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Laser Desorption/Ionization Mass Spectrometric Analysis of Methyl Violet: A New Approach to Relative Ink Age Determination

presented by

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Major professor

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LASER DESORPTION/IONIZATION MASS SPECTROMETRIC ANALYSIS OF METHYL VIOLET: A NEW APPROACH TO RELATIVE INK AGE DETERMINATION

By

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ABSTRACT

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Laser desorption mass spectrometry (LDMS) was evaluated as an analytical tool for the characterization of dyes found in inks. Experimentation revealed that colorants can be easily detected neat and on paper. The resulting mass spectra provide molecular level information, allowing for the identification of dyes and their degradation products. Industrial and forensic science applications were explored, however the focus of this work is on the forensic aspect of developing a new approach to determining the relative age of an ink on a questioned document. This method involves using LDMS to monitor the degradation of methyl violet, as an ink ages.

Dye degradation is dependent on such factors as ink formulation, paper, and storage conditions. In order to determine the relative age of a document in question, document examiners often resort to accelerated aging methods to create an "old" ink from a "new" ink. UV irradiation was found to be an effective means to accelerate the degradation of methyl violet. The same degradation products were formed as in natural aging. Natural and accelerated aging curves can be prepared, so that a correlation between natural aging and UV accelerated aging can be derived.

To my parents, Bruce Grim and Sue Salvadge, for their perseverance in teaching me the importance of determination and a strong work ethic. Their continuing love and support has given me the courage not to shy away from a challenge, and to always strive to be best that I can.

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Chapter One: Ink

Introduction

Questioned document examination encompasses not only handwriting analysis and typewriter comparisons, but also the study of ink, paper, ink-on-paper, and writing or printing instruments. As a result, when a document is called into question, numerous parameters may be subjected to scientific analysis. This work focuses on the chemical analysis of ink and ink-on-paper. Ink analysis covers a wide field, including inks found in writing instruments, printers, typewriters, stamp pads, and photocopiers. The category of writing instruments includes fountain, ballpoint, felt-tip, and rolling ball pens, to name a few. Currently in an age dominated by technology, a new emphasis on printer inks is emerging in regards to ink analysis. Laser toners and ink jet inks comprise the main printer analyses. Ballpoint pen and industrial ink jet printer inks will be the primary focus of this work, using laser desorption/ionization mass spectrometric techniques to identify ink components and monitor how they change as an ink ages.

In general, inks consist of a color component, usually a mixture of dyes or pigments, dissolved or suspended in either a water or glycol based solvent system, termed the "vehicle"(1). The name is appropriate given that the job of the vehicle is to transport the solid colorant to a substrate, commonly paper. Once on paper, solvents begin to evaporate (2). The vehicle also consists of other components, such as natural or synthetic resins, to increase the viscosity of the ink and to help the ink adhere to the paper (3). Typical synthetic resins include vinyl chloride or polyvinyl acetate (4). Additional additives, such as humectants and surfactants, are included in ink formulations to enhance the ink's visual and drying properties. Humectants act as wetting agents, by increasing

dye solubility and slowing the evaporation of the ink (5). Likewise, a surfactant's amphiphilic nature gives them the ability to decrease the surface tension of water-based inks (6), preventing the ink from drying out at the tip of a pen (1) and decreasing wettability problems with the substrate on which the ink is dispensed (6). Surfactants may also serve as defoaming agents (6). Biocides and corrosion inhibitors may be included as needed (1). Thus, ink formulations may differ significantly based on their usage. The development of improved ink analysis techniques would benefit industrial ink chemists and forensic questioned document examiners.

A Brief History of Writing Inks

Having been used for over 1000 years, iron gallotannate inks are perhaps the oldest known writing inks (4). The production of this ink involved the addition of iron salts to a fermented infusion of gall nuts (2,4,7). Gum Arabic was usually included in the suspension. The high cost of production, accompanied by oxidation problems with the ink, led to a new formulation in the 19^{th} century. The oxidation of ferric tannate was prevented by the addition of either hydrochloric or sulfuric acid, which consequently made the ink more corrosive to the writing instrument, as well as the paper on which the ink was applied. Dyes were also included in the formulation, since the ink was initially clear when applied to paper. Early dyes included indigo and aniline dyes, such as soluble blue (4).

Iron gallotannate inks are also associated with fountain pens. As time progressed, so-called "fountain pen inks" emerged as a modified iron gallotannate ink. Earlier versions were termed "blue-black permanent" ink and were characterized by a

lower iron content, and a much higher dye concentration. Later versions were dubbed "non-staining, washable inks", due to the absence of iron, and the presence of fugitive dyes, which were easily removed by oxidants (4).

Currently, ballpoint pens are probably the most widely used writing instruments. In fact, at least 80% of all questioned document examinations requiring ink analysis, were written with ballpoint pen ink (8). Ballpoint pen inks are cited as appearing on the European market in 1945, however they existed nearly 50 years before this date (4). The first ballpoint pen ink was patented in 1895 (US patent 600,299/1895), followed by another in 1898 (US patent 533,492/1898), which cited the use of lamp black suspended in castor oil (4). It is noted that there were roughly 30,000 ballpoint pens commercially available between 1935 and 1945. Due to the minimal quantity and poor quality of these pens, sources do not cite the emergence of ballpoint pens until 1945. At this time, the ink formulations were strictly oil-based. Common oils included linseed, castor, and mineral oils. Ink chemists preferred the use of linseed oil (4). These inks were characteristically neutral, and were therefore more compatible with acidic dyes, such as nitroso- or nitro-azo dyes. Around 1950, a historically significant change in ink formulations occurred, in that ink chemists switched from an oil-based medium to a glycol-based solvent system (4, 8-10). These inks were slightly acidic (pH 5-6), and were more compatible with basic dyes, including the aniline-type dyes. The introduction of the blue and green chromium and copper phthalocyanine dyes occurred around this time, in 1954 (4,9-11). Our experiments in the lab have also demonstrated that methyl violet was also introduced into ballpoint pen ink formulations around this same time period. Since the introduction of ballpoint pens, numerous types of pens have emerged, including felt tip pens (1963) (10),

roller ball pens (1970) (11), and erasable pens (1978) (11) which have characteristically different ink formulations. For instance, felt tip and rolling ball pen inks, consist of a water-based solvent system, greatly decreasing the ink's viscosity (11).

Methyl Violet or Crystal Violet?

Methyl violet was chosen as the primary focus for determining which desorption / ionization mass spectrometric technique to pursue in these studies, as well as for developing an alternative relative ink age determination method. Methyl violet is a cationic dye and is used commonly as a toner in both writing and printer inks (12). In our laboratory, the dye's presence has been detected using mass spectrometry in both blue and black pens and ink samples dating back to the early 1950's. Although methyl violet is mentioned frequently in the literature as a common dye used in inks (8,13-15), no production entry date is cited, as in the case of other common dyes, such as the blue and green phthalocyanines. However, we believe that the switch around 1950 from a strictly oil-based vehicle to a glycol-based medium influenced the types of colorants and additives, which would be miscible in the new ink formulations. As a result, methyl violet was now compatible in a glycol-based vehicle, and proved to be extremely cost effective and versatile.

Figure 1.1 is a positive ion field desorption (FD) mass spectrum of methyl violet (14). The structure of crystal violet is shown in Figure 1.1a. Crystal violet is a methylated cationic dye consisting of three phenyl groups, with six methyl ($-CH_3$) groups attached to three nitrogen atoms. This structure will be designated as C^+Me_6, representing a carbo-cation center (C^+), with six methyl groups (Me_6), and 0 H atoms

attached to the N atoms. The crystal violet cation has a m/z ratio of 372. When crystal violet is reduced, meaning that one $-CH_3$ group is replaced with a H atom, the new pentamethylated form of the dye ($C^+Me_5H_1$) is methyl violet, having a m/z ratio of 358. The mass difference between crystal violet and methyl violet is 14 amu, which is easily accounted for by the loss of the $-CH_3$ group (-15 amu) and the gain of one H atom (+1 amu). This becomes important when analyzing an ink, which is purportedly composed of "methyl violet", using mass spectrometry. In a positive ion mass spectrum of methyl violet, such as the FD mass spectrum shown in Figure 1.1 (14), the base (most intense) peak appears at m/z 372, with a small peak present at m/z 358. Dyes are usually mixtures, and methyl violet has been described as a mixture of N-hexa-methyl, penta-methyl, and tetra-methyl derivatives, with the hexa-methyl homolog predominating (14). However, quality control sets minimal limits for how much of the pure form of the dye must be present in order for the dye to still achieve its desired properties. For example, basic fuchsin, the completely demethylated homolog of crystal violet, is described as "a homologous mixture of dyes, and in any given lot any homolog may be dominant" (12). In the case of basic fuchsin, 50% of the dye mixture must be basic fuchsin, with rosaniline, magenta I, and magenta II comprising the remainder of the mixture. There appears to be no such standard for methyl violet. When methyl violet is purchased, it is essentially crystal violet. In the world of chemistry, this is very unusual in that, when you buy a 5 g sample of one compound, you get 5 g of another.

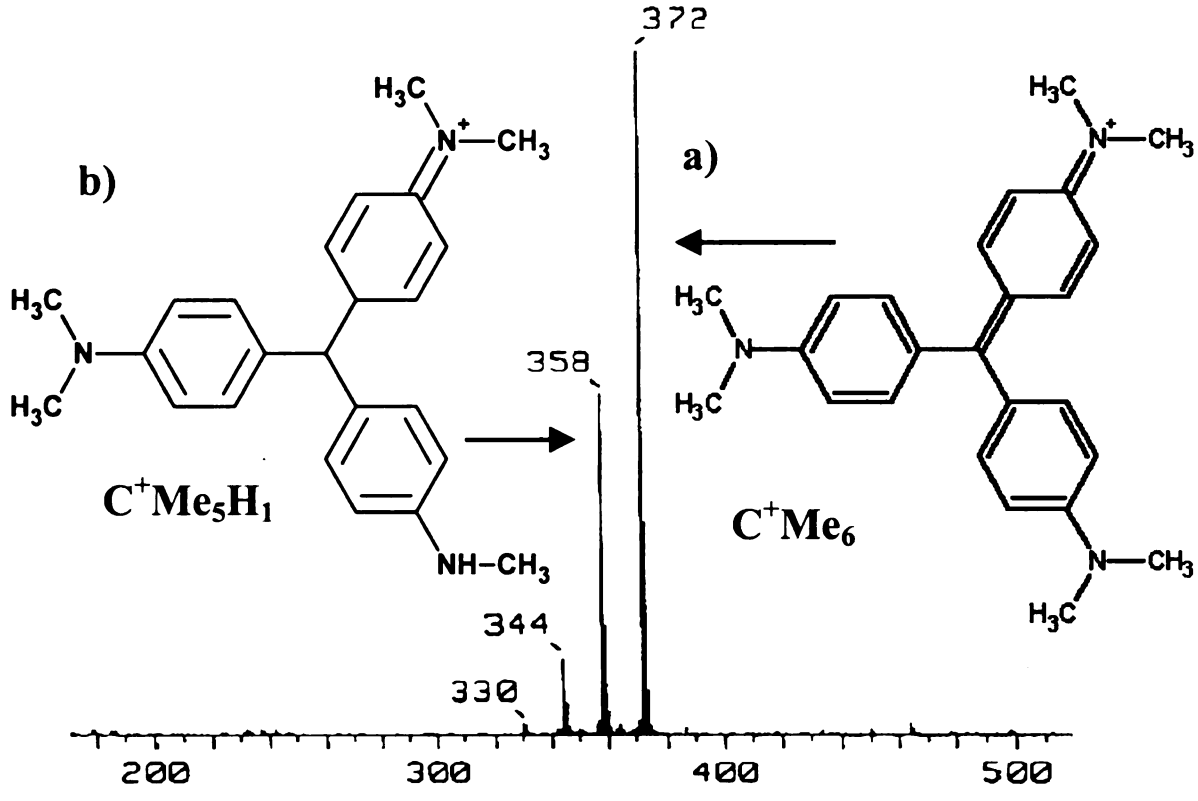

Figure 1.1: The Positive Ion Field Desorption Mass Spectrum of Methyl Violet: (a) The Structure of Crystal Violet; (b) The Structure of Methyl Violet

Another important feature of methyl violet, is the fact that it has five more $-CH_3$ groups to lose. As an ink containing methyl violet ages, each methyl group is successively lost and replaced by a H atom. This process is evident in a mass spectrum, because peaks are observed beginning at m/z 372, and continue to appear 14 amu lower than the previous peak, to m/z 288, which represents the completely demethylated form of the dye (C^+H_6). The degradation process follows an oxidative demethylation mechanism (Figure 1.2) (16). For simplicity sake, *N,N* – Dimethylaniline was used in place of the crystal violet structure, as a model to demonstrate the process (17,18). It is important to note that this is only an example of an oxidative demethylation process, which may have similarities to that which the crystal violet structure could undergo. In

this case, the sample is initially irradiated with UV-light and an electron is lost (Step 1). The resulting radical cation is attacked by either a water molecule or another base present in the ink or paper, which abstracts a proton (Step 2). The radical generated is a very good reducing agent. Consequently, the radical is oxidized forming a cationic immine complex (Step 3), which is easily hydrolyzed (Step 4). A proton transfer from the hydroxyl group to the nitrogen (Step 5) produces the final demethylated molecule and formaldehyde (Step 6). We refer to the demethylated products as "degradation products" and each are listed in Table 1.1. Again, this is just an example of an oxidative demethylation process, and not necessarily the one that crystal violet follows. For instance, free crystal violet is already a cation, therefore eliminating the need for the removal of an electron (Step 1). Furthermore, $C^{+}Me_6$ is not a radical (Step 2). Therefore, the initiating steps may be slightly different, however the chemistry of the degradation may proceed in a similar manner. The degradation mechanism can be catalyzed by the presence of oxidizers in ink or paper, such as TiO_2, a whitener used in paper production (19-21). The degree of degradation of the dye, represented in a mass spectrum can be used as a measure of how old the ink is.

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	$C^{+}H_6$

Table 1.1: Methyl Violet Degradation Products

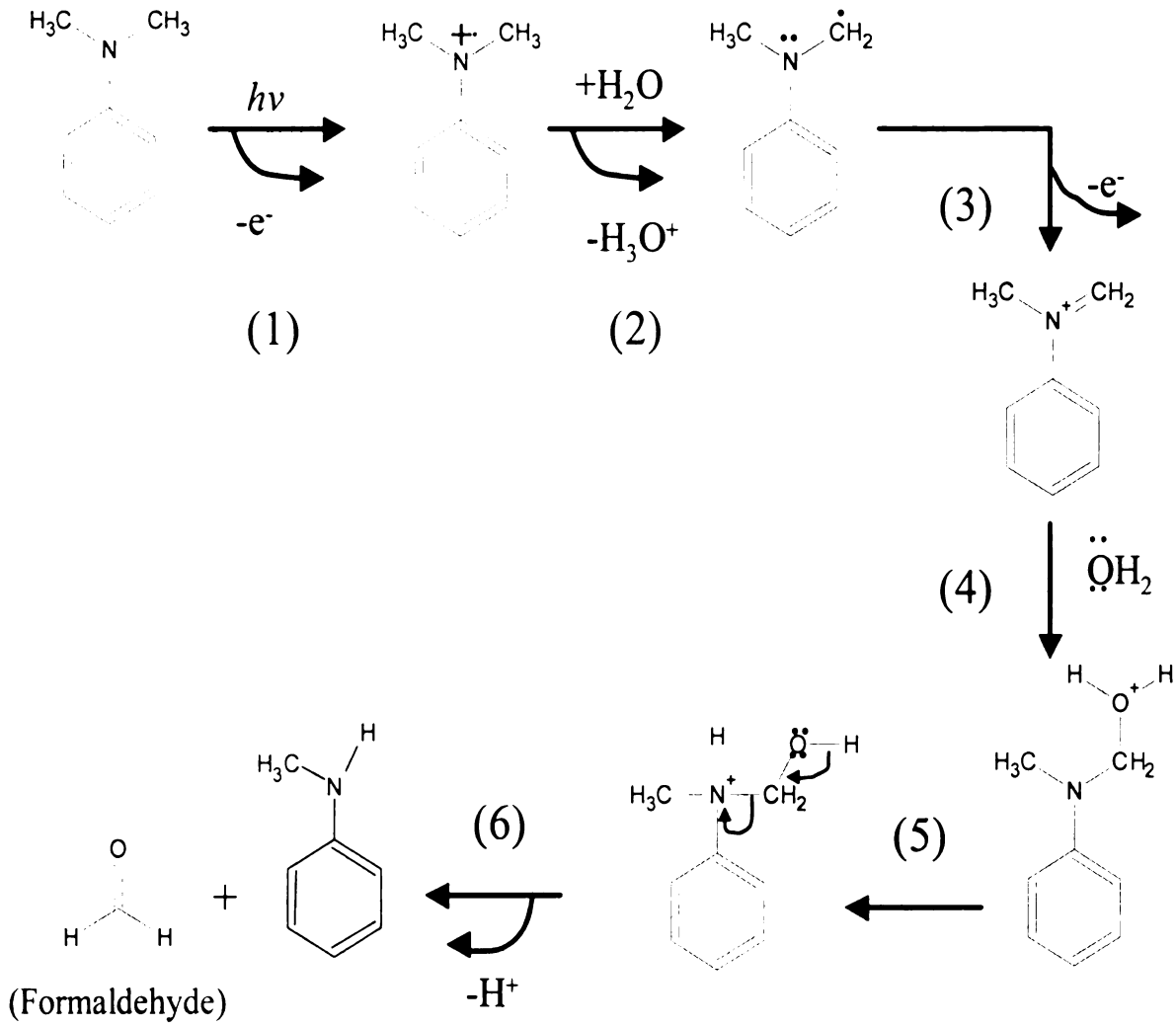

Figure 1.2: An Oxidative Demethylation Mechanism of *N,N*-Dimethlyaniline

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Chapter Two: Instrumentation

An Introduction to Desorption/Ionization Mass Spectrometry Techniques

Mass spectrometry has previously been used in the analysis of inks (1,2). A mass spectrometer is a very powerful detector following separation by gas chromatography (GC). In the GC-MS experiment, volatile components of an ink sample are separated, then ionized using electron impact ionization (EI) and subsequently analyzed by the mass spectrometer. Desorption / ionization (D/I) methods that are used with mass spectrometry have the advantage of generating ions for MS analysis from nonvolatile/thermally labile analytes. Currently there are a number of D/I methods available. These are summarized in Table 2.1. For example, field desorption (FD) can be used to generate ions from analyte molecules with molecular weights greater than 1,000 (3). In this method, analyte molecules are deposited onto a FD "emitter", which employs a carbon surface. When the surface is heated in the presence of a high electric field, ions can be generated from the analyte. FD has been used very recently for the analysis of ballpoint pen inks (4). Secondary ion mass spectrometry (SIMS) and FAB are two very similar techniques (5). In a SIMS experiment, the analyte is deposited onto a metal surface, which is inserted into the ion source of the mass spectrometer. This target is continuously bombarded with ions having several keV of kinetic energy. Secondary ions representative of species on the target surface are generated and analyzed. In the FAB experiment, the analyte is dissolved in a liquid matrix such as glycerol. This viscous solution is deposited on a metal surface, and inserted into the ion source of the mass spectrometer. This target is continuously bombarded using fast (keV) Xe atoms, typically. Both are used with magnetic sector mass analyzers. We note that both FAB

and SIMS have been used to obtain spectra of nonvolatile organic dyes (6,7, 8), and there has been one report of using SIMS to analyze colorants on paper (9). Matrix-assisted laser desorption/ionization (MALDI) (10) and LD (11) are very similar experiments, differing only in the presence of a crystalline matrix used to absorb the laser radiation in MALDI. In both techniques, a pulsed laser ionizes a solid analyte. The ions generated are separated using time-of-flight mass spectrometry. In this work, we consider FAB and LDMS as techniques for the examination of inks.

