

Matthew F. Cassidy,¹ B. App. Sc. (Hons) and
David E. Allen,¹ Ph.D.

Application of the Microdroplet Method of X-Ray Analysis to the Characterization of Inks

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ABSTRACT: This study was undertaken to investigate the application of the microdroplet method of x-ray analysis to the characterization of inks. A methodology to allow the microdroplet method to be applied to ink analysis was designed. Ethylene glycol was the preferred solvent for droplet formation. Pyridine was added to this solvent to improve ink extraction capabilities, and could then be removed selectively by vacuum evaporation. Microdroplets were delivered to a thin film support through the use of a nebuliser. Thin films of celloidin were supported on copper specimen grids which were in turn held on a specially designed carbon holder.

A number of different inks were examined using both a previously described method and the newly designed microdroplet technique. Examination of 17 inks from ballpoint pens commonly available in Australia showed that no detectable elements were present that could be used for characterization. This result was in contrast to earlier work by Harada in Japan. Four fountain pen inks were also examined and it was found that all of these inks contained detectable elements that could be used for characterization.

KEYWORDS: forensic science, questioned documents, x-ray analysis, ink

X-ray microanalysis has been proposed as a means of characterizing inks [1]. One technique that is used with x-ray microanalysis is microdroplet analysis, which enables the analysis of nanoliter to picoliter volumes of liquids. Such a method has potential for forensic investigations.

The technique of using microdroplets for x-ray microanalysis was first described by Ingram and Hogben [2]. They postulated that x-rays analysis of dried droplets could provide quantitative information about samples of nanoliter size. Subsequently it has been shown that if reproducible results are to be obtained, the droplet must be dried so as to produce amorphous or microcrystalline deposits [3-5]. This requirement may be achieved by freeze drying or by the addition of specific organic solutes to inhibit crystallization [6-7].

Further, the use of the technique with solid specimen supports results in limited sensitivity due to the Bremsstrahlung radiation arising from the support [9,10]. This restricted the method to use with Wavelength Dispersive X-ray Spectrometry (WDS). The use of thin films as supports can overcome the background problem and allows the use of Energy Dispersive X-ray Spectrometry (EDS). This method has a number of advantages over

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¹Forensic Scientists, Queensland University of Technology, Brisbane, Queensland, Australia.

WDS in that the entire spectrum of elements may be analyzed at one time with relatively short acquisition times.

Various methods of producing and delivering microdroplets to the specimen support have been described. Most use specially constructed micropipettes to deliver constant volumes [11,12]. Morgan et al. [13] described the use of a nebulizer to produce microdroplets. Solutions were sprayed onto coated grids and dried on a warm hotplate. Because this is not a constant volume technique an internal reference element of cobalt was used in order to obtain quantitative data. This internal standard enables operation parameters to be varied. All results may be calibrated to the same cobalt value for comparison.

In this investigation the advantages of the microdroplet method of x-ray analysis in the analysis of small volumes were applied to the characterization of inks.

Inks can be composed of any number of different pigments or dyes to give a particular color [14]. A number of the dyes or pigments mentioned by Stewart contain elements that may be detected by EDS. Harada [1] reported the use of EDS as a means of distinguishing different types of ballpoint pen inks. The method involved cutting out small areas of paper containing ink. These were coated with carbon in a carbon evaporator and analyzed directly. A background correction was made using an analysis of an area of paper where no ink was present. Conditions used for analysis were: magnification— $\times 100$ at 20 kilovolts (kV) for 100 s.

Harada reported that of nine black ballpoint inks tested all could be distinguished on the basis of their elemental content. Similarly nine India inks could also be differentiated on this basis.

In this study it was considered that thin film microdroplet analysis would provide a more sensitive and more accurate elemental analysis than the technique of Harada. This, combined with the ability of the method to cope with small sample sizes made the method potentially suitable for forensic investigations.

Materials and Methods

All work was carried out using a JEOL JSM 35CF Scanning Electron Microscope (SEM) fitted with a Tracor Northern Energy dispersive x-ray detector linked to a Moran Scientific x-ray analysis system. The detector was positioned at a distance of 5 cm from samples. The x-ray take off angle was 35 degrees.

Samples of 17 ballpoint pens commonly available in Australia were collected. These are listed in Table 1. Using each pen a line was drawn onto a sheet of white copy paper (Reflex 80 made in Australia by Associated pulp and paper mills). Similarly four fountain pen inks were drawn onto a sheet of the same paper (Table 2).

