J Forensic Sci, March 2009, Vol. 54, No. 2
doi: 10.1111/j.1556-4029.2008.00975.x
Available online at: www.blackwell-synergy.com

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Evaluation of the Photodegradation of Crystal Violet upon Light Exposure by Mass Spectrometric and Spectroscopic Methods

ABSTRACT: Crystal violet is a very common dye in ballpoint ink. Recent research suggests that the degradation of triarylmethane dyes gives an indication of the age of a ballpoint pen entry on a document. The main problem for the quantitative evaluation of the degradation is that it is highly dependent on the exposure to light. Moreover additional factors, such as additives and substrate play an important role in this process. The aim of this work is to compare the degradation pathways of the pure dye in water and ethanol upon exposure to xenon light by UV/VIS spectrophotometry and laser desorption ionization. Significant differences have been observed in the products and the kinetics of the degradation. N-demethylation, an expected decomposition process, was found to take place only in aqueous solution and kinetics calculations showed that the degradation occurred 2.5 times faster in ethanol compared to water. The degradation of crystal violet in inks from four ballpoint pens on paper was also studied for entries made over 2–3 years. It was observed that degradation reactions were quenched by the presence of another dye due to competitive absorption. It was also observed that the thickness of a stroke (concentration of ink) influenced the degradation process. In the absence of light only one ballpoint pen showed slight degradation. A better understanding of the influence of the paper, ink composition, and storage conditions is necessary to interpret correctly the age of an ink based on the degradation of dyes.

KEYWORDS: forensic science, laser mass spectrometry, UV–VIS spectroscopy, Matrix Assisted Laser Desorption Ionisation Mass Spectrometry, cationic triarylmethane dyes, crystal violet, aging, ink

Dye degradation has occupied scientists for a long time and has mainly been studied for industrial purposes, to find ways of quenching color fading of clothes, inks, or paper dyes without increasing the costs (1). Its neutralization has also been widely studied in environmental sciences, as it is important to photodegrade (2) or biodegrade (3) the toxic dye before releasing it in the environment.

In forensic sciences, the question of the age of a document is routinely asked and stays frequently unanswered, because actual dating methods are considered unreliable. The reason for this is that aging of ink is highly dependent on the composition of the ink and the storage conditions of the document, two variables usually unknown in forensic document examination. Dyes degradation has been lately studied for the purpose of dating documents by chromatographic or mass spectrometric methods (4–8). It has been observed that dyes degrade strongly under the influence of light, but do not or very slowly degrade in the dark. Also contradictory results on the degradation of ballpoint dyes in the absence of light have been reported (8–11). When dyes degrade, a photochemical reaction is triggered by the absorption of visible or ultraviolet light. Absorption of one or more photons of suitable energy by an organic molecule provides an electronically excited state, which is the starting point for subsequent reaction steps (12,13).

Triarylmethane dyes (e.g., crystal violet) are favored as color formers in ballpoint ink because of their low cost and strong color. They are characterized by relatively low photostability and their intensity fades with time and exposure to light (14,15). Degradation, generally resulting in color fading, is influenced by the structure of the dye, as well as by external and environmental parameters. Thus oxygen, moisture, temperature, additives such as atmosphere contaminant (sulfur dioxide and nitrogen oxides from pollution), wavelength of incident light and concentration of the dyes have an influence on the degradation speed and pathways (16–18). Moreover, the surface properties, the chemical and physical structure of the substrate, residual solvent within the substrate and porosity also influence the degradation quite significantly (15). In the textile industry, it is well known that triarylmethane dyes have poor light fastness in cotton and wool, while on the contrary they have a very strong and stable shade in acrylic fiber (1).

Photochemical degradation of the dyes can take a large variety of pathways and thus, produce a wide range of different products as a result. The deactivation pathways of the dye excited states are conditioned by interactions with the immediate environment, as many radical species originate from the solvents or the substrates upon exposure to light (15). Photodegradation of triphenylmethane dyes is accelerated by the presence of singlet oxygen sensitizers (e.g., methylene blue or titane dioxide) and retarded by singlet oxygen quenchers [e.g., β-carotene or zinc(II) and nickel(II) complexes], thus demonstrating the involvement of singlet oxygen in some degradation pathways (19). Several different degradation reactions may occur (2,3,15,16,19,20).

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Received 26 Sept. 2007; and in revised form 16 Aug. 2008; accepted 2 Nov. 2008.

- N-Demethylation (Fig. 1) has been studied by many authors, as it is easily detected. The methyl groups of the dye are sequentially replaced by hydrogens (mass difference of 14 u) upon exposure to light.
- Photooxidative cleavage of the central C-phenyl bond, probably via singlet oxygen, to give benzophenones and phenols (Fig. 2). It has been demonstrated that triarylmethane dyes produce singlet oxygen upon photolysis on paper, but not necessarily in ethanol or water (15). Ring opening by OH radicals formed by singlet oxygen in water has also been proposed (2).
- Photoreduction of an excited state dye cation to a leuco-dye form by addition of an electron to the photoexcited species or by photochemical hydrogenation of the dye (18).

