Photofading of Ballpoint Dyes Studied on Paper by LDI and MALDI MS

Céline Weyermann,* Dieter Kirsch, César Costa-Vera,†
and Bernhard Spengler
Institute of Inorganic and Analytical Chemistry, Justus Liebig University Giessen, Giessen, Germany

The determination of the age of an ink entry from a questioned document is often a major problem and a controversial issue in forensic sciences. Therefore, it is important to understand the aging process of the different components found in ink. The aim of this work is to characterize the degradation processes of methyl violet and ethyl violet, two typical ballpoint dyes by using laser desorption/ionization (LDI) and matrix-assisted laser desorption/ionization (MALDI) mass spectrometry (MS), and to evaluate the possible application of the method to forensic examination of documents. The mass spectrometric methods were first tested and were found to be adequate for the purpose of this work. Moreover, it is possible to analyze the dye from a stroke directly from the paper (LDI-MS), so the sample preparation is minimized. The degradation of the dyes methyl violet and ethyl violet in strokes from a ballpoint pen was studied under laboratory conditions influenced by different factors such as light, wavelength of light, heat, and humidity. Then, strokes from the same ballpoint were aged naturally in the dark or under the influence of light over one year and then analyzed. The results show that the degradation of these dyes strongly depends on light fluence. Humidity also increases degradation, which can be explained by the basicity of the paper. The influence of heat on the degradation process was found to be rather weak. It was also observed that the dyes from the ink strokes did not show significant degradation after one year of storage in the dark. In conclusion, the storage conditions of a questioned document and the initial composition of the dyes in the ink have to be known for correct interpretation of the age of an ink entry. Measurements over longer periods of time are necessary to follow the degradation of dyes exempt from light exposure. LDI was found adequate and very useful for the analysis of ballpoint dyes directly from paper without further pretreatment. (J Am Soc Mass Spectrom 2006, 17, 297–306) © 2006 American Society for Mass Spectrometry

In the field of forensic examination of questioned documents, the legitimacy of an ink entry is often an essential question, and the possibility of determining the age of an ink stroke would definitely help to resolve this problem. Ballpoint pens are very common scriptural instruments, the inks of which contain equivalent amounts of dyes, solvents, and resins. After deposition on paper, the ink composition begins to change qualitatively and quantitatively: the resins polymerize, the solvents evaporate, and the dyes fade. Throughout the years, many different methods were developed to measure the changes occurring in the ink with time [1–7]: decrease of extractability of ink through hardening of the resins [8–13, 16], disappearance of solvents [14–20], and degradation of dyes [18, 21–29]. The analysis of the latter compounds involve techniques such as microspectrophotometry [21], HPLC analysis [25, 26], or recently mass spectrometry [18, 22–24, 27, 28]. Laser desorption/ionization mass spectrometry (LDI-MS) is a very powerful analytical tool for the analysis of dyes [18, 24, 27, 28]. This technique allows minimal sample preparation and, thus, a minimized perturbation of sample integrity. The samples are mounted on a sample plate, introduced into the ionization chamber, and irradiated with a pulsed laser. This provokes desorption and ionization of a small layer of the sample, and the formed ions are analyzed in the mass analyzer. Mass spectral acquisition takes less than a second.

Methyl violet is a very typical dye in ballpoint inks and is relatively unstable under light influence. The typical degradation of triarylmethane dyes (widely used in ballpoint inks) is characterized by demethylation and decomposition of the conjugated structure (through opening of the aromatic ring). The degradation is a photocatalyzed process that is accelerated in the presence of titanium dioxide [29], a compound formerly widely used in paper fabrication [30]. Whitening of paper is today mostly attained by a thin-layer of calcium carbonate, which results in a somewhat basic pH of the paper [31]. Contradictory results on the

degradation of ballpoint dyes in the absence of light were reported [21, 24, 26]. These conflicting observations might be explainable by varying storage conditions (temperature, humidity), different time periods over which the studies were carried out, and different analytical methods. More specific data on the composition as well as studies on the degradation of ballpoint inks are not available from manufacturers because of confidentiality.

In spite of all the methods developed and tested, the aging of ink is still a phenomenon that is not well understood, and dating of ink entries still stays out of reach of many forensic laboratories. For this reason, the aim of this investigation was to develop methods of laser desorption/ionization (LDI) mass spectrometric analysis of dyes to evaluate the aging and degradation processes of blue ballpoint ink entries with only minimal or without preparational chemical treatment and, subsequently, to determine how such a technique would be useful in the examination of questioned documents.

Therefore, effects of the ionization step (laser irradiance) and of the employed sample preparation techniques on the degradation of the dye were evaluated first. LDI analysis was compared with matrix assisted laser desorption/ionization (MALDI), which differs from LDI in the use of a matrix mixed with the analyte before analysis [32]. The matrix is a compound that absorbs light at a given laser wavelength and allows compounds that do not absorb laser light to be desorbed and ionized without much fragmentation. Matrix addition generally improves the sensitivity of LDI-MS. The matrix protects the analyte and assists in the desorption and ionization process.

Experimental

Materials

Solvents (deionized water, ethanol, acetone) were purchased from Merck (Darmstadt, Germany), trifluoroethanol (TFE) from Flucka (Buchs, Switzerland), phenoxyethanol from Riedel-de-Haen (Seelze, Germany), dipropylene glycol, 2,5-dihydroxybenzoic acid powder (DHB, pK_s = 2.97), and ethoxyethoxyethanol from Sigma-Aldrich (Steinheim, Germany). Reference substances used were the pure dyes MV (methyl violet, Fluka) and EV (ethyl violet, Sigma-Aldrich). Blue BIC ballpoint entries (Cristal, medium blue, France) were made on a multifunction bright-white wood and chlorine free paper from Igepa Plus (80 g/m², DINA4, no. 806 A 80, Reinbeck, Germany). Subsequent analysis was carried out either after dissolution of the pure dyes in ethanol or directly from the sheet of paper.

LDI- and MALDI-TOF-MS

Mass analyses of the chemical degradation of pure dyes and of ink entries on paper were conducted on a home-built MALDI/LDI reflector time-of-flight (TOF) mass spectrometer [33]. Desorption/ionization was performed with a pulsed nitrogen laser (337 nm, 3ns, ~20 μm focus diameter). Delayed extraction was employed in the positive ion mode (allowing the analysis of cationic dyes). Mass spectra were recorded by averaging 100 individual laser pulse spectra, and the typical mass resolving power was between 3000 and 6000. The laser irradiance was regulated with an attenuator between the laser and the sample, to allow finding the operative threshold irradiance at which a sample was desorbed and ionized adequately. The following MS spectra were recorded for each point of a given aging curve:

- Pure MV/EV. Degradation values were averaged from 10 summed spectra acquired from each of 1 to 4 sample spots.
- BIC strokes. Degradation values were averaged from 4 to 6 summed spectra acquired along 2 to 3 strokes.

