

Analysis of Alizarin Dye in Accelerated Degradation Conditions

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(Received March 12, 2004 : Accepted April 30, 2004)

Abstract

The purpose of this research was to examine the degradation rate of alizarin in accelerated degradation conditions using the GC-MS quantitative analysis. Alizarin dye solution ($2.5 \times 10^{-3}M$ conc.) were kept in 150 °C oven for total of 7 days and the degradation rate was examined each day. $2.5 \times 10^{-4}M$ conc. alizarin dye solution was mixed with H_2O_2 according to $[H_2O_2]/[dye]$ ratio 40 and were kept under 365nm UV for 2 hours, analyzed after 0, 30, 60, 90, 120min using the GC-MS. Gas chromatogram showed alizarin peak at 9.96 - 10.13 min. retention time range and residual peaks in the wide range from 9.6 to 11.1 min. Oven degradation exhibited an initial decrease in the amount of alizarin, which was followed by increasing amount in 4th day. The decrease in the alizarin was significantly shown by the 7th day. Same pattern was also observed in the $H_2O_2/UV/O_2$ degradation samples and was verified by the UV-VIS spectra. The differences in the amount of alizarin between 1st day and 4th day samples, 4th day and 7th day samples, and Control and 7th day samples of the oven degradation were significant at alpha .20.

Key words : alizarin, degradation, GC-MS, quantitative analysis, thermal treatment.

I. Introduction

One of the problems in conservation and documentation of museum textiles excavated from archaeological site is the drastic loss of original color. The fading of exhumed textiles is caused by both long-term interaction with soil physico-chemical conditions and a sudden exposure to sunlight. There have been some research efforts in identifying the dyestuffs used in different museum textiles, but their analyses were highly

dependent upon the visual hue of color which permit the use of limited number of comparative standards.¹⁻³⁾ In case of a textile which completely lack its original color the analysis is much more complicated because it is impossible to select a single or a manageable number of comparative standard dye. Moreover, since the fading mechanism is the result of chemical degradation of the dye molecule itself, the comparison of dye in badly faded archaeological textile to a 'fresh' standard is itself a preposterous task. In order to pursue a valid identification of

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¹ Penelope Walton and George Taylor, "The Characterization of Dyes in Textiles from Archaeological Excavations," *Chromatography and Analysis* Vol. June (1991): 5-7.

² G. Schaefer, " The cultivation of madder," *Ciba Review* Vol. 39 (1941): 1398-1406.

³ Susana M. Halpine, "An Improved Dye and Lake Pigment Analysis Method for High-Performance Liquid Chromatography and Diode-array Detector," *Studies in Conservation* Vol. 41 (1991): 76-94.

the dye in a badly faded archaeological textile, the degradation of the dye used in the textile piece must be considered. Therefore, the experimental procedure must include some type of degradation system which can either simulate the burial context or pursue a reliable chemical degradation. As part of such research this study focused on the degradation behavior of alizarin (1,2-dihydroxyanthraquinone) which is the principal coloring matter of the madder plant. The purpose of this research was to examine the degradation rate of alizarin defined as 'the change in the amount of alizarin in the dye solution' using the GC-MS quantitative analysis. The two experimental conditions for alizarin degradation were accelerated thermal treatment in 150 °C oven and H₂O₂/UV/O₂ degradation system⁴⁾ which is often used in the degradation of dye-house wastewater. The secondary aim of this research was to test the validity of the above laboratory treatments for examining alizarin degradation.

