in fluid inks, as they were characterized by this TLC method using the standard solvent mixture and those using 1-butanol:50,

TABLE I

CHARACTERISTICS OF SOME DYESTUFFS USED IN COLORED WRITING INKS

Dyestuffs	Colour Index	R_f	H_2O	Ethanol	Acetone	HCl	$NaHCO_3$	H_2SO_4	NaOH	NH_4OH	$CHCl_3$	UV	IR	Remarks
BLUE														
Cyananthrol RXO (GAFC)	Acid Blue 47	.7	s	s	i	v	bl	v	bl	bl	i	—	—	
Victoria Blue B base (PP)		.5, .6	i	s	s	o	bl	rbr	i	i	s	—	—	
Victoria Blue Pure BO (NA)	Basic Blue 7	.6, .7	i	s	s	o	bl	ybr	i	i	s	—	—	
Victoria Blue BO base (NA)	Solvent Blue 5	.6, .7	i	s	s	o	blg	ybr	i	i	s	—	—	faint green at Rf 1.0
Victoria Pure Blue (PP)		.6, .7	i	s	s	o	bl	gry	i	i	s	—	—	
Anthraquinone Blue 3G DP	Acid Blue 3G	.6	s	i	i	v	bl	grbl	i	bl	i	—	—	
Brilliant Milling Blue (NA)	Acid Blue 15	.5	s	s	i	o	bl	o	i	bl	s	—	—	
Alphazurine 2G (NA)	Acid Blue 1	.3, .5	s	s	i	y	bl	y	bl	bl	i	—	—	
Pontamine Sky Blue 6BX (DP)	Direct Blue 1	.1	s	i	i	i	bl	bl	bl	bl	i	—	—	
Pontamine Fast Turquoise (DP)	Direct Blue 86	0	s	i	i	i	bl	gr	grbl	grbl	i	—	—	
Phthalocyan Blue (PP)		0	i	s	s	gr	bl	gr	bl	bl	i	—	+	
Alkali Blue (PP)		.2–1.0	i	s	i	i	—	r	i	i	i	—	—	
RED														
Eosin (PP)		.7 (st)	s	s	s	i	o r	y	o r	o r	s	ygr	—	
Rhodamine 6G D N (NA)	Basic Red 1	.6	s	s	s	o	v	y	i	i	s	ygr	—	
Rhodamine B (PP)		.5	s	s	dis	o	v	y	p	p	s	o	—	
Rhodamine B (NA)	Basic Violet 10	.5	s	s	s	o	v	y	p	p	s	o	—	
Safranine A (NA)	Basic Red 2	.5	s	s	i	br	r	gr	i	r	i	o	—	
Wood Stain Scarlet NS (DP)	Acid Red 73	.5	s	i	i	bl	r	v	br	rbr	i	—	—	
Brilliant Croceine 3BA-CF (GAFC)	Acid Red 73	.5	s	i	i	bl	r	v	br	rbr	i	—	—	
Fast Crimson GR Conc (NA)	Acid Red 1	.3	s	i	i	r	—	r	brr	brr	i	—	—	
Carmoisine BA Extra Conc CF (GAFC)	Acid Red 14	.3	s	i	i	r	r	v	r	r	i	—	—	
Brilliant Scarlet (NA)	Acid Red 18	.1	s	i	i	v	r	v	br	br	i	—	—	
Azo Fuchsine 6B Extra Conc (GAFC)	Acid Violet 7	0	s	i	i	r	v	r	br	br	i	—	—	
Erie Fast Orange WS (NA)	Direct Orange 102	0	s	i	i	i	r	rv	rbr	rbr	i	—	—	
Erie Fast Scarlet 4BA (NA)	Direct Red 24	0	s	i	i	i	r	v	rbr	r	i	—	—	
Solantine Red 8BL (NA)	Direct Red 79	0	s	s	i	bl	r	bl	v	v	i	—	—	