The inks were then treated as follows:

Firstly the method of Harada was repeated. Ink samples of 6 mm length were cut from the paper using a hole punch. These pieces of paper were secured onto a carbon stub using double sided adhesive. Samples were then coated with carbon in a vacuum evaporator. Samples were examined using a secondary electron image and a backscattered image before analysis of the line was carried out. The conditions for analysis were, a beam current of 0.08 by 10^{-8} Amps, by 200 magnification, at 20 kV for 100 s. The analysis was repeated using areas with no ink present for background estimation.

The same samples were then examined using the thin film microdroplet method. Thin films of celloidin (nitrocellulose, available from BDH Chemicals) were prepared by dipping a clean microscope slide into a 0.05% solution of celloidin in amyl acetate. The amyl acetate was allowed to dry, and the resultant film floated off onto water. Copper grids were then placed onto the floating film and the film with the grids picked off the water using a piece of blotting paper. Before use the coated grids were heated at 60°C in an incubator for at least 2 h. The heating step greatly increases the resistance of the

TABLE 1—Types of ballpoint pens tested.

No.	Description
1.	White Bic Ballpoint (Blue)
2.	No markings (Blue)
3.	Stabilo Scwhann (Blue)
4.	Pilot BP-S (Black)
5.	Staedler Stick 430 (Blue)
6.	Bic clear plastic (Blue)
7.	Osmer Ballpen M (Red)
8.	Osmer Ballpen M (Black)
9.	Bic clear plastic (Black)
10.	Staedler permanent (Black)
11.	Zebra Newhard N (Black)
12.	No markings (Blue)
13.	Ansell (Blue)
14.	Pental superball (Black)
15.	Parker 7FF (Black)
16.	Shwartz (Blue)
17.	Bic Metal Point (Blue)

TABLE 2—Types of fountain pen ink used for analysis.

No.	Ink description
1.	Pelikan Blue
2.	Shaeffer Blue Black
3.	Pelikan Blue Black
4.	Parker Quink Black

celloidin to electron bombardment. A specially designed carbon grid holder, acting as an electron trap was used to hold the grids in place. The sample arrangement is illustrated in Fig. 1.

To achieve evenly dried amorphous or microcrystalline deposits an anticrystallization agent was required that would also dissolve ink. Aqueous solvents that have been used

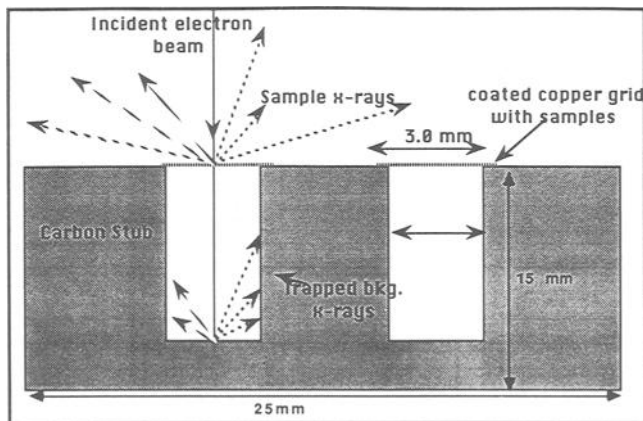


FIG. 1—Sample arrangement on specially designed carbon holder.

in the past were unsuitable because of the insolubility of most inks in aqueous systems. Ethylene glycol was found to be a suitable solvent for this purpose.

Samples of ballpoint inks were obtained by cutting a 6 mm portion of the line from the page. Extraction of the ink from the paper was found to be poor with ethylene glycol alone. Hence all further extractions were performed using a 40 μl volume of a 1:1 mixture of ethylene glycol and pyridine. The pyridine was added to improve the extraction capabilities of the method. The mixture was found to easily extract the ink from the paper. Following extraction the pyridine was selectively removed using vacuum evaporation as it was found to inhibit even drying of the samples. Five μl of a solution of cobaltous nitrate in ethylene glycol was added to 15 μl of the remaining ink/ethylene glycol solution. The cobalt was added as an internal standard and also served to visualize the droplets in the microscope. These samples were well mixed and the entire volume was loaded into a nebulizer. The nebulizer consisted of an air brush that was modified to allow spraying of small samples [15]. All samples were sprayed at a distance of 20 cm onto the celloidin coated grids. A negative control was included, this consisted of a blank piece of paper (of same size as test samples) that was subjected to the same extraction process as all the samples.

