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Review

Chromatographic and electrophoretic approaches in ink analysis

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Abstract

Inks are manufactured from a wide variety of substances that exhibit very different chemical behaviors. Inks designed for use in different writing instruments or printing methods have quite dissimilar components. Since the 1950s chromatographic and electrophoretic methods have played important roles in the analysis of inks, where compositional information may have bearing on the investigation of counterfeiting, fraud, forgery, and other crimes. Techniques such as paper chromatography and electrophoresis, thin-layer chromatography, high-performance liquid chromatography, gas chromatography, gel electrophoresis, and the relatively new technique of capillary electrophoresis have all been explored as possible avenues for the separation of components of inks. This paper reviews the components of different types of inks and applications of the above separation methods are reviewed. Published by Elsevier Science B.V.

Keywords: Reviews; Ink

Contents

1. Introduction

The forensic ink chemist faces more complex and challenging analytical problems as sophisticated writing instruments and printing technologies have evolved. Modern demands for specialized writing and printing instruments have resulted in an explosion of ink formulations, each of which may contain dozens of chemical components. The wide array of materials used in inks, coupled with the possibility of

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contamination from the writing surface, as well as chemical changes as ink ages, make separation techniques requisite for the analysis of such a complex mixture. However, chromatography and electrophoresis were not among the first techniques used for this purpose.

Prior to 1950, separation techniques were foreign to the field of ink analysis because courts were unreceptive to destructive techniques and because the methods themselves were in fledgling stages of development. Document examiners relied upon filter photography, alternate light sources, and chemical spot tests to differentiate ink samples. Nondestructive methods such as infrared and diffuse reflectance infrared spectroscopy [1–3], microspectrophotometry [4–10], visible and infrared luminescence [11–19], and dichroic filter viewing [12] remain important, valuable tools for the document examiner.

Because minimally destructive techniques are now widely applied to ink differentiation problems, chromatography and electrophoresis have been explored as potential methods for differentiation of ink samples. Described in more detail below, thin-layer chromatography (TLC) is one of the most popular methods because of its ease of use and its ability to quickly generate qualitative information unavailable through nondestructive spectroscopy. Although TLC is quite cost effective and sufficient for differentiation of many writing inks, much research has been devoted to exploring applications of instrumental techniques such as high-performance liquid chromatography (HPLC) and capillary electrophoresis (CE) for ink analysis. Gas chromatography (GC) has also been applied, often for the analysis of volatile components in ink dating procedures. It is expected that the use of these methods will further improve the chemist's ability to distinguish between ink samples due to their greater sensitivity and resolving power. It is the purpose of this paper to explore the many types of chromatography and electrophoresis that have found application in ink analysis, as well as to provide a brief introduction to common types of materials the ink chemist may encounter in an ink sample.

2. Components of major types of ink

The composition of any ink depends on its intended function. Alteration of ink components during the manufacturing process changes properties such as tackiness, drying characteristics, color, cost, fluidity, or resistance to degradation by light, heat, water and other environmental factors. In the case of writing utensils, different types such as fountain, rollerball, ballpoint, felt-tip, gel, and other pens have different mechanisms for placing writing on a page and therefore have different requirements in terms of the properties of the ink suitable for use. For example, ballpoint pen inks are thick and viscous and generally contain higher proportions of organic dyes than other pen types, such as rollerballs or felt-tip markers, that are characteristically lighter and more fluid. Naturally, differences between formulations designed for the same class of writing utensil may be quite great between manufacturers and even within a single ink manufacturer. Printing inks are similarly variable because different printing processes (such as lithography, intaglio, or digital processes such as inkjet) require inks with characteristics suitable for the process used. In any case, familiarity with the printing process used to create a document may suggest some initial compositional information. Some general categories of materials used in inks are shown in Table 1.

Ink components may be classified by their functions in the ink matrix. The liquid components are collectively referred to as the vehicle and include materials such as oil, solvents and resins that help shape the inks flow characteristics, drying mechanism, drying time, polarity, and cost. Coloring matter may be added as a combination of dyes and pigments. Dyes are distinguished by their solubility in the vehicle, whereas pigments are included as very finely ground dispersions that are not solubilised. Other components such as waxes, plasticisers, driers and other miscellaneous materials may also be added. Further information on the manufacture and composition of inks and related materials is available [20–22]. Familiarity with the processes and materials used from ink manufacture to actual writing or printing will assist the forensic chemist in selecting suitable analytical methods for ink analysis.