All these degradation reactions may occur under the same experimental conditions with different kinetics and are in competition with each other, resulting in a very complex aging process of dyes. For the reason exposed above, it is essential that the use of a dating method based on dyes degradation be thoroughly investigated.

In earlier studies, the authors (7,8) have studied the influence of external factors such as light, wavelength, heat, and humidity on the degradation of triarylmethane dyes. The present work has the purpose of determining the influence of the substrate and the ink composition on the degradation speed and pathways of the dye crystal violet (i.e., Basic Violet 3 or BB3). For this purpose, in the first experimental part, aqueous and ethanol solutions of the pure dye were exposed to light over several hours and timely measurements were carried out using Matrix Assisted Laser Desorption Ionisation Mass Spectrometry (MALDI-MS) and UV/VIS spectroscopy every hour. In the second part of this study, ink entries on paper originating from four different blue ballpoint pens containing BB3 were aged naturally over 2 years and analyzed by Laser Desorption Ionisation Mass Spectrometry (LDI-MS) every 1–2 months to compare their degradation processes.

Experimental

Materials

The solvents (water for chromatography, ethanol, acetone) were purchased from Merck (Darmstadt, Germany) and 2,5-dihydroxybenzoic acid powder (DHB, pK_s = 2.97) from Sigma-Aldrich

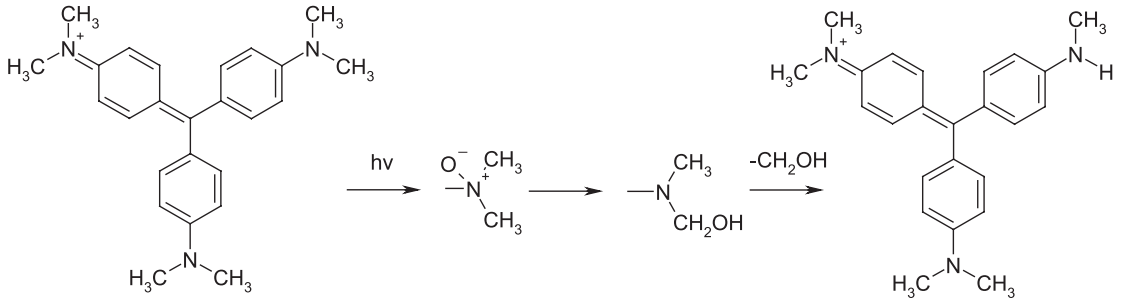

FIG. 1—*Mechanism proposed by Caine et al. (19) for the N-demethylation of crystal violet.*

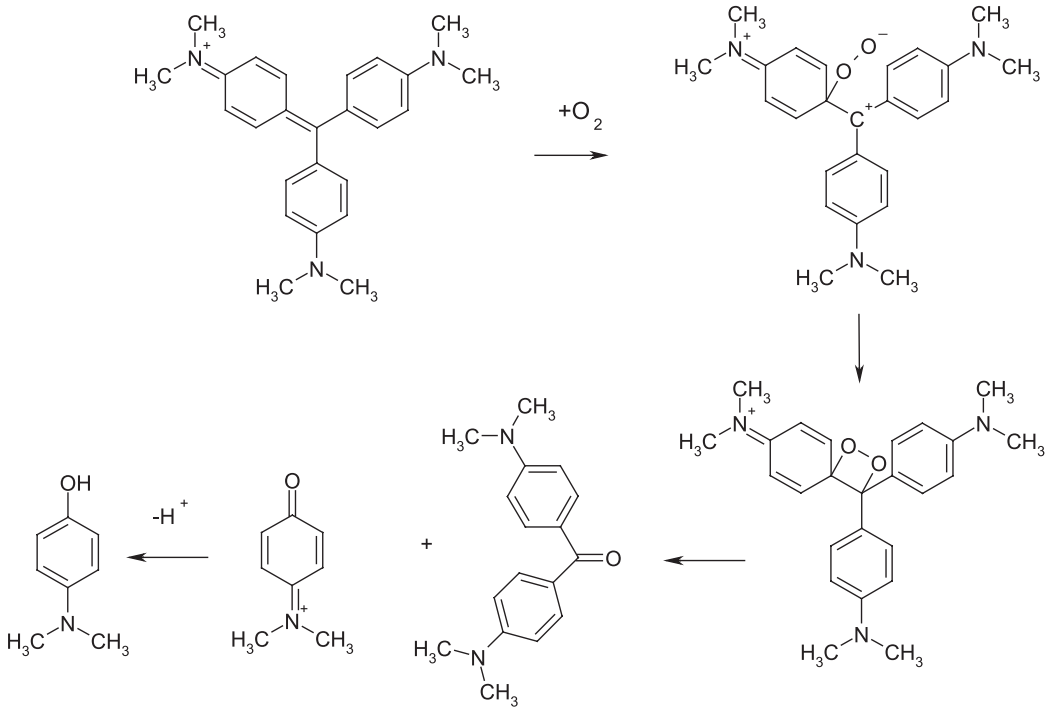

FIG. 2—*Mechanism proposed by Henriquez in 1933 (16) and redefined by Kuramoto and Kitao (20) for the degradation of crystal violet through singlet oxygen attack, which produces diemethylaminobenzophenones and dimethlyaminophenol.*

(Steinheim, Germany). The reference substance used was the pure dye BB3 (crystal violet, Fluka).

