Crystal violet: study of the photo-fading of an early synthetic dye in aqueous solution and on paper with HPLC-PDA, LC-MS and FORS

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Abstract. The photo-fading of crystal violet (CV), one of the earliest synthetic dyes and an ink component, is examined both in solution and on paper. Aqueous solutions of CV were exposed to UV light (365nm) and samples were taken at constant time intervals and analysed with a High Performance Liquid Chromatography-Photo Diode Array (HPLC-PDA) and Liquid Chromatography-Mass Spectroscopy (LC-MS). Demethylation products were positively identified. Also, deamination probably occurred. The oxidation at the central carbon likely generates Michler's ketone (MK) or its derivatives, but still needs confirmation. To study CV on paper, Whatman paper was immersed in CV and exposed to UV light. Before and after different irradiation periods, reflectance spectra were recorded with Fibre Optic Reflectance Spectrophotometry (FORS). A decrease in CV concentration and a change in aggregation type for CV molecules upon irradiation was observed. Colorimetric L*a*b* values before and during irradiation were also measured. Also, CV was extracted from paper before and after different irradiation periods and analysed with HPLC-PDA. Photo-fading of CV on paper produced the same products as in solution, at least within the first 100 hours of irradiation. Finally, a photo-fading of CV in the presence of MK on Whatman paper was performed. It was demonstrated that MK both accelerates CV degradation and is consumed during the reaction. The degradation pathway identified in this work is suitable for explaining the photo/fading of other dyes belonging to the triarylmethane group.

1. Introduction

Since the XIX century, synthetic dyes have been used as colouring matter in many inks. Triarylmethane dyes, part of a group trivially named aniline dyes, were among the first ones to be produced and marketed, Mauvein being the first synthetic aniline dye to be synthesized in 1856 by William Perkin. Due to their high colouring power and relatively inexpensive production methods, these aniline dyes initially gained great popularity, but soon their bad light fastness properties became apparent. In particular, Crystal violet (CV) or hexamethyl pararosaniline (figure 1A) with its deep purple hue has had and still has a widespread diffusion for dyeing textiles and paper, as an ingredient of writing and drawing inks and, as a pigment, consisting of the copper ferrocyanide lake, in printing

inks [1]. Methyl Violet (MV), which consists of a mixture of tetra-, penta- and hexa- methylated pararosaniline was invented in 1861 and introduced to the market in 1866. An efficient synthetic route to CV was only developed in 1883.
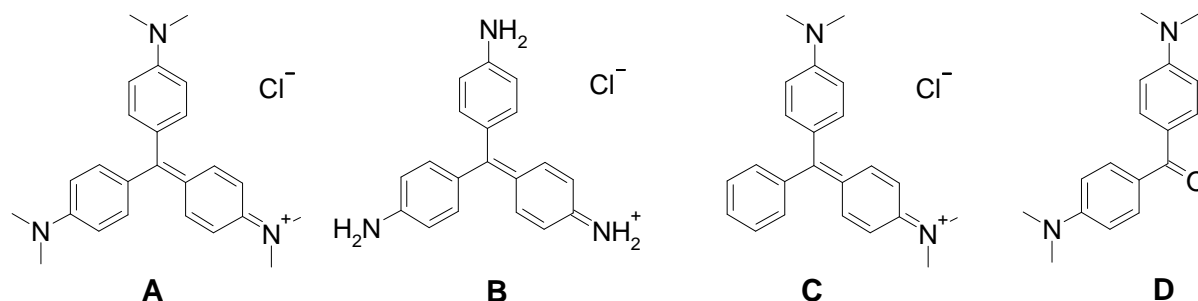

Figure 1: Structures of Crystal Violet or hexamethyl pararosaniline (A), pararosaniline (B), diamond green (or basic green 4) (C) and Michler's ketone (D).

Since then, discoloration has shown to be a crucial issue. Indeed, ink fading, leading to chromatic alterations of documents or drawings or even to a complete loss of information, was soon observed.

In particular, among Van Gogh's signed works there is a group of drawings and letters produced in 1888 in Arles and all made with a purple ink which was demonstrated to contain methyl violet, PR and other methylated derivatives whose structures are not known yet. Interestingly, a seemingly brownish drawing from the same group, entitled Montmajour (Arles, 1888), shows purple shades on the edges where the ink has been protected from light by the frame [3] (figure 2).

Figure 2: On the left: detail of Montmajour drawing (Van Gogh, Arles, 1888). On the right: enlarged view showing different purple shades (photo reproduced with permission by Van Gogh Museum -Amsterdam).

