J Forensic Sci, January 2007, Vol. 52, No. 1
doi:10.1111/j.1556-4029.2006.00299.x
Available online at: www.blackwell-synergy.com

Luc Brazeau,[1] *B.Sc., C.Chem. and Marc Gaudreau,*[2] *B.Sc.*

Ballpoint Pen Inks: The Quantitative Analysis of Ink Solvents on Paper by Solid-Phase Microextraction

ABSTRACT: We wish to describe further developments to a method previously reported on the detection of 2-phenoxyethanol in ink. The solid-phase microextraction (SPME) sampling technique, together with gas chromatography–mass spectrometry (GC-MS), has been used to quantify solvents in writing ink. In conventional approaches, the analysis of ink on documents requires some degree of destructive sampling. The methods commonly used remove ink samples from paper using a scalpel or a paper punch. To avoid document destruction, a sampling cell was constructed that allows solvents to be adsorbed directly onto the SPME fiber from the headspace above the document surface. Analytes (ink volatiles) are then desorbed from the SPME fiber on a gas chromatograph equipped with a mass selective detector (GC-MSD). With this method, it was possible to detect the presence of ink solvents on documents for a period lasting up to *c.* 2 years.

KEYWORDS: forensic science, questioned documents, ballpoint inks, solid-phase microextraction, SPME, gas chromatography, mass spectrometry, GC-MS

Establishing the approximate age of a document relative to its purported date of production is a question often raised during investigations. Excluding eyewitnesses, it is practically impossible to derive the exact time at which a document was prepared from physical or chemical evidence. There are generally two approaches that can be followed to estimate the age of a document from physical/chemical evidence (1,2).

The first is based on the evaluation of compositional or *static* characteristics of a document and how they relate to the purported date of the document. The dates of introduction or earliest availability of the paper used, the watermark, the ink from the writing instrument or printing devices, the printing technology used, as well as any additional physical or latent information that can be dated are compared with the date of the document. The earliest availability of all of the materials used must predate the date of the document. If the date of the document is earlier than the date of availability of any material used in its creation, then such an anachronism is consistent with the document having been backdated.

The second or *dynamic* approach is based on the evaluation of certain components of a document that change over time and how they relate to the purported date of a document. The state of some components of ink does not remain constant from the moment it is applied to a document. There have been a number of different methods reported in the literature for measuring the dynamic process of ink aging (3–7). To determine the solvent content of ink on a document, most techniques, if not all, require the removal of a small amount of ink from the paper, usually with a scalpel or a small hole-punch. The ink is extracted with a solvent and then analyzed by chromatographic and spectral analysis. These techniques are somewhat destructive and in some cases the analysis is rejected, due to the alteration of the original document. The development of a nondestructive technique for sampling ink would be beneficial.

The method presented here uses solid-phase microextraction (SPME) as the technique to monitor the evaporation of the ink's volatile components as the ink ages on a document. SPME is an adsorption/desorption technique that does not require the use of organic solvents to extract analytes (8–10). The adsorption procedure consists of exposing a SPME fiber coated with a thin film of a polymer having an affinity for the general class of compound being analyzed. Sampling can be conducted either in a liquid or gas phase (headspace). Our application involves sampling of the headspace over documents. In the desorption procedure, the SPME fiber is introduced into a conventional injection port of a gas chromatograph (GC) for analysis.

In our approach, we are attempting to determine whether the level of various volatiles present in ink that has been applied to paper can be quantified. To optimize the sampling method for this particular application, a number of experiments were designed to isolate the effects of various parameters on the SPME procedure. These experiments are described next.

Experimental

Selection of SPME Fiber

There are a number of sorbent materials that are used as coatings for SPME fibers. Selection of the appropriate fiber will depend on the analytes being considered (9,11). Most of the volatiles found in ballpoint pen ink are polar compounds having a high boiling point (~ 200–240°C). The volatiles being considered in this paper are 2-phenoxyethanol, benzyl alcohol, and 1-methyl-2-pyrrolidinone. An SPME fiber having a 65 μm polydimethylsiloxane/divinyl benzene film was used for our application.

[1]Laboratory and Scientific Services Directorate, Canada Borders Services Agency, 79 Bentley Avenue, Ottawa, ON, Canada K2E 6T7.
[2]Chief Forensic Sciences Division, Laboratory and Scientific Services Directorate, Canada Borders Services Agency, 79 Bentley Avenue, Ottawa, ON, Canada K2E 6T7.

Received 11 Dec. 2005; and in revised form 10 June 2006; accepted 1 July 2006; published 8 Dec. 2006.