Absorption Measurement by UV/VIS Spectrophotometry

Absorption spectra of the dyes were recorded on a UV/VIS spectrophotometer V-550 Jasco (Gross-Umstadt, Germany). The measurement range was from 990 to 190 nm at a speed of 200 nm/min and a bandwidth of 2 nm. The Hellma (Muelheim, Germany) precision cells were made of Quartz Suprasil with a light path of 10 mm.

Sample Treatments

A Heraeus (Hanau/Main, Germany) oven (220 V, 52 A, 50 Hz, 1.14 kW) at a temperature of 100 °C was used to heat the samples. To expose the sample to light, a xenon high-pressure lamp (Leitz GmbH, Wetzlar, Germany, 220/240 V, 50 Hz, XBO/CSX 450 W) was chosen because the xenon lamp irradiance covers the entire wavelength range from 250 nm to more than 1000 nm with high fluence. Samples were positioned 17 cm from the light source. Additionally, the following Schott (Mainz, Germany) Glaswerke filters (40 × 40 mm², 2 mm thickness) were used to measure the influence of the light wavelength on dye degradation: GG475 transmission above 475 nm (yellow), RG5 transmission above 650 nm (red), and additionally a Corion (Spectra-Physics, Darmstadt, Germany) SB-300-F UV band pass filter (25.4 mm diameter, 5 mm thickness). Transmission curves of the filters are shown in Figure 8a.

Sample Preparation

LDI. (a) Reference substance. Methyl violet (MV) and ethyl violet (EV) were dissolved in ethanol (~10 μg/ml). A 0.5 μl aliquot of this solution was deposited on a gold sample plate and analyzed.

(b) Ballpoint entries. Strokes were drawn with the

ballpoint pen BIC on a sheet of paper with the help of a ruler. To test the preparation method, extractions from ~2 cm strokes in ethanol, TFE, phenoxyethanol, and BIC mix (ethoxyethoxyethanol:dipropylene glycol, 1:2) [18] were carried out during 10 min at 60 °C. For the artificial aging experiments, about 4 cm strokes were made and then exposed to diverse treatments. In the case of natural aging, ~20 cm strokes were made monthly. To perform analysis on the paper, small pieces of 5 × 8 mm with 2 to 3 strokes running on the long side were cut, glued with a carbon tape to a metallic sample holder, and introduced into the MS.

MALDI. DHB matrix was prepared at a concentration of 10 mg/ml in a solution of water:ethanol (3:2), and 1 μl of this matrix solution was mixed with 0.5 μl of the dye solution (10 μg/ml) on the sample plate giving a molar ratio of 1:4836.

Spectrophotometry. MV and EV were dissolved in ethanol (~0.05 μg/ml).

Influence of the Method on the Degradation of the Dyes

Effects of the ionization procedure were first tested. A laser intensity that was too high caused dye fragmentation, as expected. Therefore, for both LDI and MALDI, the laser fluence was varied to find a setting providing adequate sensitivity for the analysis of the pure dyes. Then, the experiment was carried out on BIC ballpoint strokes on paper.

The influence of the sample preparation technique was evaluated by comparing MALDI-MS spectra of extracted ballpoint strokes in ethanol, acetone, TFE, phenoxyethanol, and BIC mix, on the one hand and with LDI and MALDI spectra of the strokes on paper, on the other hand. Following the experiments, the best ionization method with minimum alteration of the sample was chosen.

Artificial Aging

Upon aging, an ink stroke undergoes a change in its chemical composition. Artificial aging is a process in which the ink stroke is exposed to conditions (such as light, heat, water) which accelerate the normal aging process. These processes have been widely studied and discussed in forensic examination of documents [27, 29, 34–37], and it is still unclear if UV light and heat are really adequate to model natural aging. The aim in this study was to accelerate the aging of ink and to monitor if the aging occurred similarly under the different conditions.

The following artificial treatments were carried out in the laboratory. Heat and light influence on the aging of the reference substance MV and of the ballpoint pen entries were evaluated. Therefore, on the one hand,

pure MV dye solution was deposited on two gold plates. The first plate was stored in the oven during 336 h at 100 °C, while the second plate was exposed to xenon light during 6 h. MALDI measurements were carried out every 0.5 to 1 h. After 6 h only two further measurements were performed for the heated sample (t = 7 and t = 14 days). On the other hand, blue BIC ballpoint strokes on paper were stored in an oven at 100 °C or exposed to xenon light during 50 h. A reference sample was held in a dark box. LDI measurements were carried out every 5 h.

The influence of humidity was checked by holding blue BIC ballpoint strokes on paper in two hermetically sealed quartz cells exposed to xenon light during 9 h. One cell was dry while the other contained 2 ml of water below the strokes. LDI spectra measurements were performed every 1 to 1.5 h.

Lastly, the absorption curves of methyl violet and ethyl violet were measured by spectrophotometry. Then, to characterize the degradation as a function of the wavelength, three filters were chosen in relation to the wavelength of absorption of the dyes and placed between the xenon light source and the blue BIC ballpoint strokes on paper. Exposure took place during 9 h. A reference sample was processed without a filter and spectra measurements were made every 1 to 1.5 h.

Natural Aging

For natural aging, two batches of ballpoint ink entries were drawn every month during one year with the blue BIC ballpoint. One batch was stored in darkness in a drawer and the other was exposed to daylight by being attached to an outside window facing northwest. Both the reference and the sample were kept in the same room, which was located in the ground floor. There was no light-blocking construction on the opposite side of the window. Degradations of samples aged in winter or in summer were compared.