Method	**Analyte Form**	**Mechanism**	**Mass Analyzer**	**Ionization Method**
Field Desorption (FD)	Adsorbed on a carbon surface	Thermal excitation & electric-field induced emission	Magnetic Sector	Continuous
Secondary Ion Mass Spectrometry (SIMS)	Adsorbed on a metal surface	Fast atoms collide with the surface and deposit energy	Magnetic Sector	Continuous
Fast Atom Bombardment (FAB)	Dissolved in a liquid matrix	Fast atoms collide with the target and deposit energy	Magnetic Sector	Continuous
Matrix Assisted Laser Desorption/ Ionization (MALDI)	Embedded in matrix crystals	Absorb laser light from a pulsed laser	Time of Flight-MS	Pulsed
Laser Desorption (LD)	Adsorbed on a metal surface	Absorb laser light from a pulsed laser	Time of Flight-MS	Pulsed

Table 2.1: Desorption/Ionization Methods Used in Mass Spectrometry

Fast Atom Bombardment Mass Spectrometry

Instrumentation

A JEOL HX-110 (JEOL USA, Peabody, MA) double-focusing magnetic sector mass spectrometer was used to analyze the positive and negative ions formed following bombardment of samples with 10 KeV Xe atoms. A double focusing instrument consists of an electric sector and a magnetic sector. The presence of both analyzers significantly improves the resolution of the experiment. A schematic of the instrument is shown in Figure 2.1 (12). The FAB experiment uses a direct insertion probe, which introduces a planar, metal surface on which the sample is placed, into the ion source. The fast Xe atoms are generated in a JEOL charge-exchange FAB gun (Figure 2.2) (12). Xe gas is allowed to flow into the gas chamber, where Xe^+ ions are formed. The Xe atoms are ionized so that they can be accelerated by the wire mesh anode. The fast Xe^+ ions are focused through a series of lenses, which must be optimized by the user, into a gas chamber, where they are neutralized by Xe gas molecules. Bombardment of the sample with fast Xe atoms, results in desorption and ionization of the analyte. The desorbed analyte ions leave the ionization source, and enter the electric sector. In this instrument, the electric sector is placed before the magnetic sector, although the setup can be reversed. In the electric sector, an electric field (E) is applied across two plates. The electric field has two functions. The beam of ions will exit the ion source with a significant energy spread. As the beam passes through the electric sector in a circular path, stray ions are deflected away, leaving a more focused ion beam, with a smaller energy spread. Secondly, the electric field disperses ions according to their kinetic energy, based on the principle that ions of varying energies will travel paths of different

radii (13). The focused ion beam then enters the magnetic sector mass analyzer, where the ions experience a magnetic field. The ions travel circular paths of different radii, and are separated on the basis of their momentum. Momentum is the product of the mass of the ion and its velocity. The "double focused" ions reach the detector, which is an electron multiplier (13)

The electron multiplier is made of a copper-beryllium alloy, which has the ability to sputter electrons when bombarded with ions. For example, if one fast ion collides with the copper-beryllium surface, 10 electrons may be sputtered off. The 10 ejected electrons are then accelerated to another copper-beryllium surface, which is held at a slightly larger potential, where each one of the 10 electrons sputters off 10 more electrons. The process continues, until the signal is amplified 10^5-10^6 times (14).

Experimental

FAB analyses are usually performed using 1 nmol of analyte dissolved in 1-2 microliters of glycerol, the viscous liquid matrix. Here we will show that inks can be analyzed directly by FAB. In some cases, viscous solvents in the ink serve the role of matrix for the FAB experiment. To directly analyze ink on a paper surface target, several approaches were considered including saturating the paper in a glycerol matrix. However, we found that FAB will generate ions directly from a dry paper target ("dry-FAB"). This approach is used here.

The instrument was calibrated using Ultramark 4000 (PCR Incorporated, Gainesville, FL), a standard calibration compound. Analyses of ink, neat and on paper, were performed. In the first experiment, ink was applied directly to the probe tip, without

a matrix. The mass spectrometer was set for mid-field positive ion analysis. Fast atoms were generated from the JEOL fast atom gun using a potential of 12 kV and an emission current of 5 mA. These conditions produce informative spectra for the determination of ink composition. In the second experiment, the ink-on-paper sample (prepared as described in the laser desorption section) was applied to the probe tip using double stick tape. In these dry-FAB experiments, short signal durations were encountered. When a paper sample is introduced and bombarded under standard operating conditions, ions are generated and detected by the mass spectrometer, but only for a period of 3-5 seconds. Much more time is required to optimize instrument parameters and obtain a spectrum. If the flux of fast atoms is decreased, the sample is desorbed more slowly from the target, resulting in more long-lived signals. For this instrument, one way to accomplish this is to defocus the FAB beam. This decreases the number of fast atoms striking the target per unit area per second. While this decreases signal intensity, it allows for the continuous generation of ions for periods of minutes instead of seconds, so that spectra can be obtained. Both positive and negative ions can be generated and analyzed in FAB MS.

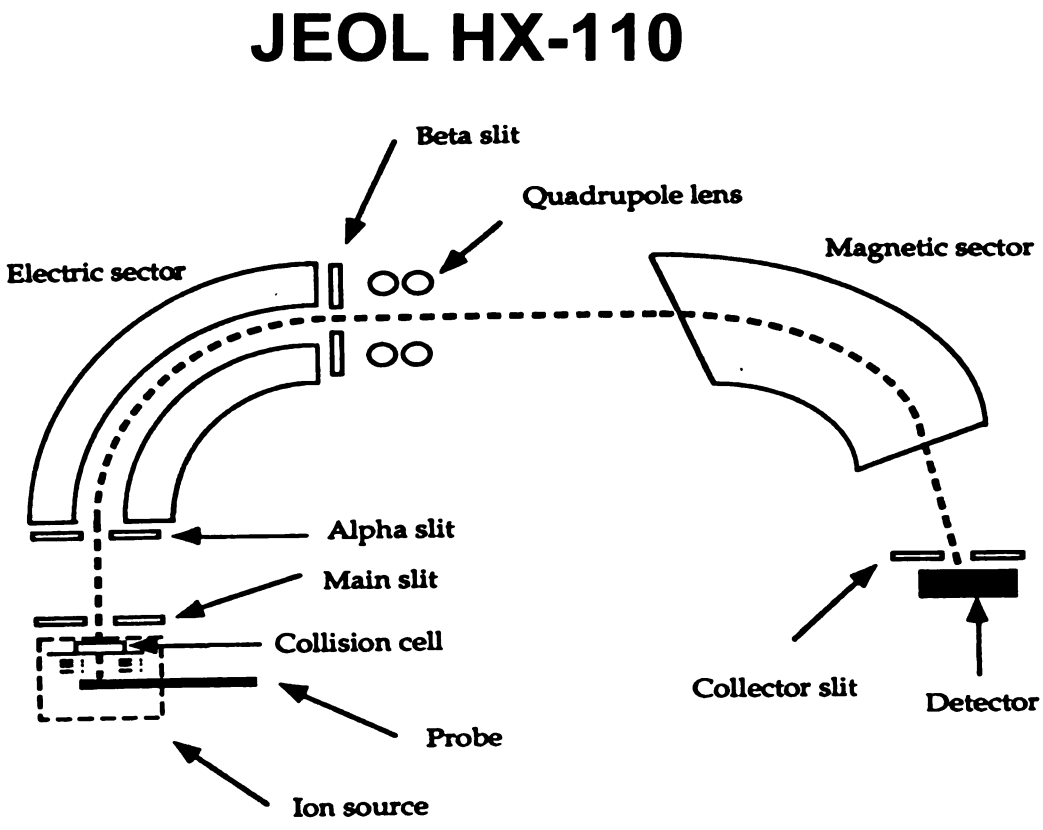

Figure 2.1: Fast Atom Bombardment Double Focusing Magnetic Sector Mass Spectrometer

JEOL Charge Exchange FAB Gun

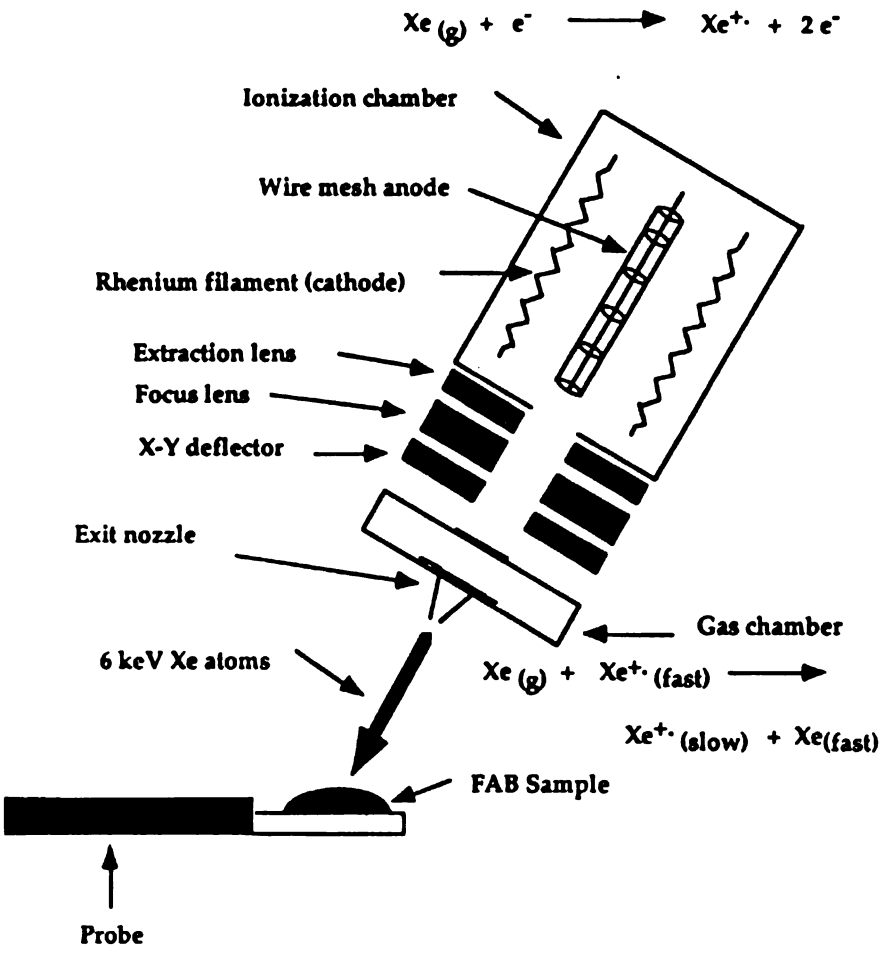

Figure 2.2: Charge-Exchange FAB Gun

Laser Desorption/Ionization Mass Spectrometry
Instrumentation

Positive and negative ion spectra of ink samples were obtained using a PE Biosystems Voyager DE instrument (Framingham, MA) (Figure 2.3). LD MS was performed on an instrument designed for MALDI MS. In the current MALDI technology, sample plates containing 100-400 "wells" are introduced into the instrument. One microliter of a sample solution can be placed in a well. The solvent evaporates, leaving a solid sample target in the well for analysis. The planar target is moved, using mechanical X-Y positioning devices, so that a pulsed laser can be focused onto various positions. For our purposes, this plate can be easily modified such that a piece of paper containing ink is introduced, and the paper moved so that the laser irradiates one or more locations where ink is present, generating ions for subsequent MS analysis.

Once the sample is introduced into the ion source, the instrument utilizes a pulsed nitrogen laser (337 nm, 3 ns, 3 Hz) to desorb and ionize the sample. The ionized molecules are accelerated and analyzed using a linear time-of-flight mass spectrometer. The guide wire located in the time-of-flight tube attracts the ions to keep them on a focused route to the detector. In the time-of-flight tube, ions of different masses accelerated with the same kinetic energy, will travel at different velocities. As a result, ions with different m/z values will reach the detector at different times. This allows for separation of the ions according to velocity (13). An electron multiplier, which was described in FAB section, is employed as the detector (14). The following user-selected parameters were employed: for analysis of the positive (negative) ions formed by LD, the sample plate was held at a voltage of 20,000 V (-15,000 V), an intermediate acceleration grid was held at 94.5% (94.5%) of the plate voltage, and a delay time of 150

ns was used between laser irradiation and ion acceleration. The manufacturer supplies a sample plate that does not contain wells, but is machined such that a polyacrylamide gel can be attached. When paper is taped onto this metal sample plate, and spectra are generated at a rate of 3 Hz, full resolution of the instrument is realized using the conditions cited here. Care must be taken to maintain a flat target.

Calibration

For TOF MS experiments, ion flight times are measured, and m/z values must be computed. To calibrate LD spectra, a known spectrum containing peaks with known m/z values is first obtained. To do so, a saturated solution of CsI (99.9%; Aldrich, Milwaukee, WI) was pipetted onto paper and allowed to dry. Laser irradiation of this target yields positive ions including Cs^+, and ions with the formula $Cs_{(n+1)}I_n^+$, such as Cs_2I^+. These were used for calibrating the instrument, so that flight times for other ions could be converted into m/z values in the mass spectra generated. Similarly, for neat ink analysis, 2 μL of the same CsI solution were pipetted onto a gold plate containing wells, allowed to dry, and used for generating spectra for calibration.

Analysis of Ink on Paper

Two industrial ink-jet inks not yet on the market, identified here as Ink A and Ink B, were supplied by an ink manufacturer, along with their compositions. Ink A and Ink B were applied to paper using an air brush (Badger Air-Brush Co, Franklin Park, Il. model 250). This method allowed for minimal, uniform sample loading. In preliminary experiments, the ink from a commercially available ballpoint pen was applied to paper by

drawing lines across the paper as evenly as possible, covering a 1 in^2 surface area. Minimizing the space between each line ensured that the laser was irradiating dye-covered paper. With experience, the laser could be focused on regular written pen strokes, such as signatures, and spectra obtained. In UV accelerated aging studies, the sample was irradiated using a UV-lamp (254 nm, 760 microwatts/cm^2; UVP Inc., San Gabriel, CA, model UVGL-58) and analyzed at various intervals. For the various experiments described, the instrument settings remained the same, with moderate changes to the sample preparation. Any differences in the sample or in its preparation will be noted when appropriate.

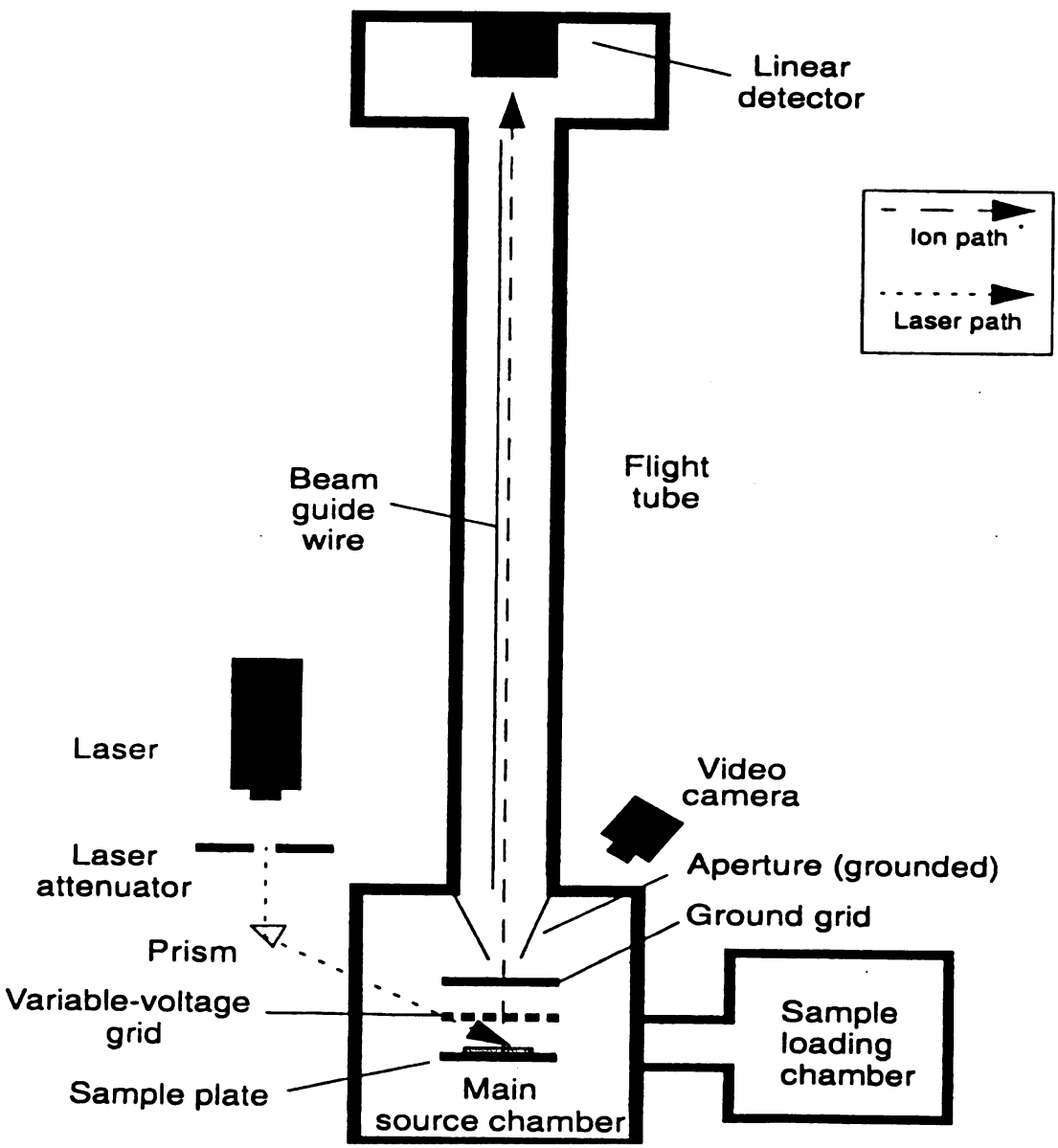

Figure 2.3: Matrix-Assisted Laser Desorption Time-of-Flight Mass Spectrometer (Voyager-DE Mass Spectrometer)

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Chapter Three: Industrial Applications

An Introduction to Ink Properties and Testing

Industrial ink chemists are continuously formulating new inks and refining old inks, driven by the consumer demand for cheap, but attractive inks. While doing so, they must also be careful not to sacrifice other important properties, such as drying time and the shelf-life of the ink. While some changes in formulations may be slight, others may have a serious impact on the types of constituents that may be used. Writing inks in particular have experienced a dramatic change since the early twentieth century. Early writing inks were composed of iron gallotanate dissolved in an oil based medium (1-3). Mid-century, ink formulators switched to a glycol based vehicle, which completely changed the types of dyes which could be included in the formulation (1,4-6).

Before an ink can be released on the market, ink chemists perform a rigorous shelf-life test (7). In this test, the ink's initial properties are measured and the ink is swabbed across a piece of paper, for a color sample. The ink is then subjected to a thermal accelerated aging study. The sample is placed in an oven (140° F) and monitored weekly for 2 months. At each weekly interval, the ink's surface tension, density, and viscosity are remeasured, and the ink is swabbed on the same paper, to detect any fading. Eight weeks at 140° F, is believed to be equivalent to 2 years of natural aging (8). A two-year natural aging study is also performed. In this study, the ink is placed on a shelf at room temperature and the same properties are monitored at monthly intervals. However, if the ink undergoes the accelerated aging technique with little to no changes in properties, the ink will be in production before the natural aging study is completed.

Lightfastness is another crucial property, that concerns ink chemists, as well as dye and pigment manufacturers. Lightfastness is the ability of ink to withstand fading when exposed to light. Some ink industries, such as the ink jet business, may not be as concerned with this property as others. Ink jet ink is frequently used for "junk mail" or printing on cardboard packages. In this case, ink chemists formulate the least expensive, fastest drying inks, with little regard to whether or not the ink will fade in a couple of years.

Mass Spectrometric Analysis of Industrial Ink Jet Printer Inks

When a solid precipitates out of ink after only two weeks into an accelerated aging study, or if a significant degree of fading is noted following 3 days exposure to UV-light, what has chemically occurred? Ink formulators are interested in knowing if an ink's components are reacting and, if so, what the products are. Desorption/ionization mass spectrometric techniques have been shown to provide molecular information about ink components. Specifically, mass spectrometry can be used to detect the initial components, as well as the products of reactions that occur. Two industrial ink jet printer inks, designated Ink A and Ink B, were supplied to us by an ink manufacturer (Graphic Controls, Inc., Cherry Hill, NJ), which were not yet released on the market. The inks would be used to print labels directly onto cardboard boxes. Consequently, the inks needed to be extremely fast drying, since an employee would be at the end of the conveyor belt to handle the boxes as the freshly printed boxes came off. The complete ink formulations were provided to us and are listed in Table 3.1. Ink A and Ink B were

analyzed in positive and negative ion modes using fast atom bombardment (FAB) and direct laser desorption mass spectrometry (LDMS).