II. Theoretical Framework

According to Salmon and Cass⁵⁾ and Grosjean, Salmon, and Cass⁶⁾ color fading is the result of loss of chromophore in the dye substance which arises from a number of different

oxidation pathways. In their experimental study of fading of alizarin by nitric acid they suggested that oxidation of alizarin produced trihydroxyanthraquinone and quinone as the degradation products. In another study, they found that fading of alizarin by ozone produced phthalic acid or phthalic anhydride as major product, and methyl phthalate as one of the minor products.⁷⁾ Similarly, pigment alizarin, the alizarin crimson, showed phthalic anhydride or phthalic acid and small amount of benzoic acid as its degradation products.⁸⁾ In author's recent research, alizarin was degraded using a series of different thermal conditions and also by H₂O₂/UV/O₂ system often used in the dye wastewater research.⁹⁾ It was found that similar products suggested by Grosjean et al.^{10,11)} were repeatedly detected in both degradation systems and that their percent amounts increased with the degradation progression. Inversely, it is expected that the amount of alizarin will decrease in accordance with the formation and increasing amount of the above degradation products. Thus, it is suggested that the quantitative analysis of alizarin under degradation is another way of examining its the degradation behavior.

Burial induced fading of exhumed textiles has been the subject of limited research due to the complexity in burial microclimate. Among the

⁴ Cheunsoon Ahn and S. Kay Obendorf, "Toward the Study of Dyes in Archaeological Textiles: Analysis of Alizarin and Its Degradation Products," *Textile Research Journal*, in press.

⁵ Lynn G. Salmon and Glen R. Cass, "The Fading of Artists' Colorants by Exposure to Atmospheric Nitric Acid," *Studies in Conservation* Vol. 38 (1993): 73-91.

⁶ Daniel Grosjean, Lynn G. Salmon, and Glen R. Cass, "Fading of Organic Artists' Colorants by Atmospheric Nitric Acid: Reaction Products and Mechanisms," *Environmental Science and Technology* Vol. 26 No. 5 (1992): 952-959.

⁷ Daniel Grosjean, Paul M. Whitmore, C. Pamela De Moor, and Glen R. Cass, "Fading of Alizarin and Related Artists' Pigments by Atmospheric Ozone: Reaction Products and Mechanisms," *Environmental Science and Technology* Vol. 21 No. 7 (1987): 635-643.

⁸ *Ibid.*, 635-643.

⁹ Cheunsoon Ahn and S. Kay Obendorf, "Toward the Study of Dyes in Archaeological Textiles: Analysis of Alizarin and Its Degradation Products," *Textile Research Journal* (in press).

¹⁰ Daniel Grosjean, et al., *Op. cit.*, (1992), 952-959.

¹¹ Daniel Grosjean, et al., *Op. cit.*, (1987), 635-643.

few directly related are Needles et al.¹²⁾ who examined burial induced color and strength change of wool and silk fabric dyed with different natural dyestuffs and mordants, and Peacock¹³⁾ who simulated the waterlogged burial context by burying undyed cotton, linen, silk, and wool in sandy loam and garden peat separately. However, due to the complexities in the burial environment and the uncontrollable soil microclimate, a valid and consistent simulation of burial context is difficult.

Thermal treatment in oven, however, has been used as a successful laboratory treatment for examining the accelerated color change as well as the accelerated polymer degradation. Peacock¹⁴⁾ combined the acid hydrolysis using agents such as deionized water or methyl carbonate and the moist-heat aging of linen at 70°C and 50% RH for 21 days and suggested that combined treatment simulated some of the properties of naturally aged linen. The degraded linen samples lost weight, discolored, and lost tensile strength. Block and Kim,¹⁵⁾ in examining the effect of tetrahydridoborate reduction on the strength and color retention of cellulosic fiber upon ageing, treated cotton fabrics in oven at temperature

ranging from 100°C to 150°C. Needles and Nowak¹⁶⁾ (1989) investigated the ageing behavior of linen dyed with red, violet, or blue type synthetic dyes when it was kept at 180°C forced draft oven for 1-10 hours. They reported that significant loss of tensile strength, and color change toward darkening and yellowing occurred. Based on the previous literature, 150°C oven temperature was selected as one of the experimental condition.