Results and Discussion

Mass Spectra and Calculation of Aging Curves

MALDI mass spectra of the pure dyes MV and EV are shown in Figure 1a. The corresponding spectra are characterized by the presence of the molecular ions, M^+ = 372.2 u and M^+ = 456.3 u, respectively. The typical degradation of MV and EV under light influence is characterized by a progressive loss of C_nH_{2n} groups (Figure 1b). MV showed six degradation products (Δ = 14 u) at m/z = 358.1 u, 344.1 u, 330.1 u, 316.1 u, 302.0 u, and 288.0 u. On the other hand, EV presented the degradation products (Δ = 28 u) at m/z = 428.2 u, 400.2 u, 372.2 u, 344.1 u, 316.1 u, and 288.0 u. Between these peaks another set of less intense peaks was observed (Δ = 14 u) at m/z = 442.2 u, 414.2 u, 386.2 u, 358.1 u, 330.2 u, and 302.1 u.
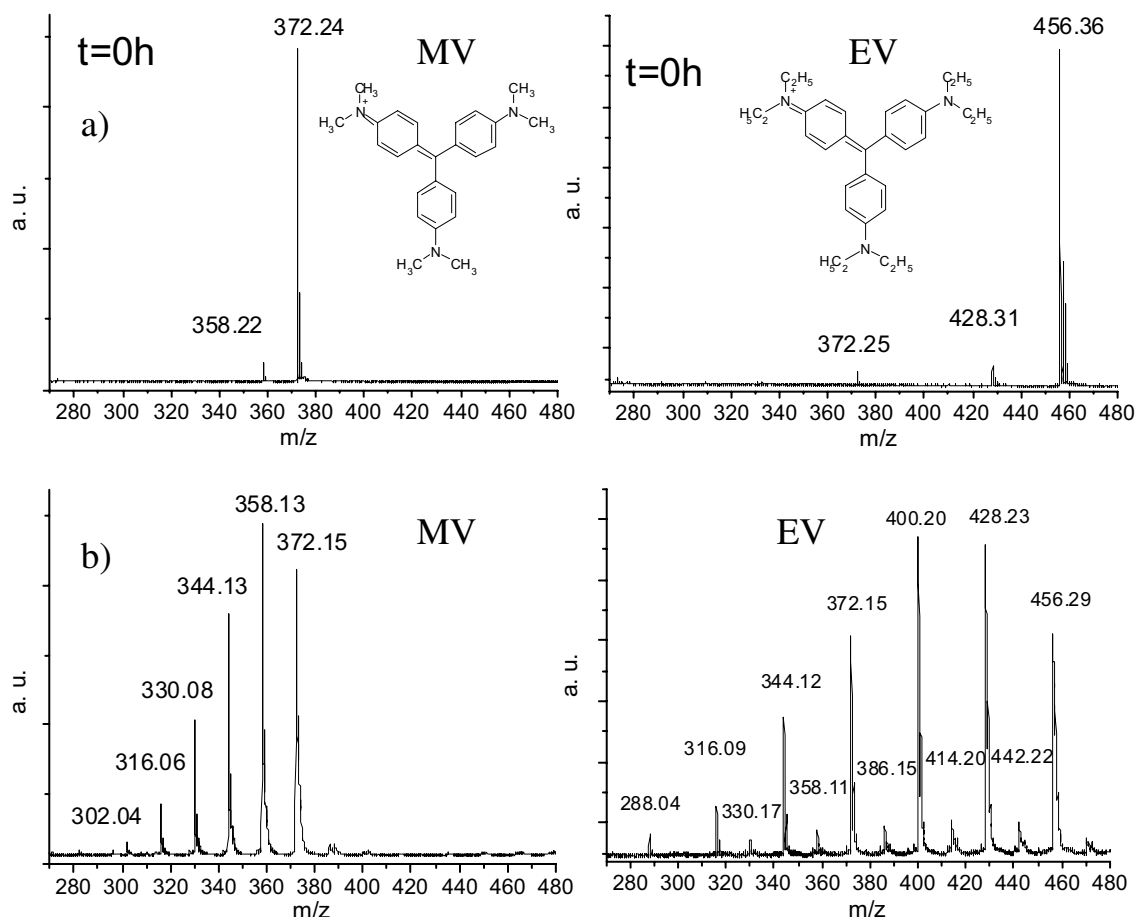
Figure 1. (a) MALDI-MS spectra and structure of MV (methyl violet; M^+ = 372.2 u) and EV (ethyl violet; M^+ = 456.3 u) solved in ethanol (~10 μg/ml). (b) MALDI-MS spectra of MV and EV solved in ethanol (~10 μg/ml) after 6 h exposure to a high-pressure xenon lamp: signals from five of six degradation products of MV (D = 14 u) were observed. The sixth degradation product at m/z = 288.0 u was observed after longer exposure. For EV, signals from six degradation products (D = 28 u) and another set of five less intense signals from six degradation products (D = 14 u) were observed.

This degradation is suitably quantified by the relative peak area (RPA) in each case, defined as

$$RPA_i = \frac{A_i}{A_{tot}} \cdot 100\% \qquad (1)$$

where A_i is the area of the signal at m/z = i and A_{tot} is the total area of all the signals (molecular ion and degradation products) of the dye. With this definition, it is possible to define aging curves of RPA_i as a function of time. The most important of those for the case of MV are

$RPA_{372} = A_{372}/(A_{372} + A_{358} + A_{344} + A_{330} + A_{316} + A_{302} + A_{288})$
$RPA_{358} = A_{358}/(A_{372} + A_{358} + A_{344} + A_{330} + A_{316} + A_{302} + A_{288})$
$RPA_{344} = A_{344}/(A_{372} + A_{358} + A_{344} + A_{330} + A_{316} + A_{302} + A_{288})$

which characterize the degradation of the molecular ion (m/z = 372.2 u), the production of the first

degradation product (m/z = 358.2 u), and the second degradation product (m/z = 344.2 u). The aging curves were then fitted to an exponential function of the form

$$y = y_0 + A \cdot e^{(-x/\tau)} \qquad (2)$$

where x is the time, y is the RPA value and y_0, A, τ are constants. Ballpoint ink compositions change from pen to pen and from batch to batch even within samples of the same company. We observed, for instance, that two blue ballpoint BIC pens bought at an interval of a few months had different dye compositions. From a selection of 18 blue ballpoint pens from different companies, 16 contained MV as a mixture of the hexamethylated form (m/z = 372.2 g/mol) and the pentamethylated form (m/z = 358.2 g/mol), while two did not contain MV. Consequently at time zero, i.e., when the aging has not yet occurred, the RPA_{372} value of the mixture can already be significantly below 100%. RPA_{372} values from the 18 ballpoint pens determined before aging diverged between 53 and 92% and, therefore, their
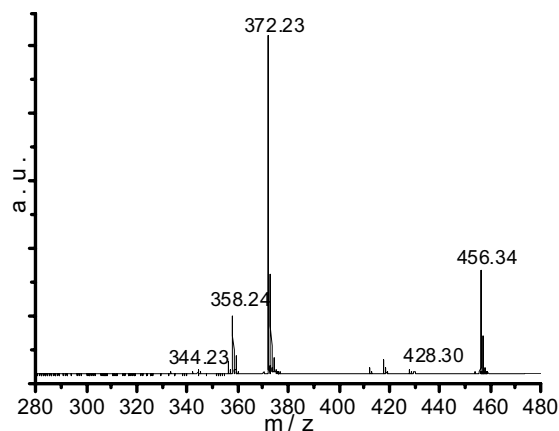
Figure 2. LDI-MS spectrum of a blue ballpoint BIC stroke on paper at time t = 0. The ink contains the dyes methyl violet (M^+ = 372.2 u) and ethyl violet (M^+ = 456.3 u). Smaller signals at m/z = 428.4 u, 358.3 u probably represent degradation products of the molecular ions and/or additional compounds contained in the ink paste composition.

aging behaviors differed from one another. In other words, the initial composition of an ink has to be known for interpretation of mass spectra.