3. Chromatographic techniques

3.1. Paper chromatography

Dating from the early 1950s, the first chromato-

Table 1
Components of inks

Ink component	Characteristics	Properties affected
Coloring material:		Appearance
Dyes	Classified as acidic, basic, solvent, etc. depending on characteristics Soluble in vehicle	
Pigments	Consist of finely ground multimolecular granules Insoluble in vehicle	
Vehicle:		Flow and drying characteristics
Oils	Can be linseed, soy, mineral or other type of oil Classified as drying, non-drying, or combination, depending on degree of unsaturation of oil	
Solvents	Can be any of several organic solvents, or water Analyzed in many ink dating procedures	
Resins	Noncrystalline material of high molecular weight May be natural or synthetic	
Other additives:		
Driers	Catalyze oxidation of drying oils Many are inorganic salts	Drying characteristics
Plasticisers	Reduces brittleness of ink Consist of solvents with low volatility	Stability of ink film
Surfactants	Changes surface tension of ink Typically consist of soaps or detergents	Wetting ability
Waxes	Increase flexibility and reduce brittleness May be hydrocarbon waxes, greases such as petroleum jelly	Hardness/flexibility

graphic method used to analyze inks was paper chromatography. These efforts were driven in part by the increased production of inks based on organic dyes, to which chromatographic methods are particularly appropriate. The earlier iron-gallotannate inks are not easily separated by chromatography.

Among the first published articles on the topic were methods of separating liquid and dried ink components on filter paper strips using distilled water [23] or organic solvents [24]. In 1954 [25], a method was described for the analysis of fluid and dry ballpoint pen inks by horizontal paper chromatography, in which separation was carried out on a circular piece of filter paper with dots of ink arranged in a circle near the center of the paper. The mobile phase flowed by capillary action through a paper ‘wick’ onto the center of the filter paper, where it spread radially and enacted the separation, yielding a colorful array of separated dye components. Further work on ballpoint pen inks was done that combined horizontal paper chromatography in various organic solvents with chemical spot tests to attempt identification of individual dye components in liquid inks [26]. A wide variety of liquid and dry inks, including among others, fountain pen inks, ballpoint pen inks, and stamp pad inks, were also analyzed by horizontal paper chromatography [27].

Although it remained a popular method of discriminating inks into the 1960s, paper chromatography has been effectively replaced by TLC because of the latter’s superior resolution. Inability to completely distinguish ink samples was a frequent problem encountered by researchers in paper chromatography. Extraction solvent selection and extraction of sufficient ink were also difficult problems, and the procedure was tedious. Nonetheless, in 1989 Lederer and Schudel [28] proposed a paper chromatographic system for ink analysis suitable for small police agencies with minimal laboratory facilities. The system suggested, although limited in sophistication, is extremely cost-effective, generates no hazardous waste, and requires minimal space in which to operate.

3.2. Thin-layer chromatography

As chromatographic science developed, thin layers of silica gel, cellulose, or other materials gradually replaced filter paper as the stationary phase of choice. One precursor to modern TLC is disk chromatography [29], that was developed in the early 1950s. The questioned ink sample was extracted from the document using a suitable solvent and placed at the center of a circular disk of dry talc slurry deposited on a microscope slide. Drops of solvent were gradually added to the center of the slurry, creating a circular chromatogram as the solvent flowed from the center of the disk outward. Since then, TLC techniques have undergone many developments. Much research has been devoted to optimizing solvent systems for TLC of inks, and a wide variety have been suggested for use with different stationary phases.

Brunelle describes two solvent systems in his suggested ink identification procedures [30]. These include ethyl acetate–solute ethanol–distilled water (70:35:30) and *n*-butanol–ethanol–distilled water (50:10:15), with 30-min development of spotted ink samples completed on Eastman precoated silica gel sheets. Another standard method proposed by Kelly and Cantu [31] utilized silica and cellulose stationary phases and two mobile phases, consisting of *n*-butanol–isopropanol–distilled water (2:1:1) and *n*-butanol–ethanol–10% aqueous oxalic acid (50:10:15). Tappolet has reported optimized HPTLC separation conditions for various colors of fountain, ballpoint and fiber-tipped pens [32]. TLC separation conditions suggested by various researchers are indicated in Table 2.

Among the many improvements suggested are reductions in the amount of ink sample necessary to perform TLC [33,34], improved separation of dyes with similar Rf values [35], on-line equilibrium sandwich chamber extraction [36], and suggestions for quality control [37]. Furthermore, higher resolutions have been demonstrated with the application of high-performance thin-layer chromatography (HPTLC), in which the particles composing the thin layer are of substantially smaller size than in conventional TLC [32,38].

TLC has been shown to be effective for the analysis of ballpoint inks [39–41], typewriter ribbon inks [42], fiber-tipped pen inks [43], felt markers [41] and inks of other types. However, solvent systems designed for writing inks are often unsuitable for India inks and printing inks because they contain many insoluble pigments that are not well suited to separation by TLC. This problem has been addressed by separation of synthetic organic pigments using a large number of solvent systems [44], each applicable to separation of different combinations of pigments, and, more recently, using three solvents run sequentially to consecutively shorter distances on the same plate [45]. Like paper chromatography, TLC of inks produces a colorful separation of dye components, but the noncolored components remain invisible without additional visualisation procedures.