Blue BIC® ballpoint entries (Cristal™ and Velocity™ medium blue, France), blue Parker® ballpoint pen entries (Medium, ISO 12757-2, England) and blue Herlitz® ballpoint pen entries (Medium, Germany) were made on multifunction bright-white, wood and chlorine free paper from Igepa Plus (80 g/m^2, DINA4, nr. 806 A 80, Reinbeck, Germany). Subsequent analysis was carried out either after dissolution of the pure dye in ethanol or water, or directly from the sheet of paper.

Instrumentation

LDI- and MALDI-TOF-MS—Mass analyses of the chemical degradation of the pure dyes and of ink entries on paper were conducted on a laboratory-built MALDI/LDI reflector time-of-flight (TOF) mass spectrometer. Desorption/ionization was performed with a pulsed nitrogen laser (337 nm, 3 ns, *c.* 20 μm focus diameter). Delayed ion extraction and a dual-stage ion mirror were used to improve mass resolution. Positive ions were recorded for the analysis of cationic dyes. Mass spectra were recorded by signal averaging 100 individual single-shot spectra, with the typical mass resolving power between 3000 and 6000. Laser fluence was regulated with a variable attenuator placed between the laser and the sample, which allowed finding an optimal operative irradiance. Each point of a given aging curve was calculated from averages taken from several averaged spectra:

- *Pure BB3*: Degradation values were calculated from 10 averaged spectra acquired over 1 or 2 sample spots.
- *Ballpoint pen entries*: Degradation values were calculated from 6 to 10 averaged spectra acquired along two to three different strokes.

UV/VIS Spectrophotometry—Absorption spectra of the dyes in solution were recorded on a UV/VIS spectrophotometer V-550 Jasco. The measurement range was from 190 to 900 nm at a scan rate of 200 nm/min and a resolution bandwidth of 2 nm. Quartz Suprasil® Hellma® precision cells with a light path of 10 mm were used.

Dye Degradation by Exposure to Light—For light exposure, 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 wavelengths from 250 nm to more than 1000 nm with high fluence.

Procedures and Treatments

Pure Dye BB3—About 0.01 mg/mL pure BB3 was dissolved in water or ethanol and exposed to xenon light for several hours in a quartz cell for spectrophotometry. Degradation of the dye was studied by UV/VIS spectrophotometry and MALDI-MS until the dye signals completely disappeared. Measurements were taken every hour. For MALDI-MS analysis, the DHB matrix was dissolved at a concentration of 10 mg/mL in a solution of water:ethanol (3:2). 1 μL of this matrix solution was then mixed with an aliquot of 0.5 μL of the illuminated dye solution on the gold coated stainless steel sample plate giving an optimal molar ratio of about 1:1000 for analysis. As the solvents evaporated during the illumination, an additional experiment with volume adjustment was repeated to determine the kinetics of degradation.

Ballpoint Pen Entries—Strokes were drawn with the ballpoint pens on a sheet of paper with the help of a ruler. Two batches of entries of *c.*15 cm each for every pen were made monthly. One was stored in darkness in a drawer and the other was attached to a window north-west and exposed to daylight. Both the reference and the sample were kept in the same room and the experiment was carried over about 2 years.

To perform analysis on the paper, small pieces of about 5 × 8 mm with 2–3 strokes running on the long side, were cut, glued with a carbon tape onto a metallic sample holder, and introduced into the mass spectrometer.

Results and Discussions

In the following presentation of the results, the error bars shown in the figures correspond to the mean standard deviation of the measurements.

Mass Spectra and Aging Curves Calculation

MALDI mass spectra of the pure dye BB3 are characterized by the presence of the molecular ions M^+ = 372.2 u. In an earlier paper (7), the authors determined that the typical degradation of BB3 under light influence is characterized by a progressive loss of CH_2 groups. Consequently, BB3 has six degradation products (Δ = 14 u) at m/z = 358.1 u, 344.1 u, 330.1 u, 316.1 u and 302.0 u, and 288.0 u, which are easily detected by MALDI-MS. This degradation is suitably quantified by the definition of the percentual Relative Peak Area (RPA) defined earlier as:

$$\mathrm{RPA}_i = \frac{A_i}{A_{\mathrm{tot}}} \cdot 100 \qquad (1)$$

where A_i is the area of the peak 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 as a plot of RPA_i as a function of time. For example, the relations