Consequently, the question of whether other drawings and letters by Van Gogh which are now brown-coloured were originally purple, needs to be answered.

Therefore the knowledge of synthetic dyes degradation mechanisms and the possible identification of original dyes from their degradation products in historical samples are gaining more and more importance in the field of cultural heritage study and conservation.

CV shows poor light fastness, in particular on paper and on natural fibers such as cotton, silk and wool. Moreover these substrates are not inert to light, and the reactivity of the dye-substrate system is complicated by the degradation of the substrate when exposed to light. For this reason, many studies have dealt with the reactivity of the dye in solution (aqueous solution or solutions mimicking the substrates) in an attempt to simplify the complex reactivity of the dye-substrate system. Of course, when dealing with a real object such as a drawing or a print, it is not possible to neglect the substrate reactivity [4] and a study of model systems for the dye on paper is necessary [5].

The objective of this work is to study the photo-fading of CV via HPLC-PDA, LC-MS and FORS both in aqueous solution and on paper in order to use the information obtained from the former system to best interpret the results of the study of the degradation of the dye on paper. The final goals are:

- to determine the composition of the purple ink used by Van Gogh;
- to shed light on the discoloration mechanism of purple drawings and letters by Van Gogh and contemporaries;
- to obtain information on the original colour of discoloured inks on paper.

2. Experimental

2.1. Sample preparation

Two aqueous solutions of CV (Acros) 5×10^{-5} M were prepared using ultrapure water (Simplicity system Millipore). Afterwards, both solutions were exposed to UV light (365 nm) from a Spectroline® Super High Intensity black light lamp (SB-100/F Spectronics Corporation, USA) for at least 100 hours and samples were periodically taken.

CV was applied to Whatman paper discs (Cat No 1003 125) by soaking them for 10 minutes in an aqueous solution of CV (5×10^{-4}M) or in an ethanol solution of CV (5×10^{-4}M) and MK (1×10^{-3}M). Afterwards, the discs were left to dry horizontally.

Samples (1x0.5 cm strips of paper) were analyzed before and after different UV irradiation intervals. The dye was extracted from each sample with 200 μl methanol (Fluka, for HPLC) at 70 °C for 10 minutes. Samples of un-dyed paper were treated in the same way and used as a reference.

Pararosaniline (Acros) was used as received and Diamond Green B was obtained from the Dyes Reference Collection of ICN.

2.2. HPLC-PDA analysis

HPLC analysis was performed with equipment from Waters Chromatography BV (Etten-Leur, The Netherlands). Mobile phase was delivered at a flow rate of 0.2 mL/min by a 616 LC pump, controlled by a 600S controller. An in-line degasser degassed all solvents used. Samples were injected by a 717 autosampler. Detection was performed with a 996 Photo Diode Array (PDA) detector equipped with a 10 μL detector cell. The equipment was controlled by a computer with Empower software from Waters Chromatography BV; the same system was used for data acquisition. Separation was performed on a Luna C18 (2) column (150mm x 2mm id) protected by a security C18 guard column, both supplied by Phenomenex (Torrance, CA, USA). Mobile phase consists of a gradient of water (purified by a Simplicity system Millipore, Amsterdam, The Netherlands), methanol (Fluka, Zwijndrecht, The Netherlands) and 5% phosphoric acid in water (ACS reagent, Sigma, Zwijndrecht, The Netherlands). The composition of the solvents and the gradient profile is given in Table 1. Identification is based on both PDA spectra and retention time of the components which are compared with those of known reference materials.

Table 1. Gradient profile for the HPLC system.

Time/min	%A[1]	%B[2]	%C[3]
0	74	16	10
15	0	90	10
20	0	90	10
23	0	100	0
27	0	100	0
30	74	16	10
50	74	16	10

[1] A=10% methanol/water (v/v)
[2] B=100% methanol
[3] C=5% phosphoric acid (w/v)

2.3. LC-MS analysis

LC-MS analysis was performed with equipment from Thermo Electronic corporation. Mobile phase was delivered at a flow rate of 1 mL/min by a Finnigan surveyor LC Pump plus. Samples were injected by a Finnigan surveyor Autosampler plus. Detection was performed with a Finnigan surveyor PDA Plus detector. Separation was performed on a Intersil ODS-3 column (4.6 × 50mm). The mobile phase consists of a gradient of water, formic acid and acetonitrile. Mass analysis was performed with the use of a Finnigan LXQ Ion Trap apparatus.