Although spectral methods are frequently applied to intact inks prior to extraction from paper, some researchers found it effective to use alternate light sources [46], infrared luminescence [47,48], or microspectrophotometry [49] to characterize components following separation by TLC.

3.3. High-performance liquid chromatography

While TLC remains the most widely used chromatographic technique for the analysis of inks, because of its ease of use and low cost, its resolution is limited. It is often incapable of differentiating between ink formulations or distinguishing between different batches of ink of the same general formula. Furthermore, TLC does not permit ready viewing of noncolored components of inks. In such cases, HPLC offers more resolving power and may generate quantitative information on colored or noncolored components. HPLC has successfully differentiated many ink samples indistinguishable by TLC. Nonetheless, HPLC requires greater technical skill on the part of the operator, a larger ink sample, and a greater monetary investment than TLC. Consequently, it remains less popular than the latter for routine analyses.

In the first published application of HPLC to ink analysis, Lyter used a normal-phase silica column to successfully distinguish a number of ballpoint pens with a mobile phase of dichoroethane–ethanol–formamide (89:10:1) [50]. Since then most researchers

Table 2
Solvent systems for TLC of inks

Researcher(s)	Solvent system(s)			Reference
	Number or ink type	Composition	Parts	
Brunelle and Pro	(1)	Ethyl acetate	70	
		Absolute ethanol	35	[30]
		Distilled water	30	
	(2)	*n*-Butanol	50	
		Ethanol	10	
		Distilled water	15	
Kelly and Cantu	(1)	*n*-Butanol	2	
		Isopropanol	1	[31]
		Distilled water	1	
	(2)	*n*-Butanol	50	
		Ethanol	10	
		10% Oxalic acid	15	
Milovanovic, Ristic-Solajic, Janjic	Synthetic organic pigments in artists' paints	Many different solvent systems are suggested for separation of various combinations of pigments	See Ref.	[44]
Tappolet	*Fountain pen inks*:			
	Black, blue-black, royal blue, or blue	Isobutanol	20	[32]
		Ethanol	5	
		99% Acetic Acid	5	
	Red inks	Distilled water	10	
		Isopropanol	12	
		1-Pentanol	22	
		Distilled water	6	
	Green inks	Conc. formic acid	3	
		Butanol (saturated with water)	97	
	Ballpoint pen inks:			
	Black and blue	Ethyl Acetate	70	
		Ethanol	35	
		Distilled water	30	
	Blue and red	Methanol	2	
		n-Propanol	10	
		I-Pentanol	26	
		Distilled water	4	
	Fiber-tip pen inks:			
	Black and blue	Isopropanol	10	
		1-Pentanol	25	
		Distilled water	5	
	Black	Isobutanol	23	
		Isopropanol	10	
		Distilled water	7	
Aginsky	Printing inks, artists' paints, or copy toners	Sequentially:		
		(1) Chloroform (10 cm)	–	[45]
		(2) Ethyl acetate (7 cm)	30	
		Isopropanol	15	
		Water	10	
		Acetic acid	1	
		(3) Conc. H_2SO_4 (2 cm)	–	

have adopted reversed-phase systems. Later, Lyter separated ten ballpoint pen inks not distinguishable by TLC [51,52]. He reported that HPLC was capable of detecting subtle batch-to-batch differences. The procedure used a C-18 column with a mobile phase consisting of acetonitrile–water (80:20) with 5 m*M* Pic B-7 reagent. Keto presented a method of analyzing alkali blue in printing ink, one of the extractable components of such inks [53]. He used a gradient elution system consisting of mixtures of methanol and water, with a C-18 column for the analysis of alkali blue. In all of these examples, detection was accomplished with one or two wavelengths in the ultraviolet region.

Other researchers utilized multiwavelength detectors, that are particularly suitable because they generate spectral as well as chromatographic data that may assist in the identification of separated components. White and Wheals' procedure for ballpoint inks used a rotating disk detector capable of rapidly scanning four wavelengths that provided additional spectral information while requiring minimal data storage capacity [54]. They tested three reversed-phase columns and an optimized eluent consisting of acetonitrile–tetrahydrofuran–water (924:432:644). Tebbett et al. found that a diode-array detector scanning from 200 to 800 nm was effective in differentiating closely related inks [55]. They tested five combinations of mobile and stationary phases and the most effective was a Spherisorb 5 μm ODS column with a mobile phase of acetonitrile–water (80:20) with 0.005 *M* heptanesulfonic acid at pH 4.7. Analysis was possible using a much smaller sample of ink (on the order of picograms) than previously necessary. In 1993, Lofgren and Andrasko reported a reversed-phase method that employed three detectors (visible, UV and fluorescence) connected in series [56]. In addition, they describe use of sequential solvent extractions for removing printing inks from paper, a troublesome problem because the pigments often become trapped in the drying vehicle.

