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Talanta 67 (2005) 425–429
Talanta
www.elsevier.com/locate/talanta

The use of laser desorption/ionization mass spectrometry in the analysis of inks in questioned documents

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Received 7 February 2005; received in revised form 28 February 2005; accepted 1 March 2005
Available online 23 May 2005

Abstract

Determination of the age of a handwritten or ink printed questioned document can be an important consideration in forensic cases. Most often the age of a document is determined by the chemical behavior of the dyes that make up the ink. Exposure of the dyes to environmental factors such as oxygen and ultraviolet or visible light cause them to degrade. Often this degradation can be correlated to the time since the exposure of the ink to the elements began. A number of methods have been used to track the aging of inks on paper. This paper reports the use of laser desorption mass spectrometry as a valuable tool in not only elucidating the structures of dyes used in inks but tracking the change in their chemistry as they age. This study also explores methods for artificially aging documents using ultraviolet and visible light.

Keywords: MALDI, Laser desorption mass spectrometry; Ink analysis; Artificial aging of inks; Ball point pen; Methyl violet

1. Introduction

All of the following ink and dye research was supported by a National Institute of Justice Grant #2002-RB-CX-K002, John Allison and Jay Siegel, PIs. Some of the results can be found on our database at http://poohbah.cem.msu.edu/peninks/pens_main.htm.

Questioned document examiners in a forensic setting are called upon to perform many types of examinations on paper and other document media. For forensic purposes, a document is any medium that contains printed or handwritten markings whose source or authenticity is in doubt. Many of these examinations involve the ink that the document was written with. Such issues may involve the identity of the particular writing instrument used to write a questioned document. For example, there may be a question about a set of observations concerning a patient in a hospital. These are written in a chart when they are noted and a question may arise as to whether entries were all written with the same pen. A more complex situation arises when the issue is when a particular document or part of a document was written. In a recent case, which did not process to court, a physician was accused of malpractice by the family of a patient who died while under his care. One of the issues concerning the suit was a handwritten entry that the physician allegedly made in the patient's chart concerning an important medical test that he claimed he did, but that the family claimed that he did not do and was therefore negligent. He said that he made an entry in the chart right after doing the test, whereas the family claimed that he made the entry only after the patient died to cover up his malpractice. The forensic issue was then; was the ink in the entry in question older than the entry immediately after it in the chart and younger than the one before it. In recent years the use of computers to produce documents has changed because many documents such as wills are no longer handwritten. This has provided increased challenges

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doi:10.1016/j.talanta.2005.03.028

to document examiners because the nature of inks and toners used in computer printers is more complicated than pens.

A number of analytical techniques have been used in the characterization of inks on paper [1,2]. These include chromatographic and spectroscopic methods. Determination of the composition of an ink sample is usually pretty straightforward. The determination of the age of ink may be more complex although there are some basic extraction-based methods that are commonly in use [3–8].

Mass spectrometry has been used for a number of years in the analysis of ink [6,9]. The earliest methods involved GC/MS where the quadrapole or ion trap mass spectrometer acts as a detector for the GC. The volatile components of the ink are separated by the GC and then ionized by electron impact (IE). More recently desorption/ionization mass spectrometric methods have been employed. In this research we focused on matrix assisted laser desorption ionization or MALDI [10] and laser desorption or LD [11]. The two techniques differ in that in MALDI the analyte is impregnated with a crystal matrix that absorbs the ionizing energy and then transfers it to the analyte. In both techniques, a pulsed laser is used to supply the ionizing energy and the ions that are generated are separated by time-of-flight (TOF) mass spectrometry. One of the advantages of MALDI and LD is that very little decomposition of the molecular ion takes place and the resulting mass spectra are generally uncomplicated.

A number of approaches have been taken in the determination of the age of ink [2,3,7,8]. In order to accomplish this one needs to have ink samples of various known ages. There are a couple of ink libraries available but their samples are limited and basic research sometimes requires large amounts of analyte. The major approach to artificial aging of ink is to heat the sample in an oven at 100 °C and then use an extraction-based method for age determination [9,12–14]. In our studies we have found that light can achieve similar results. We started with UV light but found that incandescent light works just as well. We are also interested in using an oxygen-rich atmosphere in the dark but have not tried that yet. The most important consideration in implementing an artificial aging technique is that it causes the same chemical changes in the ink that natural aging does. Clearly natural aging is a complex process that involves light, heat, oxygen and paper.

2. Experimental

All of our mass spectrometry was performed on a PE Biosystems Voyager DE instrument (Framingham, MA). The pulsed nitrogen laser emits at 337 nm, 3 ns, 3 Hz. The following conditions were used:

For positive ions	20000 V
For negative ions	−15000 V
Intermediate acceleration grid	94.5% of plate voltage
Delay time between laser irradiation and ion acceleration	150 ns

The metal sample plate supplied by the manufacturer was used to hold the paper and ink samples. These are irradiated directly with the laser.