$$\mathrm{RPA}_{372} = A_{372}/(A_{372} + A_{358} + A_{344} + A_{330} + A_{316} + A_{302} + A_{288})$$

$$\mathrm{RPA}_{358} = A_{358}/(A_{372} + A_{358} + A_{344} + A_{330} + A_{316} + A_{302} + A_{288})$$

characterize the degradation of the molecular ion with m/z = 372.2 u as well as the production of the first degradation product with m/z = 358.2 u. The BB3 powder that had been purchased already contained traces of the degradation products M^+ = 358.2 u and M^+ = 344.2 u, in addition to the pure dye M^+ = 372.2 u. The definition above was also used to follow aging of BB3 from ballpoint pen entries on paper. It has to be noted that some inks are composed of additional dyes or pigments, whose signals may interfere in the spectra.

Degradation of Pure BB3 upon Light Exposure in Water and in Ethanol

For this experiment, aqueous and ethanol solutions of BB3 (0.01 mg/mL) were exposed to light in spectroscopic quartz cells. Every hour a UV/VIS-spectrum was recorded and a 0.5 μL aliquot was taken for MALDI-MS analysis. Degradation occurred noticeably differently in both solvents.

In water, both a decrease and a shift of the absorption maximum, corresponding to the N-demethylation degradation products

mentioned above were observed by UV/VIS (Fig. 3*a*). The disappearance of the BB3 signal and the sequential formation and decay of the degradation products was followed by MALDI analysis (Fig. 3*b*). BB3 shows an absorption maximum at 590 nm in water and a large signal at m/z = 372 in the MALDI mass spectra. The maximum shifted to 580 nm after 12 h exposure to xenon light (explained by the formation of the degradation product with a mass of 358 g/mol), then to 571 nm after 19 h (explained by the formation of the degradation product with a mass of m/z = 344 u), to 566 nm after 26 h (explained by the formation of the degradation product with a mass of m/z = 330 u), to 556 nm after 34 h (explained by the formation of the degradation product with a mass of m/z = 316 u), to 548 nm after 38 h (explained by the formation of the degradation product with a mass of m/z = 302 u) and finally to 540 nm after 42 h (explained by the formation of the degradation product with a mass of m/z = 288 u). The strong absorption features and the MS signals disappear after about 54 h from the spectra.

Quite differently, no shift of the absorption maximum in the visible range was observed for the ethanolic solution of BB3. This maximum shows in this case a wavelength slightly different from that observed for absorption in water at 588 nm (see Fig. 4*a*). This

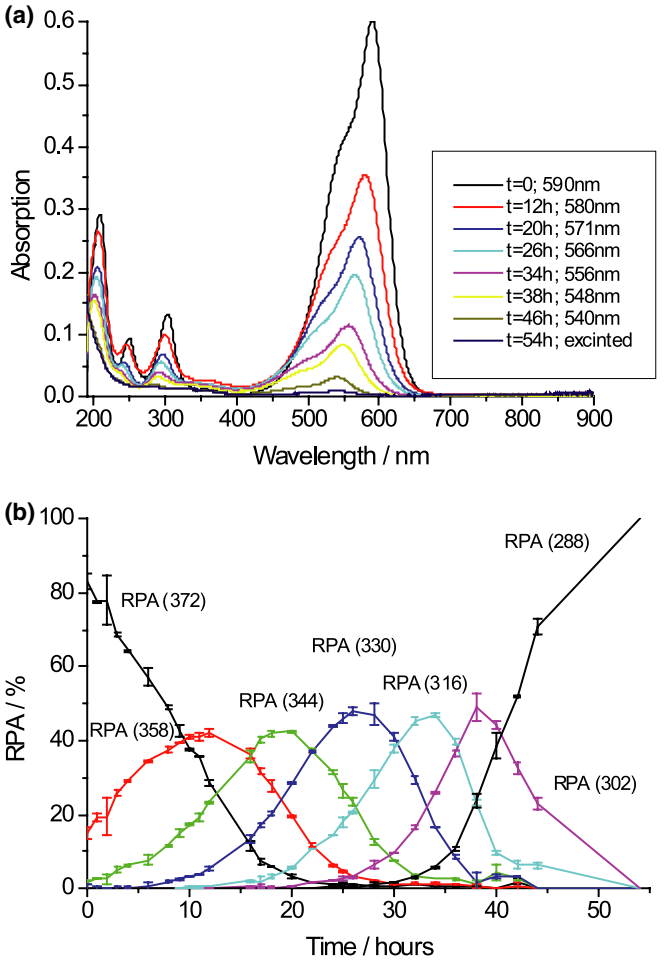

FIG. 3—*Exposure of a 0.009 mg/mL aqueous solution of BB3 to xenon light (a) UV/VIS absorption spectra for different illumination times and (b) Relative Peak Area (RPA) values calculated from the MALDI mass spectra as a function of time, for different ionic species. RPA values characterize the degradation of the dye and the production of the degradation products. The legend in the box (a) lists the wavelength of maximum absorbance at measurement times.*