The BIC ballpoint pen used for the experiment contained both MV and EV. The LDI mass spectrum is shown in Figure 2. Therefore, the degradation product signals of EV overlapped with MV signals, and instead of A_{tot}, only the products that were clearly separated from MV were taken into account for calculation of RPA values of EV. The most important RPA values used for strong degradation stages, i.e., when the degradation products of the two dyes overlapped, were consequently modified to

$$RPA_{456} = A_{456}/(A_{456}+A_{442}+A_{428}+A_{414}+A_{400})$$
$$RPA_{428} = A_{428}/(A_{456}+A_{442}+A_{428}+A_{414}+A_{400})$$
$$RPA_{400} = A_{400}/(A_{456}+A_{442}+A_{428}+A_{414}+A_{400})$$

which characterize the degradation of the molecular ion (m/z = 456.3 u) and the production of the second and of the fourth degradation products (m/z = 428.3 u and 400.3 u) of EV.

The reproducibility of the results was found to be better for the LDI measurements of strokes on paper than for the measurements of the pure dyes. Mean standard deviations of the RPA values were calculated and were used as error bars in the graphics.

Influence of the Method on the Degradation of the Dyes

To evaluate the fragmentation effects of the ionization process, samples of pure MV and EV deposited on a gold target from an ethanol solution were analyzed with LDI and MALDI. For MALDI, the samples were mixed with matrix directly on the plate. Measurements were started at a high fluence which was then stepwise decreased down to the threshold fluence, defined as the

lowest laser irradiance at which a sufficient signal was obtained. The threshold fluence was found to be higher for LDI than for MALDI.

It was expected and observed that the fragmentation of the molecular ion increased with the laser irradiance (RPA_{372} and RPA_{456} decreased) and the degradation product signals increased (RPA_{358}, RPA_{344}... and RPA_{442}, RPA_{428}, RPA_{372}..., respectively). The curves in Figure 3 show similar effects for MALDI and LDI, but stronger fragmentation of the molecular ion of EV compared to MV. The threshold fluence at which a minimal signal was observed was not the operative fluence at which good measurements could be taken. Therefore, the RPA values increased until the operative fluence was reached and then decreased again because fragments were produced during the desorption/ionization process. Moreover new fragments appeared in the spectra at higher fluence (such as RPA_{356}, RPA_{357}, RPA_{368}, RPA_{413}, RPA_{415}...) which were not taken into account in the RPA definition. This effect was found to be stronger in the case of LDI measurements compared to MALDI. The operative fluence to threshold fluence ratio was chosen as 1.5 which is equivalent to an operative fluence of 3×10^3 J/m^2.

In the second part of this experiment, the influence of the laser fluence on fragmentation was tested for ballpoint strokes on paper instead of the pure dye solutions. Laser irradiance also provoked fragmentation of the dyes, and for this particular ink the dye MV was found to be less stable to laser irradiance compared to the dye EV. It was also observed that older samples generally had higher threshold laser irradiance than freshly prepared samples. This observation can be explained by solvent evaporation that takes place after the
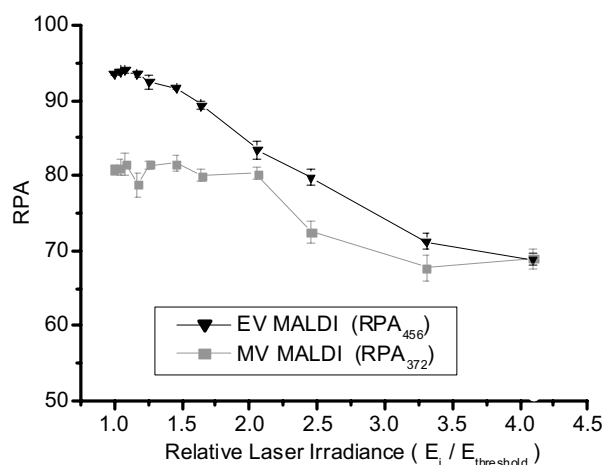

Figure 3. Fragmentation as function of the laser irradiance for MV and EV. RPA_{372} and RPA_{456} values that are the relative signal areas of the molecular ion (M^+ = 372.2 u and M^+ = 456.3 u) and a measure of the degradation of the dyes were calculated from the MALDI-MS spectra of MV and EV solved in ethanol (~10 mg/ml). High laser fluence promoted the degradation of the molecular ion; therefore, measurements had to be performed with fluence near threshold. For measurements, a relative value of 1.5 was chosen as operative fluence.

Table 1. RPA_{456} (EV) and RPA_{372} (MV) values from LDI and MALDI mass spectra of ballpoint BIC® strokes on paper and from MALDI mass spectra of extraction of the paste from ≈2 cm ballpoint BIC strokes in diverse solvents. The best results were obtained for the measurements performed directly on the paper (high RPA values)

Preparation method	EV (RPA_{456})	MV (RPA_{372})
Paper(LDI)	79.9 ± 1.0	86.7 ± 0.4
Paper(MALDI)	78.1 ± 1.1	87.1 ± 0.7
Ethanol	31.2 ± 1.8	39.2 ± 1.1
Acetone	30.4 ± 1.5	40.3 ± 0.8
TFE	31.7 ± 2.7	62.3 ± 1.3
Phenoxyethanol	52.3 ± 1.0	80.6 ± 0.2
BIC mix	46.0 ± 6.0	72.3 ± 2.5

application of the ink on paper. Resins and dyes are then expected to form a more compact surface [1].

Since the laser irradiance has a measurable influence on the results, it is important that analysis of aged samples is performed at a given fluence to allow any comparison. We chose to record our results at an operative fluence (relative value of 1.5) close to the threshold value of old inks.

The chemical influence of the matrix on the degradation of the pure dyes using ethanol as solvent was tested by MALDI experiments. The results showed that the molecular ions of the dyes degraded already during the preparation and/or desorption/ionization steps of the experiment. RPA_{372} and RPA_{456} values were found to be significantly below 100% (Figure 3).