2.1. Analysis of ink on paper

Ink samples wesre applied directly to paper from the pens. A straight line was drawn with a single stroke of a pen. The paper was then cut into small strips, which were taped to the sample holder of the inlet of the MS. Each strip was estimated to contain a few milligrams of ink. A solid matrix had to be used with a few of the inks to get them to ionize. The instrument was calibrated using a saturated solution of CsI on paper. Laser desorption of CsI yields $(Cs_nI_{(n-1)})^+$ ions. Mass spectra from 50 laser shots at each location were gathered and averaged to yield a final mass spectrum. For most ink samples, spectra were obtained at three points along the line. Standard deviations were computed from all of the spectra for each sample.

2.2. Aging studies

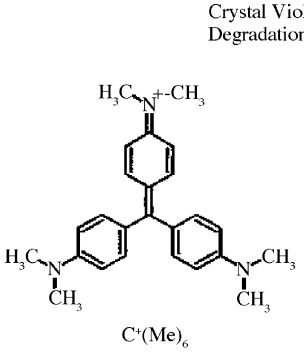
Two natural aging studies were conducted in this research. In the first study, documented ink samples were supplied by Speckin Forensic Science Labs (Okemos, MI). The samples, aged under controlled conditions came from a Bic®—STK BP black B-460 fine point pen, purchased in April of 1991. The Speckin collection was made in the following manner. A single piece of 8 in. × 11 in. bond and printer paper was used for each ink. Each month, a line was made on the paper and dated. In between these markings, the paper was stored by itself in an envelope in a drawer. The paper was not folded and light was prevented from reaching the ink. The other study was not controlled. Numerous samples of writing that have been stored in the basement of the Chemistry building for many years were obtained. Each sample had information on it that indicated when it had been written (but not how old the pen was).

3. Results and discussion

3.1. Aging studies of blue and black ball point pen inks

Much of the early work in this study focused on ball point pens, specifically black and blue pens [14,15]. These pens all had the same dye in them, *methyl violet*, which has been used as a dye in such pens since about 1950. Methyl violet is derived from *crystal violet* . Fig. 1 shows the structure of crystal violet and the weights of its degradation products. The loss of one methyl group from crystal violet yields methyl violet, the principle dye in these pens.

During the natural aging of this dye, successive methyl groups are lost and are replaced by solvent protons, resulting in the loss of 14 mass units each time. The older the ink, the more methyl groups are lost. Fig. 2 is a series of three mass



m/z	Structure
372	$C^+(Me)_6$
358	$C^+(Me)_5H_1$
344	$C^+(Me)_4H_2$
330	$C^+(Me)_3H_3$
316	$C^+(Me)_2H_4$
302	$C^+(Me)_1H_5$
288	C^+H_6

Fig. 1. Crystal violet and its photodegradation products.

spectra of blue inks taken from the vaults in the basement of the department of chemistry at Michigan State University. The inks were taken from three different documents. The dates of the documents are confirmed in the writing. This is an uncontrolled experiment in that there are no controls on the paper and the manner of storage can only be assumed to be similar for all three documents during the period since they were written. Of course, it is quite likely that the pens were all different too and it is not known how old the pens were when they made the marks. In spite of this, the results are quite reasonable. As can be seen in Fig. 2, the first ink, taken in 1996, has one major peak at *m*/*e* 372, which is crystal violet. There is a very small peak at 358, corresponding to methyl violet. In the second document, written in 1974, the base peak is still 372, but now there is a prominent peak at 358 and one at 344, corresponding to the loss of two methyl groups, and a small peak at 330 (MH^+–$3CH_3$]. Finally in the third document, written in 1961, the base peak is methyl violet and there are peak clusters representing the loss of up to four methyl groups.

In the artificial aging studies that were carried out in this research, UV light was used. Fig. 3 shows an artificial aging

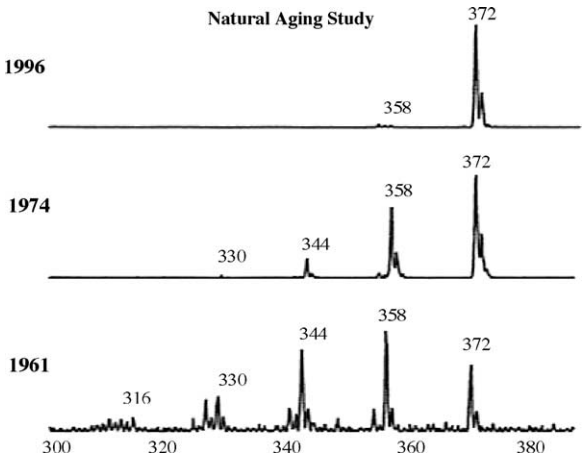


Fig. 2. A natural aging study on blue ink. These three documents were obtained from the archives of the Department of Chemistry at Michigan State University.