Ink B	Ink A
Solvent Black - 12%	Methyl Violet - 5%
Triethylene Glycol - 56%	Nigrosine - 7%
Pyrollidone - 20%	Oleic Acid - 60%
Alcohol - 2%	Diethylene Glycol - 10%
Surfactant - 10%	1-Hexanol - 10%
	Surfactant - 8%

Table 3.1: The Chemical Composition of Two Ink Jet Printer Inks: Ink A and Ink B

FAB MS of Ink Directly

Positive and negative ion spectra were obtained of Ink A and Ink B using FAB. In a typical FAB experiment, 1 μL of a viscous, non-volatile liquid, usually glycerol or nitrobenzyl alcohol, is applied to the probe tip, in addition to 1 μL of the liquid analyte. The viscous liquid serves as the matrix for the analyte. The matrix absorbs and transfers the energy from the fast Xe atoms to the analyte, so that it may be desorbed and ionized. Ink A and Ink B already contain highly viscous solvents, which in a sense, act as a matrix. Therefore, in these experiments, an additional matrix was not necessary, and the inks were analyzed directly.

Figure 3.1 shows two portions of the positive ion FAB mass spectrum of Ink A. From this spectrum, a number of components were identified. The region of the

spectrum below m/z 300 (Figure 3.1a) suggests the presence of oleic acid, $C_{17}H_{33}COOH$. In positive ion FAB, neutral analyte molecules are frequently desorbed in protonated form. The neutral molecule M is generated in the gas phase as a protonated molecule, $[M+H]^+$. The peak at m/z 283 in the spectrum represents these ions. The protonated molecule efficiently loses water to form a major peak at m/z 265. Lower mass peaks at m/z 55, 69, 83, etc. are indicative of the hydrocarbon portion of oleic acid. Similar peaks are observed in EI spectra of alkanes and alkenes. A FAB spectrum of pure oleic acid (not shown) was obtained, which matches this portion of the spectrum. Oleic acid is a viscous organic acid, which is the primary solvent of Ink A's vehicle.

Figure 3.1b represents the higher mass region of the positive ion FAB spectrum of Ink A, which indicates that one of the dye components is Methyl Violet. The dye is introduces as a salt (C^+A^-). The cation is desorbed directly from the sample and C^+ is observed in the spectrum (m/z 358). As discussed in chapter 1, dyes are frequently mixtures. For this particular dye, the hexamethylated form is more abundant than the pentamethylated form (9). Note, the manufacturer used methyl violet, but the mass spectrum shows it was really crystal violet. This becomes evident when examining the ink's spectrum. The base peak (largest peak) in Figure 3.1b is seen at m/z 372, which corresponds to the hexamethylated structure, whereas the peak at m/z 358 represents the pentamethylated form of the molecule. The homologue with four methyl groups yields the peak at m/z 344. The peak at m/z 368 remains unassigned. Key at this point is the fact that the peaks in the region of the spectrum shown in Figure 3.1b are indicative of the presence of this cationic dye, and that ink components can be detected using FAB.

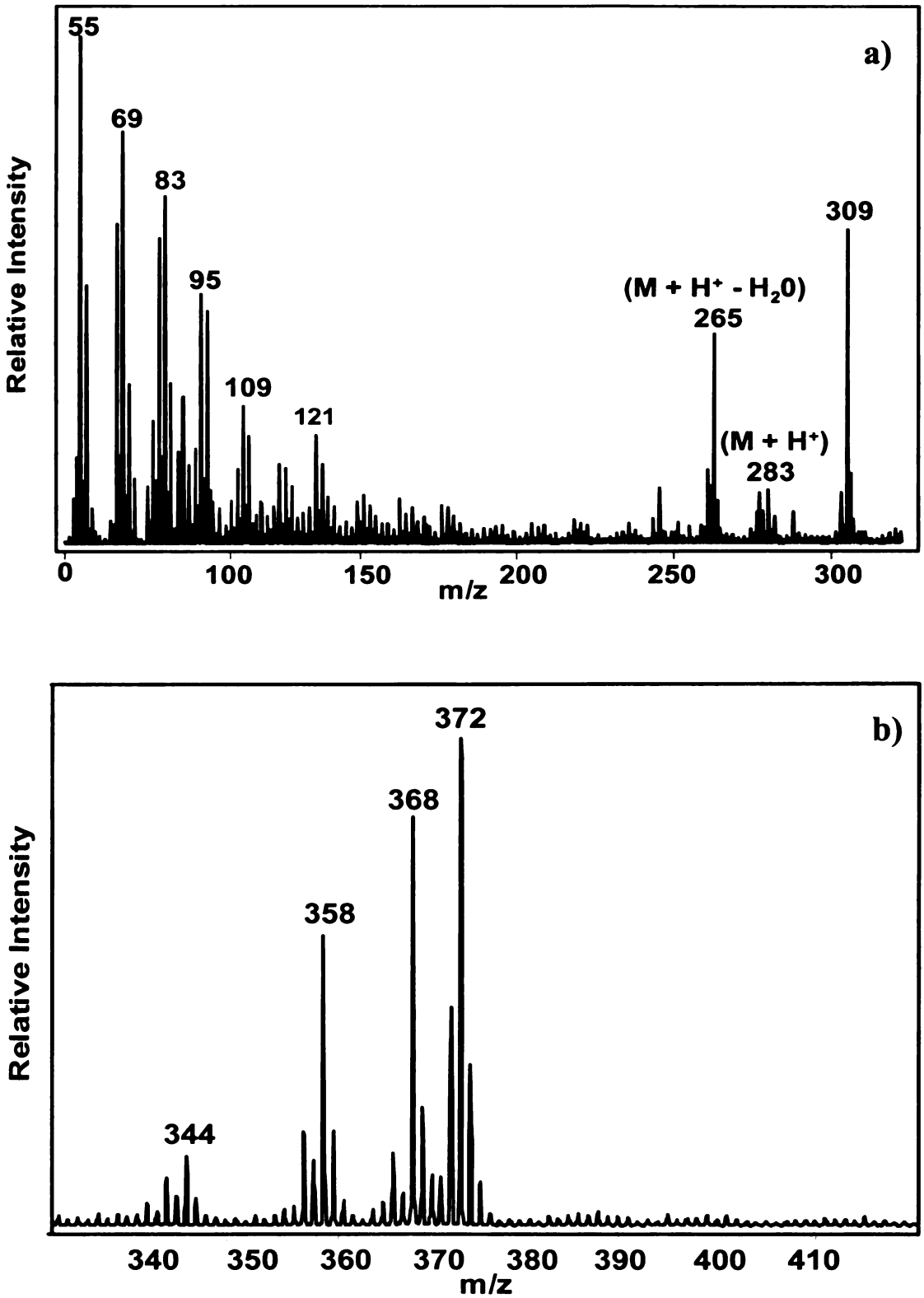

Figure 3.1: The positive ion FAB mass spectrum of Ink A: a) low mass region; b) high mass region

In contrast, Ink B contains a very different dye, Solvent Black 29. The dye consists of a Cr^{3+} center with two identical ligands, each carrying a 2- charge, attached to it. The structure (Figure 3.2) carries an overall negative charge, making it anionic. As with the cationic dye, an anionic dye is introduced as a salt C^+A^-. When detecting negative ions, the dye should be detected as A^-. In contrast to ionic materials, neutral analyte molecules are desorbed in deprotonated form in negative ion FAB, meaning that the neutral molecule M is detected in the gas phase as $[M-H]^-$. Figure 3.3a shows the low mass region of the negative ion FAB spectrum of Ink B. The primary solvent, triethylene glycol, is detected in deprotonated form at m/z 149. In Figure 3.3b, a higher m/z portion of the same spectrum, the peak at m/z 666 corresponds to the major anionic dye component (A^-), Solvent Black 29.

The FAB spectra, Figures 3.1 and 3.3, show that components of inks, especially ionic dyes, are easily detected using FAB. Stable aromatic molecular species such as dyes are desorbed/ionized in a single form – yielding a single, intense peak with no fragmentation. In contrast, oleic acid, which is made up mostly of single bonds, fragments extensively.

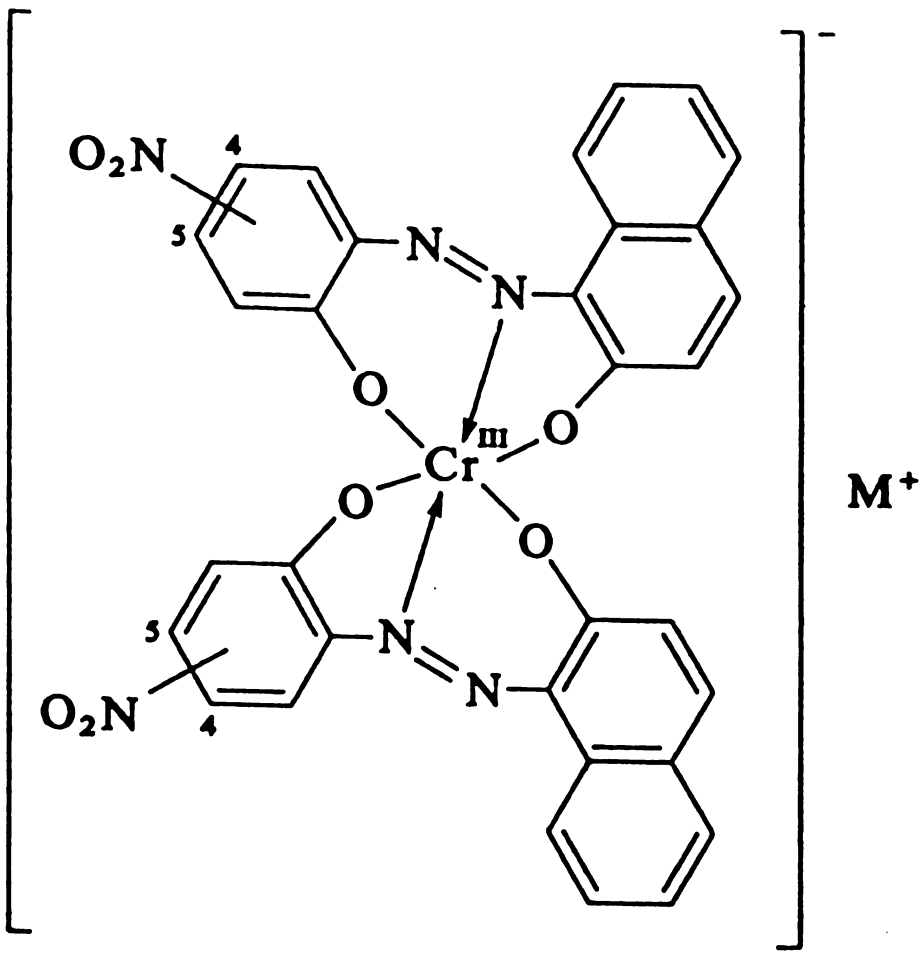

Figure 3.2: Structure of Solvent Black 29

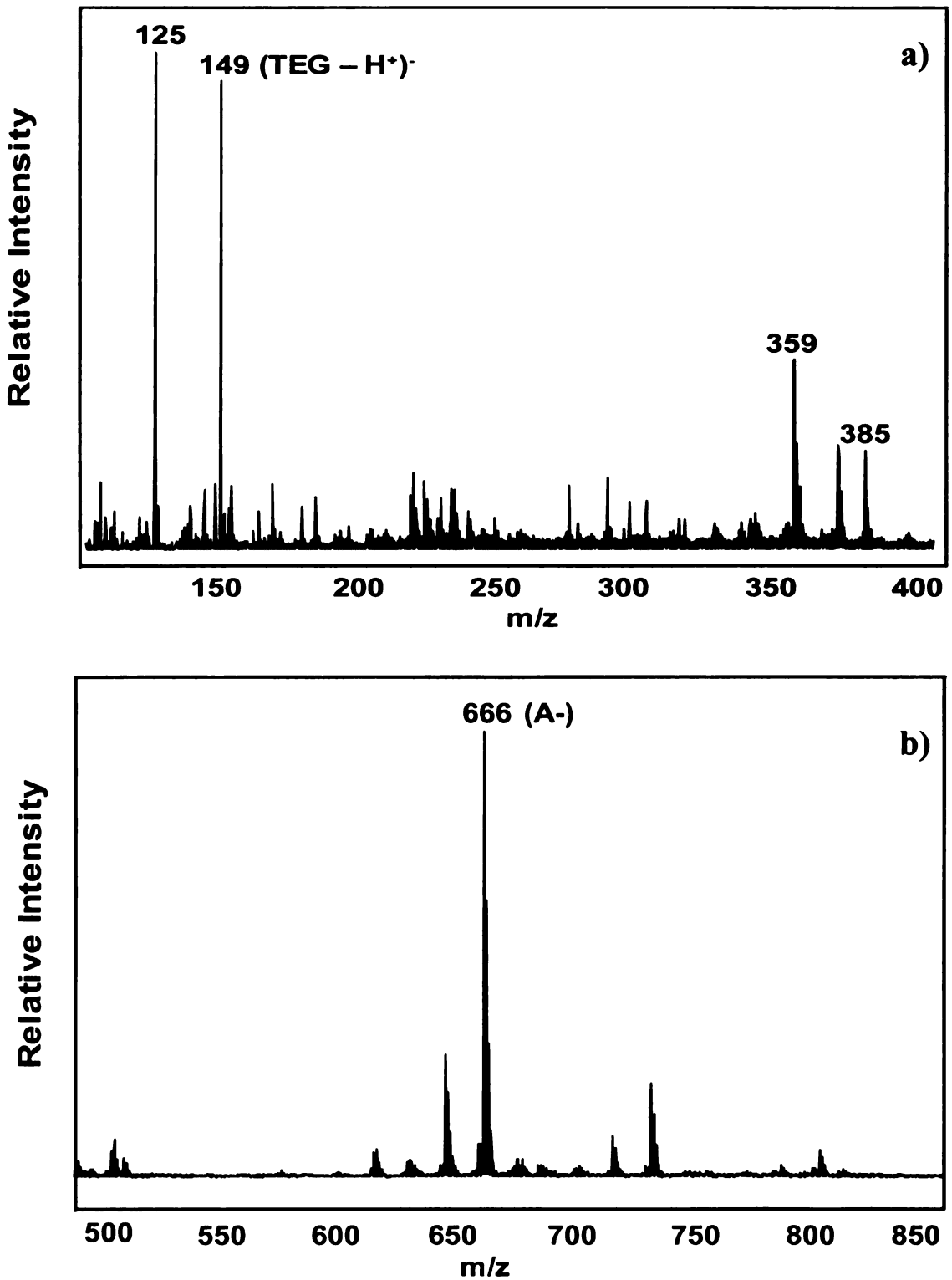

Figure 3.3: Negative ion FAB mass spectrum of Ink B: a) low mass region; evidence of triethylene glycol (TEG); b) high mass region

LDMS of Ink Directly

To evaluate the utility of various D/I methods for this field, the same ink samples were analyzed using LDMS. As an example, Figure 3.4a shows the positive ion LDMS spectrum of Ink A. Only peaks representing the dye are observed. If the spectra shown in Figures 3.1 and 3.4 are compared, it is clear that more components can be identified using FAB. This is reasonable based on the mechanism of desorption/ionization mechanism for these methods. In FAB, the mixture is bombarded with fast atoms and all species present absorb energy, and can be desorbed/ionized. If a species is ionic, it will be desorbed directly. If it is neutral, it will be detected if chemistry can occur during the FAB process that will protonate or deprotonated the molecule (resulting in positive or negative ions). In contrast, LD is more of an optical technique. Here, the sample is irradiated with 337 nm light from a nitrogen laser. Only those molecules that absorb light at this wavelength will be affected. In the case of ink, most of the components are transparent at this wavelength; only the dye absorbs and is transformed into gas phase ions. Thus, for mixture analysis of this type, FAB would yield information on a larger number of components. The situation becomes much different when the target for analysis is ink on paper, since dyes become the main component present.

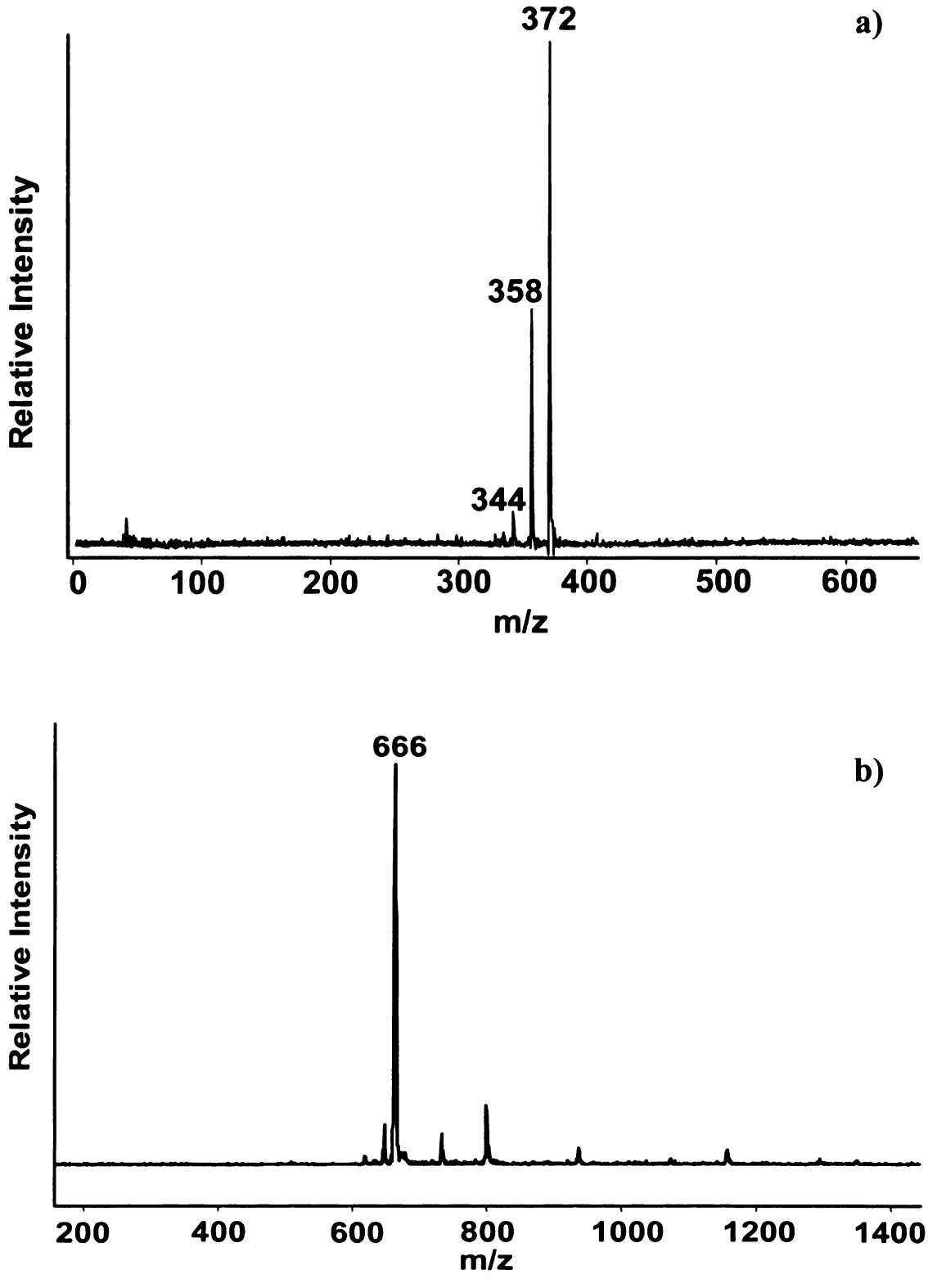

Figure 3.4: a) Positive ion LD mass spectrum of Ink A; b) Negative ion LD mass spectrum of Ink B

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Chapter Four: Forensic Science Applications

Introduction to Questioned Document Examination

"The ability to determine when a document was written would rate as one of the major breakthroughs in forensic science, having a significant impact on the detection of all kinds of fraud. This would result in a corresponding financial benefit to both State and Federal administration."(1)

Questioned document examination encompasses a vast range of analyses. Wills and hospital records are perhaps some of the more common types of documents called into question. In the case of a will being challenged, a handwriting analyst may be employed to determine if the signature is authentic or a forgery. The examination requires a trained eye to detect slight differences between the questioned signature and an examplar provided. Other types of documents require a more technical analysis. A hospital record may be called into question concerning a dated entry, which introduces the complex area of relative ink age determination.