Another way to approach the burial-induced fading of dye is to apply the method used in the biodegradation of dyehouse wastewater. White-rot fungi such as *Phanerochaete chrysosporium* and *Trametes versicolor*¹⁷⁾ were found to be effective degraders and decolorizers of dye wastewater by the production of lignin peroxidase or manganese peroxidase enzymes. The mechanism of microbial decomposition of dye wastewater is reproduced synthetically by the use of Advanced Oxidation Process involving H₂O₂/UV/O₂ system.¹⁸⁾ And the method has been successfully applied in detecting the degradation products of alizarin.¹⁹⁾ In addition to the thermal treatment, this study also utilized the H₂O₂/UV/O₂ to examine the degradation behavior of alizarin.

¹²⁾ H. L. Needles, V. Cassman, and M. J. Collins, "Mordanted, Natural-dyed Wool and Silk Fabrics: Light and Burial-induced Changes in the Color and Tensile Properties," In *ACS Symposium Series 212*, H. Needles and S. H. Zeronian eds. (Washington, D.C.: American Chemical Society, 1986): 199-210.

¹³⁾ Elizabeth E. Peacock, "Characterization and Simulation of Water-degraded Archaeological Textiles: A Review," *International Biodeterioration & Biodegradation* (1996): 35-47.

¹⁴⁾ Elizabeth E. Peacock, "Deacidification of Degraded Linen," *Studies in Conservation* Vol. 28 (1983): 8-14.

¹⁵⁾ Ira Block and Hye Kyung Kim, "Accelerated Aging of Cellulosic Textiles at Different Temperatures: The Effect of Tetrahydridoborate Reduction," In *Historic Textile and Paper Materials: Conservation and Characterization, Advances in Chemistry Series 212* (Washington D.C.: American Chemical Society, 1986): 411-424.

¹⁶⁾ Howard L. Needles and Kimberly Claudia J. Nowak, "Heat-induced Aging of Linen," *Historic Textile and Paper Materials II: Conservation and Characterization. ACS Symposium Series 410* (Washington D.C.: American Chemical Society, 1989): 159-167.

¹⁷⁾ Anna Jarosz-Wilkolazka, Janina Kochmanska-Rdest, Elzbieta Malarczyk, Wladyslaw Wardas, and Andrzej Leonowicz, "Fungi and Their Ability to Decolourize Azo and Anthraquinonic Dyes," *Enzyme and Microbial Technology* Vol. 30 (2002): 566-572.

¹⁸⁾ Gian Maria Colonna, Tullio Caronna, and Bruno Marcandalli, "Oxidative Degradation of Dyes by Ultraviolet Radiation in the Presence of Hydrogen Peroxide," *Dyes and Pigments* 41 (1999): 211-220.

¹⁹⁾ Cheunsoon Ahn and S. Kay Obendorf, *Op. cit.*

III. Materials and Methods

1. Materials

Alizarin at 97% purity (1,2-dihydroxyanthraquinone) was purchased from Sigma Aldrich (Milwaukee, WI) and used without further purification. Methanol (HPLC grade) was purchased from Mallinckrodt Baker (Paris, KY). A 0.45 μm glass fiber enhanced syringe filter (Alltech, Deerfield, IL) was used for filtering samples for GC-MS analysis.

2. Methods

Thermal degradation was carried out during the total of 7 days period in 150°C oven. The degradation behavior of alizarin was examined during 1st to 7th day degradation time slots. Five vials were allotted for each degradation time slots and the dye solution was fixed to $2.5 \times 10^{-3}\text{M}$ conc. for each vial. The evaporated samples were taken in methanol for GC-MS analysis.

$\text{H}_2\text{O}_2/\text{UV}/\text{O}_2$ degradation system was simulated as such: Dye solution was fixed to $2.5 \times 10^{-4}\text{M}$ conc. and mixed with H_2O_2 according to $[\text{H}_2\text{O}_2]/[\text{dye}]$ ratio 40 for each vial. The vials were kept under the UV lamp (365 nm, UVL-18, UVP, Upland CA) for 2 hours and analyzed after 0, 30, 60, 90, 120min using the GC-MS. Two vials each were examined for each time slot.