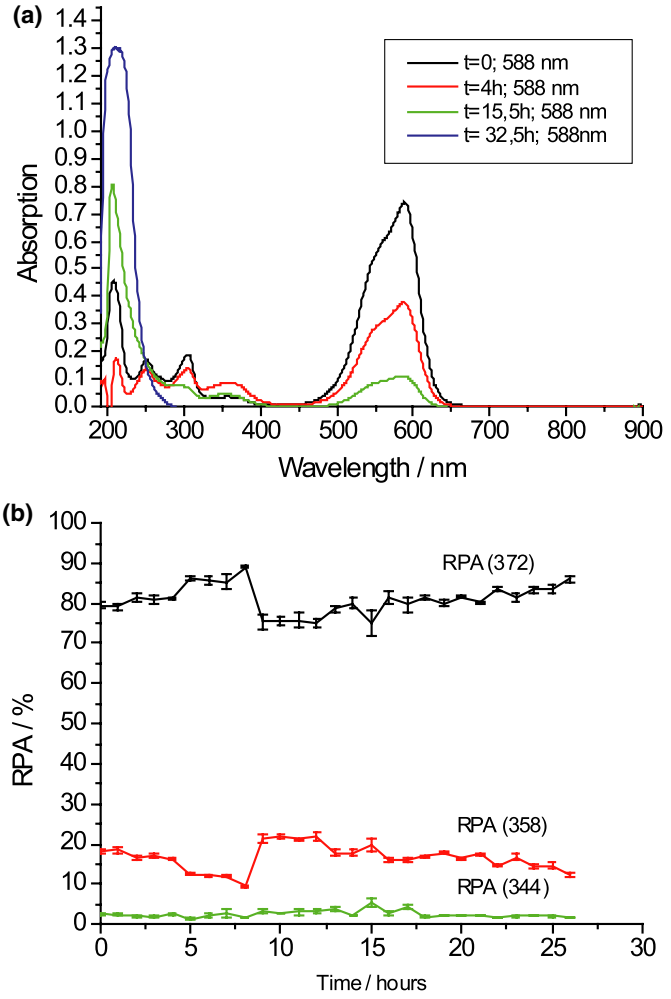

FIG. 4—*Exposure of an 0.013 mg/mL ethanol solution of BB3 to xenon light (a) UV/VIS absorption spectra after various illumination times and (b) Relative Peak Area (RPA) values calculated from the MALDI mass spectra as a function of time. RPA values confirm that no N-demethylation took place. The legend in the box (a) lists the wavelength of maximum absorbance at measurement times.*

situation indicates different interactions of the substance with the solvents, which probably results in a slightly different electronic configuration, and thus absorption profile. The absorption decreases upon illumination and a semi-reversible change (also called phototropy) is detected in the UV range, absorption at 202 nm increases during exposure to light and decreases slightly again after storage in the absence of light for some time. MALDI mass spectra show no additional formation of the degradation products (Fig. 4*b*), thus demonstrating that degradation pathways in ethanolic solution do not include the N-demethylation of the dye. Fading in ethanol was found to be completed after about 32 h.

As the ethanolic solution considerably evaporated during the illumination, a second experiment with volume adjustment was carried out to determine the kinetics of the experiment. About 0.001 mg/mL aqueous and ethanolic solutions of BB3 were exposed to light over 9 h and absorption measurements were carried out every hour. The decrease of absorption, as observed for the absorption maximum of the dye mixture (BB3 and its degradation products), was plotted against time in order to determine the reaction velocity constant. For this purpose the extinction coefficient of the dye was experimentally obtained by measuring the absorption at six different concentrations and using Beer's law:

$$A = \log_{10}\frac{I_o}{I} = \varepsilon \cdot l \cdot c \quad (2)$$

where I_o and I are the intensities of the incident and transmitted light respectively, l is the path length of the absorbing solution (cm), and c is the concentration (mol/L). The value $\log_{10} I_o/I$ is called the absorbance A and ε is the molar extinction coefficient (L/mol/cm). From the experimental data, linear regression fits resulted in the following two expressions (R^2 is the correlation coefficient):

$$\text{Dye in water: } A = 23079\,\text{L/mol/cm} \cdot l \cdot c \quad R^2 = 0,9894 \quad (3)$$

$$\text{Dye in ethanol: } A = 20446\,\text{L/mol/cm} \cdot l \cdot c \quad R^2 = 0,9965 \quad (4)$$

The decrease in absorption for the dye mixture (total amount of the dye in all its cationic triphenylmethane forms) corresponds to a simple exponential process and thus, the reaction can be considered to be of first order. For this reason only the decrease of the absorption maximum was taken into account to determine the chemical kinetics (17). Figure 5 represents the negative logarithm of the concentration as a function of time:

$$c = c_0\,\mathrm{e}^{-kt} \quad (5)$$

$$-\ln(c) = -\ln(c_0) + kt \quad (6)$$

The slopes give the velocity constant k (s^{-1}) of the degradation of the dye mixture in both solutions (21):