Document examiners typically employ two main approaches to determine the relative age of an ink on paper: the static approach and the dynamic approach (2-4). The static approach is rather straightforward. It involves identifying inconsistencies in the composition of the document, including in both the ink and in the paper (2). A document examiner is searching for components, that should not be included in the formulation, since they were either not available or not used at the alleged time of production. In a classic case, the Hitler Diaries were proven to be fake by the identification of a specific

whitener in the paper, which was not used in paper production at the alleged date that the diaries were written (5). Thin layer chromatography (TLC), microscopy, solubility tests, infrared reflectance and luminescence, and UV fluorescence are commonly employed analytical techniques used to determine an ink's chemical and physical properties (6,7). The ink's compositional profile is then compared to a reference library. In 1968, Brunelle initiated a comprehensive ink library at the Bureau of Alcohol, Tobacco, and Firearms. The collection, which maintains over 5000 different ink formulations and is complete for inks in production since 1958, is now maintained by the United States Secret Service (2,5-7). The "Ink Library" approach is obviously quite limited, since samples are not easily accessible to examiners outside of the Secret Service.

A more sophisticated static approach for ink age determination was introduced in the mid-1970's, which eliminated the need to identify the ink formula or the manufacturer. The ink tagging system, initiated in 1975, required ink formulators to include a marker, which would be unique only to that ink (2,5-7). The tags were changed yearly, so that the year of manufacture could be positively identified. Samples of the inks and a record of the included tags were kept in the International Ink Library, which is maintained by the United States Secret Service. The tags were usually trace quantities of rare earth organometallic compounds or trace amounts of optical whiteners. The former could be detected by performing an x-ray optical fluorescence analysis on a prepared $SrWO_4$ phosphor from an organic extract of the tag. The whiteners could be analyzed using a TLC method, which would separate the tags, without separating the dyes (2). The response was initially overwhelming, with 50% of all US writing inks tagged by 1978. (6). However the large influx of foreign inks, compounded with ink manufacturers'

frequent ink formulation modifications, resulted in a rather short lived program. Consequently, document examiners sought additional ways to identify inks, and to determine their relative age.

The dynamic approach to relative ink age determination involves the complex evaluation of the aging process. As ink ages on paper, solvents evaporate, resins polymerize, and dyes degrade. Numerous methods have been explored which monitor these different aspects of the ink. Though these methods differ significantly, they all share a set of common limitations. For instance, in order for two inks to be accurately compared, the inks must be on the same paper, of the same formula, and stored under the same environmental conditions (relative humidity, temperature, light conditions, etc.) (3,6). If the ink samples are on separate pages, the storage history of both documents must be known. Additionally, the parameter studied should be independent of the amount of ink sampled (6). Establishing the accuracy of the experiment, is also a key factor. Ink aging curves behave exponentially, meaning that the initial rate of aging proceeds very quickly, and then slowly, asymptotically levels off (3,4,6,8). The rate of aging and point of leveling off are dependent on the ink formulation. Furthermore, the accuracy of the technique significantly decreases at this stage of the aging process. Lastly, one of the two inks must be of a known date. Often times, a document examiner does not have access to a comprehensive ink library, such as the International Ink Library, to compare his or her questioned sample to inks of known age. Consequently, accelerated aging techniques have been developed to create old ink from new ink, in a very short amount of time (9-11). The following sections will briefly outline some of the notable contributions from the veterans in the field of forensic document examination.

Notable Contributions to the Field

Early ink dating methods, developed in the 1930's, were used to determine the age of iron gallotannate inks. These methods, termed ion migration tests, were credited to Mitchell (England) and Hess (Germany) (3,5-7,12). Their studies focused on the migration of ions, specifically chloride (Cl^-) and sulfate (SO_4^{2-}) ions, assumed to be present in the ink. It was believed that over time these ions diffused into the paper, away from the initial ink line. However, the ions were not visible, and therefore needed to be developed chemically to visualize the "ion diffusion picture". Figure 4.1 illustrates a Cl^- ion migration test. Figure 4.1a represents an ink sample on paper at the initial time the ink was applied to paper. Cl^- ions are assumed to be present in the ink. Over time, the Cl^- ions migrate into the paper, away from the ink line (Figure 4.1b). The ion migration test begins by removing a small piece of paper (approximately $1cm^2$) with ink from the document and placing it in a vial. Silver nitrate ($AgNO_3$) is added to the vial, and silver chloride (AgCl) is formed (Figure 4.1c). Addition of a reducing agent, such as formalin, results in the formation of elemental silver from the AgCl. The Ag metal produces a "black shadow" on the questioned document (Figure 4.1d). The distance the Cl^- ions migrated is measured as a function of age. The greater the distance traveled, the older the ink. The damaged sample is placed back into the document. SO_4^{2-} ions may also be monitored, however the chemistry of the ion migration test is significantly different, employing lead perchlorate and potassium permanganate solutions (3,12). Besides the high destructiveness of this technique, ion migration tests have serious limitations. Cl^- and SO_4^{2-} ions were believed to cease diffusing into the paper after only one or two years, which significantly limited the time span for age determination (3,12). The low

accuracy of the experiment, combined with the presence of too many assumptions, prompted document examiners to develop a more scientific approach.

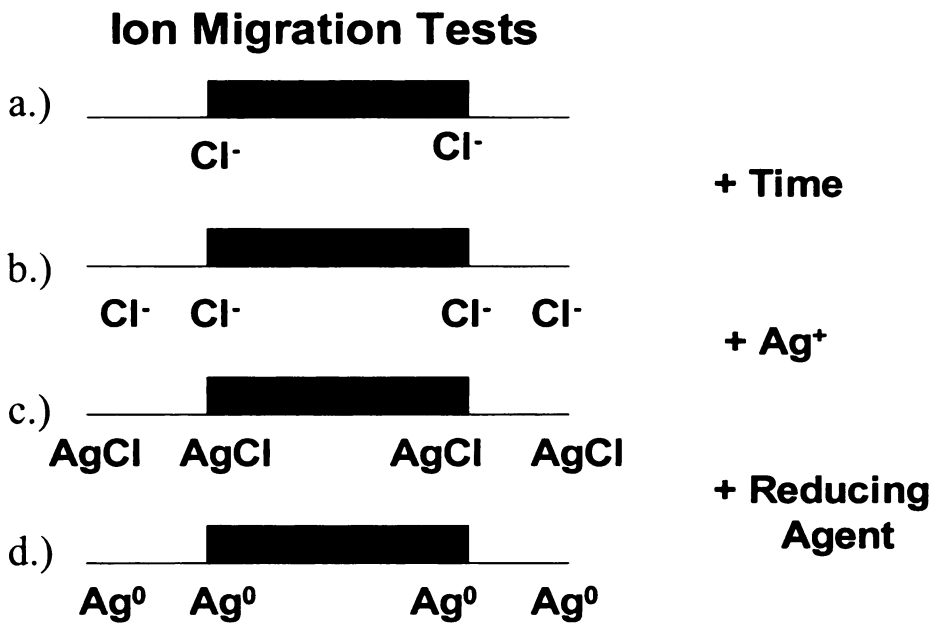

Figure 4.1: The Cl^- Ion Migration Test

In the 1950's, Kikuchi developed the foundation of the well-accepted and employed solvent extraction methods (3,6,7). Like Mitchell and Hess, Kikuchi studied the aging of iron gallotannate ink. She noted that following the application of solvents to a document, older inks took longer to disperse into the paper than newer inks. She concluded that an ink's solubility decreases with age, and worked towards using this parameter as a measurement of the relative age of an ink. In her experiment, a drop of dilute oxalic acid was placed on an ink stroke. Using a microscope, she watched the ink disperse into the oxalic acid, and recorded the time at which the ink stopped dispersing (3). This proved to be the development of a very primitive relative ink aging curve.

Kikuchi's work extended to ballpoint pen ink analysis, using dilute HCl, as in place of the oxalic acid.

On the same principle as Kikuchi's work, Witte focused on the disappearance of the solvent system as a function of age. His work concentrated on using the ink's "copying power" as an indication of the ink's age (6). Newer inks are not completely dry. When a piece of paper is pressed on top of the questioned sample, some ink will be transferred to the other paper. Volatile components evaporate quickly as an ink ages on paper, ultimately decreasing a document's copying ability. Witte concluded that the amount of ink transferred will decrease with the age of the document (6).

Quite a few years later in 1984, McNeil extended the ion migration test of Mitchell and Hess, to include the migration of Fe^{2+} ions (7). Using scanning Auger microscopy, McNeil was able to detect the extent of the migration of these ions. He concluded that Fe^{2+} ions moved 1 micron every 29 years. McNeil had hoped that this would help to alleviate the short time frame limitation of the Cl^- and SO_4^{2-} ion migration tests, however his method proved to be rather inaccurate. Additionally, McNeil was not able to reproduce his technique using accelerated aging techniques, the importance of which will be discussed in a later section.

As the scientific field became more technologically advanced, questioned document examiners sought the use of advanced analytical instrumentation to more efficiently quantitate ink aging parameters, such as the evaporation of the solvent system or the degradation of dye components. Fourier transform infrared spectrophotometry (FTIR), gas chromatography (GC), and microspectrophotometry have been evaluated as tools for analyzing parameters of the ink aging process.

Humecki is given credit for studying ink aging using FTIR (3,6,13). In this experiment, a 0.25 ink-on-paper sample is removed from the document in question. The ink is extracted from the paper with pyridine, and the solution is transferred onto a salt window. Humecki observed differences in the IR spectra of new and old ink. Specifically, he noted the hydroxyl (OH), methyl (CH), and carbonyl (CO) absorption bands changing with time, which he related to the solvent evaporating and the nonvolatile components oxidizing. Humecki concluded that plotting the ratio of the OH absorption band to the CH absorption band versus time was a fair representation of ink aging. The resulting aging curve had the characteristic exponential shape, and leveled off after ten years. The ratio between the OH and CO infrared absorption bands was considered as a function of age. However, the results were not as consistent as using the ratio of the OH and CH absorption bands, meaning that the plot OH/CO ratio versus time sometimes changed direction, while the plot of OH/CH ratio did not. A decrease in the ratio was believed to be related to an older ink. The use of a ratio rendered this technique to be mass invariant.

In 1985, Stewart utilized GC to study the disappearance of the solvent system as an ink ages (3,6,7). Generally, solvents are believed to remain in the paper for up to 1 to two years (6). Some may argue that the solvents are always present, since the volatile molecules may become trapped in the polymerized resins (14). Based on this premise, Stewart used strong solvents to extract the volatile components of the solvent system, and analyzed them using a GC. He reported a significant decrease in the volatile components, for up to year, as the ink ages (7).

Extensive work has been completed by Aginsky in developing microspectrophotometry for monitoring both the solvent system and the nonvolatile components during ink aging (3,14-16). One area of interest involved the study of the sensitivity of inks to the exposure to certain gases, such as benzylamine and piperidine (3,14). He observed that a number of fresh inks undergo reversible color changes. Aginsky measured the reflectance values of the inks as they changed color, using a microspectrophotometer. He then related the rate of color change to the age of the ink, noting that the rate of color change decreased as a function of age. Aginsky justified the depressed rates as a result of the decrease in the available molecules to react with the gases, since many molecules become trapped in polymerized portions of the ink film.

The study of optical surface changes in inks as they age was also pursued using a polarizing microspectrophotometer (16). During this experiment, Aginsky observed that the ratio of dyes present in the ink change on the outer surface layers of the ink film, as the result of oxidation. Specifically, he studied copper phthalocyanine and methyl violet. Spectral reflectance measurements of the surface layer were first taken under non-polarized light, followed by diffuse reflectance measurements of the interior layer under cross polarized light. The cross polarized light prevented the surface spectral reflectance from interfering with the diffuse reflectance measurements. The measurements were taken at two different wavelengths, determined by the dyes present in the ink. A surface reflectance ratio (R_1) and a diffuse reflectance ratio (R_2) of the interior surface for the two dyes were computed.

$$R_1 = \frac{\text{outer reflectance}_{(\text{dye 1})}}{\text{outer reflectance}_{(\text{dye 2})}} \qquad R_2 = \frac{\text{inner diffuse reflectance}_{(\text{dye 1})}}{\text{inner diffuse reflectance}_{(\text{dye 2})}}$$

The data analysis is a bit complicated, in that the aging parameter actually studied is a ratio of the two ratios:

$$\frac{R_1}{R_2}$$

This ratio was observed to decrease with age, leveling off in approximately six years (16). The technique is mass invariant, however large errors are associated with the aging curve.

Though all of these techniques have merit, not one has been more embraced by the field of questioned document examination than the solvent extraction methods developed by Cantu and Brunelle (3,4,6-8,17). Fairly recently, Brunelle proposed a sequential multiple approach for determining the relative age of writing inks (17). The approach involves the three most commonly used techniques, which include the R-Ratio, the sequential extraction, and the Dye-Ratio methods.

The premise behind the solvent extraction technique lies in the fact that as an ink ages, the vehicle slowly evaporates. In theory, a relatively new ink sample on paper will not be completely dry. Consequently, some of the solvent system will still be present in the paper, making extraction of the ink using weak solvents easier. An older ink sample on paper will have dried to a greater extent, making the rate and extent of extraction much lower. Extraction curves are prepared based on two principles: the R-ratio and the L^{th} extraction time (percent extraction) (3,6,17). The R-ratio is a measure of the ease and rate of extraction, whereas the L^{th} extraction time is related to the time needed to extract a certain percentage of the ink. For instance, a value of L = 0.90 is the time at which the

extraction is 90% complete. The percent extraction technique has been determined to be a more efficient means to date ink, as compared to the R-ratio method.

R-Ratio

In 1979, Cantu developed a technique, based on Kikuchi's work, which could be used to measure the rate of the extraction of ink into weak solvents. To begin the technique, samples are obtained from the document using a blunted or hollow 20-gauge boring hypodermic needle; 10-15 micro-plugs (approximately 1 mm in diameter) are removed (13,18,19). Alternatively, a 1 cm line can be removed with a scalpel. The micro-plugs are placed in 3-5 μL of a weak solvent, typically isopropanol, toluene, or n-butanol for ballpoint pen inks, or a 1:1 solution of ethanol and water for other pens (18). When ink on paper is extracted using a weak solvent, the color of the solvent becomes darker, indicating that the ink is dissolving. The sample is agitated for a couple of minutes to ensure that the extraction is complete. The solution containing the extracted ink is spotted on a TLC plate. The concentration of ink present is determined using a densitometer. Concentration measurements are taken at various points during the extraction, with the last measurement taken at or near the completion of the extraction.

An alternative method is to dissolve the ink on the punched out discs directly in a cuvette for either a UV-visible or a fluorimetry experiment (17). The concentration is calculated using Beer's Law, $A = \varepsilon bc$. A is the maximum absorbance value, ε is the molar absorptivity constant, b is the path length, and c is the concentration of the analyte (ink).

The R-ratio is defined as C_1/C_2. C_1 is the concentration of the ink in the extracting solvent in the initial minutes of the extraction, which is representative of the highest rate of extraction. C_2 is the concentration of the ink in the extracting solvent taken at the completion or near completion of the extraction of the ink from paper. The "newer" inks will theoretically have larger R-ratios than the older inks, since more ink can be extracted from an ink when most of the solvent is still present. Thus, the R-ratio is expected to decrease as the ink ages. Unfortunately, this is not true for every ink formula. Since it has been observed for the R-ratio of some inks to actually increase over time, the ink in question must first be tested by artificially aging a portion of the questioned document to see in which direction the R-ratio moves as a function of age (17).

The Sequential Solvent Extraction Method (L^{th} Extraction)

The sequential solvent extraction technique is an extended version of the single extraction (R-ratio) method previously described. The first part of this experiment is identical to the R-ratio method, in which the sample is obtained and extracted in the same manner. However, following the first extraction in the weak solvent, the punched out discs are placed in a stronger solvent, typically pyridine, so that the remainder of the ink can be extracted. A percent extraction of ink in a particular solvent after t minutes is then calculated using the following equation:

$$P = [v_1C_1/(v_1C_1 + v_2C_2)] \times 100$$

C_1 and v_1 refer to the concentration of ink in the first extracting solvent and the volume of weak extracting solvent used, respectively. Likewise, C_2 and v_2 refer to the concentration

of ink in the stronger extracting solvent and the volume of that solvent used, respectively. If equal volumes for solvents 1 and 2 are used, the equation may be reduced to:

$$P = [C_1/(C_1 + C_2)] \times 100$$

This equation may be used if TLC-densitometry is used as the analytical technique for determining concentration. However, if fluorescence is used a modified version of the equation is considered:

$$P = [v_1/(v_1+v_2)] \times (F_1/F_2),$$

where F is equal to the fluorescence measurements. Figure 4.2 (6) is representative of a sequential solvent extraction experiment, in which toluene was used as the weak solvent and benzyl alcohol was used as the stronger second solvent. Figure 4.3 (6) represents results from two different single extraction experiments using 2-propanol performed on the same day. The near overlapping curves suggest a high reproducibility factor for the solvent extraction method.

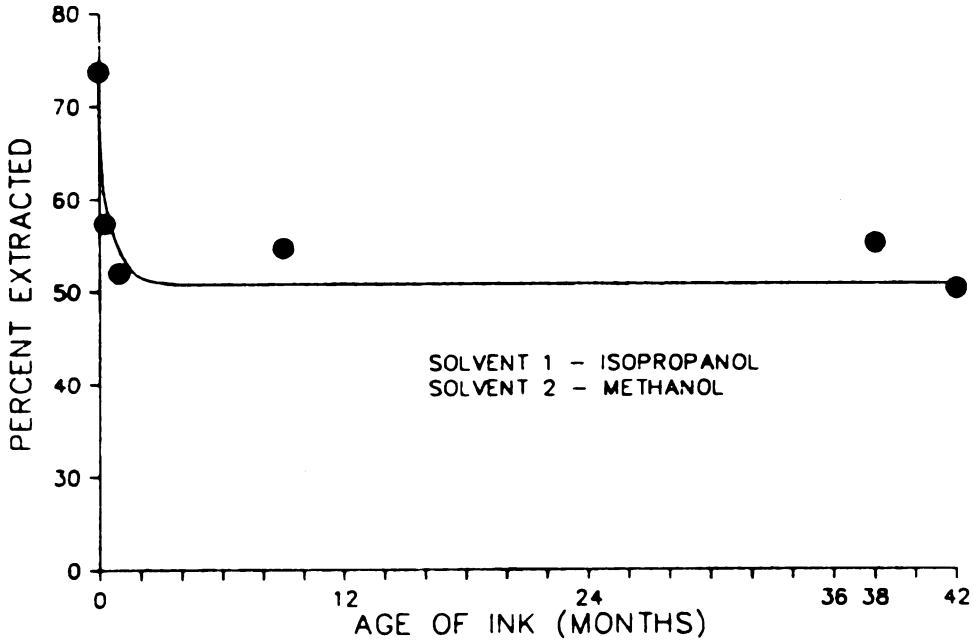

Figure 4.2: Aging of ballpoint pen ink (sequential solvent extraction) with Anja M-311

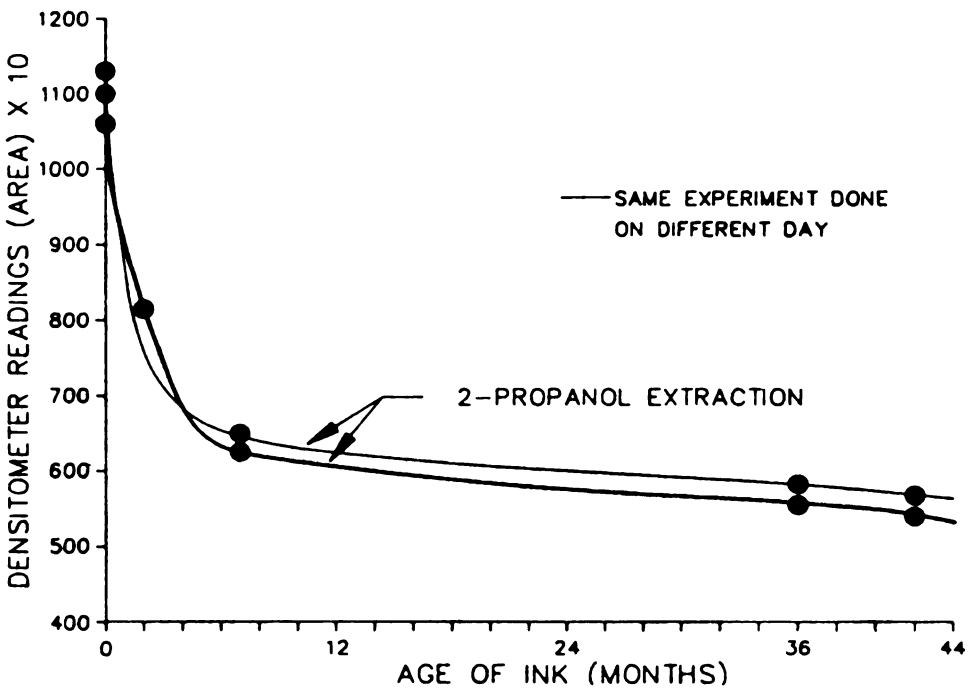

Figure 4.3: Aging of ballpoint pen ink (single solvent extraction) with Anja M-311

Questioned document examinerstend to prefer using a percent extraction value as opposed to considering only the rate of reaction (R-ratio). However, this method also has its limitations. For instance choosing the second solvent sometimes can be a troublesome task. Figure 4.4 (6) represents a sequential solvent extraction curve in which the second solvent was not a wise choice, since n-butanol and pyridine are not miscible.