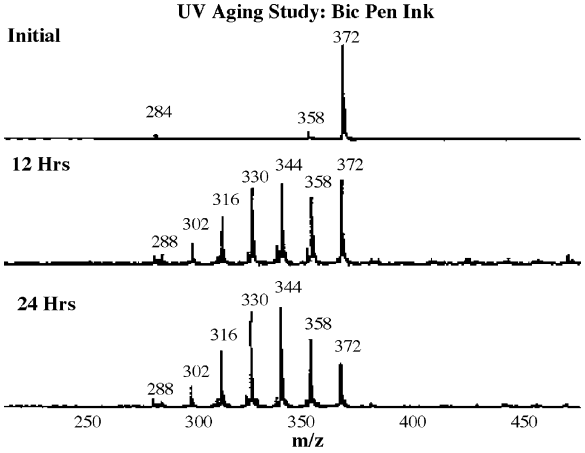


Fig. 3. An accelerated aging study. The three mass spectra were obtained from the same pen.

of the ink in a Bic blue ball point pen. Initially, the major peak in the mass spectrum corresponds to crystal violet with a very small methyl violet peak. After 12 h of irradiation, there are seven peaks present, each one representing the loss of an additional methyl group including the one at 288 where the dye has been completely demethylated. The two tallest peaks are at 372 and at 344, where two methyl groups have been lost. There is also a strong peak for methyl violet at 358. After 24 h of continuous UV irradiation, there has not been as dramatic a change in the mass spectrum. There is much less crystal violet but the rest of the spectrum is not changed very much. This is probably due to the fact that the loss of two or three methyl groups yields fairly stable compounds that resist further oxidative demethylation.

3.2. Aging of ink within a ball point pen cartridge

In the determination of the age of an ink sample in a ball point pen which has been used to write a questioned document, the assumption is currently made that the age characteristics of the ink in the pen cartridge are the same throughout and that the ink does not undergo chemical aging inside the cartridge. Assume that a document was written in 1990 with a Bic blue ball point pen and the LD mass spectrum is taken. Ten years later, the pen is retested after half of the ink in the cartridge has been used. The LD mass spectrum is taken again. If the ink had not aged in the cartridge, then both mass spectra should look the same. The research studies reported here indicate that this is not the case [16]. Fig. 4 shows such a case. It consists of three mass spectra taken from the cartridge of a pen of unknown manufacturer. Spectrum (a) is a sample of ink taken from the end of the cartridge nearest the pen tip. Spectrum (b) is taken from the middle of the cartridge and spectrum (c) is taken from the other end of the cartridge which is open to the atmosphere. A couple of things are important here. First, the large amount of the compounds at 358 and 344, indicate that this ink is aged. A new ink would have its only significant peak at 372. Comparison with known inks in

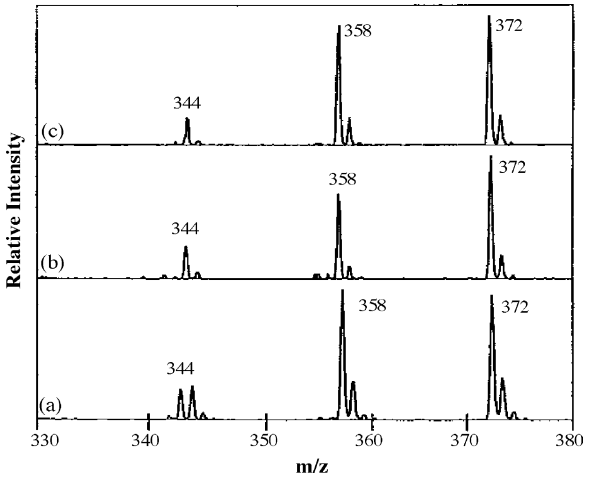


Fig. 4. The positive ion LD mass spectra of ink in a ball point pen cartridge.

an ink library at Speckin labs, indicates that this pen might be as old as 35 years. Second, although the differences among the ink samples in Fig. 5 are not great, they are significant. Note especially the differences between the 358 peaks in the middle of the cartridge compared to those on either end. The ends would be expected to be exposed to more oxygen and thus show more aging. This could be important in trying to determine the age of an ink sample that is old. One must be at least aware that the ink inside a cartridge ages with time and that it may age unequally.