2.4. FORS analysis

FORS analysis was performed with a home-assembled equipment composed of a halogen lamp (AvaLight-Hal, Avantes), a reflection integrating sphere (ISP-50-8-REFL, Avantes) and a spectrophotometer (AvaSpec-2048, Avantes). The equipment was controlled by AvaSoft 6.1 software. Reflectance spectra were collected in the range 380-800 nm, with an integration time of 200 ms and averaging 5 scans. Absorption spectra were calculated from reflectance spectra using Kubelka-Munk transform. Reflectance spectra for CV were recorded before and after exposure to UV light of a dyed disc of paper. Colorimetric L*a*b* values were calculated from reflectance spectra for a D65 illuminant and a 10° Standard Observer.

3. Results and discussion

3.1. CV in aqueous solutions

3.1.1 *HPLC-PDA and LC-MS analysis.* A colour shift from purple to reddish was observed upon irradiation of an aqueous solution of CV. The HPLC chromatograms for CV in aqueous solution before and after prolonged irradiation (115 hours) are shown in figure 3.

Before irradiation only two peaks are visible at around 21.1 min (relative area >98%) and 20.7 min (relative area <1%), due to CV and mono-demethylated CV respectively (as it will be demonstrated later).
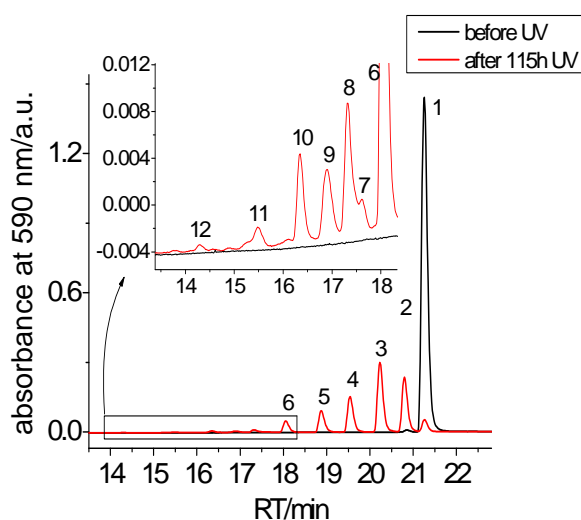

Figure 3: Chromatograms of CV(aq), before and after prolonged irradiation.
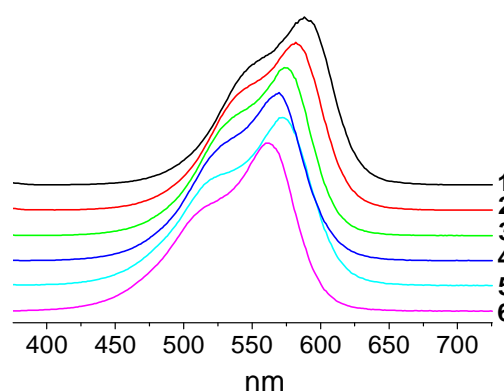
Figure 4: Normalized absorption spectra for peaks 1 to 6 in the chromatogram of irradiated CV(aq) .

Upon irradiation, a decrease of the CV concentration and the formation of at least 11 degradation products were observed.

Peaks 2, 3, 4 and 5 were identified by LC-MS analysis as mono-, bis-, tri-, tetra- demethylated CV. Incidentally, mono-demethylated CV is penta-methylated pararosaniline, bis-demethylated CV is tetra-methylated pararosaniline, tri-demethylated CV is tri-methylated pararosaniline and tetra-demethylated CV is bis-methylated pararosaniline. Absorption spectra for CV and for HPLC peaks 2,3,4,5 are shown in figure 4 together with the spectrum of peak 6 that is believed to be penta-demethylated CV. Demethylation causes a blue shift in the absorption spectra (with the exception of peak 5) and a decrease in retention time, the latter effect being probably due to an increased polarity of the molecules formed. LC-MS analysis revealed the presence of two isomers both for bis- and tri-demethylated CV.

A comparison between the absorption spectra of pararosaniline (RT=14.07 min; λmax=543nm) and of peak 12 in the chromatogram of CV after 115 hours of irradiation (figure 5) enabled to positively identify peak 12 as fully-demethylated CV (pararosaniline). The absorption spectrum of HPLC peak 10 looks very similar to that of pararosaniline, although its maximum has shifted 11 nm to the red (figure 5).