Aqueous solution:

$$-\ln(c) = 13.2026 + 2.1708 \times 10^{-5} s^{-1} \cdot t$$

$$R^2 = 0,9969$$

$$k_{aqu} = 2.17 \times 10^{-5}\ s^{-1}$$

Ethanol solution:

$$-\ln(c) = 12.4546 + 4.6358 \times 10^{-5} s^{-1} \cdot t$$

$$R^2 = 0,9935$$

$$k_{eth} = 4.64 \times 10^{-5}\ s^{-1}$$

Accordingly, the fading reaction of the dye mixture upon exposure to xenon light runs 2.14 times faster in ethanol than in water.

The results show that significantly different pathways and kinetics are in concurrence for the degradation of the tryarylmethane dye mixture in aqueous and ethanolic solution.

The signals peak area in the MALDI mass spectra decreased systematically with the exposure to light and no specific peaks could be identified after the dye completely degraded.

Preliminary electrospray (ESI) MS and MS/MS data obtained from an ion trap instrument demonstrated a great potential for the analysis of degradation pathways of dyes. A large variety of oxidation and degradation products could be detected after illumination of dye solvents. A systematic study of these products including solvent effects will be performed in a future investigation. Crystal violet interacts extensively with the solvents, and therefore, it is also used as a pH indicator for basic and acidic changes. Brezová et al. (15) showed that an aqueous solution of the photoexcited triarylmethane dyes in contact with air produces singlet oxygen and ·OH radicals, while in the ethanol solution the production of $\cdot OC_2H_2$ and $\cdot O_2H$ radicals was demonstrated. For this reason, the N-demethylation in water is probably linked to the hydroxyl radicals, which attack the amino groups. In ethanol, the produced radicals should give way to a photooxidative electron/proton transfer mechanism. The role of singlet oxygen in these processes has been proven, but its production in ethanol or water is not demonstrated, albeit nor excluded (15).

Degradation of BB3 in Ballpoint Ink Entries upon Light Exposure on Paper

For samples on paper, comparison of the data obtained in the LDI mass spectra shows that the initial RPA values differ from one pen to another (Fig. 6). At time $t = 0$, RPA_{372} of BB3 from the ballpoint pens BIC1a, BIC1b, Herlitz, and Parker are about 92%, 62%, 69%, and 79%, respectively. This means that the initial concentration of the pentamethylated form (m/z = 358 u) of the dye differed for these four inks. No degradation upon storage in the dark was measured for BIC1a after 3 years and for BIC1b and Parker after 2 years; however the BB3 from Herlitz pen entries show a slight degradation in absence of light, as the RPA decreased to 55% after about 2 years.

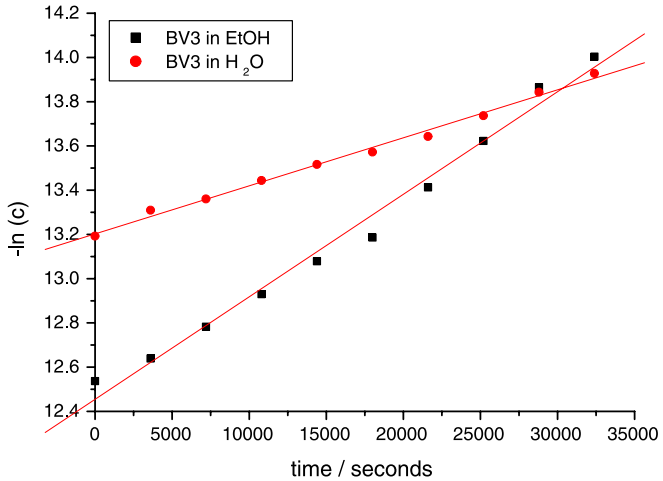

FIG. 5—*Representation of negative logarithm of the concentration of BB3 in water and ethanol as a function of time in seconds. The slopes are obtained from the linear regression equations, providing the velocity constant* k *[s^{-1}] of the pseudo 1st order reaction. Degradation goes 2.14 times faster in ethanol than in water.*