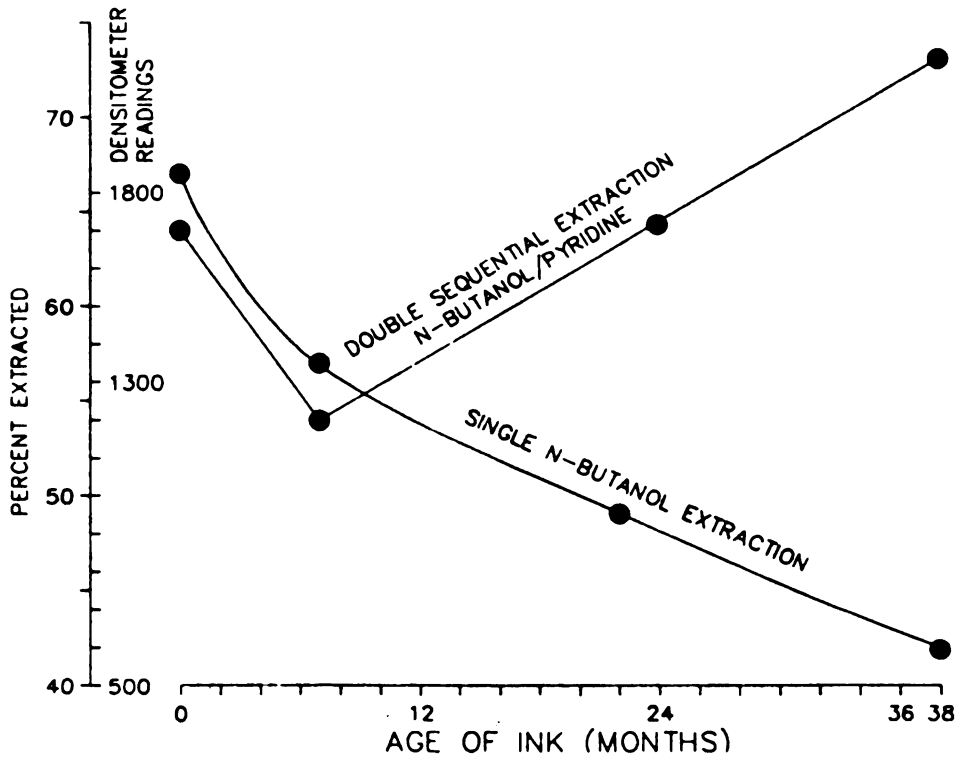

Figure 4.4: Aging of ballpoint pen ink with Formulab 587

Other disadvantages, as in other ink dating methods, include differences in drying rates of the inks. Most inks dry completely within two years, with some inks drying in less than two months. Therefore, this technique could not be applied to cases in which the age of the ink in question exceeds this time frame. Also, as the aging curve becomes asymptotic (reaching its leveling off point), the accuracy of the age determination decreases

significantly. This leads to the question of whether or not using the disappearance of the solvent system is the best option for ink age determination.

Limitations exist for both the R-ratio and L^{th} extraction time interpretations of the extraction curve. First of all, numerous points are required on the curve in order to obtain optimal accuracy. Also, many errors may be introduced during the actual sample preparation. For instance if TLC is used, extreme caution must be taken to ensure that equal amounts of sample are spotted on the plate. If fluorimetry is used, the ink must contain a dye that fluoresces, and if not, a known standard (rhodamine-type dyes) must be added which is known to fluoresce (17). In this case, a measurement of the fluorescence "robbed" by the non-fluorescent extracting solvent is taken. The most obvious limitation deals with the fact that the inks need to be first tested to see in which direction their R-ratio or L^{th} extraction values are going to migrate over time. This suggests that a complete understanding of the chemistry that is occurring between the ink and the paper over time has not yet been obtained.

Dye-Ratio

The Dye-Ratio method measures the change in the relative concentration of dyes in the ink extracted in a weak solvent (7). As previously mentioned, inks are usually composed of a mixture of dyes or pigments. Ink samples are first extracted and spotted on a TLC plate as previously described. Typical TLC plate developing solutions for ballpoint pen inks, include ethyl acetate/ethanol/water (70:35:30) and n-butanol/ethanol/water (50:10:30) (1). Once the dyes are separated, their concentrations are obtained using a densitometer. All possible dye ratios are considered and monitored

over time. In a typical blue ballpoint pen ink, the dye composition consists of Cu phthalocyanine blue (dye 1), as the primary dye, and methyl violet (dye 2), as a toner. It has been noted that the ratio of dye 2/dye 1 will decrease as an ink ages. Methyl violet is significantly less stable than Cu phthalocyanine blue, therefore this would be expected. It has been noted that no work has ever been attempted for the purpose of explaining the degradation of the dyes or the dynamics of the Dye-Ratio method. Our work explores the dynamics of methyl violet degradation and how it can be used as an alternative to the solvent extraction techniques for determining the relative age of questioned ink.

Accelerated Aging – The Thermal Approach

In order to determine the relative age of an unknown ink, there needs to be a known standard to compare it to. Using artificial aging techniques, a new ink can be made to appear chemically as an old ink. The artificial aging process is monitored so that aging curves may be developed. Data from the unknown ink sample is compared to the artificial aging curve, and its age is subsequently determined. Currently, the most accepted method for accelerated aging is using heat, which is based on the Q_{10} theory. The Q_{10} theory states that for a 10°C increase in temperature, the rate of a reaction (decomposition) doubles (20). Interestingly, there does exist a huge disagreement amongst questioned document examiners as to the correlation between the length of time the ink sample is heated as a function of its age. For instance, it has been documented that heating the sample in an oven at 100° C for 4 minutes is equivalent to 8 days of natural aging at 25° C (21). On the other hand, another source states that exposing the ink sample to the same conditions is equivalent to 20 days of natural aging at 22° C (11). Cantu considered both of these claims and proceeded to perform his own accelerated

aging analysis. His studies indicated that 90 days of natural aging at 25° C is the same as heating the sample at 100° C for 4 minutes (11). This discrepancy between the correlation of natural aging and accelerating aging introduces the possibilities of significant errors when using this artificial aging method to set up standards for comparison to a questioned ink sample. However, it is important to note that these accelerated aging correlations are based on paper aging studies (10). Ink, paper, and ink on paper, all age quite differently. Thus, there exists an absence of a true understanding of the dynamics of ink aging on paper. Furthermore, it has been cited that many of these correlations are pertinent only to a defined set of conditions, such as a particular ink on a specific type of paper (10). We encountered the same discrepancies using an alternative method of UV-light to accelerate a very different aging parameter – dye decomposition.

Summary

In summary, a "perfect" method for determining the age of a questioned document may not exist. There are numerous variables involved in this type of analysis, which can not be controlled. Such variables include the various types of media the inks are printed on, storage conditions (temperature and the presence or absence of light), the writing instrument, etc. Another defect is developing the best accelerating aging technique. Heating definitely has its flaws, and UV irradiation has not yet been proven. Perhaps, there is a more efficient technique not yet tested, such as creating an oxygen enriched environment using ozone to accelerate the oxidative demethylation process of methyl violet. Lastly, it is possible that both the disappearance of the solvent system, as well as the degradation of the dye components need to be considered concurrently.

Studies would need to be performed to determine if these two techniques compliment each other. Overall, the sequential solvent technique is sufficient for current analyses of questioned documents, however there is always room for improvements or more efficient methods.

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Chapter Five: LDMS Analysis of Methyl Violet on Paper

Introduction – The Detection of Ink on Paper

In Chapter 3, the ease of detecting methyl violet neat in FAB MS and LDMS experiments was demonstrated. While industrial ink chemists would use results from the analysis of bulk ink, forensic chemists would benefit more from the analysis of ink on paper. An important question is related to the sample sizes involved. Is there a sufficient surface concentration of dye deposited on paper during writing or printing to be detected in a FAB or LD experiment? Consider a typical ballpoint pen cartridge, which contains about 0.6 g of ink (1). For the pen we used, the written line was 0.36 mm wide. We assumed that an ink contains 20% dye by weight. In the case of methyl violet (molecular weight of 372 g/mol), the ink cartridge would contain 0.3 mmol of dye. Also, considering that one ink cartridge can write a line approximately 3000 m long (1), this correlates to about 0.3 mmol/m^2 of sample, as a molar surface coverage per unit area. In a FAB experiment, a nanomole of analyte is typically dissolved in microliter of a viscous liquid matrix and deposited on the end of a direct insertion probe tip. This correlates to approximately 0.3 mmol/m^2 as an analyte surface coverage, suggesting that there will be enough dye sample present on paper to detect in a FAB experiment. In MALDI/MS, picomolar amounts of sample are used, corresponding to a molar surface coverage of 0.3 μmol/m^2, however in the MALDI experiment, the analyte is not absorbing laser energy directly. Matrix surface concentrations are larger. Considering the fact that laser-based methods have been cited as having extremely how detection limits, in the range of 10^{-18} to 10^{-19} g, it is likely that the surface coverage of dye deposited on paper during writing and printing will be sufficient for direct laser desorption. Based on the ease of analysis

and flexibility of the LDMS experiment, compared to FAB MS, and the simplicity of the instrumentation, the decision was made to focus on LDMS, rather than FAB, for the analysis of ink on paper. We note that FAB spectra were successfully obtained, however short signal durations (see Chapter 2) made the approach less than ideal.

Figure 5.1a shows a portion of the positive ion LD mass spectrum of the positive ion LD mass spectrum of Ink A on paper. The peaks shown from m/z 344-372, separated by 14 amu, represent the components of the cationic dye, methyl violet. These are the dominant peaks in the mass spectrum. This is very similar to field desorption mass spectrum reported by Sakayanagi, *et al.* for the same dye (2). To ensure that all of the peaks present in the spectrum are indeed from the ink and not from the paper, both positive and negative ion LD mass spectra were taken of the paper itself. The positive ion LD mass spectrum of the paper substrate used in all of our analyses of ink on paper, is shown in Figure 5.1b. When paper is subjected to UV laser irradiation in this experiment, alkali ions are usually observed as intense peaks at m/z 23 (Na^+) and m/z 39 (K^+). Two peaks, at m/z 284 and m/z 575 are consistently formed. One source cited the peak at m/z 284 as stearic anhydride, an additive used in paper production (3). The peak at m/z 574 has an isotopic pattern consistent with that of copper phthalocyanine blue. Sometimes, blue dyes may be added to increase the paper's brightness, however there has been no confirmation that copper phthalocyanine is present in the paper. The key point is that the peaks are not representative of ink components. These two peaks representing the paper are not always observed when an ink sample is being studied. This may depend on the extent of coverage of the paper by the dye. For example, in the experiment that resulted in the spectrum shown in Figure 5.1a, no paper-related peaks were observed.

Ink may also be detected on paper in negative ion mode using LDMS. Figure 5.1c shows a portion of the negative ion LD mass spectrum of Ink B on paper. The peak at m/z 666, the largest peak in the spectrum, shows that Solvent Black 29 can clearly be detected. Many spectra can be generated at high signal-to-noise ratios, indicating that LDMS can be used for the direct detection and analysis of inks-on-paper, at the surface concentration typically encountered in written documents. Obviously, for an ink sample, an analyst may not know if an anionic or cationic dye may be present. However, it has been our experience that sufficient dye is present in ink to perform both positive and negative ion LDMS analyses.

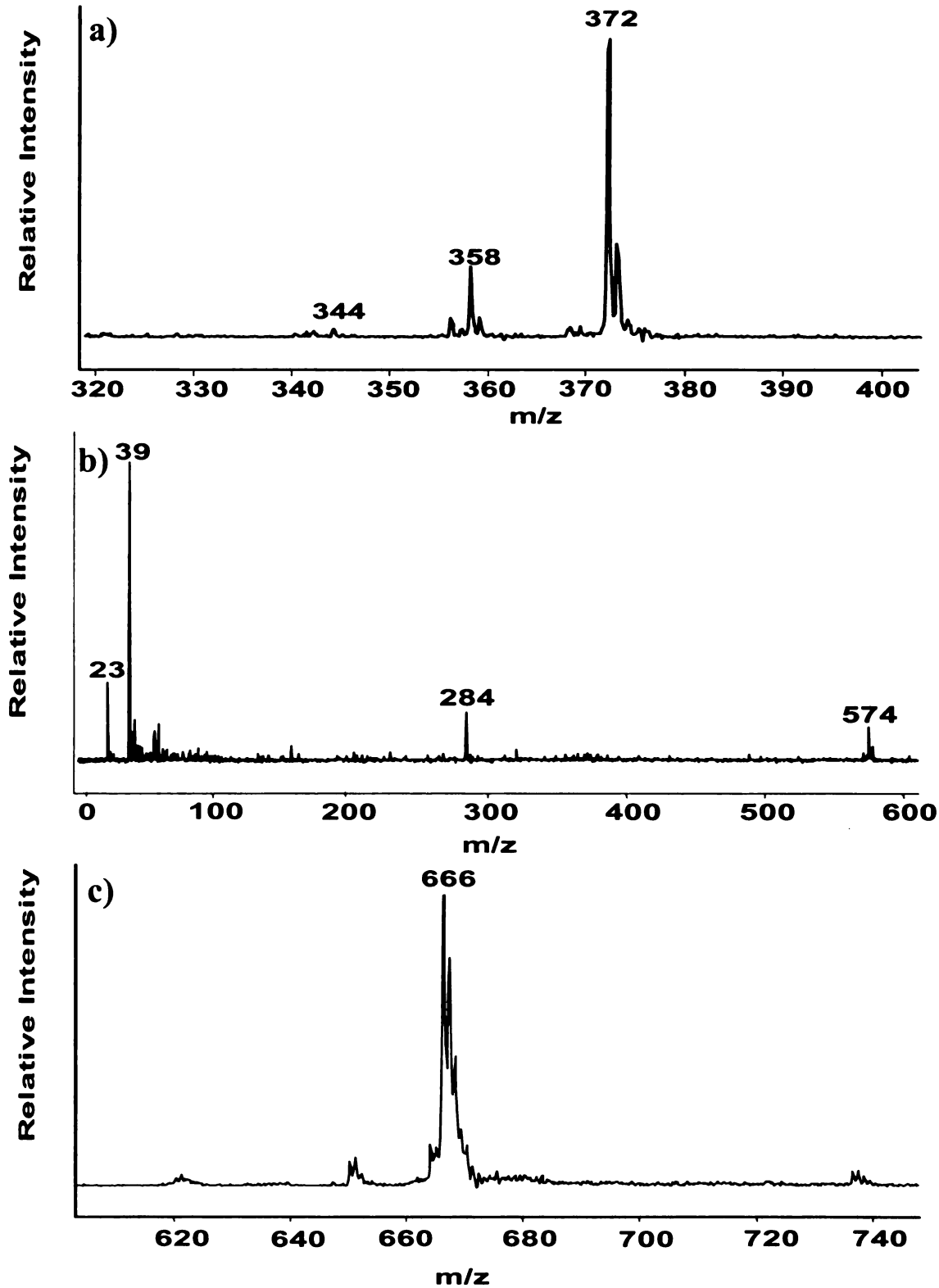

Figure 5.1: a) Partial positive ion LD mass spectrum of Ink A on paper; b) Positive ion LD mass spectrum of paper; c) Partial negative ion LD mass spectrum of Ink B on paper

UV-Accelerated Aging

Since LDMS can directly detect dyes on paper from ink, the decision was made to analyze ink from a ballpoint pen. Over time, ink on paper changes in appearance. This suggests that the chemical composition of the dye molecules is changing. Possibly, the dye could polymerize. If this occurred, the LD mass spectrum of aged ink on paper would show new peaks in the higher mass region. Perhaps, there would be a decrease in the molecular ion peak intensity and the appearance of peaks in the lower mass region, indicating that the dye is degrading. If the dye is extremely stable, there is the chance that the spectrum would not change. In an attempt to generate an aged ballpoint pen sample to compare with a fresh sample, accelerated aging techniques were considered. Commonly accepted thermal accelerated aging methods were discussed in Chapter 4. When deciding on which accelerated aging technique to use, one needs to consider what component of the ink is being measured as a function of the age of the ink. In the case of the well-accepted solvent-extraction technique, the amount of dye present in solution is measured using a densitometer, following an extraction with a mixture of weak solvents. The premise behind this technique is that it is harder to extract ink from older documents, since the majority of the solvent system has evaporated. Hence, a less concentrated extraction solution is indicative of an older ink. This method is indirectly measuring the solvent content left in the paper. Therefore, applying heat to a new sample makes sense, in order to evaporate the solvent more quickly. Thermal methods were attempted, in which our ink sample was placed in an oven at 100°C, for several hours. No changes in the initial spectrum were noted, which was not completely unexpected. Dye degradation is a chemical change, not a physical change like that of a solvent evaporating. UV

irradiation is commonly employed in the dye manufacturing industry to induce fading, which also occurs with age (4,5). Therefore, UV light was considered as an alternative accelerated aging method.

A preliminary UV-aging study was performed using Bic black ballpoint pen ink, containing the dye methyl violet. The ink was applied uniformly to piece of 4 in^2 paper. An initial positive ion LD mass spectrum was obtained. Approximately one third of the sample was covered and the entire sample was irradiated using a UV lamp, placed directly on top of the sample for 12 hours. Another third of the sample was masked off and the sample was irradiated for another 12 hours. This experiment demonstrated that significant fading was noted following 12 or more hours of intense UV irradiation. Also, it showed that by using masks, an array of UV aged ink on a single piece of paper could be easily generated, introduced as a single sample into the mass spectrometer, and spectra for each region obtained. For this preliminary work, the simplicity of UV aging made it an ideal choice. We acknowledge that there is currently no accepted irradiation /age correlation for this experiment.

The preliminary UV accelerated aging data are presented in Figure 5.2. Positive ion LD mass spectra are shown of methyl violet dye as originally deposited by the ballpoint pen onto paper, and the same portion of the mass spectrum is shown following 12 and 24 hours of UV irradiation. After irradiating the sample for 12 hours, degradation product peaks are clearly present – a series of peaks separated by 14 amu. The m/z 372 peak remains the most intense peak. The spectrum shows that six new compounds, lower-mass forms of Methyl Violet, are now in abundance. Irradiating the sample for another 12 hours causes the initial base peak to further decrease in intensity, such that the

degradation product peaks dominate. Andrasko noted similar results using high performance liquid chromatography (HPLC) to study ballpoint pen inks stored under various light conditions (6.)

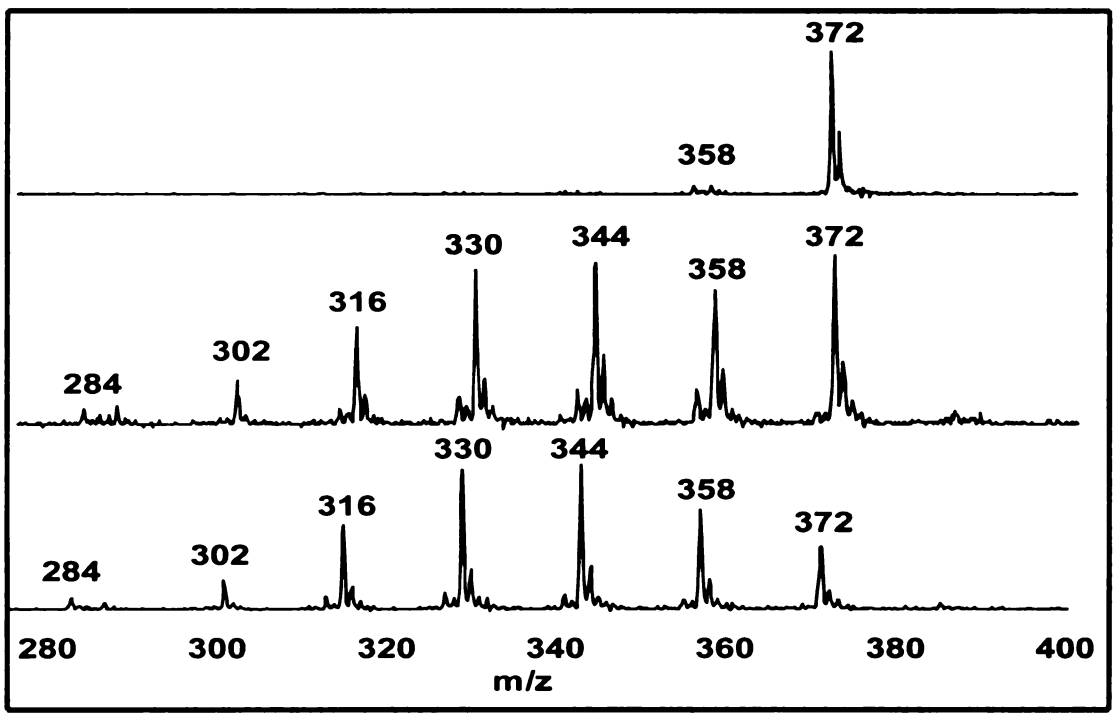

Figure 5.2: Preliminary UV-Accelerated Aging Study: Bic Ballpoint Pen Ink on Paper

When comparing these results to naturally aged samples (shown and discussed in the following section), we realized that we had artificially aged the samples past that which occurs naturally. Aginsky stated the importance of ensuring that the accelerated aging method mimics natural aging as closely as possible, meaning that the technique should not induce any chemical changes that would not happen naturally (7). Consequently, the experimental setup was adjusted by elevating the UV lamp 6 cm above the sample, and by monitoring the irradiated sample at much shorter time intervals (every

15 minutes). This allowed for us to visualize the dye degradation process more clearly, as well as to mimic the natural aging process more effectively.