The absorption spectra of peaks 7, 9 and 11 might be due to the products of deamination reaction, such as diamond green which was detected in faded samples of CV [6]. Peaks 7, 9 and 11 have very similar absorption spectra (figure 6), the main difference being a blue shift in the absorption maximum in going from peak 7 to 11 as it was observed for the different products of CV demethylation. Moreover, absorption spectrum for peak 7 is similar to that of diamond green B (figure 1C) although there is no retention time matching. All this suggests the possible formation of various demethylated diamond green-like compounds during CV degradation.
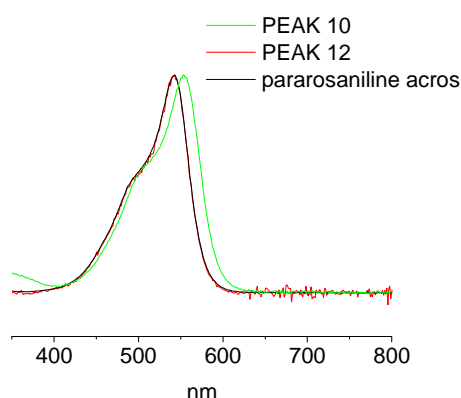

Figure 5: Normalized absorption spectra for pararosaniline and for peaks 10 and 12 in the chromatogram of CV(aq) after irradiation.
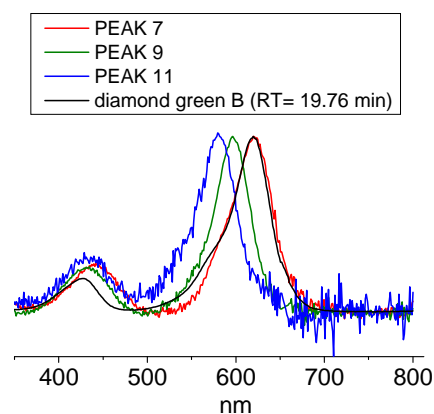
Figure 6: Normalized absorption spectra for peaks 7, 9 and 11 in the chromatogram for irradiated CV(aq) and for diamond green B.

Another hypothesis for the identification of peaks 7, 9 and 11 is reported by Duxbury [7]: in this work it was reported that the absorption spectra of N-oxide derivatives of CV were very similar to the spectrum of diamond green. Moreover, the higher polarity for N-oxides would explain the lower retention times obtained for peaks 7, 9 and 11 with respect to diamond green.

Finally, the absorption spectrum of peak 8 (not shown) has not been identified yet but it is clearly due to the superimposition of at least two spectra as is suggested by the change in shape of the spectrum during irradiation. Table 2 summarizes all positive and possible attributions for HPLC peaks detected after irradiation of an aqueous solution of CV (chromatograms in figure 3).

Table 2: positive and possible attributions for the HPLC peaks detected after irradiation of an aqueous solution of CV.

Peak Number	RT/min	λ_{max}/nm	Positive Attribution	Possible Attribution
1	21.26	588	CV	
2	20.79	582	Mono-demethylated CV	
3	20.23	573	Bis-demethylated CV	
4	19.54	570	Tri-demethylated CV	
5	18.87	572	Tetra-demethylated CV	
6	18.05	562		Penta-demethylated CV
7	17.61	620		Diamond green-like compound or N-oxide derivative of CV
8	17.32	---	superimposition of two spectra	
9	16.89	597		Diamond green-like compound or N-oxide derivative of CV
10	16.34	554		Pararosaniline-like compound
11	15.48	579		Diamond green-like compound or N-oxide derivative of CV
12	14.29	543	pararosaniline	

3.2. CV on paper

3.2.1 *HPLC-PDA analysis.* Irradiation caused an evident fading of the colour of dyed paper. A quantification for the colour change, in terms of colorimetric L*a*b* values, is given later.

Chromatograms for CV extracted from paper before and after 340 hours of irradiation are shown in figure 7. Peak numbering is consistent with the one in figure 3. After 340 hours of irradiation a significant decrease in CV concentration was observed and other peaks had increased in intensity, showing that degradation products were being formed.
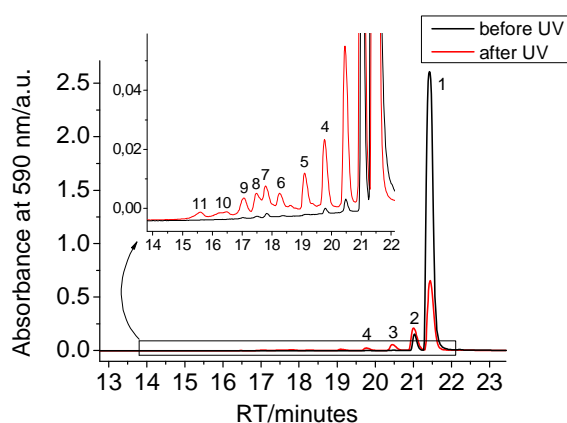