A further experiment compared MALDI and LDI analyses of strokes directly on paper and MALDI analyses of different extraction solutions of ballpoint strokes (Table 1). Extraction by ethanol, acetone, and TFE, and dilution in a matrix solution (DHB in water/ethanol 60:40 vol/vol) resulted in values below 60% for RPA_{372} and below 40% for RPA_{456}. Phenoxyethanol and the BIC mix (ethoxyethoxyethanol:dipropylene glycol, 1:2) are typical ballpoint pen compounds [18, 20, 38]. They are good extraction solvents for ballpoint dyes. Values were between 70 to 80% for RPA_{372} and between 50 to 60% for RPA_{456}. Nonetheless, LDI and MALDI analyses of the strokes on paper still gave more intense signals with minimal degradation of the molecular ions of MV and EV. The RPA_{372} values were 85% and RPA_{456} values were 80%. These results clearly show the influence of extraction procedures and matrix sample preparation on the determination of the degradation status. As the dyes readily absorb the laser light and are easily ionized, the use of a matrix did not improve the measurements of ink strokes. Moreover, the matrix is an acid that could chemically influence the organic dyes. Therefore, for best results, we suggest LDI analysis of the ballpoint strokes directly on paper close to threshold laser irradiance. This has the advantage of reducing the sample preparation steps and, thus, any possible alteration of the samples to a minimum.

Artificial Aging of Pure Methyl Violet and Blue Ballpoint BIC

The preliminary study of the influence of light and heat on pure MV confirmed that the degradation of the dye strongly depends on the quality and duration of light fluence. After 6 h exposure to the xenon light, the RPA_{372} value calculated from the MALDI-MS spectra had decreased to 25% (Figure 4). The influence of exposure to heat was found to be weaker but also provoked degradation. After one week of exposure, the RPA_{372} value calculated from the MALDI-MS spectra had dropped to about 40% (Figure 4). This indicates that degradation of the dyes proceeds even in the absence of light. The study, however, did not address the question of degradation of the dye upon very long exposure to heat and whether it would occur to a similar extent as upon light exposure. We additionally observed that the aging curves seem to level off after longer exposures to light or heat, meaning that the degradation process reaches a limit or is slowing down significantly.

The experiments on ink strokes gave similar results. An LDI spectrum of the blue ballpoint BIC at time t = 0 is shown in Figure 2. No obvious difference was observed in the spectrum taken after 50 h storage in an oven at 100 °C (Figure 5a). The degradation product signals of both EV and MV, however, became very strong after 50 h exposure to the light of a xenon lamp (Figure 5b). Aging curves confirm these observations (Figure 6). The aging curve of a standard, which was held in a dark box under normal conditions, is flat as no change is measured after 50 h. The corresponding curve for the strokes exposed to 100 °C shows a very weak degradation of the molecular ion of EV, while the curve for the strokes exposed to the xenon lamp shows a steep
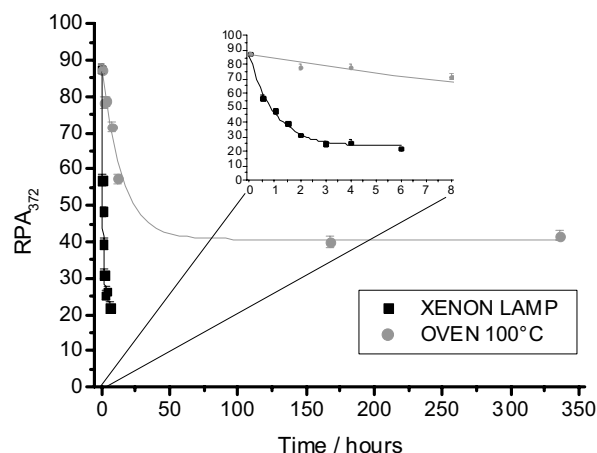

Figure 4. Comparison of the degradation of methyl violet (~1 mg/ml in ethanol) provoked by a high-pressure xenon lamp (d = 17 cm) over 6 h and by an oven at 100 °C over 336 h (14 days). Upon exposure to light, RPA_{372} values dropped from ~97 to 25% after 3 h exposure. The RPA_{372} value of samples stored in the oven attained ~40% after 7 days of treatment and stayed roughly constant upon further exposure.
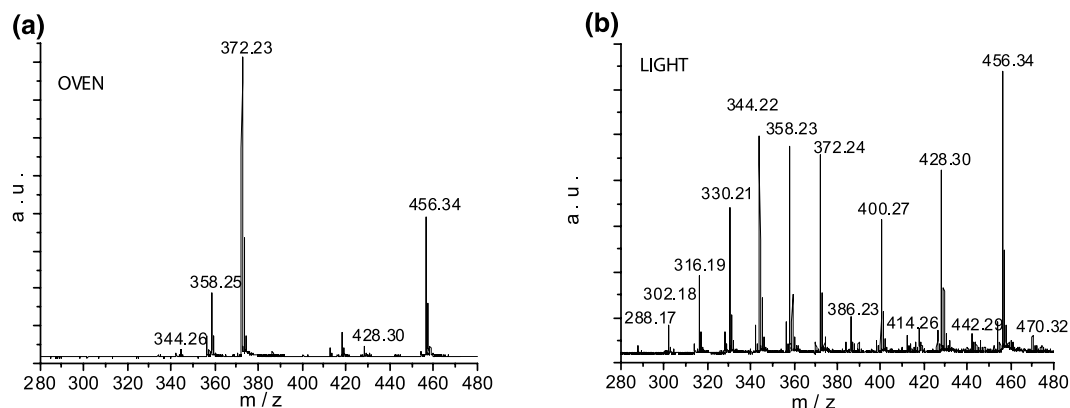
Figure 5. LDI-MS spectra of a blue ballpoint BIC stroke on paper at time t = 50 h: (**a**) Spectrum of ink entries stored in an oven at 100 °C and (**b**) spectrum of ink entries exposed to a high-pressure xenon lamp.

decrease of the signal of the molecular ion of EV. RPA_{456} attained 30% after 50 h, while RPA_{428} increased to over 20% already after 10 h, and RPA_{400} increased linearly to 20%. No levelling off of the degradation process was observed after 50 h exposure.

Another interesting observation is that degradation of the dyes MV and EV in the ink strokes upon light exposure is enhanced by humidity (Figure 7). After 9 h exposure to light, the LDI mass spectra of dry samples led to $RPA_{372} \sim 60\%$ and $RPA_{456} \sim 80\%$, while the LDI mass spectra of humid samples gave $RPA_{372} \sim 30\%$ $RPA_{456} \sim 60\%$. The pH of the paper may play an increasing role under high humidity conditions since paper shows a basic reaction with water (pH $\sim$ 8).