Chemicals, Materials, and Samples

Benzyl alcohol 99.8% anhydrous, 1-methyl-2-pyrrolidinone 99+% ACS reagent, and 2-phenoxyethanol 98% were purchased from Sigma-Aldrich Canada (Oakville, ON, Canada). Acetonitrile HPLC grade was purchased from Caledon (Georgetown, ON, Canada). Methanol HPLC grade was purchased from J. T. Baker (Phillipsburg, NJ). Fiber holder and SPME fiber assemblies from Supelco. Black ink from a ballpoint pen containing 2-phenoxyethanol in the ink formulation was purchased from a local office supplies store. A regular paper punch having a 7 mm cutting diameter was also purchased from the local office supplies store. A Harris micropunch having a 1.2 mm diameter was purchased from Whatman (Florham, NJ). Silylalized 2 mL samples vials were purchased from Supelco (Bellefonte, PA) and plain normal 2 mL vials were purchased from Chromatographic Specialties (Brockville, ON, Canada). The paper used was Buntin Read Photocopy Paper 10M P4, 75 g/m^2, long grain. A neodymium iron boron magnet 1 in. disk, 0.25 in. thick, was purchased from Edmund Scientific Company (Barrington, NJ).

Instrumentation

The headspace was analyzed with an Agilent 6890A GC equipped with a 5973N mass selective detector (MSD) equipped with electronic pressure control capability and a split/splitless injector (250°C). Samples were handled by a Multipurpose Combi Pal autosampler equipped with a multi-vial incubator with headspace and SPME capability. The GC/MS is from Agilent, and the auto-sampler from CTC analytics (Zwingen, Switzerland). The column used was a DB-5 (J&W Scientific, Folsom, CA) 30 m × 0.25 mm × 0.25 µm. The oven was programmed from 70°C (1.5 min hold) to 250°C (8 min postrun) at 25°C/min. The total cycle time was 21 min. Helium (99.995%) was used as a carrier gas (inlet pressure 9.8 psi) with a constant flow of 1.2 mL/min. Column and detection conditions were kept constant during and throughout the study. The system is controlled by the Agilent Chemstation version G1701A data system revision C.00.00, and the auto-sampler by the Cycle Composer software version 1.4.2.

Sampling Cell

A sampling cell was designed to allow the nondestructive sampling of ink volatiles from headspace above the surface of a document. The bottom of a vial was cut and a magnetic metal flange was fitted flush with the bottom edge of the vial (Figs. 1 and 2).

FIG. 1—*Sampling cell.*

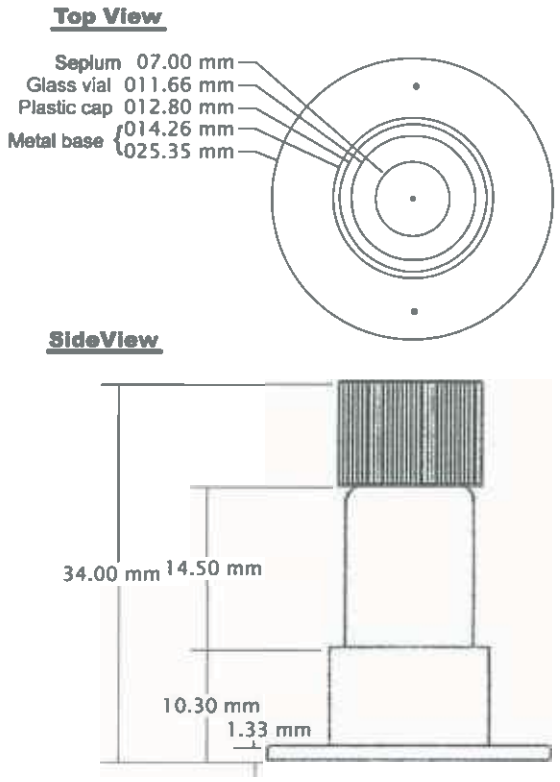

FIG. 2—*Sampling cell design.*

Placing a magnet below the document surface being sampled will secure the vial firmly in place for SPME extraction. Stainless-steel 416 was used to make the metal flange due to its high machinability. The 400 series stainless-steel is also relatively inert and is magnetic (12). Epoxy was used to bond the glass vial to the metal flange part. A heating jacket for the sampling cell was made from an aluminum bar stock; three holes were made into it to incorporate two heaters and a thermistance to monitor the temperature (Figs. 3 and 4). To control the temperature of the sampling cell, the instrument temperature controller from Valco Instrument (Houston, TX) was utilized with two pencil heaters and a thermocouple sensor.