UV accelerated aging mimics natural aging from a dye perspective, and can be characterized by LDMS. Figure 5.3a represents a portion of the positive ion LD mass spectrum of new black Bic ballpoint pen ink on paper. New ink is characterized by a large peak at m/z 372, representing the non-degraded dye molecule ($C^{+}Me_6$), with a very small peak at m/z 358 ($C^{+}Me_5H_1$). Figure 5.3b is the positive ion LD mass spectrum of a 38-month old naturally aged Bic black ballpoint pen ink on printer paper. As an ink ages, lower mass peaks appear in the spectrum representing the molecules that are referred to as degradation products, accompanied by a decrease in the relative intensity of the original m/z 372 base peak, representing the original intact dye molecule. The number and amount of degradation products present is a function of the age of the ink, but if this were ink on a questioned document, how could the age be determined from the spectrum? As in other ink dating methods, there must either be spectra from naturally aged samples for comparison purposes, or there must be a calibrated method for accelerating the aging of a new sample of similar ink that can be used to create a sample that yields the same spectrum. One goal of this work is to develop a calibrated UV accelerated aging method. It has previously been demonstrated that this means of artificially aging ink produces the same chemical changes in terms of the degradation of methyl violet, which occurs naturally as a document ages. Irradiating Bic black ballpoint pen ink-on-paper, for 6.25 hours with UV light, produces a very similar mass spectrum (Figure 5.3c) as that of the naturally aged 38-month old document. Based on this data alone, a calibration for the UV method can be estimated. Irradiation for 6.25 hours produces the same extent of

degradation as what occurs naturally over a period of 38 months. Thus, every hour of UV irradiation accelerates the aging by approximately 182 days. If a different sample was in question, the ink could be irradiated until the same extent of degradation was observed, and from the irradiation time required, the corresponding natural age could be calculated. This approach, along with other insights into the variables that influence the rates of dye degradation, are studied in this work.

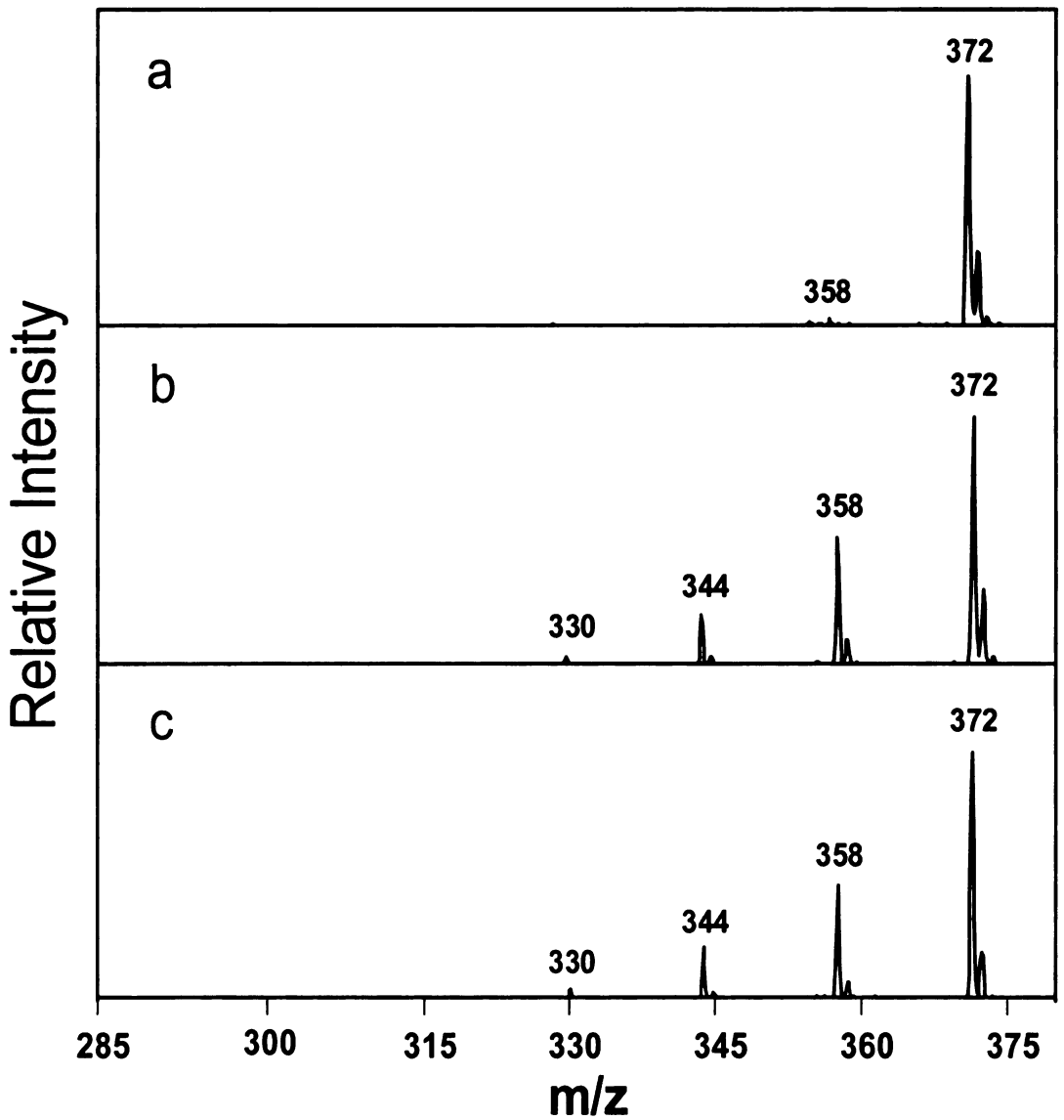

Figure 5.3: A portion of the positive ion LD mass spectrum of Bic black ballpoint pen ink-on-paper: a) ink from a new document; b) a 38-month old controlled, naturally aged document; c) ink from a document irradiated for 6.25 hours with UV light

Developing a Relationship Between Natural Aging and UV-Accelerated Aging

Methyl violet can be efficiently degraded on paper with UV irradiation. Ink samples on paper were subjected to UV radiation, followed by LDMS analysis, for periods of up to 8 hours. In these time periods, more than 50% of the dye molecules can be converted into degradation products. Figure 5.4 is a plot of the normalized relative intensities for each of three mass spectral peaks representing the dye or degradation product as a function of time for the UV accelerated aging study of Bic© black ballpoint pen ink-on- paper. The relative intensity is a measure of the concentration of a particular species in comparison to the other components detected in the sample mixture. In Figure 5.4, the relative abundance of the intact dye molecule (m/z 372) decreases as the relative abundances of the degradation products (m/z 358, 344) increase with irradiation time, which is an indication of the dye degrading. How can the data be used to develop a determination of an ink's age? There are many options. For instance, the age correlation may involve the relative intensity of the m/z 372 peak, revealing the amount of dye remaining in its original form. Another possibility would be to take a ratio of two peaks, such as the ratio of the m/z 358 peak to the m/z 372 peak, since these two peaks are always present, and seem to experience the most significant changes as the ink ages. Ideally, a function would be identified, in which a single value could be computed that incorporates information on all of the degradation products present. One way to accomplish this is to compute the average molecular weight for the dye at each time interval. Figure 5.5 shows the plot of the average molecular weight versus the time the document was irradiated in the UV accelerated aging study of Bic black ballpoint pen ink on paper. The average molecular weight (MW_{avg}) was calculated by multiplying the

normalized intensity of each peak by the nominal mass of that peak, summing all of the products, and dividing by the sum of the relative intensities of all of the peaks:

$$MW_{avg} = \frac{[RI(m/z\ 372) \times 372] + [RI(m/z\ 358) \times 358] + [RI(m/z\ 344) \times 344] + ...}{RI(m/z372) + RI(m/z358) + RI(m/z344) + ...}$$

This value was computed for each of the five spectra acquired per sample, and the five molecular weights were averaged. Scatter in the data is noted, however a distinct trend is established in which an increase in the degradation of the dye, i.e., a decrease in the average molecular weight, occurs over time. The use of MW_{avg} lends some physical meaning to the experiment. For instance, when the dye is initially deposited on paper, its average molecular weight would be nearly 372 Daltons, since it is essentially only crystal violet. If a sample is analyzed that shows an average molecular weight of 364 Daltons, it is understood that the degradation products are dominating the spectrum, indicating that the dye has degraded. The lower MW_{avg} limit for this experiment is 288 Daltons, which would occur if all of the C^+Me_6 were converted to C^+H_6. We have not yet found any naturally aged samples in which degradation has been this extensive. Figure 5.5, shows that the average molecular weight falls to 361 Daltons following 450 minutes of UV irradiation.

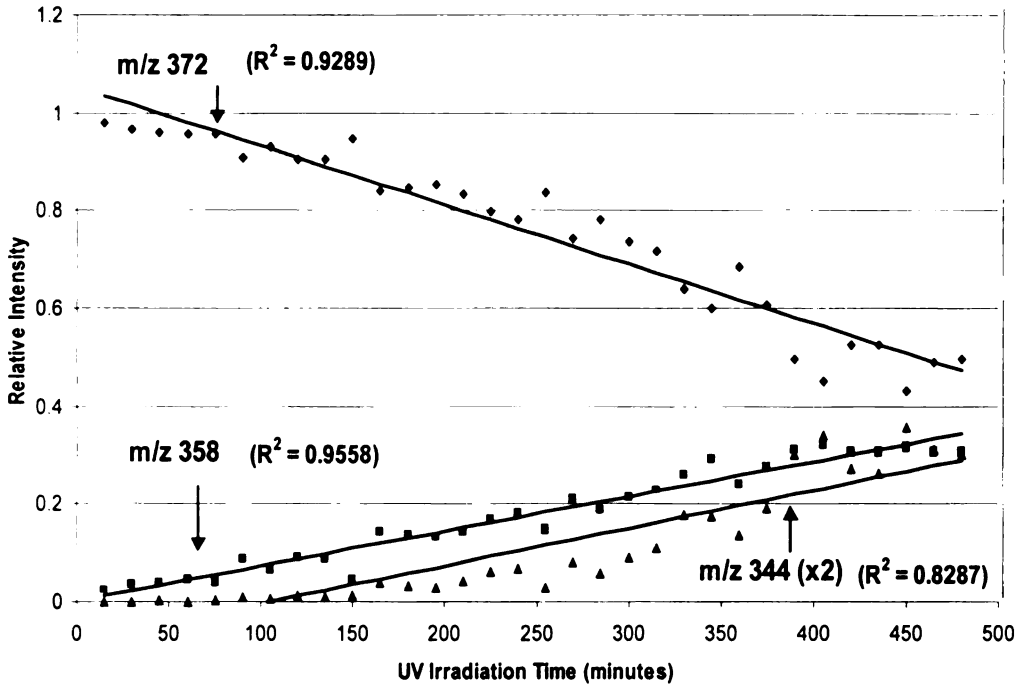

Figure 5.4: UV accelerated aging study data for new Bic black ballpoint pen ink on printer paper: a plot of the relative intensity of the m/z 372, 358, and 344 (x2) peaks versus irradiation time

Similar dye degradations occur naturally. In order to relate the UV accelerated aging curve in Figure 5.5 to aging which occurs naturally, a natural ink aging curve (Figure 5.6) was constructed using a set of controlled ink library samples from Speckin Laboratories, Okemos, MI. The samples were written with the same pen, on the same paper, and stored under the same conditions. Again, from LDMS spectra, the average molecular weights were computed. According to the UV accelerated aging curve prepared under the outlined experimental conditions, 500 minutes (8.3 hrs) of UV irradiation degraded methyl violet to an average molecular weight of approximately 360 Daltons. Referring to the straight line fit of the controlled natural aging data (Figure 5.6), an average molecular weight of 360 Daltons is equivalent to 52 months of natural aging

for this particular ink and paper. By combining the information in Figures 5.5 and 5.6, it appears that 500 minutes of UV irradiation (at the conditions used here) would age a document by approximately 52 months (~1560 days). Therefore, in this particular study, for every hour of UV irradiation, the document was aged roughly 187 days. This compares favorably with the initial estimate of 182 days per hour determined from the initial data shown in Figure 5.3.

A comment should be made concerning the linearity of the two best fit lines in Figures 5.5 and 5.6. The linear least squares fit to the data n Figure 5.5 has an R2 value of 0.8845. The R2 value is a measure of the strength of the linear relationship. The closer the R2 value is to unity, the stronger the relation. The linear regression line for the data in Figure 5.6 has an R2 value of 0.6331, indicating that the data generated during the UV accelerating aging study correlated better to a straight line fit than the data accumulated from the controlled natural aging study. This is not unexpected considering the time frames of the experiments. The UV accelerated aging study was completed in the matter of hours, whereas the controlled natural aging study occurred over ten years. During the UV accelerated aging study, variables affecting the aging process, such as temperature and humidity fluctuations, were minimal, resulting in a more constant rate of aging.

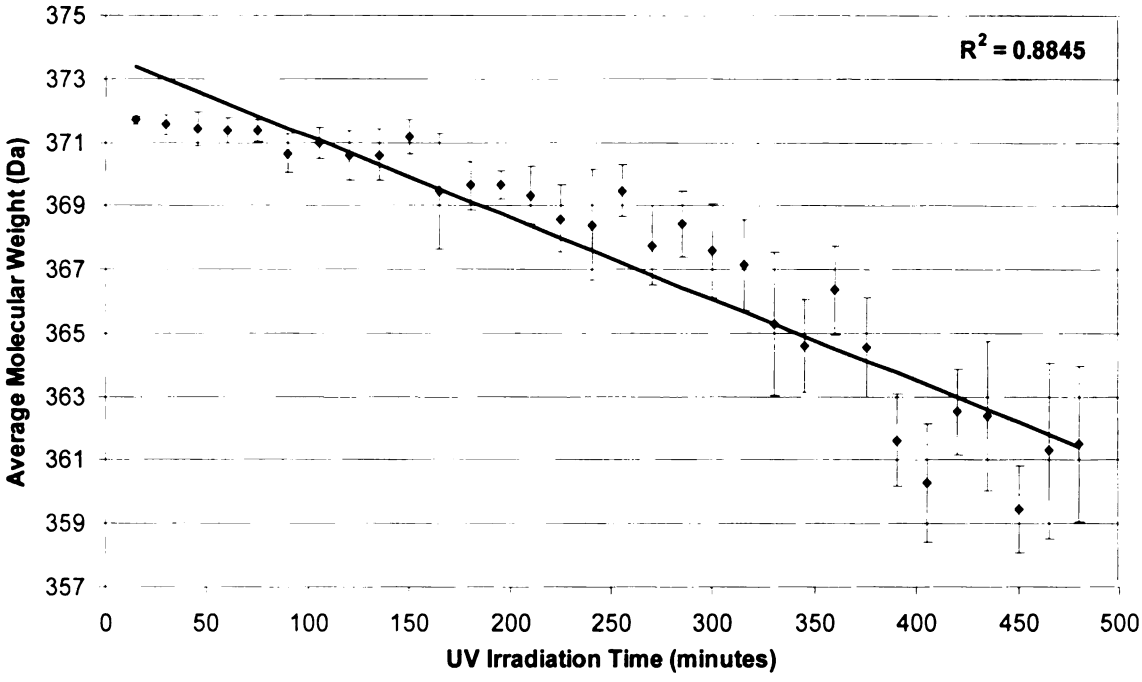

Figure 5.5: UV accelerated aging study data for new Bic black ballpoint pen ink on printer paper: a plot of the average molecular weight of the dye, methyl violet, versus minutes of UV irradiation.

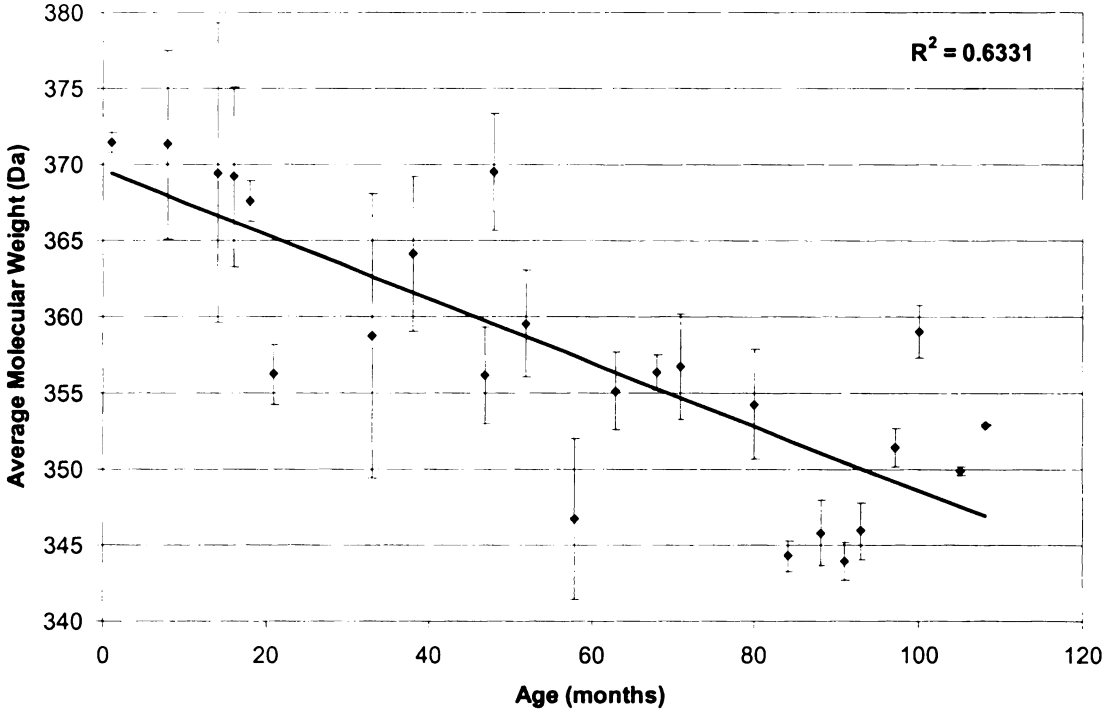

Figure 5.6: Controlled, natural aging study data for Bic black ballpoint pen ink on printer paper: a plot of the average molecular weight of the dye, methyl violet, versus the age of the document.