The product search in the GC-MS analysis was conducted using the SCAN mode, and the quantitative analysis of alizarin was carried out using the SIM mode. The GC-MS instrument used was the Hewlett-Packard GC 6890 Series coupled to the Agilent Technologies 5973N MSD system. Operating conditions were as follows: Front inlet was kept at splitless mode with initial temperature at 250°C. The products were separated on a Hewlett Packard 190915-433 capil-

lary column (30m \times 250 μm i.d., 0.25 μm nominal film thickness) programmed from 50°C to 305°C,²⁰⁾ run time 20.12min, column flow 1.3ml/min at initial temperature. Initial temperature at MSD was 310°C. Mass spectra were recorded at scan range 80–250 m/z for both SCAN and SIM mode. The threshold for the scan mode was 200. The assignment of products was based on match with the known spectrum in GC-MSD library database. Perkin Elmer Lambda 2 UV-VIS Spectrometer was used to measure the color change of the samples.

The degradation rate in terms of the change in the amount of alizarin in the dye solution was calculated using the concentration vs. relative abundance equation [$y=8\text{E}-11x + 0.014$, $R^2=0.9997$], where y is the concentration of alizarin(g) in MeOH(g), and x is the corrected sum of area of the alizarin peak at 10.03 r.t. obtained through the GC-MS SIM program.²¹⁾ The calculated concentration was normalized according to the actual mass of MeOH solution. The differences in the amount of alizarin between the samples were compared with the paired samples T-test using the SPSS 9.0 Version for Windows software.

IV. Results and Discussion

Gas chromatogram of alizarin control showed a strong alizarin peak at 9.96–10.13min. retention time range and residual alizarin peaks in the wide range from 9.6 to 11.1 min. The mass spectrum of alizarin control (Fig. 1) showed a clear match with that of the NIST MS library standard and the corresponding ion fragmentation pattern. Within several trial runs, small amount of impurities (1.5% of the total area %) were detected at all times at 9.07 and 10.71 min. r.t. which were assigned as 9,10 anthracenedione ($\text{C}_{14}\text{H}_8\text{O}_2$ =M.W. 208) and diisocetyl

²⁰ Daniele Fabbri, Giuseppe Chiavavi, and He Ling, "Analysis of Anthraquinoid and Indigoid Dyes Used in Ancient Artistic Works by Thermally Assisted Hydrolysis and Methylation in the Presence of Tetramethylammonium Hydroxide, *J. Anal. Appl. Pyrolysis* Vol. 56 (2000): 167-178.

²¹ Statistical Handbook, Hewlett-Packard GC 6890 Series Manual.