GREEN														
Brill. Mill. Green B. (DP).....	Acid Green 9	.4	s	s	i	y	blgr	y	i	bgr	i	–	–	blue-green
Acid Green L. Extra (NA)..	Acid Green 3	.4	s	s	i	o	gr	o	dis	dis	i	–	–	blue-green
Alkali Fast Green (NA).....	Acid Green 16	.3	s	s	s	o	gr	y	gr	gr	i	–	–	bright-green
Pontamine Green 2GB (DP).....	Direct Green 12	.1–.5 (st)	i	s	i	i	i	v	blbk	blbk	i	–	–	forest-green
Pontamine Green BXN (DP).....	Direct Green 6	.1–.5 (st)	i	i	i	i	i	blbk	gr	gr	i	–	–	forest-green
Naphthol Green B (GAFC).....	Acid Green 1	.1	s	i	i	y	gr	y	gr	gr	i	–	+	olive-green
Naphthol Green B (NA).....	Acid Green 1	.1	s	i	i	y	gr	y	gr	gr	i	–	+	olive-green
Naphthol Green B Extra Conc. (DP).....	Acid Green 1	.1	s	i	i	y	gr	y	gr	gr	i	–	+	olive-green
PURPLE VIOLET														
Wool Blue (DP).....	Acid Blue 59	.8	s	s	s	i	dis	gr	i	v	i	–	–	
Methyl Violet Base 4BN (PP)....		.5–.7	s	s	s	o	v	o	i	v	s	–	–	
Methyl Violet 2B (NA).....	Basic Violet 1	.5–.7	s	s	s	o	v	o	i	v	s	–	–	
Crystal Violet 6B (NA).....	Basic Violet 3	.5–.7	s	s	s	o	v	o	i	v	s	–	–	
Methyl Violet Base (NA).....	Solvent Violet 8	.5–.7	i	s	s	brgr	v	brgr	i	i	s	–	–	
Paper Blue R (DP).....	Basic Violet 3	.5–.7	s	s	s	o	v	o	i	v	s	–	–	
Wool Violet 4BN (NA).....	Acid Violet 49	.4–.6	s	s	i	o	v	o	i	v	i	–	–	
Pontacyl Violet S4B (DP).....	Acid Violet 17	.4–.6	s	s	i	o	v	o	i	v	i	–	–	
Pontacyl Blue Black SX (DP)....	Acid Black 1	.6	s	s	i	br	blbk	blgr	bl	bl	i	–	–	
Naphthylamine Black 10BR (GAFC)	Acid Black 1	.3	s	s	i	i	i	blgr	bl	bl	i	–	–	
Buffalo Black NBR (NA).....	Acid Black 1	.3	s	s	i	br	i	blgr	bl	bl	i	–	–	
Pontamine Brill. Violet B. (DP)...	Direct Violet 9	.2 (st)	s	s	i	bl	v	gr	i	v	i	–	–	
Pontamine Violet N. (DP).....	Direct Violet 1	.1 (st)	s	i	i	i	dis	bl	v	v	i	–	–	
Fast Wool Blue R. (NA).....	Acid Blue 92	.1, .2	s	i	i	i	v	blgr	brr	brr	i	–	–	
ORANGE														
Acid Orange (GSFC).....		.6	s	i	i	r	o	r	o	o	i	–	–	
Croceine Orange Y (NA).....	Acid Orange 12	.6	s	i	i	o	o	yo	o	o	i	–	–	
Wool Orange 2G (NA).....	Acid Orange 10	.3	s	i	i	o	–	o	brr	o	i	–	–	
YELLOW														
Quinoline yellow (NA).....	Acid Yellow 3	.7	s	i	i	y	y	y	i	y	i	–	–	
Auramine O (NA).....	Basic Yellow 2	.7	s	s	i	dis	y	dis	i	i	s	–	–	
Tartrazine C Extra CF (GAFC)...	Acid Yellow 23	0	s	i	i	y	y	y	y	y	i	–	–	
Wool Yellow Ex (NA).....	Acid Yellow 23	0	s	i	i	y	y	y	y	y	i	–	–	

Legend: s—soluble, i—insoluble, bl—blue; o—orange, r—red, gr—green, bk—black, br—brown, y—yellow, v—violet/purple, p—pink, dis—discolor, st—streak
GAFC—General Aniline & Film Corp., N.A.—National Aniline Div., Allied Chemical Corp., DP—DuPont DeNemour Co., PP—Pilot Pen Co., Japan.

10% oxalic acid:15, absolute ethanol:10; and 1-butanol:50, 6 N ammonium hydroxide:15, absolute ethanol:10, clearly revealed that the more popular blue "fountain pen" inks such as Parker, Script, Pilot, etc., contained seven or more blue dye components and in some cases red and green dyes.

Cartridge inks of various popular American and Japanese brands were found to contain, by the present TLC method, the same dyes as their bottled ink counterparts. For example, Parker blue Quink in both bottle and cartridge form had the same dye components.

In testing fluid inks by TLC, there was some difficulty in completely removing the ink from discs punctured from an ink line. A number of solvents including acids, alkali, and salts including potassium binoxalate (4) were tested, but none afforded complete removal; some residual ink, presumably pigments, remained in the fibers. Fluid ink also had a tendency of penetrating into the silica gel layer leaving only a light spot on the surface. These losses seriously hampered micro-application such as described here for ball pen ink. Further study is being conducted to establish a micro-application method for fluid inks.

A number of commercial silica gel preparations were tested, and it was found that the amount of binder substance, calcium sulfate, had an appreciable effect on the resolution of ink dyes. The more commonly used "Silica Gel-G" containing 13% calcium sulfate, although offering comparable results, did not resolve some of the ball point ink samples in the manner effected by "Wakogel B-5" which has a 5% calcium sulfate inclusion. A silica gel preparation containing no binder, "Wakogel B-0", offered results comparable to those of "Wakogel B-5" indicating that the amount of calcium sulfate does affect resolution of ink dyes on TLC.

The advantage of the TLC method is in the high resolution of dyestuffs as compared with that of paper chromatography, for example, methyl violet will resolve characteristically into a formation of three or four spots (Fig. 2) strung like violet beads. None of the paper chromatography systems (1, 3, 4, 8) which we have tested make this characterization. The separation is sufficiently discrete to allow examiners to remove small portions from dye spots for micro-tests. Also, this procedure precludes having to bleed the ink writing with acid directly on the document for dye identification such as done by Crown, et al (2). This will prevent further damage to the writing and hence to the document.

Summary

A thin-layer chromatography system is described for characterizing small amounts of blue and blue-black ball point inks collected from writings. A procedure is described for removing portions of the separated dyes for further characterization by spot testing. A table listing commercial dyestuffs used in the manufacture of colored writing inks, their R_f values, solubility and color reaction in acids and bases are reported.

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