Figure 7: Chromatograms of CV extracted from paper before and after 340 hours of irradiation.
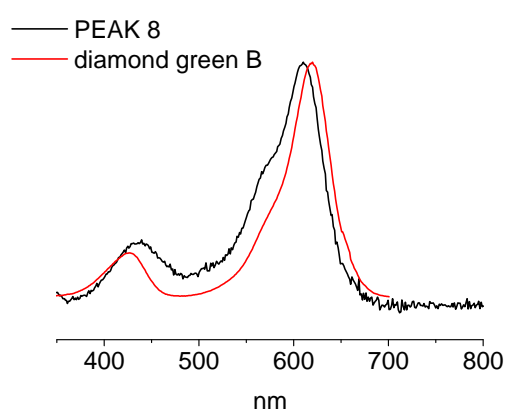

Figure 8: Normalized absorption spectra for peak 8 and diamond green B in the chromatogram of CV extracted from paper after 340 hours of irradiation.

The chromatogram of the extract obtained after irradiation of CV on paper was compared to that obtained after irradiation of CV in aqueous solution.

Except for the peaks having retention times between 14 and 15 minutes, the same peaks were obtained whether CV was irradiated in solution or applied to paper, indicating that the same degradation products are formed. With the only exception of peak 8, a comparison of absorption spectra for all the peaks confirms the analogy.

The absorption spectrum belonging to peak 8 (for CV on paper after irradiation) is similar to that of for diamond green B, but it has a shoulder. In figure 8 the absorption spectra belonging to peak 8 (RT=17.65 min) and diamond green (RT=19.76 min) are shown. In this case peak 8 is not due to the superposition of two different species as instead was the case in aqueous solution. Based on the retention times measured, the species responsible for peak 8 in figure 7 should be more polar than diamond green. However, due to the similarity of absorption spectra, they could be diamond green-like compounds: the higher polarity could be possibly due to a higher degree of demethylation of the species responsible for peak 8. The blue shift in the absorption maximum is consistent with this explanation.

To summarize, apart from peak 8, the same considerations made for CV in solution about peak 1 to 11 apply also for CV on paper (table 2).

Among the possible intermediates in the synthesis of CV and methyl violet there is Michler's ketone (figure 1D), which is of particular interest [8]. As a reagent in the synthesis of the dye, impurities of Michler's ketone could be present in the ink from the beginning. Moreover, Michler's ketone is believed to be a degradation product of CV and methyl violet and therefore it could also be formed on paper. Michler's ketone can accelerate CV degradation and for this reason the effect of this ketone on CV degradation has been investigated in our work. In the presence of Michler's ketone the consumption of CV during irradiation was higher. Moreover, MK is not a catalyst but is consumed during the photo-fading of CV. This is therefore a possible explanation for the absence of MK in the chromatograms for faded CV on model paper samples.

3.2.2. *FORS analysis.* A substantial discoloration was observed for CV on paper during irradiation as can be seen from the colorimetric L*a*b* values reported in table 3.

Table 3: L*, a*, b* values for CV on paper at different irradiation times.

	Before UV	16 h UV	33 h UV	79 h UV	102 h UV	126 h UV	149 h UV	176 h UV	200 h UV
L*	52,96	53,85	56,58	62,38	64,67	66,77	70,13	70,84	71,60
a*	21,08	19,99	13,53	8,98	6,55	6,09	4,50	3,10	3,03
b*	-62,10	-42,64	-37,98	-31,03	-29,08	-29,00	-25,65	-23,96	-23,30

The a* component decreased whereas b* and L* components increased upon irradiation giving a duller and lighter colour. Absorption spectra for CV on paper before and after irradiation are shown in figure 9. Upon irradiation both a decrease in absorption intensity and a change in spectral features were observed.
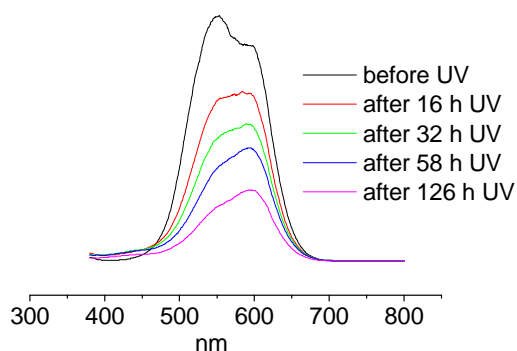

Figure 9: Absorption spectra for CV on paper before and after irradiation.