The influence of the wavelength of the incident light on the degradation of the dyes in ink entries was also studied. The spectrum of the incident light was filtered, leading to degradation within restricted wavelength ranges. The two dyes have their maximum absorption at 588 and 592 nm, respectively, and additionally absorb in the UV (Figure 8a). The three chosen filter transmission curves are shown in the same figure: the yellow filter (GG475) transmits the visible light, UV filter (SB-300-F) transmits between 260 and 360 nm, and the red filter (RG5) cuts off both the UV and the maximum of absorption. The experiment was carried out over 9 h for each filter or without filter respectively. The corresponding results are shown in Figure 8b. The strongest degradation occurred when no filters were used ($RPA_{372} \sim 55\%$ and $RPA_{456} \sim 70\%$). When both the UV part of the light and the region endorsing the absorption peak of the dye were blocked (red filter, $\lambda > 650$ nm), no considerable degradation was observed after 9 h exposure to the xenon lamp ($RPA_{372} \sim 90\%$ and $RPA_{456} \sim 95\%$). Moreover, the results show that wavelengths in the UV ($\lambda < 360$ nm) and within the absorption maximum of the dye (yellow filter, $\lambda > 450$ nm) are both effective with respect to degradation ($RPA_{372} \sim 75\%$ and $RPA_{456} \sim 90\%$). This holds for each of these two regions independently, although with slightly different intensity. It is reasonable to assume that the
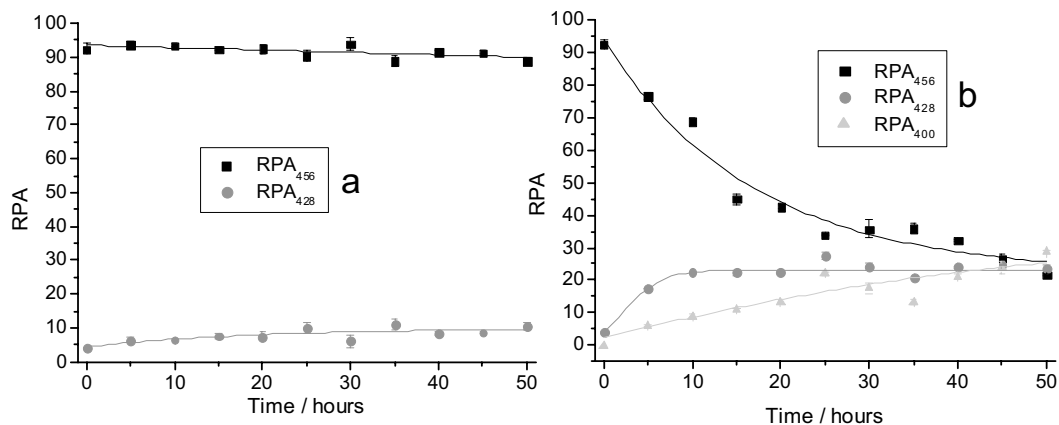

Figure 6. Artificial aging of EV contained in ink entries from a ballpoint pen BIC upon the following treatments over 50 h: (**a**) storage in an oven 100 °C and (**b**) exposure to a high-pressure xenon lamp. The aging curves show that heat has a very small effect on the degradation of ethyl violet while light has a strong effect, which can already be observed after a few hours exposure.
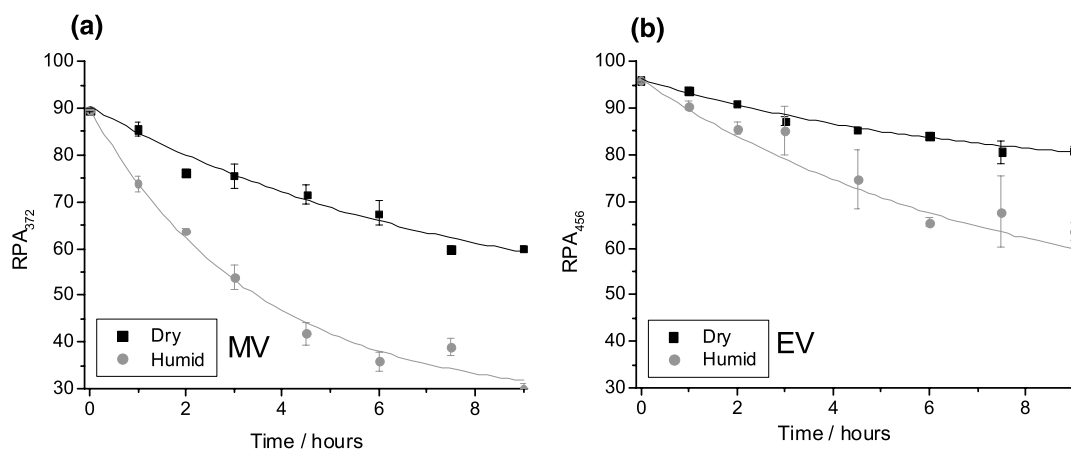
Figure 7. Influence of humidity on the degradation of MV and EV from ink entries of a ballpoint pen BIC upon 9 h exposure to a high-pressure xenon lamp. The curves show that the degradation of both dyes upon light exposure is stronger in a humid atmosphere compared with a dry atmosphere.

fragmentation mechanisms leading to identical degradation products in each of these two wavelength regions are not the same, attributable to the different physico-chemical behavior of the dyes upon excitation by these wavelengths.