A Note on UV-Accelerated Aging

UV accelerated aging is more complex to perform than thermal aging. The details of the UV-aging experimental set-up can influence the resulting aging curve. Although the exact correlation between natural aging and artificial (thermal) accelerated aging may still be unknown, a thermal aging technique is very straightforward. For instance, one source suggests that, if an ink sample placed in an oven at 100° C for 4 minutes, the age of the sample increases by 3 months (8). UV aging is not as straight forward and easy to control. The distance between the UV lamp and the sample, as well as the age of the light source in the lamp affect the flux (# photons / area*time) of UV photons and consequently, the aging rate. Furthermore, the power of UV lamps may vary the longer the lamp is in use. In the preliminary UV aging studies, the lamp was placed directly on top of the paper. As a consequence, the degradation process proceeded at very high rates. The UV lamp should not be placed too close to the sample, to ensure that the temperature of the sample does not increase significantly, and to maintain a moderate photon flux from the UV lamp. To make certain that heat did not influence the rate of degradation, the samples were not continuously irradiated. Rather, the lamp was turned off at hourly intervals for 15 minutes throughout the experiment, to ensure that the sample was not being heated by the lamp to any appreciable extent. After experimenting with distances between the lamp and the sample, as well as altering the time increments between acquiring spectra of the samples in order to obtain a clear description of the gradual degradation of the dye, it was found that having the lamp 6 cm above the sample surface, allowing the sample to cool every hour for 15 minutes, and taking LDMS spectra at 15 minutes intervals, allows for generation of an acceptable aging curve. Others attempting

to use this aging technique would therefore need to first develop their own aging curve, using their own defined experimental geometry and lamp. In other words, the experiment cannot be defined as simply as "heat the sample in an oven at 100° C for 4 minutes to age the sample 3 months". This method is laboratory dependent. The accelerated aging rate of 180-190 days/hour is for this particular system, not to be considered as applicable in general.

Testing the Influence of Variables in Natural Ink Aging

The rate of the natural aging of methyl violet is dependent on many variables. Certainly, it would be ideal if the chemical degradation of methyl violet was insensitive to the common variables involved in ink aging. These variables, mentioned previously, include ink formulations, the type of paper, relative humidity, and environmental storage conditions. To determine whether dye aging is sensitive to some of these variables, naturally aged ink samples were obtained from the departmental archives. The samples gathered were written in both blue and black ink, and were therefore of different ink formulations, and were obviously not from the same pen. Also, the types of paper selected varied significantly. Positive ion LD mass spectra were obtained for more than 25 samples, that spanned a 50 year period. The average molecular weight of the dye was calculated as previously described, and then plotted versus age (Figure 5.7). The graph shows differences from the aging curves of the artificial aging study (Figure 5.5) and the controlled ink library study (Figure 5.6). Substantial scatter is evident in the data, and it appears as though, after roughly 15 years, all of the samples produce very similar spectra. Therefore, this study suggests that the generally accepted theory that ink aging (from a

solvent content standpoint) will asymptotically level off and eventually stop, may be true in the case of dye degradation, as well. Another interesting observation is that the leveling off occurs at an average molecular weight of approximately 365 Daltons. It has already been demonstrated that UV accelerated aging can surpass this value (Figure 5.5). What is even more surprising is that the natural aging ink library samples had degraded to lower average molecular weights (Figure 5.6) than what is seen in Figure 5.7. A series of experiments were designed to test some of the variables, which were thought to be causing the deviations (scatter) in the "real life" samples (Figure 5.7). There is also interest in why the naturally aged samples appear to stop aging, while the accelerated aging and controlled ink library samples have aged to a greater extent and appear to be still aging significantly.

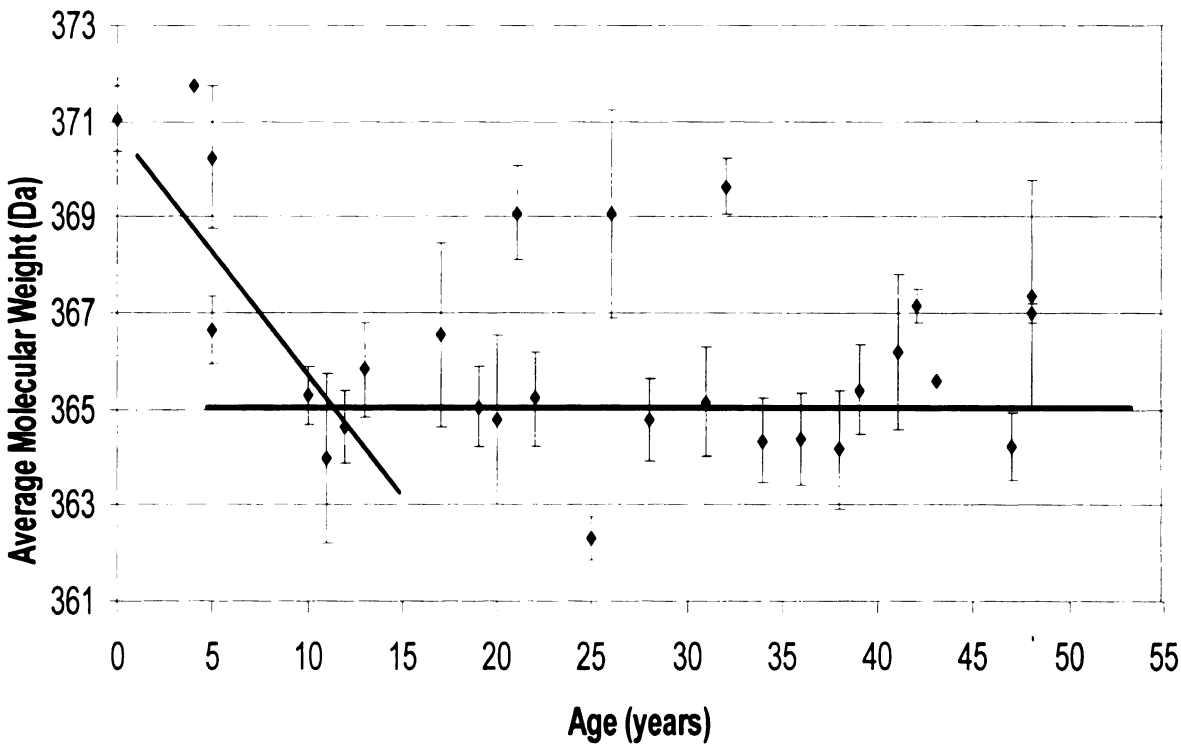

Figure 5.7: Uncontrolled, natural aging study data for various inks on different types of paper: a plot of the average molecular weight of the dye, methyl violet, versus the age of the document

The type of paper influences both natural and accelerated aging of methyl violet. To test the influence of the paper on the degradation of methyl violet, an additional set of samples was provided by the ink library of Speckin Forensic Laboratories. This set was written with the same pen and stored under identical conditions as the first set, however this second set was written on bond paper as opposed to printer paper. Positive ion LD mass spectra of the samples were obtained and a natural aging curve of the average molecular weight of methyl violet versus time was prepared (Figure 5.8). While there is significant scatter in the data, the dye appears to be aging at a slower rate than that of the first set of ink library samples (Figure 5.6). For instance in 10 years, dye in the first set has degraded to an average molecular weight of 347 Daltons, while the second set, the dye has only degraded to 366 Daltons. In the data from Figure 5.7, spectra from 10 year old documents led to a dye MW_{avg} of 365 Daltons. So, it is clear that natural aging as reflected by dye degradation products is paper dependent. It is important to note that the artificial UV aging study performed in this laboratory was conducted on the same type of paper as the first set of ink library samples, which is the basis for our initial correlation that 1 hour of UV irradiation is equivalent to 187 days of naturally aging. However, the studies were not performed with the same pen, but with the same company's ink. It is important to note that most of the samples in documents selected for the study in Figure 5.7 are "professional correspondence", mostly on letterhead paper.

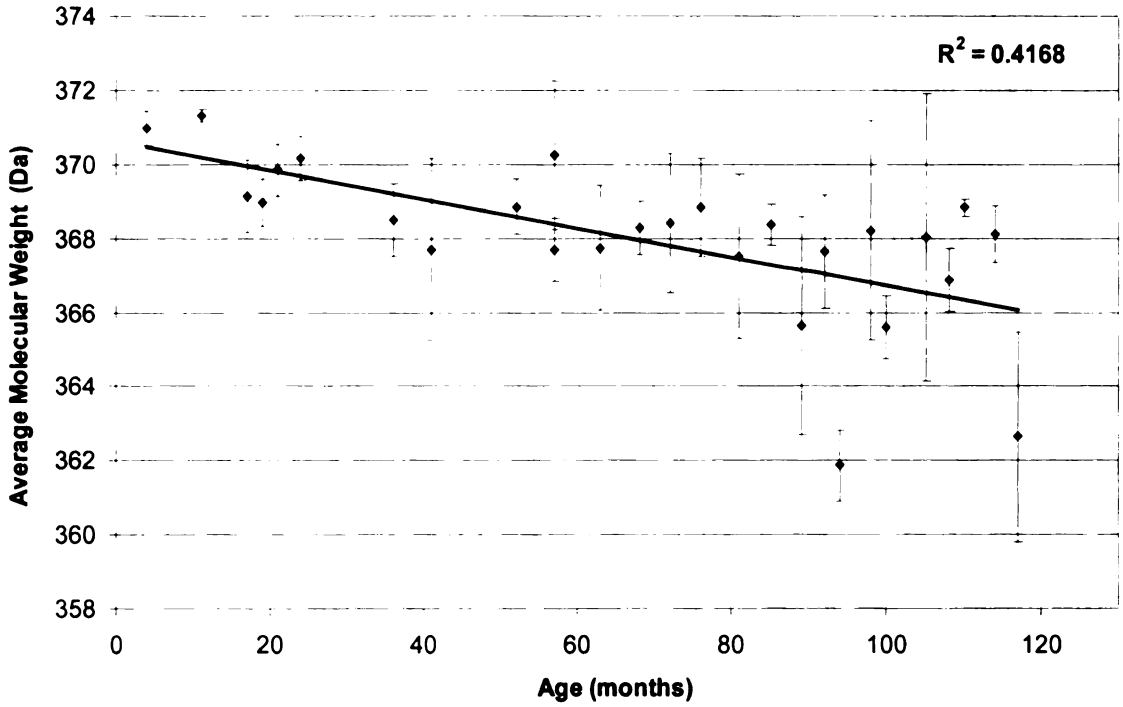

Figure 5.8: Controlled, natural aging study data for Bic black ballpoint pen ink on bond paper: a plot of the average molecular weight of the dye, methyl violet, versus the age of the document.

Having established that the rate of natural degradation of the dye is paper-dependent, the decision was made to investigate whether accelerated aging is paper dependent from data generated from non-white paper. A legal pad, which consisted of tan paper with a green design on each page, was used for this study. Ink was written on the different colors of the page, and artificially aged under the same conditions previously described. There are certain inorganic compounds which are known to catalyze the degradation of methyl violet in solution (i.e., TiO_2), which have been used in the manufacture of white paper. Colored paper, having a different composition (varying amounts of whiteners and dyes) than typical white paper, may cause the dye to age at a different rate. The results of this experiment (not shown), suggested that the artificial ink

aging process on the colored and white papers proceeded at similar rates. If ranked, they would fall in the following order: degradation rate: (tan paper) > (green paper) > (white paper). In other words, the original hypothesis that degradation on non-white paper would proceed at a slower rate was incorrect, but one can conclude that the type and composition of the paper certainly influences the aging process.

Methyl violet in blue ink degrades faster than in black ink. One other factor to consider when attempting to rationalize the scatter appearing in the uncontrolled natural aging study (Figure 5.7) involves the influence of ink formulations. Certainly, one would expect the presence of different dye components, and as a result, changes in the vehicle, for different ink colors. In the data presented to this point, all of the experiments were conducted using Bic black ballpoint pen ink. Additional accelerated UV aging studies using Bic blue ballpoint pen ink were performed and the results are presented in Figure 5.9. Comparing this data to that shown in Figure 5.5, it appears as though components present in the blue ink cause it to degrade much more rapidly. At 250 minutes of UV irradiation, for example, it has already surpassed the extent of degradation observed for the black ink at 500 minutes of UV irradiation. Therefore, if there are components in blue ink, which are not commonly used in black ink, and they cause the methyl violet to degrade much faster, this would help explain the scatter present in the uncontrolled, naturally aged samples (Figure 5.7). Further investigations into the other dyes present in blue, black, and red ballpoint pen ink, are currently being conducted. Evidence has already been collected which indicates that other dyes undergo similar oxidative alkyl degradation processes.

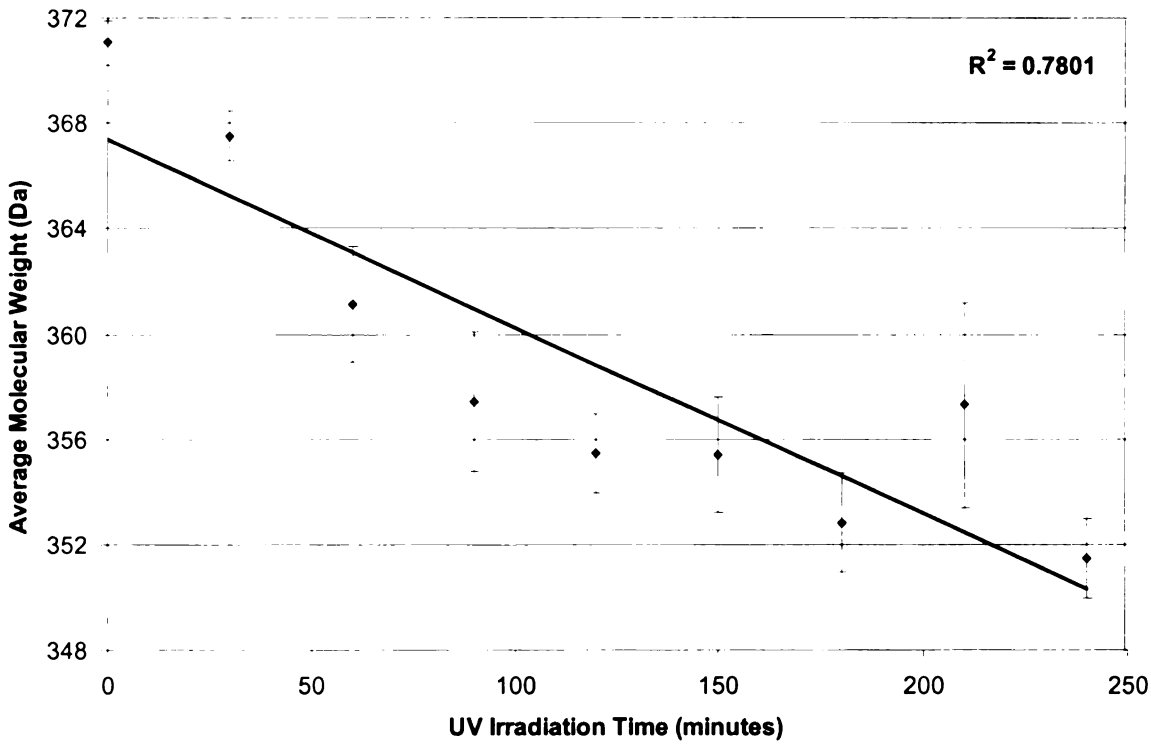

Figure 5.9: UV accelerated aging study data for new Bic blue ballpoint pen ink on printer paper: a plot of the average molecular weight of the dye, methyl violet, versus minutes of UV irradiation.

Insight into the Uncontrolled Natural Aging Curve

Although the extent of dye degradation in samples 20-50 years old are similar, this does not necessarily suggest that degradation ceases to occur after 15 years. One explanation for the data in Figure 5.7 is that, after 15 years on paper, dye molecules do not continue to react and form degradation products. There could be multiple explanations for the data. First, if the dye reacts with solvent molecules, the concentration of the solvent in the paper could be sufficiently small after 15 years that the reaction essentially ceases. Alternatively, the reaction may involve water, which could

always be provided by the relative humidity of the environment in which the document is stored, but the dye molecules may become immobile over time. They may become permanently bound to the paper, trapped in hardened resins, and may be unable to diffuse to reactive molecules and form products. If these possibilities are correct, then old samples should not age if one were to subject them to UV accelerated aging. To test this, a 1958 sample from the naturally aged set was taken and exposed to UV irradiation. The results of this experiment, which was conducted using the same UV accelerated aging parameters outlined previously, are shown in Figure 5.10. Initially, the average molecular weight of the 42-year-old sample was roughly 367 Daltons. Irradiating the sample with UV light decreased the average molecular weight to approximately 363 Daltons. It is obvious that the ink aging process continued. One interpretation is that methyl violet on documents that are more than 15 years old can and does continue to form degradation products, but more slowly than on documents created more recently. Certainly, in the past 50 years there have been considerable changes in both ink formulations and in paper chemistry. Ink chemists are learning how to create thinner films, with different vehicle systems. Older vehicles may form more of a non-reactive, protective coating on the dye molecules than do newer solvent systems. It would certainly not be surprising that a document written 40 years ago with a pen that contained methyl violet in the ink would be a very different chemical system than one created recently.

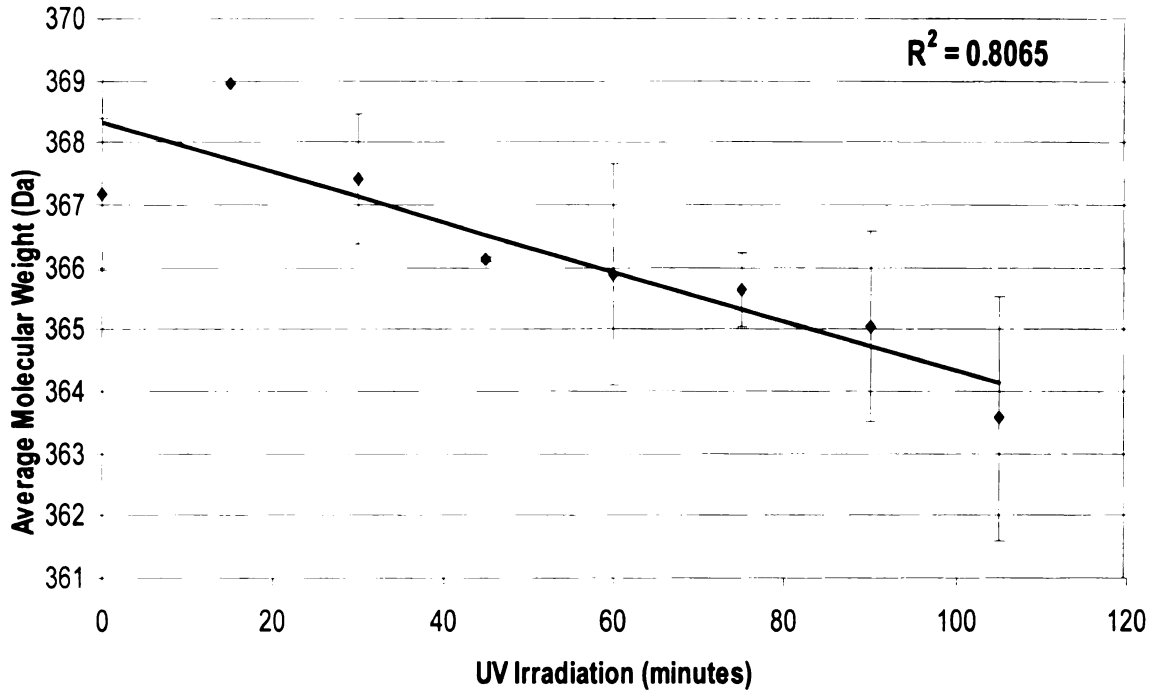

Figure 5.10: UV accelerated aging study for ink on a (1958) old document: a plot of the average molecular weight of the dye, methyl violet, versus minutes UV of irradiation.

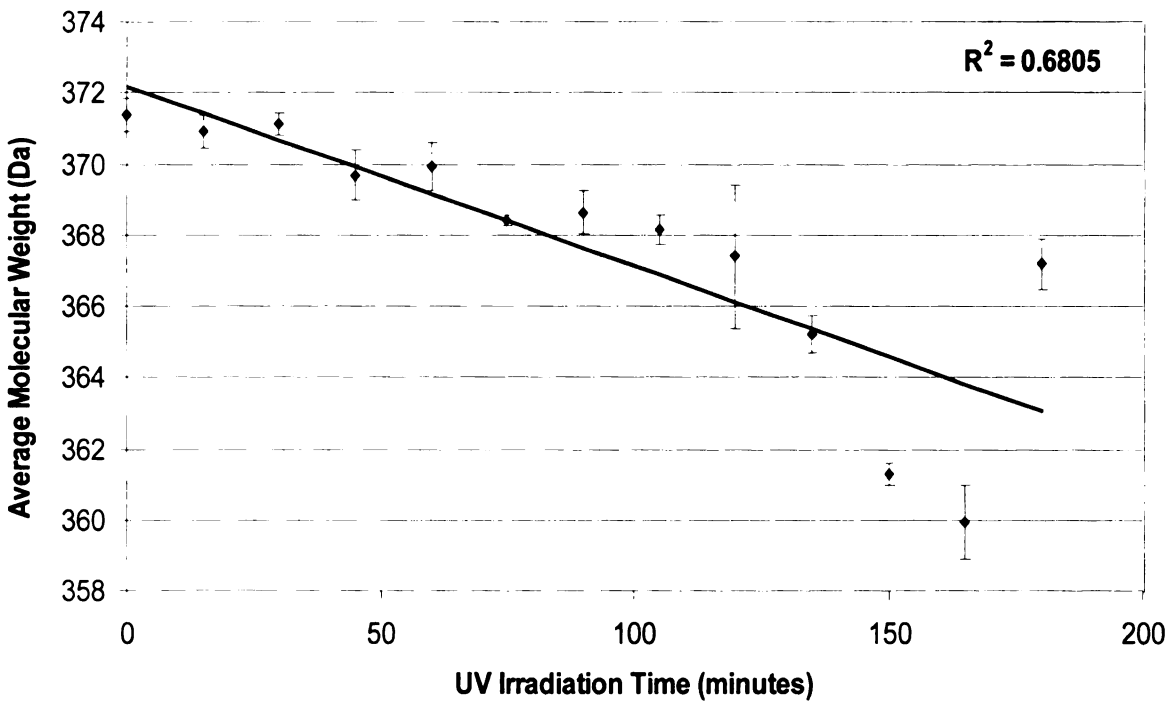

Figure 5.11: UV accelerated aging study data for thermally aged 20 year old ink on printer paper: a plot of the average molecular weight of the dye, methyl violet, versus minutes of UV irradiation.