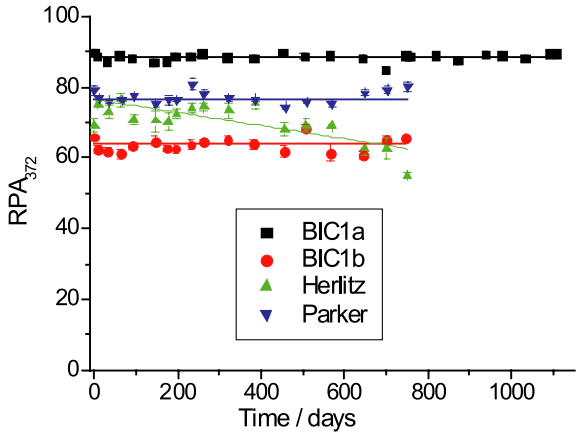

FIG. 6—*The relative peak areas (RPA_{372}) for mass m/z = 372 u as a function of the time in days allow to compare the degradation of crystal violet from four ballpoint pens on paper upon storage in the dark. Measurements have been carried out during 3 years for the BIC1a (no degradation) and during 2 years for the BIC1b (no degradation), Herlitz (slight degradation), and Parker (no degradation).*

The degradation of these different inks on paper was also studied upon exposure to day light through a window. By LDI-MS, the N-demethylation reactions were detected for each of the four ballpoint pens used. Their kinetics, however, differed widely (Fig. 7).

The appearance and disappearance of degradation products in these spectra were not very distinct, unlike the situation seen for the degradation of BB3 in water (Fig. 3). These products were generated simultaneously, and their RPA values did not reach levels above 30%. The time required for BB3 degradation under identical storage conditions was found to be between 95 and 148 days for Herlitz, between 260 and 320 days for BIC1b, beyond 752 days for Parker, and between 977 and 1032 days for BIC1a. The degradation rates differed quite significantly between the inks. The aging curves shown were best fitted to an exponential function of the form of

$$\mathrm{RPA}_{372} = y_0 + A \cdot \mathrm{e}^{\left(\frac{-t}{\tau}\right)} \quad (7)$$

where t is the time and y_0, A, τ are constants (see Table 1). However, the actual decays do not exactly fit the exponential function, because the degradation was not a regular process. For example, it occurred very differently on a sunny day compared to a cloudy day.

The half-life period of the dye BB3 (symbolized $t_{1/2}$) is defined as the lapse of time necessary for the decay of half of the dye initially present. From Eq. (7), given that RPA = RPA_0 and $t = t_{1/2}$, then:

$$t_{1/2} = 1.44 \cdot \tau \quad (8)$$

The calculated half-life periods in decreasing order are then: $t_{1/2}$ (Parker) *c.* 27.2 days, $t_{1/2}$ (BIC1a) *c.* 21.2 days, $t_{1/2}$ (BIC1b) *c.* 20.8 days, and $t_{1/2}$ (Herlitz) *c.* 5.9 days. The LDI mass spectra of the inks from ballpoint pen BIC1b and Herlitz contained signals of BB3 only, while the BIC1a spectra also contained a signal at m/z = 456 (ethyl violet), and the Parker spectra had an additional signal at m/z = 470 (solvent blue 26). The two triarylmethane dyes have maximum absorption peaks very close to that of BB3, at 596 nm for ethyl violet in water and at 599 nm for solvent blue 26 in ethanol. These two substances also absorb light in the UV range. For these reasons they competed with BB3 in the absorption of light and quenched the degradation of BB3. Their degradation through the loss of CH_2 groups was also detected by LDI-MS. Moreover, blue pigments were detected by HPTLC in BIC1a, BIC1b, and Parker ink entries (22). This could clarify why BB3 degradation in BIC1b ink entries was not any stronger, since pigments can also act as degradation quenching substances.

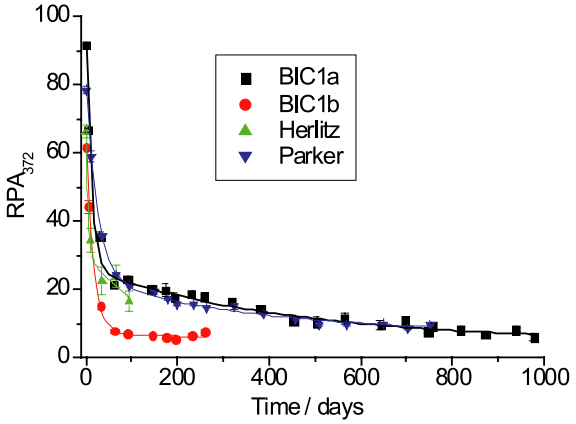

FIG. 7—*Relative peak areas (RPA_{372}) plotted as functions of time provide a basis for comparison of the degradation of crystal violet from four ballpoint pens on paper during exposure to daylight. Measurements were carried out over a period of 3 years for BIC1a (degradation g completed after 977 to 1032 days), and over a period of 2 years for BIC1b (degradation completed after 260 to 320 days), for Herlitz (degradation completed after 95 to 148 days), and for Parker (degradation not completed after 752 days). The curves were best fitted with an exponential function.*

TABLE 1—*Exponential fits of the aging curves of four blue ballpoint inks.*

	BIC1a	BIC1b	Herlitz	Parker
y_0 (%)	89.55	60.92	66.23	75.84
τ (days)	14.71	14.45	4.12	18.86
R^2	0.99	1.00	0.98	0.99

Ink Thickness—The Parker ballpoint pen slightly smeared during writing, leaving ink blots on the ink entries that were thicker than the entries themselves. An analysis by LDI-MS after 2 years of exposure to daylight showed that aging was not as advanced for ink in these blots as in the rest of the entries (Fig. 8).