3.3. Photodegradation of red inks

We also did some limited studies of some red inks [17,18]. Red ball point pens used today are known to contain one of (or a combination of) two dyes: Rhodamine 6B or Rhodamine 6G. The structures of these two dyes are shown in Fig. 5.

Note that Rhodamine B has four ethyl groups attached as side chains to the aromatic amines analogous to the methyl groups in crystal violet and its analogues. It would be expected therefore, that photodegradation of this dye would result in the loss of an ethyl group which would be replaced by a proton from the solvent, giving a net loss of 28 mass units. Likewise, Rhodamine 6G has two ethyl groups and two methyl groups that can be shed in an analogous manner. Figs. 6 and 7 are LD mass spectra of Rhodamine B and Rhodamine 6G. The (a) spectra are the dye before exposure to incandescent light and the (b) spectra are after 12 h of exposure to incandescent light.

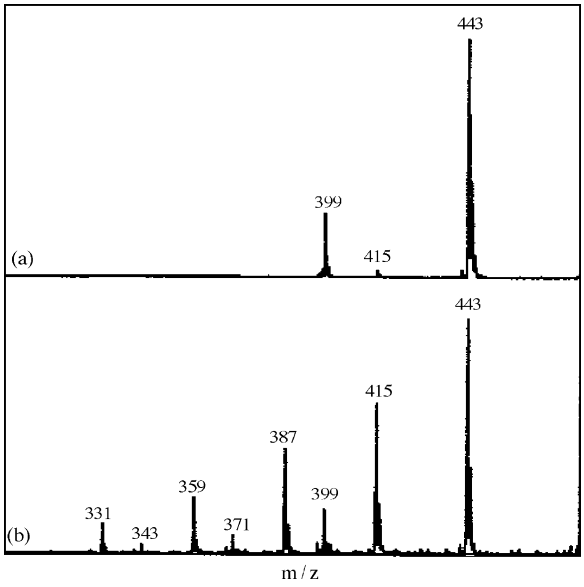


Fig. 6. Positive ion LD mass spectra of Rhodamine B: (a) is with no irradiation and (b) is after exposure for 12 h with incandescent light.

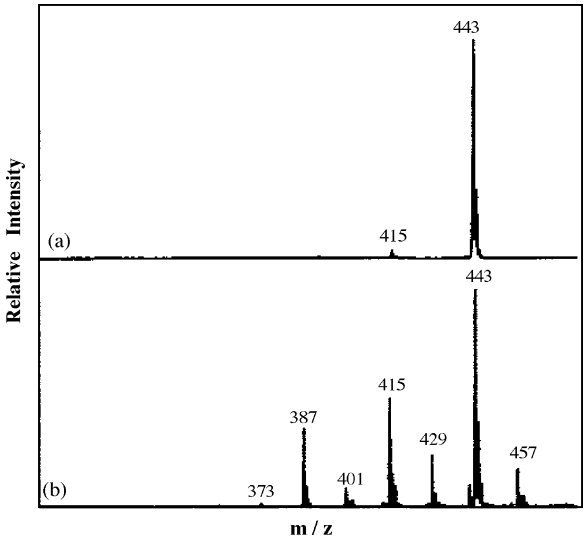


Fig. 7. Positive ion LD mass spectra of Rhodamine 6G: (a) is with no irradiation and (b) is after exposure for 12 h with incandescent light.

In both figures, the molecular weight is shown to be 443; the dyes are isomers. The peak at 399 in Fig. 6 is the result of the loss of CO_2 from Rhodamine 6B. In the photodegraded sample of this dye, there are peaks that repre-

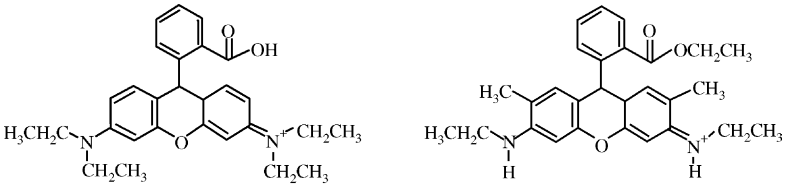


Fig. 5. The chemical structures of Rhodamine B and Rhodamine 6G.

sent the loss of four successive ethyl groups. In Fig. 7, fragments resulting from the loss of ethyl and methyl groups can be seen.

4. Summary and further work

Laser desorption mass spectrometry has been shown to be an effective technique for the characterization of ball point pen dyes. Using UV or incandescent light, ink dyes can be artificially aged in the same manner as they age naturally. This shows promise in determining the approximate age of a questioned document. This method has also been evaluated on other types of dyes such as those present in the exploding currency packets given bank robbers, and certain paint pigments.

We are now in the process of evaluating LDMS on pigmented inks where the colorants are suspended in solvents rather than dissolved. These are widely used in gel pens and computer ink jet printers.

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