A second aspect that is appropriate to point out is that, while UV aging forms the same degradation products for methyl violet (and other dyes) as natural aging, the mechanisms are likely not the same. UV radiation forms electronic excited states that may be much more reactive than ground state molecules. Certainly, between and even within natural and accelerated aging studies, there may be multiple mechanisms that can lead to the same set of degradation products.

Solvent molecules from the ink are not the only possible compounds that react with dye molecules to form degradation products. An additional experiment was conducted to test the solvent dependency of the dye degradation process. A new sample was aged in the oven at 100° C for 320 minutes, which is equivalent to 20 years of natural aging according to a thermal method of aging. Most of the ink solvents should have evaporated. LDMS spectra of the sample were taken following this aging process. The spectra showed that the sample was still "new" from a dye degradation standpoint. This sample was then exposed to the UV accelerated aging experiment. If most of the solvent was gone, and the degradation of methyl violet was solvent-dependent, then no further aging would take place. The results of this experiment are shown in Figure 5.11, and they clearly demonstrate that the aging process can still occur. In fact, the aging process continues at a rate similar to that observed in Figure 5.4. This may suggest the –H donor that reacts with the dye molecules may not be a volatile ink component, but may be a component of the paper or even another dye molecule. Another possibility is that, since paper is hydrophilic, it quickly absorbs some amount of water vapor from the room, and can quickly restore sufficient amounts of reactive water molecules in the paper to allow reaction to occur. Clearly, the chemistry of dye molecules on paper is complex, and

much work remains to determine the actual mechanisms through which degradation occurs.

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Chapter Six: Ink Aging Inside of a Pen

Aging inside of a pen could contribute to a significant error in relative ink age determination. A common assumption amongst questioned document examiners is that ink does not begin the aging process until it is applied to paper. The pen cartridge is considered a "closed system", while the ink-on-paper is considered an "open system" (1). Many ink dating methods are somehow related to the evaporation of the solvent present in ink, as a function of age (1-11). Consequently, the hypothesis is that only a negligible amount of ink is evaporated from the open ends of the pen cartridge over time. However, there is a notable absence of any published scientific studies supporting this hypothesis. Dye degradation is very different than solvent evaporation. Therefore, ink inside of old pens were subjected to laser desorption/mass spectrometric analysis.

In respect to analyzing the dye component to determine the age of an ink, the difference between a "new" or fresh ink, and an "old" or aged ink, in terms of mass spectral data, has been established in the Chapter 5. Figure 6.1 shows the positive ion LD mass spectrum of ink on paper from a 20-year-old pen (pen #1). The base peak at m/z 372 represents the cationic dye, crystal violet. Thus, the mass spectrum shown if Figure 1 is typical of what would be obtained for a new ink, and is characterized by a large peak at m/z 372, with a small or absent peak at m/z 358. This suggests that these dye molecules are stable in this pen, and have not "aged".

For these experiments, a variety of old pens were gathered which were known to be 5 to 20 years old. Positive ion LD mass spectra were obtained of the pen lines which were drawn using them. Pen #1, used to obtain the data in Figure 1, was roughly 20 years old, and was known not to have been used for years. The positive ion LD mass

spectra obtained from any point along the pen lines were consistent with the spectrum in Figure 6.1, indicative of new ink. Other pens tested generated the same results, lending support to the assumption that ink does not age inside of the pen. However, some of the old pens produced spectra indicative of old ink, which required a more in depth analysis.

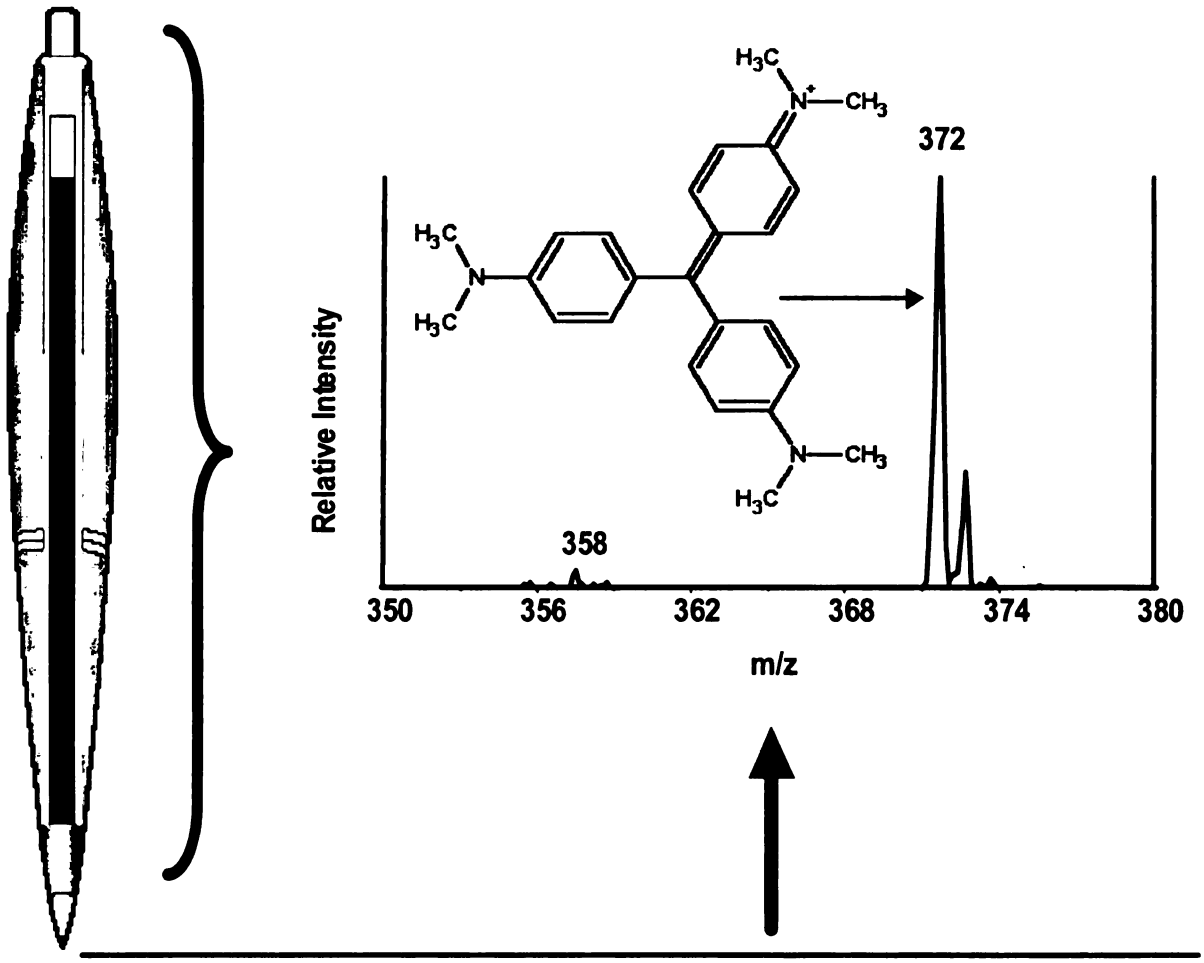

Figure 6.1: The positive ion LD mass spectrum of ink on paper from Pen #1.

A second old pen, pen #2, yielded ink that was clearly "aged" ink. Figure 6.2 shows the positive ion LD mass spectra of the ink from pen #2, which were taken at various points along the length of the pen line. As the dye degrades, the crystal violet dye molecules continue to lose $-CH_3$ groups. Evidence for this process appears in each of the mass spectra of Figure 6.2. A significant increase in the intensity of the m/z 358 peak, and the appearance of a small peak at m/z 344 are noted. The presence of the degradation products is an indication that pen #2's ink has been aging in the pen. All three spectra are very similar and the dyes have degraded, but to what extent have they degraded, when compared to naturally aged ink-on-paper samples?

The calculation of the average molecular weight of the dye and its degradation products from LD mass spectral data was recently proposed as an effective way to determine the extent of dye degradation. Furthermore, a controlled natural aging curve was created from ink library samples (Speckin Forensic Laboratories, Okemos, MI), which related the actual age of the ink to the average molecular weight of the dye computed from mass spectral data. From the mass spectral data in Figure 6.2, the average molecular weight of the dye from each spectrum was approximately 363 amu. It is important to note that the visual variations among the three spectra did not have a significant effect on the calculation of the average molecular weight. Compared to the natural aging of ink on paper, a dye with an average molecular weight of 363 corresponds to roughly 35-year-old ink. So, in this particular pen, the ink aged faster than if it had been on paper.

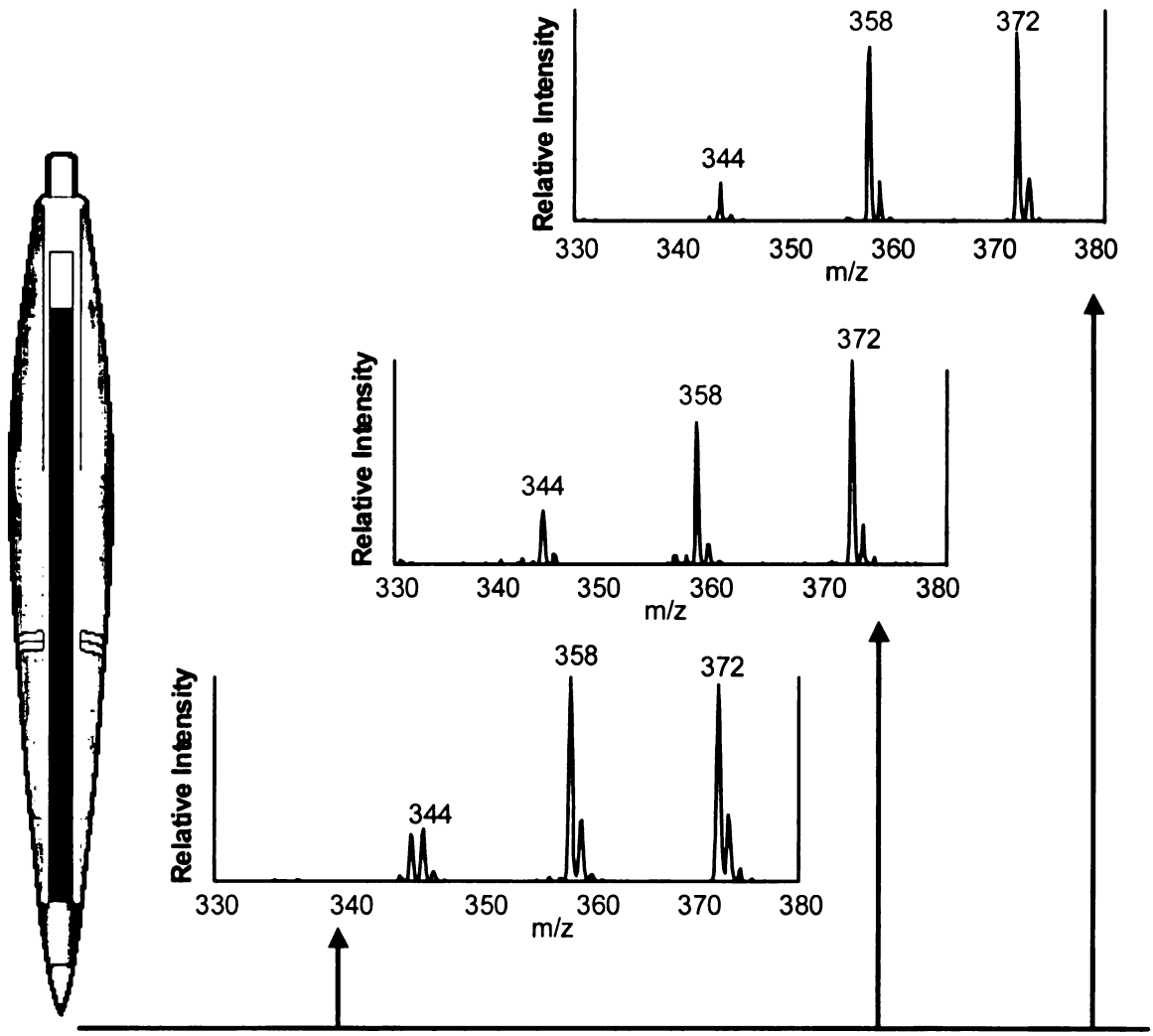

Figure 6.2: The positive ion LD mass spectra of ink along a written line from Pen #2.

Considering that a pen cartridge holds enough ink to write a line approximately 3000 m long (1), the sample quantity of ink analyzed in the experiment is not a fair representation of the ink throughout the entire pen cartridge. The ink may only have degraded near the tip of the pen, since it is more exposed to atmospheric gases and light, which may catalyze the oxidative demethylation process. Thus, some pens were disassembled to investigate the issue further.

Ink from pen #1, taken from the region closest to the metal pen tip, the middle region of the cartridge, and the open, far end of the cartridge, generated LD mass spectra indicative of new ink (Identical to that shown in Figure 6.1). The data indicate that the ink remained stable and intact throughout the entire ink cartridge. Figure 6.3 shows the positive ion LD mass spectra of ink from pen #2, taken from three regions inside of the ink cartridge. As in Figure 6.2, the spectra in Figure 6.3 are all very similar, and representative of a dye with an average molecular weight of 363 amu. The slight differences realized within the cartridge itself are small. The significance arises when trying to determine the relative age of a questioned document, when the pen used contained dye that, from a degradation standpoint, is already 35 years old.

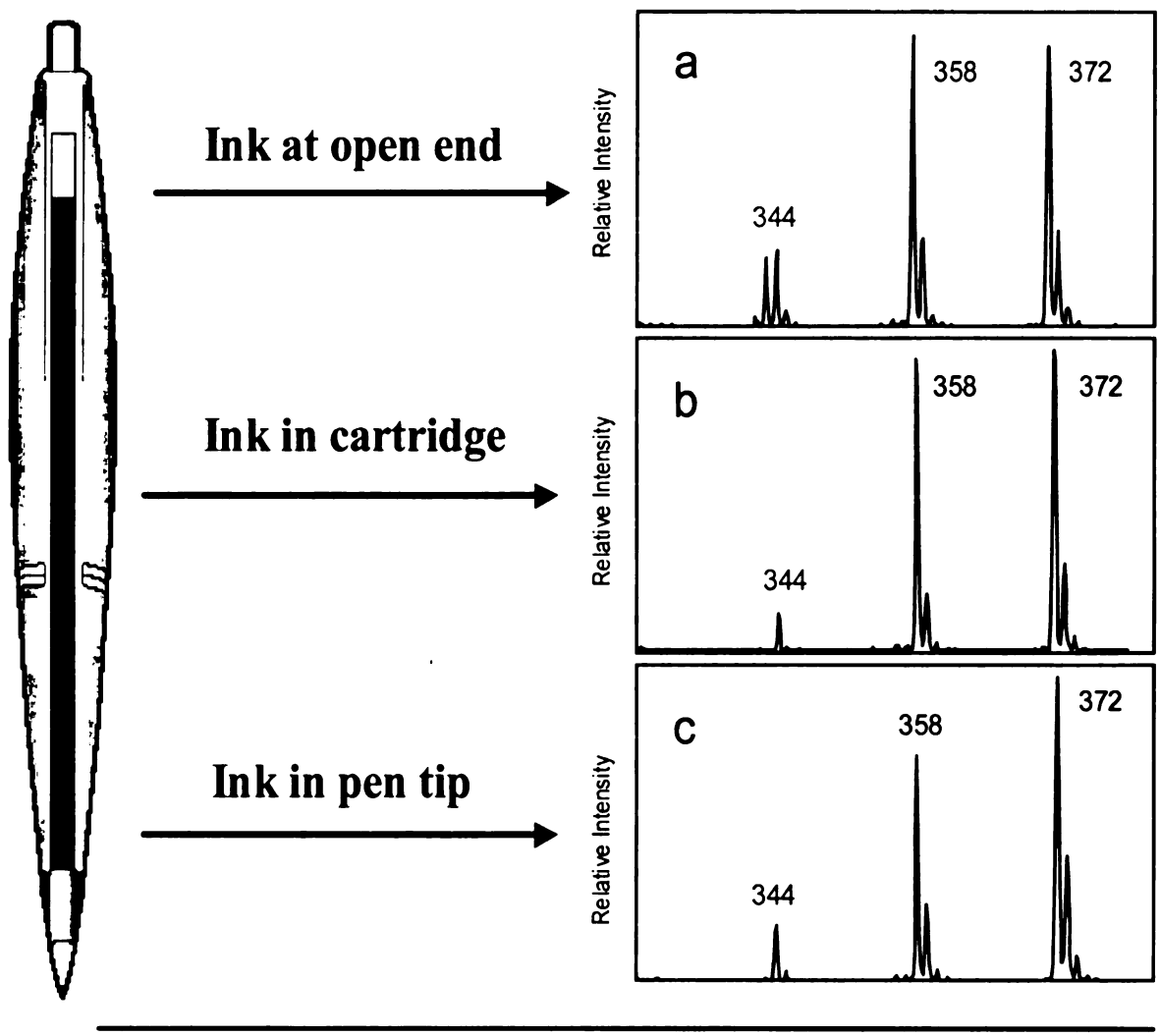

Figure 6.3: The positive ion LD mass spectra of ink within the cartridge of Pen #2 : a) ink removed from open end of cartridge; b) ink removed from middle of cartridge; c) ink removed from pen tip.

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Chapter Seven: Conclusions & Future Work

Laser desorption mass spectrometry (LDMS) is a sensitive analytical tool for the detection of intact dye molecules and their degradation products directly from a paper substrate. This molecular level information can be used for simply identifying a specific dye component present in the ink, or possibly for determining the relative age of an ink on a questioned document, based on the extent a dye has degraded. As many other relative ink age determination techniques, our method based on the analysis of dye degradation products, also has its limitations. All ink dating methods are in some way dependent on uncontrollable variables, such as ink formulation, type of paper, and storage conditions. In a typical questioned document forensic science case, storage conditions most often need to be inferred, and the original pen used to write the document will most likely never be recovered. Furthermore, documents aged in the "real world" under natural conditions are very different from those produced artificially in the laboratory. Although great measures have been taken to make the two conditions the same, there is no exact correlation between the two, and perhaps only approximations can be derived. These limitations have driven some skeptics to propose that relative ink age determination by any method is impossible (1,2). Up to this point, the emphasis has been on solvent extraction methods, with minimal research into the different possibilities available from the analysis of dyes (3). Perhaps, the solution to developing the "perfect" relative ink age determination involves a combination of solvent extraction methods and dye degradation product analyses.

While this work focused on one dye, methyl violet, there are other common dyes, such as the rhodamine-type dyes, found in inks. In the case of Rhodamine B and

Rhodamine 6G, both dyes have the same molecular weight, but one has four ethyl groups attached to N atoms, while the other has two ethyl groups, respectively. When exposed to a light source, the ethyl groups are lost and replaced by H atoms, similar to what occurs with methyl violet. LDMS can be used to distinguish between the two dyes, by identifying the number of peaks present in the mass spectrum resulting from the loss of ethyl groups. Thus, accelerated aging is being explored as a means to degrade dyes for characterization purposes, as opposed to ink dating purposes. Other light sources, such as incandescent light bulbs and fluorescent lamps, are also being explored as an alternative to UV light. Overall, the full potential of using LDMS for the analysis of questioned documents has not yet been obtained.

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