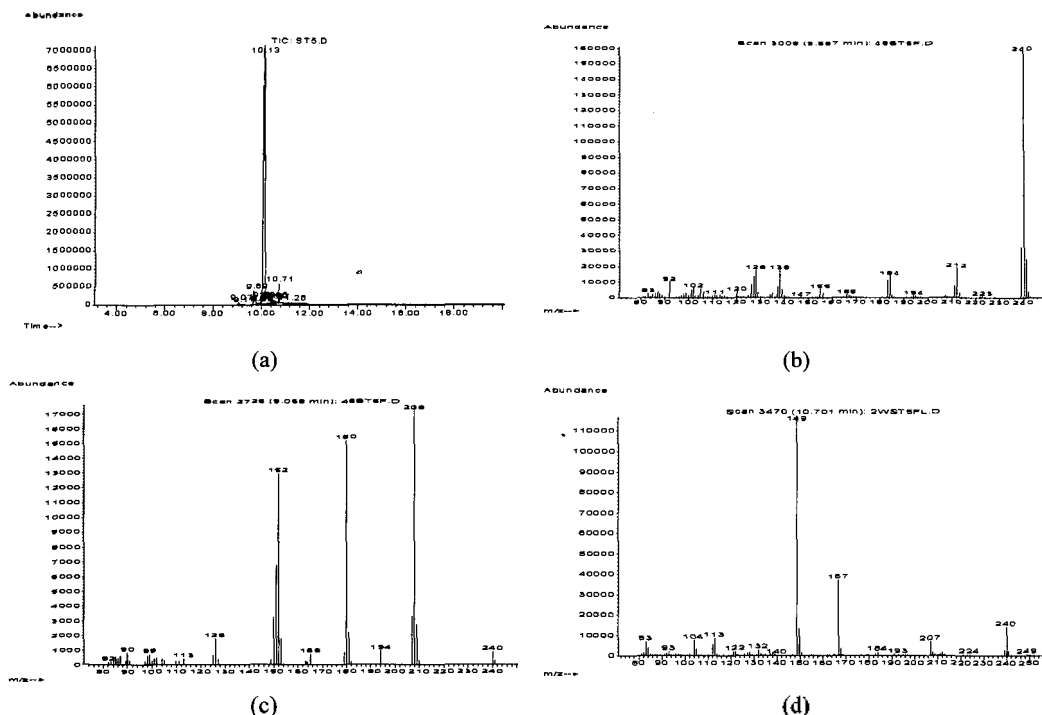


Fig. 1 Chromatogram of Alizarin Control (a) and the MS Spectra of Products: (b) Alizarin, (c) Assigned as 9,10-Anthracenedione, (d) Assigned as Diisooctyl ester phthalic acid.

ester phthalic acid ($C_{24}H_{38}O_4$ =M.W. 390) respectively by the MS library. Due to the small residual peaks of ion fragment at 240 m/z which are not characteristic of the compounds' standard MS spectra the product assignment following the MS library could not be fixed at this point of analysis. However, same products were repeatedly detected with each sample in the 7th day oven degradation system.

Fig. 2 illustrates the change in the amount of alizarin in oven degradation samples. A major decrease in the amount of alizarin was observed in the 1st day sample. However, it was followed by a continuous increase in the 2nd and 3rd day samples. In the 4th day sample, the amount reached the maximum level. It was only after the 4th day that the expected decrease in the amount of alizarin was observed. Paired sample t-test conducted on these degradation samples indicated that the differences in the amount of

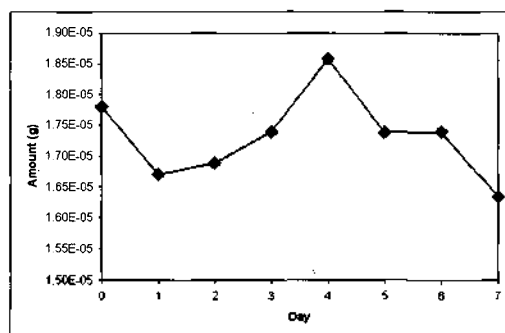
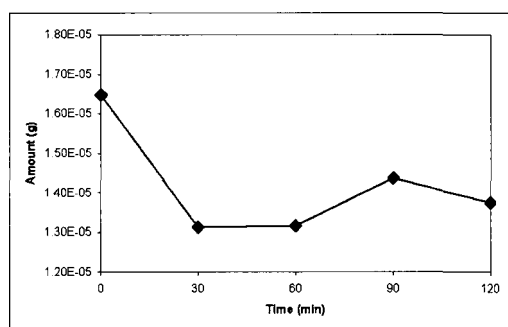


Fig. 2 Change in the Amount of Alizarin in the Oven Degradation.

alizarin between 1st day and 4th day samples, 4th day and 7th day samples, and Control and 7th day samples were significant at alpha .20 (Table 1). Within the H_2O_2 /UV/ O_2 degradation, the lowest amount of alizarin was detected at 30 min, and 60 min time slots after which an increase (90 min) and the following decrease (120 min) was

observed (Fig. 3). The statistical comparison for the $H_2O_2/UV/O_2$ degradation series was inappropriate due to small sample size.