FIG. 3—*Heater about to be placed over the sampling cell on paper.*

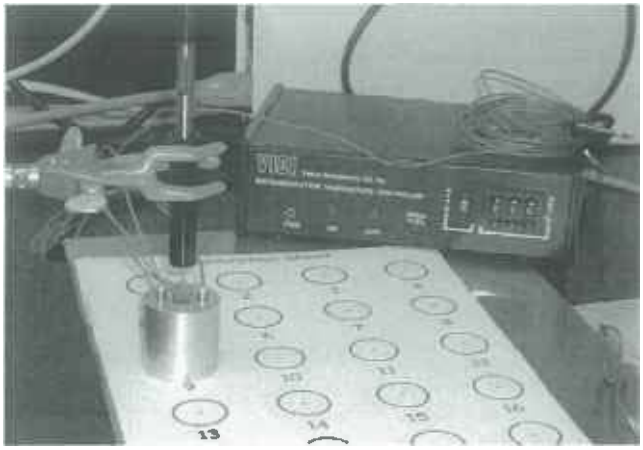

FIG. 4—*Controller, sampling cell, and solid-phase microextraction holder over calibration paper sheet.*

General Sample Preparation and Analysis

Small paper disks were cut from a sheet of plain photocopy paper ($8\frac{1}{2} \times 11$ in. 40 M) with either a Harris micropunch (plug size about 1.2 mm in diameter) or hole punch (office equipment large punch about 7 mm in diameter). The instrument used was previously cleaned with methanol, dried with Kimwipes. Blank paper disks (10) were also cut and discarded to ensure clean conditions. For a Harris micropunch, 20–30 disks were cut and discarded. Large paper disks from new plain photocopy paper were placed at the bottom of the closed vials using tweezers. Known amounts of 2-phenoxyethanol were taken from standard solutions and slowly transferred to a paper disk by contacting the needle of a microsyringe to the paper and monitoring the rate of absorption into the paper as to prevent any liquid loss. The syringe was washed five times before and after sampling with acetonitrile. The paper with the solution was allowed to air dry for 5 min. The vials were tightly sealed by hand and placed in the auto-sampler for headspace analysis by SPME.

All samples were prepared at a 21-min interval and then placed in the incubator. Twenty-one minutes is the instrument cycle time. In a separate experiment, a ballpoint pen containing 2-phenoxyethanol was used to draw lines as evenly as possible on white photocopy paper. A hole punch was used to cut 7 mm paper disks. The disks were placed in a 2-mL auto-sampler vial for headspace analysis by SPME/GC/MS.

Optimization of Absorption Time

The absorption time is the amount of time the SPME fiber is left inside the vial for analyte adsorption. This time period was varied from 2.5 to 30 min. Ten nanograms of 2-phenoxyethanol was added to a 7 mm paper disk in a vial as described above. The sampling temperature was kept constant at 50° (Fig. 5).

Optimization of Sampling and Equilibrium Temperature Conditions

Ten nanograms of 2-phenoxyethanol was added to a 7 mm paper disk in a vial as described above. Samples were run in a similar way, keeping the absorption time constant and only varying the time at which the fiber was introduced into the vial. The results were plotted as intensity versus sampling time (Fig. 6). The process was repeated for different temperatures and graphed. In a second experiment, the same test was performed using the optimum temperature and varying the absorption time. The optimal sampling time was determined by plotting the intensity against absorption time (Fig. 7). By doing this, we can determine what is the shortest sampling time, and still giving high sensitivity. After optimizing both the absorption and equilibrium times for a given temperature, the experiment was repeated using different solvent concentrations in the vial and generating new intensity versus concentration (Fig. 8) data to determine the range of linearity of the method. 2-phenoxyethanol concentrations ranging from 1 to 150 ng were analyzed. After optimizing for 2-phenoxyethanol, the same procedure was applied for benzyl alcohol and 1-methyl-2-pyrrolidinone.

Calibration

There are a number of reported approaches of SPME calibration (13–15). Two types of calibration were conducted: one in which a known quantity of 2-phenoxyethanol was added directly into a