Natural Aging of Blue Ballpoint BIC

Consistent with the above mentioned facts, the natural aging of the ink entries was found to be accelerated when exposed to daylight entering through a
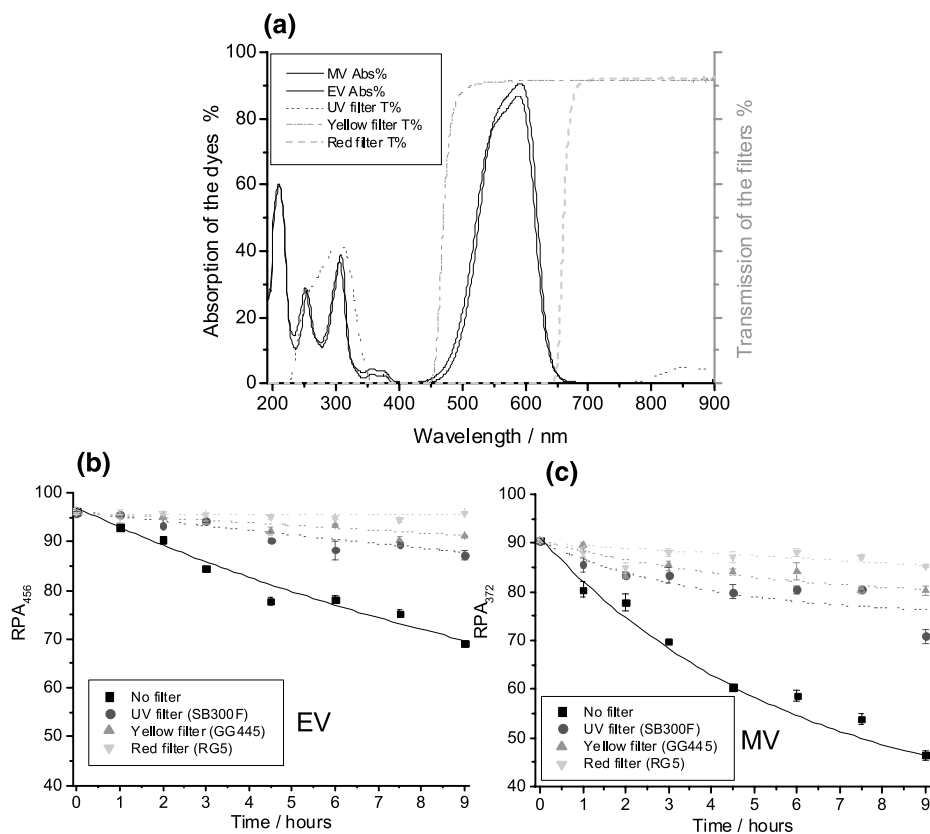

Figure 8. (**a**) Spectral absorption of MV and EV and spectral transmission curves of three filters: UV (SB300F), yellow (GG445) and red (RG5). (**b**) Influence of the wavelength of the incident light on the degradation of MV and EV from ballpoint pen BIC entries upon 9 h exposure to a high-pressure xenon lamp. The spectrum of the incident light was filtered using three filters, leading to degradation within restricted wavelength windows.

regular house window. The BIC spectra showed the unchanged conditions of the reference sample at t = 0 and t = 349 days. In contrast, after 349 days exposure to daylight, strong signals of the degradation products of MV and EV were observed.

The aging curves (data not shown) confirm these observations. No measurable change was found for the ink strokes stored in the dark after one year ($RPA_{456} \sim 95\%$, $RPA_{428} \sim 5\%$, and $RPA_{400} \sim 0\%$), whereas a significant degradation was observed after already three days of exposure to daylight ($RPA_{456} \sim 35\%$, $RPA_{428} \sim 30\%$, and $RPA_{400} \sim 30\%$). Moreover, the aging curve for ink entries exposed to daylight level off after 150 days and a change of color is also visible to the naked eye; that is, the blue color turned to a green-turquoise shade.

The measurements of ink entries exposed to daylight up to six months during the winter and summer season were compared. As expected, the degradation of the dye occurs in a shorter time during the spring/summer season because of longer daily exposure to light. The degradation reached a limiting value after 100 days. In autumn and winter, the exposure to light was shorter and, additionally, direct sunshine reached the window only after 5.00 pm; therefore the paper sheets with the strokes were irradiated mostly by scattered light in this season. Degradation has not yet reached a limiting value after 180 days, and measurements are still ongoing.

Conclusions

The results demonstrate that using MALDI- and LDI-MS provide valuable information on the degradation of inks, provoked by a number of processes. High laser fluence upon mass analysis promotes additional fragmentation of the molecular ion. Consequently, we have used fluence near the threshold of ion detection in our experiments. Additionally, we have found that extraction of analyte with an adequate solvent can induce unwanted effects. Therefore, analysis of the ink strokes was performed directly from the paper. The method requires no special sample preparation procedure and is only semi-destructive. The laser focus of about 20 μm in diameter ablates only a small layer of the ink stroke, giving a measurable signal even after hundreds of laser pulses. The aging curves used are reliable and informative tools to characterize degradation processes of dyes. The results are reproducible within a small error range.

We have observed that the studied degradation of dyes strongly depends on the storage conditions under which a given sample is kept. Degradation is caused mostly by absorption of light with wavelengths in the UV and within the absorption maximum of the dyes. Questioned documents should then be stored preferably in the darkness or under illumination with light sources in which these wavelength regions are not present. Additionally, since high humidity conditions

in the presence of light promote dye degradation, a dry environment should be used to store samples. Heat has a weaker influence on degradation of the dye, but should also be taken into account, particularly for high-temperature exposure of 100 °C. Strokes held in the dark at room temperature didn't show any degradation after one year. Therefore, precise storage conditions have to be known for correct forensic interpretation of dye age.

The initial composition of an ink should also be known, as the RPA of a dye can be significantly under 100% before aging took place. At this stage, we can reproducibly and valuably compare strokes made with the same pen and kept at the same environmental conditions. The assessment of the age of an ink entry by dye degradation analysis is much less reliable when these two conditions are not fulfilled.