Fountain pen inks could not be extracted from paper using the described method. In fact no suitable solvent was found to effectively extract the fountain pen inks that were used in this study. Solvents tested included pyridine, methanol, chloroform, distilled water, butanol, isopropanol, hexane, ethanol, and acetone. Extraction was also attempted after oxidation with concentrated nitric acid but, while this decolorized the samples it failed to produce soluble components. This finding contradicts the manufacturers advice that fountain pen inks were soluble in distilled water (Parker Pen, UK). However it is recognized that the fountain pen inks used in this study were the nonwashable type. Accordingly, fountain pen ink samples were tested using 20 μl samples from the stock bottles. This volume of each ink was again sprayed at a distance of 20 cm from the support film.

Results

The ballpoint inks examined could not be differentiated using the method of Harada. Examination of the samples with a secondary electron image showed an indent where the line was present but no difference in image contrast. Similarly, a backscattered image revealed no differences between areas with ink and those without. No significant difference was seen upon examination of the spectra obtained for the blank paper and those obtained for inked areas. Figure 2 typifies all the spectra obtained for this experiment.

Fountain pen inks also could not be characterized using this method. The spectra obtained for the inks had a slightly increased sulphur peak, this rise was not sufficient to be of use for identification.

When examined by the thin film microdroplet technique, no significant elemental peaks were observed. Figure 3 shows a typical spectrum of an extracted ballpoint ink. The small chlorine potassium and calcium peaks could not be differentiated from similar peaks in the negative controls. These peaks did not significantly change between any of the samples examined. The large cobalt peak represents the internal standard that was at a concentration of 9 mmole/L. All spectra obtained contained peaks assigned to copper. Although the inks may have contained copper, due to the presence of copper phthalocyanine dyes, this could not be reliably established because of copper x-ray emission from the microscope column. Masking of the specimen chamber of the SEM has been attempted however the peaks still occurred and are thought to arise from the backscatter detector, which is composed of brass. Simple background subtraction methods could not resolve this prob-

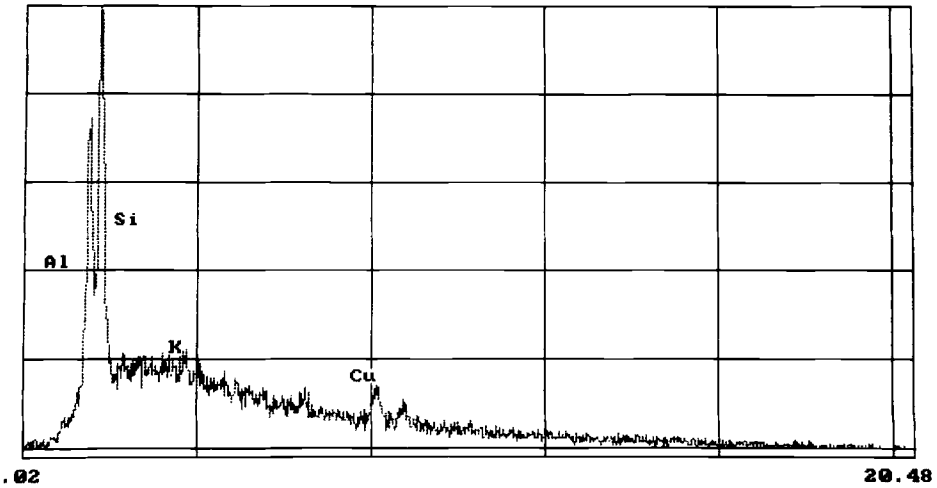


FIG. 2—Method of Harada (full scale = 489).