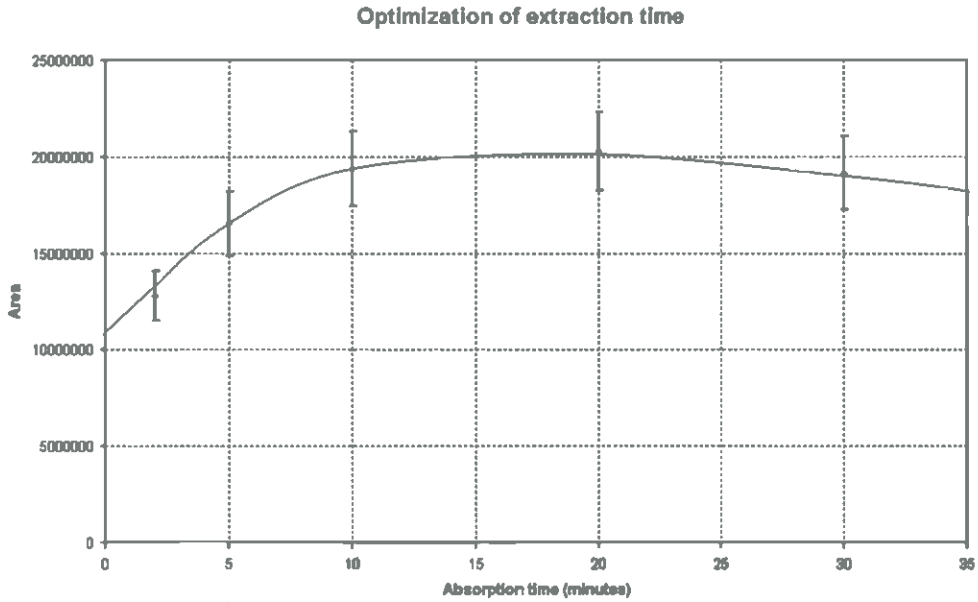

FIG. 5—*Optimization of absorption time at 50°C (no equilibrium).*

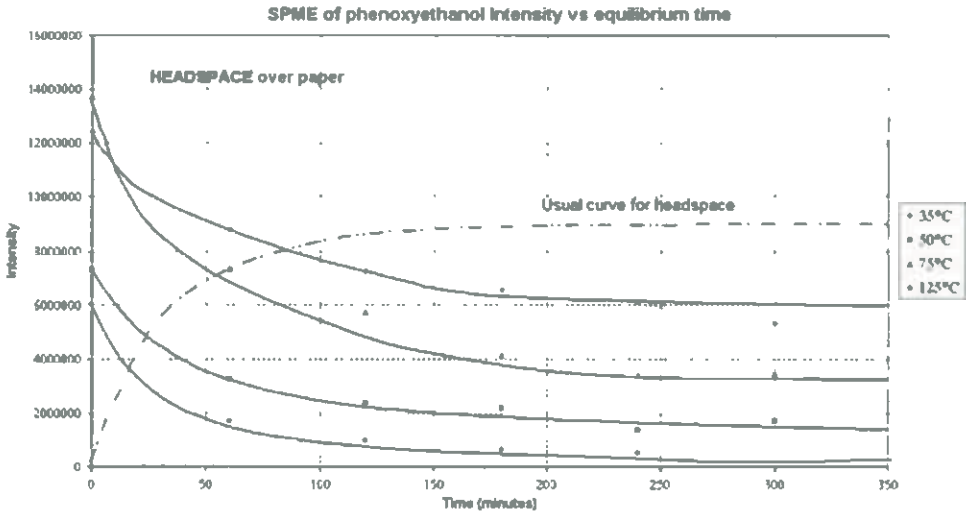

FIG. 6—*Time to achieve equilibrium (exposure time of fiber 15 min).*

vial, and the second, where a known quantity of 2-phenoxyethanol was added to paper disks at the bottom of a vial. The calibration range for both was from 1 to 150 ng.

Effect of Paper Surface on Analyte Adsorbtion on SPME Fiber

Known quantities of 2-phenoxyethanol were placed directly in the vial and run at an optimized temperature; to check whether the paper will have an effect on the concentration measure, a small plug of paper was added and later on a larger plug was added to the vial and run. Comparing result runs with the same amount of solvent and varying the surface of paper, we can find whether the surface of the paper does have an effect on the intensity or peak area.

Sampling Vial Effects

The same sample amount was placed in 10 individual vials and run for comparison. The same experiment was repeated with the silylalized vial.

Application of Methodology to the New Nondestructive Sampling Cell

To determine whether the nondestructive sampling cell would reproduce the results obtained in the autosampler experiments, the same calibration procedure was performed from 1 to 150 ng using the sampling cell over a sheet of paper. The standard solutions were placed directly on a sheet of paper in a marked area. The sheet of paper was placed on an aluminum sheet with a magnet under this sheet beneath the area being sampled. The heating block is placed over the cell and the SPME fiber is introduced into the cell. The timing is kept constant in this procedure (Fig. 3).