Acknowledgments

References

1. Brunelle, R. L.; Cantu, A. A. A Critical Evaluation of Current Ink Dating Techniques. *J. Forensic Sci.* **1987**, *32*(6), 1522–1536.
2. Steward L. F.; Guertin S. *Current Status of Ink Age Determination*; International Criminal Police Review (March/April): 1991; pp 9–13.
3. Brunelle, R. L.; Lee, H. Ink Dating—The State of the Art. *J Forensic Sci.* **1992**, *37*, 113–124.
4. Hicks, T. Determination de l'age des encres–Possibilites de standardisation, Universite de Lausanne, Institut de Police Scientifique, Seminaire de 3eme annee, 1993.
5. Aginsky V. N. Some New Ideas for Dating Ballpoint Inks—A Feasibility Study. *J. Forensic Sci.* **1993**, *38*(5), 1134–1150.
6. Cantu, A. A. A Sketch of Analytical Methods for Document Dating Part II. The Dynamic Approach: Determining Age Dependent Analytical Profiles. *Int. J. Forensic Documents Examiners* **1996**, *2*(3), 192–208.
7. Dormann, P. Les differentes techniques et la controverse sur la datation des encres, Universite de Lausanne, Institut de Police Scientifique, Seminaire de 2eme annee, 2000.
8. Brunelle, R. L.; Breedlove, C. H.; Midkiff, C. R. Determining the Relative Age of Ballpoint Inks Using a Single-Solvent Extraction Technique. *J. Forensic Sci.* **1987**, *32*(6), 1511–1521.
9. Cantu, A. A.; Prough, R. S. On the Relative Aging of Ink. *J. Forensic Sci.* **1987**, *32*, 1151–1174.
10. Aginsky, V. N. Determination of the Age of Ballpoint Pen Inks by Gas and Densitometric Thin-Layer Chromatography. *J. Chromatogr. A* **1994**, *678*, 119–125.
11. Aginsky, V. N. A New Version of the Internal Standard Method in Quantitative Thin Layer Chromatography. *J. Planar Chromatogr.* **1994**, *7*, 309–314.
12. Brunelle, R. L. A Sequential Multiple Approach to Determining the Relative Age of Writing Inks (TLC, Solvent Extraction and Relative Dating). *Int. J. Forensic Documents Examiners* **1995**, *1*(2), 94–98.
13. Andermann T.; Neri R. Solvent Extraction Techniques—Possibilities for Dating Ballpoint Pen Inks. *Int. J. Forensic Documents Examiners* **1998**, *4*(3), 231–239.
14. Steward, L. F. Ballpoint Ink Age Determination by Volatile Component Comparison—A Preliminary Study. *J. Forensic Sci.* **1985**, *30*(2), 405–411.
15. Aginsky, V. N. Dating and Characterizing Writing, Stamp Pad, and Jet Printer Inks by Gas Chromatography/Mass Spectrometry. *Int. J. Forensic Document Examiners* **1996**, *2*(2), 103–116.
16. Aginsky, V. N. Measuring Ink Extractibilty as a Function of Age—Why the Relative Aging Approach is Unreliable and Why it is More Correct to Measure Ink Volatile Components than Dyes. *Int. J. Forensic Document Examiners* **1998**, *4*(3), 214–230.
17. Fortini, A. Datation des encres: Tester la reproductibilite des measures de l'une des methods developpee par Aginsky (GC/MS), Universite de Lausanne, Institut de Police Scientifique, Seminaire de 4eme annee, 2000.
18. Weyermann, C.; Kirsch, D.; Costa-Vera, C.; Spengler, B. Dating of Ink Entries by MALDI/LDI-MS and GC-MS Analysis: Reality or Utopia? *Proceedings of the 3rd European Academy of Forensic Science Meeting*; Forensic Science International: Istanbul, September 2003; pp 73–74.

19. Andrasko J. *Proceedings of the 3rd European Academy of Forensic Science Meeting*; Forensic Science International: Istanbul, September 2003; pp 73–74.

20. Lociciro, S.; Mazella, W.; Dujourdy, L.; Lock, E.; Margot, P. Dynamic of the Aging of Ballpoint Pen Inks. *Science & Justice* **2004**, *44(3)*, 165–171.

21. Aginsky, V. N. A Microspectrophotometric Method for Dating Ballpoint Inks—A Feasibility Study. *J. Forensic Sci.* **1995**, *40*, 475–478.

22. Sakayanagi, M.; Komuro, J.; Konda, Y.; Watanabe, K.; Hirigaya, Y.; Analysis of Ballpoint Pen Inks by Field Desorption Mass Spectrometry. *J. Forensic Sci.* **1999**, *44*(6), 1204–1214.

23. Lyter, A. H. In Situ Molecular Analysis of Sequential Ink Writings by TOF-SIMS. Questioned Documents Section (Abtract). IAFS Meeting, Los Angeles, CA, August 1999.

24. Grim, D. M.; Siegel, J.; Allison, J. Evaluation of Desorption/Ionization Mass Spectrometric Methods in the Forensic Applications of the Analysis of Inks on Paper. *J. Forensic Sci.* **2001**, *46*(6), 1411–1420.

25. Andrasko, J. HPLC Analysis of Ballpoint Pen Inks Stored at Different Light Conditions. *J. Forensic Sci.* **2001**, *46*(1), pp 21–30.

26. Andrasko, J. Changes in Composition of Ballpoint Pen Inks on Aging in Darkness. *J. Forensic Sci.* **2001**, *47*(2), 324–327.

27. Grim, M. D.; Siegel, J.; Allison, J. Evaluation of Laser Desorption Mass Spectrometry and UV Accelerated Aging of Dyes on Paper as Tools for the Evaluation of a Questioned Document. *J. Forensic Sci.* **2002**, *47*(6), 1265–1273.

28. Weyermann, C.; Kirsch, D.; Andermann, T.; Spengler, B. Analysis of the Degradation Process of Ballpoint Ink by Mass Spectrometry. *Poster Contribution at the 50th Annual Conference on Mass Spectrometry and Allied Topics*; Orlando, FL, June, 2002; extended Abstract.

29. Li, X.; Liu, G.; Zhao J. Two Competitive primary Processes in the Photodegradation of Cationic Triarylmethane Dyes Under Visibile Irradiation in TiO_2 Dispersions. *New J. Chem.* **1999**, *23*, 1193–1196.

30. Hess, J. The Black and White Report. *Ink World*; June 2000 pp 53–54.

31. Levinson, J. Questioned Documents: A Lawyer's Handbook; Academic Press: London, 2001; pp 128–129

32. Karas, M.; Bachmann, D.; Bahr, U.; Hillenkamp, F. Matrix-Assisted Ultraviolet Laser Desorption of Nonvolatile Compounds. *Int. J. Mass Spectrom. Ion Processes* **1987**, *78*, 53–68.

33. Chaurand, P.; Luetzenkirchen, F.; Spengler B. Peptide and Protein Identification by Matrix-Assisted Laser Desorption Ionization (MALDI) and MALDI-Post-Source Decay Time-of-Flight Mass Spectrometry. *J. Am. Soc. Mass. Spectrom.* **1999**, *10*, 91–103.

34. Stewart, L. F. Artificial Aging of Documents. *J. Forensic Sci.* **1982**, *27*, 450–453.

35. Cantu, A. A. Comments on the Accelerated Aging of Documents. *J. Forensic Sci.* **1988**, *33*, 744–750.

36. Aginsky, V. N. Accelerated Aging—Its Use in Methods for Dating Ink, Letters. *Int. J. Forensic Documents Examiners* **1996**, *2*(3), 179–181.

37. Stewart, L. F. Fortunato, S. L. Distinguishing Between Relative Ink Age Determinations and the Accelerated Aging Techniques. *Int. J. Forensic Document Examiners* **1996**, *2*(1), 10–15.

38. Weyermann, C.; Costa-Vera, C.; Kirsch, D.; Spengler, B. A Study of the Aging Processes of Ballpoint Dyes and Solvents by LDI-MS and GC/MS. *Oral Presentation at the 17th International Symposium on the Forensic Sciences*; Wellington, New Zealand, March, 2004; Abstract.