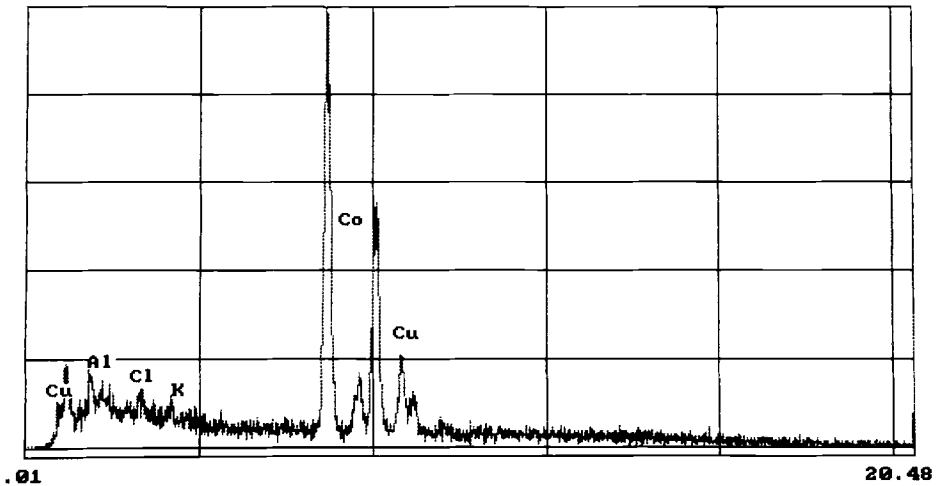
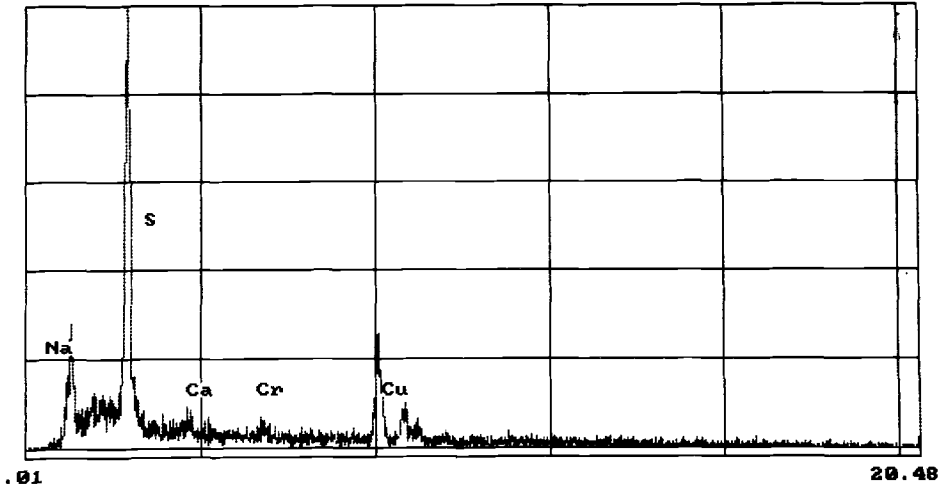
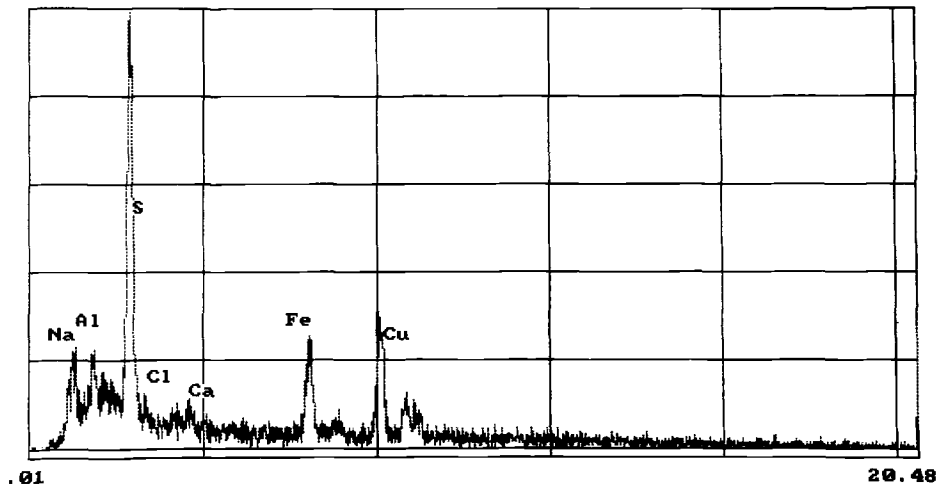


FIG. 3—Extracted ink (full scale = 351).

lem because of the variation of droplet size and hence analytical parameters such as beam current and magnification.

To ensure that the lack of distinctive peaks was not a problem relating to low concentration of the inks following extraction, inks were also analyzed using concentrated solutions of ink in ethylene glycol. No additional information was obtained from these samples.

Fountain pen inks tested by the microdroplet method as described were found to contain significant levels of detectable elements as shown in Figs. 4-7. All inks could be distinguished by the elemental distributions revealed. All of the inks contained sulphur as the major element. The result shows that the method has a much increased sensitivity over the method described by Harada.

FIG. 4—*Pelikan blue* (full scale = 270).FIG. 5—*Shaeffer blue black* (full scale = 373).

Discussion

No evidence was found upon examination of the 17 ballpoint pen inks to suggest that they could be characterized by their elemental content using EDS as presently achievable sensitivity levels. As explained the presence of copper could not be ruled out in the ballpoint ink samples. However it is doubtful whether the reliable detection of copper would change the findings of this study. The presence of only a single element would be insufficient for identification of inks because of the difficulty in standardizing the amount of ink extracted. Although the presence or absence of copper could be used to broadly categorize inks this can be achieved by other methods. Also, spectra obtained after extraction from paper suggested that the type of ink had no effect on the level of elements extracted from the paper.