Results and Discussion

Qualifying Ions: MS single ion monitoring (SIM) of Ion m/z 79, 94, and 99

Benzyl alcohol, 2-phenoxyethanol, and 1-methyl-2-pyrrolidinone represent a large proportion of solvents used as vehicles for ballpoint inks and other applications. These compounds are readily

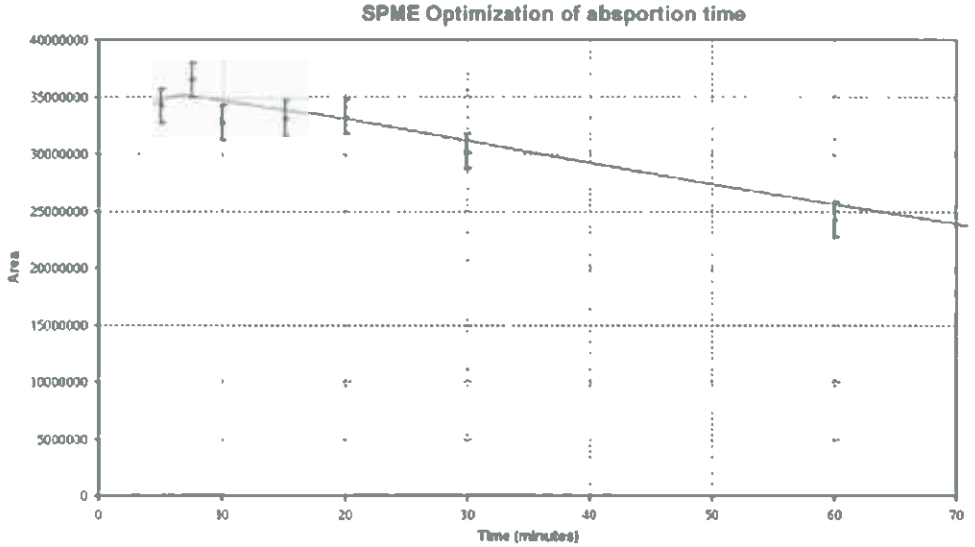

FIG. 7—*Optimization of absorption time at 115°C (no equilibrium).*

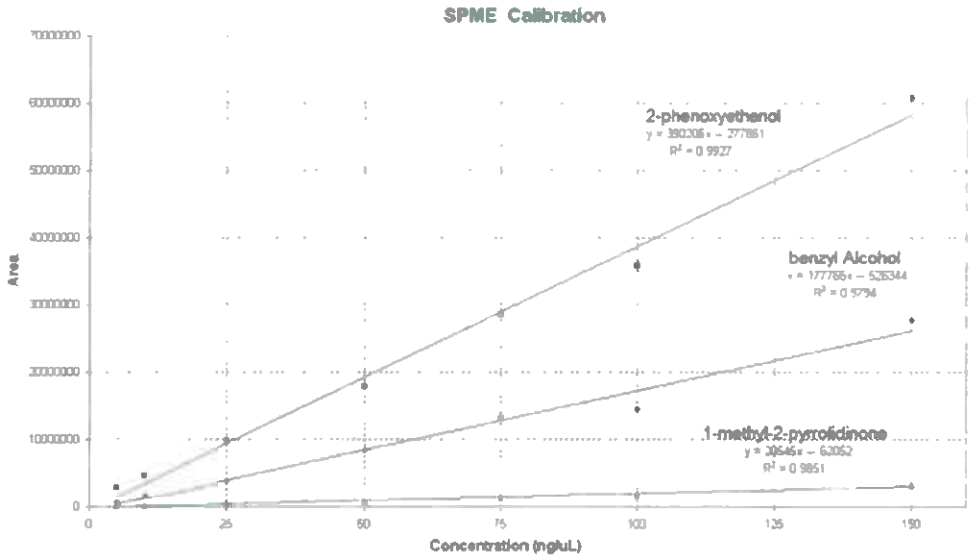

FIG. 8—*Calibration by solid-phase microextraction (SPME) for various solvents found in ballpoint pen ink (absorption time 7.5 min).*

identified by GC-MS. Working in SIM mode for benzyl alcohol masses *m/z* 77, 79, 107, 108; for 1-methyl-2-pyrrolidinone *m/z* 71, 98, 99; and for 2-phenoxyethanol *m/z* 77, 94, 107, 138 are used to track and quantify benzyl alcohol, 1-methyl-2-pyrrolidinone, and 2-phenoxyethanol, respectively.

Using different columns under various conditions, it was difficult to obtain good peak separation between benzyl alcohol and 1-methyl-2-pyrrolidinone. However, in the SIM mode, they can be discriminated by monitoring mass 79 for benzyl alcohol and mass 99 for 1-methyl-2-pyrrolidinone. The signal response for these masses is good for the quantitation of each compound without having any interference.

Sampling for Headspace Equilibrium, Optimization, and Calibration Studies

The adsorption of analytes on the polymer film coating of the SPME fiber is a dynamic process that, at equilibrium, is directly related to the concentration of the analyte in the matrix; the thickness; porosity and volume of the SPME fiber; and the volume of the sampling cell. In our experiment, the equilibrium is affected by the various partitions between: matrix and vapor phase; vapor phase-SPME fiber; vapor–cell wall interactions; and the partition coefficient for the analyte between coating and sample matrix (16,17).