The RPA_{372} in the ink entry had a value of 9%, while the same values in the two blots were 34 and 44% (Fig. 9). Thin ink entries usually reached such values after 11 to 36 days. These results indicate that degradation depends on the local dye concentration (thickness of the ink entry on paper) or the protective interaction with aromatic resin compounds. Degradation occurred faster in traces having a smaller local dye concentration.

Conclusion

With the help of mass spectrometric and spectroscopic methods, it was possible to gain a better understanding of the degradation of the dye crystal violet for forensic purposes. With the aid of MALDI-MS and UV/VIS spectrophotometry, we demonstrated that the degradation pathways of the dye under exposure to xenon light are significantly different in water and ethanol. The proposed N-demethylation mechanism happened only in aqueous solution. As demonstrated by Brezová et al. (15), reaction of the dyes with the paper substrates upon exposure to light and oxygen yield the

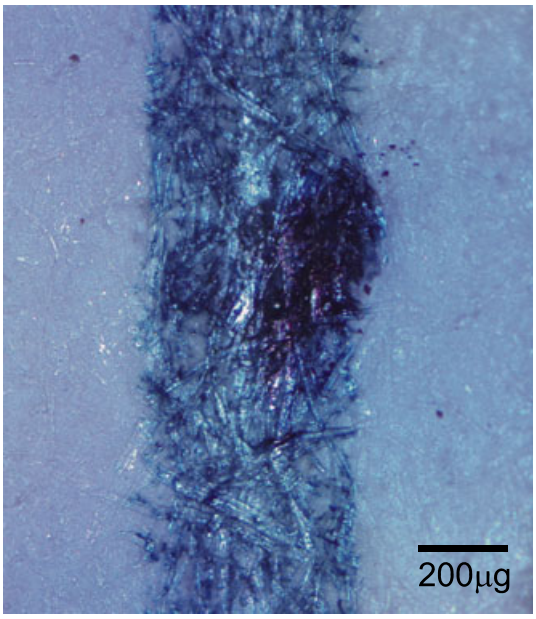

FIG. 8—*Photograph of a Parker ballpoint entry 2 years old: a small blot produced during writing is thicker than the rest of the entry.*

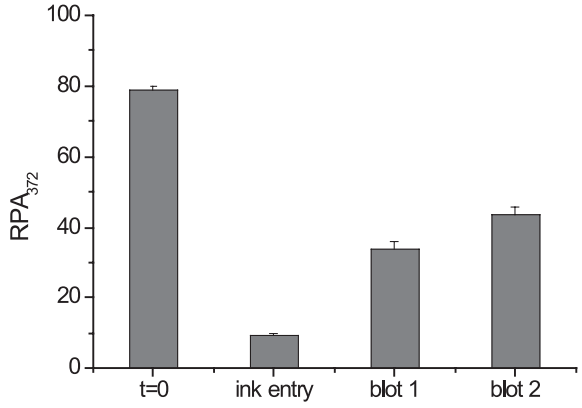

FIG. 9—*The Parker ballpoint pen during writing produced small blots that were thicker than the ink entries. Their analyses by LDI-MS after a time of about 2 years produced higher RPA_{372} values in the blots than in the rest of the entry, demonstrating the influence of the local amount (or concentration) of the dye on degradation during exposure to light.*

same kind of reaction and induce dye and paper degradation. Therefore the paper influence should be thoroughly studied in future works. Studies of the degradation kinetics showed that degradation occurred about two times faster in ethanol than in water solutions.

A second reported set of experiments addressed the degradation process under natural irradiation of ink on paper over 2–3 years using entries from four different pens. LDI mass spectrometry from these samples allowed demonstrating the influence of the ink composition on the degradation process. Two of the ballpoint pens contained additional dyes, whose spectral absorption competed with that of crystal violet, thus quenching the degradation. An effect of the total local concentration of the ink on an entry was also observed: after 2 years' exposure to daylight, several thick ink blots showed a significantly weaker degradation than the rest of the entry. Finally, all pens showed a high stability in the absence of light. Only one of them showed a slight degradation after 2 years' hold in the darkness.

These reported results have a significant bearing in the forensic dating of questioned documents, because they implicate that scientists should exercise extreme carefulness when attempting to date ink by measuring dye degradation, and that peculiarities such as storage conditions, ink composition, and thickness of entries must be taken into account before a legal statement is produced. In fact the type of paper certainly has a non-negligible influence on aging and more experiments are needed to fully understand the role of that factor on the degradation of ballpoint dyes. Very encouraging preliminary tests indicate the great potential of ESI-MS/MS for the study of dye degradation products and their chemical structures. This will be the subject of a following publication.

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