The initial increase of alizarin in both oven and $H_2O_2/UV/O_2$ samples can be explained as the result of earlier oxidation of smaller molecules in alizarin sample as trace impurities. The disappearance of the smaller molecules might in turn have caused an increasing effect on the relative abundance of alizarin in the GC quan-



⟨Fig. 3⟩ Change in the Amount of Alizarin in the $H_2O_2/u.v./O_2$ Degradation.

tative analysis. Higher increase in the amount of alizarin within the oven degradation supports such proposal in that the high temperature in oven is likely to have a faster oxidation effect on the more volatile smaller species. The increase of alizarin during the initial degradation period tested in this experiment can be verified by the trend in the UV-VIS absorption of the oven degradation samples (Fig. 4). According to their UV-VIS spectra the Control sample showed the highest absorption followed by the 7th day samples in the order of 1st, 6th, 4th, 2nd, 5th, 3rd, 7th days. Although the absorption pattern does not exactly follow the order of the amount of alizarin in ⟨Fig. 2⟩, it does verify that samples during the middle stage of degradation show higher absorption than expected and that the least absorption is observed during the final stage of the degradation period. The above result of the change in the amount of alizarin in both thermal and $H_2O_2/UV/O_2$ degradation series imply that when the degradation time is extended to longer time period, such as in archaeologi-

⟨Table 1⟩ Result of Paired *t*-tests of Oven Degradation Samples

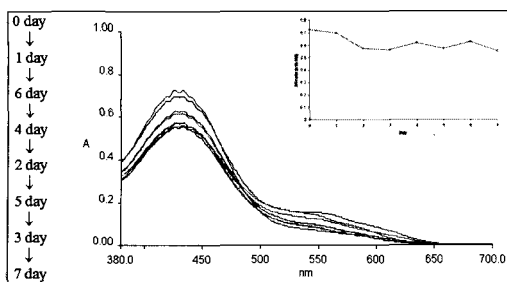
	Mean	SD	SE of Mean	<i>t</i>	Sig.
Control ~ 1 st Day	1.098E - 06	2.665E - 06	1.192E - 06	.922	.409
1 st Day ~ 4 th Day	-1.87 E - 06	2.630E - 06	1.176E - 06	-1.591*	.187
4 th Day ~ 7 th Day	2.235E - 06	2.846E - 06	1.273E - 06	1.756*	.154
Control ~ 7 th Day	1.462E - 06	1.780E - 06	7.962E - 07	1.836*	.140

Note: *t*-test results with * are significant at alpha .20.

⟨Table 2⟩ Result of Paired *t*-tests of $H_2O_2/u.v./O_2$ Samples

	Mean	SD	SE of Mean	<i>t</i>	Sig.
Control ~ 30 min	3.347E - 06	8.729E - 07	6.172E - 07	5.423*	.116
30 min ~ 90 min	-1.24 E - 06	1.599E - 06	1.131E - 06	-1.093	.472
90 min ~ 120 min	6.183E - 07	1.821E - 06	.1.287E - 06	.480	.715
Control ~ 120 min	2.729E - 06	1.094E - 06	7.739E - 07	3.526*	.176

Note: *t*-test results with * are significant at alpha .20.



(Fig. 4) UV-VIS Spectral Change of Alizarin in Oven Degradation Series.
(Inset: Concentration Change of the Corresponding Spectra).

cal textiles, the continuing decrease of alizarin amount is expected.

V. Conclusion

The major purpose of this research was to investigate the degradation behavior of alizarin, which is the major chromophore of madder plant, using GC-MS quantitative analysis. Both thermal and $H_2O_2/UV/O_2$ degradation treatment showed an initial decrease in the amount of alizarin at the early stage of degradation. It was followed by an unexpected increase and then exhibited a decrease with degradation progression. The present findings imply that when the degradation time is extended to longer time period such as in archaeological textiles the continuing decrease in the amount of alizarin would occur, and as a result remaining chromophore within the textile will either be extremely small or none. This study therefore supports the premise that it is inappropriate to analyze the dye in archaeological textiles using 'fresh' chromophoric chemical as the comparative standard. However, it is recommended that further research into the change in the amount of alizarin using longer degradation time is necessary as future research effort.

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