The first step was to determine the optimal absorption time. This is the time that the fiber is exposed to the headspace. The fiber is inserted at the beginning and exposed for a fixed period of time. The time of exposure was varied from 2.5 to 30 min. The maximum sensitivity was found with an exposure time of *c.* 15 min (Fig. 5). This step was performed to gain an approximate idea of how long the fiber should be exposed at 50°C. Later, we will see that the optimum temperature is 115°C.

The plot of area over time for different temperatures shows the same headspace equilibrium profile. As can be seen in (Fig. 6), this is quite different from the profile for conventional headspace analysis over liquid (shown in dashed lines). When a liquid phase is present, there is an overabundant supply of analyte to the gas phase. In this experiment, the supply of analyte to the gas phase from the paper surface is much more limited. Because of this, the interaction between analyte and paper, the effects of paper, vial surface, and volume are also more significant. During initial sampling, values are high as the analyte are primarily adsorbed onto the SPME fiber in a nonequilibrium environment. As time passes, the value decrease as condensation of the analyte on the vial wall as well as equilibration of the distribution of the analyte in the entire gas phase occurs. The fact that the intensity increases with temperature further supports the effects of condensation. The values obtained tend to reach equilibrium after a period of *c.* 4 h. It was also noted that by increasing the temperature, and adding more sample into the vapor phase, however, the curve was still similarly showing a reduction with time. By increasing the temperature, sensitivity is increased but at a temperature above 125°C, there is leakage from the vial caps, and also some desorption from the fiber may start occurring. We found that the vial caps retain the gas phase well at a temperature of 115°C. Hence, the temperature was lowered to 115°C to obtain the best sensitivity.

A second optimization was performed at 115°C to determine the optimal absorption time. We found that there is no significant difference between 7.5 and 20 min; therefore, the shortest time was selected (Fig. 7).

Running samples from 1 to 150 ng generated a near-linear calibration curve. Running samples in triplicates reduced the error, bringing the *R* (correlation coefficient) from 0.96 to 0.99 for 2-phenoxyethanol (Fig. 8).

Effect of Paper Surface

For the same amount of solvent added to the vial, varying the paper surface area will have a proportional effect on the intensity. The greater the surface, the lower the intensity. The lowest intensity result was achieved for a paper surface completely covering the bottom of the vial (*c.* 11.5 mm in diameter). Hence, when working in headspace with very small amounts of analyte, it is critical that physical parameters such as paper surface, sampling cell as well as any other system component surfaces remain constant.

Glass Effects and Silation of Vials

It was shown that the glass vial could have chemically active sites. Samples were analyzed in both normal and silylalized vials.

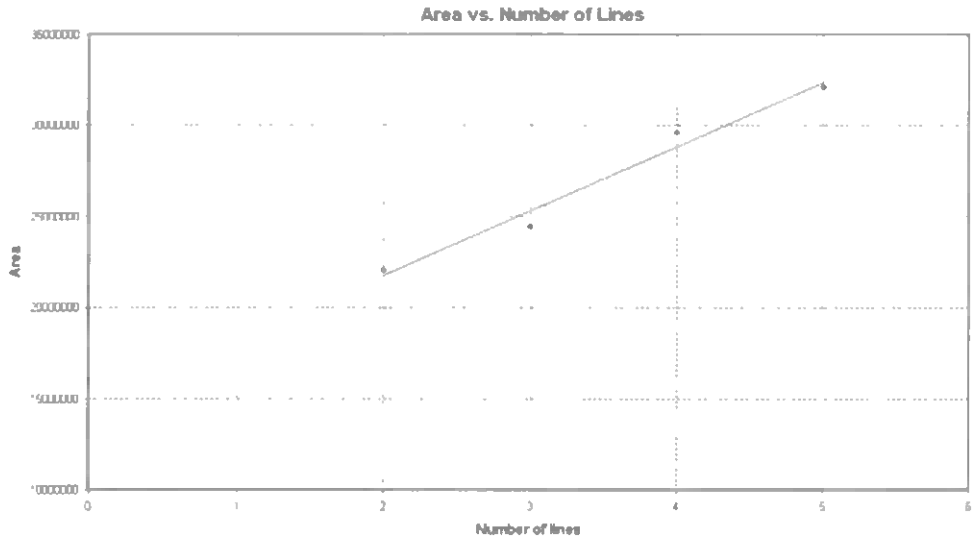

FIG. 9—*Linearity in relation of ink surface on paper.*

The change in conditions shows a small but significant increase in the average intensity for silylalyzed vials (*c.* 10% increase).