3.4. Gas chromatography

Because most visible components of ink are virtually nonvolatile, GC has been primarily applied to the dating of inks by analysis of the volatile components that comprise the vehicle. Treatment of this subject follows in the section of dating of ink. Pyrolysis GC has been used more in the non-dating chemical analysis of photocopy toners [57–61] than ink, though one application has been reported for ink and paper samples [62].

4. Electrophoretic techniques

4.1. Traditional electrophoresis

In 1954 Brown and Kirk reported an early application of paper electrophoresis to ink analysis and its comparison to paper chromatography [63]. These researchers presented a diethylbarbiturate buffer and an inexpensive, commercially available electrophoresis apparatus for the separation of more than 50 blue, blue-black and black writing inks. They achieved chromatographic separation using horizontal paper chromatography and two solvent systems: n-butanol–acetic acid–water (52:13:35 parts by volume) and n-butanol–acetone–water (4:2:1 parts by volume). The authors concluded that paper electrophoresis exhibited many advantages over paper chromatography, including speed of analysis, quality of separation and improved reproducibility. The authors suggested that the two methods were complementary and would be effectively used in tandem. In 1963, Crown detailed construction of power sources for electrophoresis using commonly available electronic equipment, such as radios [64]. Soon afterwards, Thompson included aluminum citrate or acetic acid–butyric acid–water electrophoretic buffers, describing results superior to electrophoretic separations obtained using weak acidic or basic buffers or paper chromatography [65]. Moon found electrophoresis effective for separation of felt-tipped pen inks, but noted that electrophoresis requires considerably more ink than TLC and a greater investment in time and equipment [66]. Electrophoresis has not attracted as much attention as the various chromatographic techniques and many researchers consider it inferior to chromatography for ink separations.

4.2. Capillary electrophoresis

Although it is a relatively new analytical technique, CE has already found many applications in the forensic sciences due to its many advantages over traditional modes of separation. These advantages include high resolution, ability to quantitate, and short analysis time. The novelty of the method prompts a concise review of its operation and capabilities.

Unlike many other analytical methods, the basic hardware common to CE instrumentation can be adapted to suit a wide variety of analytes without great cost or difficulty. This is intriguing because different ink types contain materials with widely varying properties, making it difficult to identify a single separation method suitable for all components of a single ink, let alone a general method suitable for all types of inks. Most importantly, CE has extraordinarily small sample requirements and it is possible to enact a separation with nanogram (or even picogram) quantities. This is advantageous in minimizing the destruction of the document being tested.

In 1991, Fanali and Schudel reported the first CE analysis of inks, describing a series of liquid black inks and red water-soluble fiber-tipped pen inks that were found to be qualitatively distinguishable [67]. CE was also applied to 26 black rollerball pen and marker inks extracted from paper by Tsutsumi and Ohga [68]. The procedure readily differentiated inks originating from different manufacturers and gave reproducible migration times and relative peak areas. In recent research, Rhode et al. obtained baseline separation for components of several liquid fountain pen inks, that enabled all the tested inks to be qualitatively differentiated [69]. Preliminary results concerning the analysis of liquid black rollerball pen inks have been reported by Zlotnick and Smith [70].

5. Ink dating

The dating of inks has been a topic of sustained interest, with reports by Brunelle and Cantu [71,72]. Initially, methods of dating inks were not aimed at determining the absolute age of writing or printing on paper, but rather at detecting backdating by demonstrating that the ink on a document was not available until after the document was purportedly produced. An example of such an approach was the assembly of a standard ink library, where questioned inks were matched to known inks as occurred in such high-profile cases as the Howard Hughes Mormon will [73]. More recently, Cantu and Prough [74], Brunelle et al. [75], and Brunelle and Lee [76] developed techniques based on the relative rates of extraction of questioned and known inks by various solvents. Stewart reported methods utilizing GC for determination of volatile components [77], as has Aginsky using GC/TLC [78].

6. Conclusion

Application of separation methods has advanced the field of ink analysis since the first uses of paper chromatography and electrophoresis. Separation methods provide compositional information rarely available through use of nondestructive techniques, and TLC has been successfully used for ink analysis in the forensic environment for many years. In the future, instrumental applications such as HPLC and CE are expected to lend even greater accuracy to ink determinations due to their quantitative abilities and higher resolving powers.

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