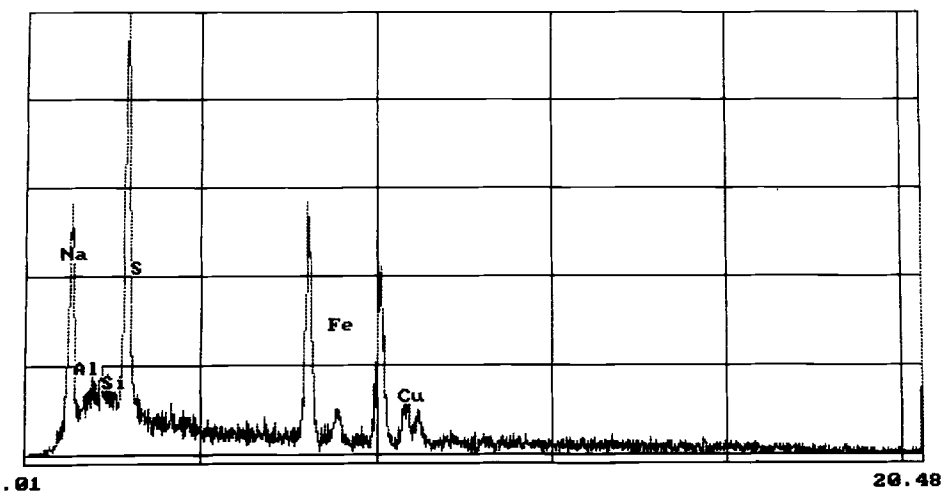


FIG. 6—Pelikan blue black (full scale = 225).

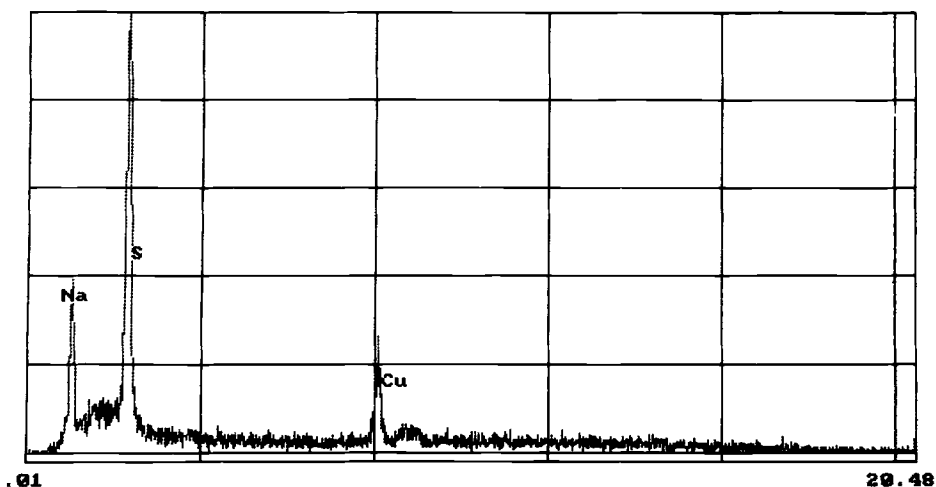


FIG. 7—Parker Quink black (full scale = 344).

The results obtained differ from the findings of Harada. The report by Harada specifies that inks were taken from poor quality paper but does not include any estimates of reproducibility. Clay materials are often used to give paper specific properties, it is possible that the variations seen by Harada may have been due to variations in these clay materials. This possibility is supported by the large variations seen for these background elements as shown in Tables 3 and 4 in the paper by Harada [1]. It should be noted that the inks used by Harada are not commonly available in Australia and hence were not used in this study.

It was found that fountain pen inks contained characteristic elements detectable by EDS. However these inks were unable to be distinguished by the method described by Harada despite the fact that they contained significant levels of elements. This further supports the theory that the variations seen by Harada were not due to variations in ink elements.

No suitable method was found to extract the fountain pen inks once they had dried onto paper. The solvents used in the preparation of these inks are propriety information, but it is likely that the inks contain colloidal particles of pigment such as Prussian Blue. This material is only soluble in oxalic acid when freshly precipitated (Merck Index). They may also contain iron-gallotannate, which becomes insoluble on oxidation [16]. Accordingly, while these inks are potentially identifiable by microanalysis this cannot be realized until a suitable extraction protocol can be developed.

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Address requests for reprints or additional information to
 Matthew F. Cassidy
 Analytical Electron Microscopy Facility
 QUT
 P.O. Box 2434
 Brisbane, 4001
 Australia