Nonequilibrium Sampling

Working at room temperature and at equilibrium, the presence of the three solvents placed on paper from fresh to about 2 months old can be detected. Samples containing solvents older than 2 months and of a similar surface area cannot be detected easily. This is more or less equivalent to the amount of solvent present in an ink that has been applied to paper for a period of less than 2 months preceding the analysis. The benefits of working at non-equilibrium are an increase in sensitivity and detection of samples that can range up to 2 years. The drawback is that the timing of the extraction is more critical as it can induce more error than working at equilibrium.

Contamination

It is important to run blank paper samples near the ink to confirm that no additional or other extraneous solvents are present. It is known that some of the solvents contained in ink may also be found in low quantities in other products such has hand creams, shampoo, etc.

Test with Ink

To determine whether the SPME technique had a linear response to the surface area of ink on paper, test sheets having in-

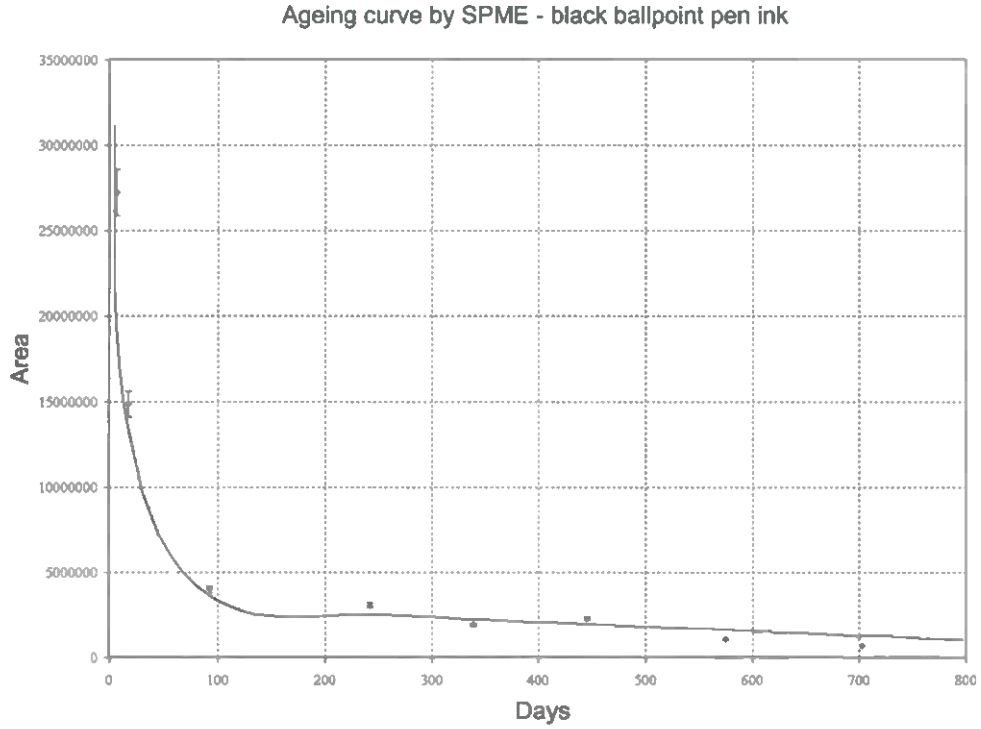

FIG. 10—*Aging curve of black ink.*

creasing number of ink lines were made, each having a length of about 4 mm. Figure 9 shows the response for two to five lines of ink on paper. As can readily be observed, volatility detection is directly proportional to the amount of surface sampled. The curve does not pass through or intercept the origin. This is due to area/density variation from line to line and also to a carry-over effect from the cell.

Nondestructive Surface Sampling Cell

The data obtained with the nondestructive sampling cell demonstrated the same linearity as the results obtained with closed incubated vials. A slight difference in intensity was noted. This was due to differences in paper surface area.

After developing the current method, the nondestructive sampling cell was used to measure 2-phenoxyethanol on sheets of paper bearing ink lines of known age varying from fresh to about 2 years. Although we were able to detect the presence of solvent over the entire period, the intensity of 2-phenoxyethanol detected becomes very faint after *c.* 1 year (see Fig. 10).