Before irradiation the spectrum shows an absorption maximum at around 550 nm and a pronounced shoulder at around 600 nm. Upon irradiation there is an increase of relative intensity of the band at 600 nm. This behaviour has already been observed by many authors for CV in solution [7,9,10] and it has been explained as an effect of the aggregation of dye molecules, the absorptions around 590 nm (α-band) and around 550 nm (β-band) being attributed to single monomers and dimers of CV respectively. At higher dye concentrations larger aggregates are formed, causing a shift in the absorption maximum toward shorter wavelengths. FORS therefore enabled both to measure the decrease in CV concentration on paper upon irradiation and to evaluate the aggregation type for the dye. The latter, besides influencing spectral features, plays a role also in determining the photochemical behaviour of dyes and for this reason the possibility of determining the aggregation type for CV on solid substrates with FORS renders this technique very interesting from a conservation point of view.

3.2.3. *HPLC analysis of 'Montmajour' and reliability of model samples.* Based on a previous HPLC analysis, the purple dye derived from the drawing 'Montmajour', made by Van Gogh (figure 2) consists of a mixture of methyl violet and fuchsine. Such a mixture can be explained either as the result of CV degradation on paper or as the result of the use of an ink already containing the mentioned dyes. To this regard, historically accurate reconstructions would be needed in order to verify these two hypotheses.

Finally, the result of HPLC analysis of a model sample of CV faded on paper was compared to the result of HPLC analysis of the sample coming from the drawing 'Montmajour' (figure 10). Interestingly, peak positions were very reproducible for the historical sample and the model sample (except for the RT range 22-25 minutes) giving evidence of the reliability of the model samples used.
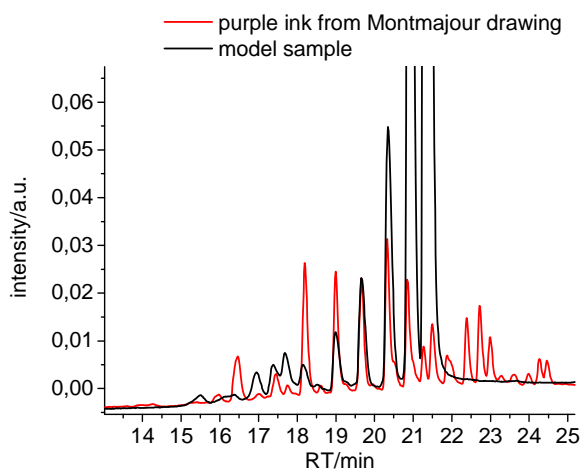

Figure 10: chromatograms for a model sample of CV faded on paper and for a sample of purple ink coming from the drawing 'Montmajour'.

4. Conclusion

The photo-induced degradation of CV was studied both in solution and on solid substrate via HPLC-PDA, LC-MS and FORS.

UV irradiation of CV in water caused a colour shift from purple to red. HPLC-PDA combined with LC-MS enabled to detect degradation products for CV in water. A series of demethylation products, from CV to pararosaniline (fully-demethylated CV) were detected. Moreover, diamond green-like spectra were observed which could be due to demethylation products of diamond green or N-oxides derivatives of CV.

With the applied experimental conditions, degradation products detected for CV on paper were the same as for CV in solution.

The photo-fading of CV in the presence of MK confirmed that the latter accelerates CV degradation and showed how MK is consumed during photo-fading.

Reflectance spectra enabled to express in term of colorimetric L*a*b* values the change in colour of CV on paper. Moreover, the changes in reflectance spectra during irradiation were attributed to changes in CV concentration and in CV aggregation type.

The comparison between the chromatogram for a model sample of CV faded on paper and for a sample of purple dye coming from 'Montmajour' drawing showed a very good consistency between results obtained from artificially aged model samples and those obtained from a naturally aged historical sample. Therefore, the method of ageing and analysis is reliable.

To conclude, the knowledge of the products formed during CV fading and their concentration ratios provides information about the original dye used for preparing the ink and consequently its original colour and its estimated date of production. It is however important to bear in mind that CV was often used in a mixture with various demethylated derivatives and it would be necessary to know the historical ink recipes and the production method of the dye in order not to misinterpret data on composition of historical ink samples.

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