Conclusions

The results of this investigation show that the volatile components of ballpoint pen inks can be quantified by the analysis of the headspace above the paper surface using the SPME technique. By using the modified sampling cell described, this method allows the nondestructive analysis of ink volatiles on paper for the first time. The analysis requires minimum sample manipulation and can be repeated on different areas of a document. Our results are consistent with a similar study reported, where the method can be applied to detect whether inks are less than 6 months old (18). The effects of type of paper cannot be ignored especially if the results from analyses carried out on different papers need to be compared. Ideally, a calibration curve could be generated from the surface of any paper being investigated (using a reference ink or pure 2-phenoxyethanol, which would allow a more accurate interpretation of the data for a questioned ink). In the next phase of our research, we aim to develop a method in which the approximate age of an ink could be inferred from one or a series of SPME readings.

Additionally, this nondestructive approach can potentially be applied to a wide variety of other applications and material surfaces (walls, automotive parts, printed materials, toys, etc.) for sampling volatile compounds emanating from clear coats, paints, varnishes, conditioners, etc.

Acknowledgments

The authors thank Dr. Rachel Ng at the Laboratory and Scientific Services Directorate, and Dr. Tony Cantu at the U.S. Secret Services for initiating the project and their general support. Special thanks are due to Pierre Lafontaine and Michel Hupé for their constructive comments. The technical help of Ms. Tanya Pazniak, Ms. Helen Prochazka, and Mr. Ognjen Panic, is greatly appreciated. This work was partly funded by the Technical Support Working Group under the Memorandum of understanding for Counter-Terrorism Research and Development.

References

1. Brazeau L, Chauhan M, Gaudreau M. The use of solid phase micro extraction (SPME) in the development of a method to determine the aging characteristics of inks. Poster presented at: Proceeding of 58th Annual Meeting ASQDE, August 24–29, 2000; Ottawa, Ontario, Canada. Ottawa: American Society Questioned Document Examiner, 2000.
2. Cantu AA. A sketch of analytical methods for document dating Part II: the dynamic approach determining age dependent analytical profile. Int J Forensic Doc Exam 1996;2(3):192–208.
3. Brunelle RL, Cantu AA. A critical evaluation of current ink dating techniques. J Forensic Sci 1987;32(6):1522–36.
4. Stewart LF. Ballpoint ink age determination by volatile component comparison; a preliminary study. J Forensic Sci 1985;30:405–11.
5. Aginski VN. Dating and characterizing writing, stamp pad and jet printer inks by gas chromatography/mass spectrometry. Int J Forensic Doc Exam 1996;2(2):103–16.
6. Aginski VN. Some new ideas for dating ballpoint inks—a feasibility study. J Forensic Sci 1993;38(5):1134–50.
7. Andrasko J. HPLC analysis of ballpoint pen inks stored at different light conditions. J Forensic Sci 2001;46(1):21–30.
8. Sheppers W, Sue A. Solid phase microextraction a practical guide. New York: Marcel Dekker, 1999.
9. http://www.sigmaaldrich.com/Graphics/Supelco/objects/4600/4547.pdf
10. Pawliszyn J., editor. Applications of solid phase microextraction. London: The Royal Society of Chemistry, 1999.
11. http://www.sigmaaldrich.com/Graphics/Supelco/objects/11200/11143.pdf
12. Kirk-Othmer encyclopedia of chemical technology, Vol. 15, 3rd ed. New York: John Wiley & Sons, 1981:541.
13. Namiesnik J, Gorlo D, Wolska L, Zygmunt B. Analysis of selected gaseous organic micro-pollutants in air by means of solid phase microextraction-gas chromatography-mass spectrometry. Calibration problems. Analysis 1998;26:170–4.
14. Martos PA, Pawliszyn J. Calibration of solid phase microextraction for air analyses based on physical chemical properties of the coating. Anal Chem 1997;69:206–15.
15. Gorlo D, Wolska L, Zygmunt B, Namiesnik J. Calibration procedure for solid phase microextraction-gas chromatographic analysis of organic vapours in air. Talanta 1997;44:1543–50.
16. Ai J. Headspace solid phase microextraction: dynamics and quantitative analysis before reaching a partition equilibrium. Anal Chem 1997;69:3260–6.
17. Oomen AG, Mayer P, Tolls J. Non-equilibrium solid phase microextraction (SPME) for determination of the freely dissolved concentration of hydrophobic organic compounds: matrix effects and limitation. Anal Chem 2000;72:2802–8.
18. Andrasko J. A simple method for distinguishing between fresh and old ballpoint pen ink entries. Proceeding of 3rd European Academy for Forensic Science Meeting; September 22–27, 2003; Istanbul, Turkey. Forensic Sci Int 2003;136:80.

Additional information and reprint requests:
Luc Brazeau, B.Sc., C.Chem
Laboratory and Scientific Services Directorate
Canada Borders Services Agency
79 Bentley Avenue
Ottawa
ON Canada K2E 6T7
E-mail: Luc.brazeau@cbsa-asfc.gc.ca