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# A NOTE ON CHARACTERIZATION OF THE COCHINEAL DYESTUFF ON WOOL USING MICROSPECTROPHOTOMETRY

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**Abstract**

**The use of microspectrophotometry to characterize cochineal in dyed wool is described. The effect of mordants and adjuvants on the absorbance and emission spectra was measured. Chromium, copper, and iron mordants and bases cause a red shift in absorbance spectra while tin and aluminum mordants and acids cause a blue shift. Cochineal mordanted with tin and aluminum fluoresces while chromium, copper, and iron mordants quench fluorescence. There is a great deal of overlap in the absorbance and emission spectra of the different formulations. A plot of all three emission peak maxima discriminates among substantive, mordanted, and acid-treated unmordanted cochineal.**

## 1 Introduction

Many natural organic red colorants have been used as dyes and pigments. Their chemical composition falls into several classes including xanthanes (e.g., rhubarb), flavonoids (e.g., weld), and quinones. Of the quinones, a subset of anthraquinone pigments and dyes can be prepared from either plant or animal sources (e.g., madder and cochineal, respectively). These have a long history of use.[1-5] The cochineal dye is extracted from the insect *Dactylopius coccus* (American cochineal) which lives on *Opuntia* cacti. The main colorant is carminic acid. Cochineal can give many colors depending on the use of mordants and adjuvants such as organic acids and ammonia (Figure 1).[6] Adjuvants are dye bath additives used as color modifiers. The colors that can be produced range from orange to purple, and even deep browns-used for mourning colors.[7]

Identification of the specific colorant used in a work provides important information on the technology available to the artist or craftsperson who made it, evidence for trade in materials and know-how, and is valuable in assessing condition and specifying conservation protocols.

Arguably, high performance liquid chromatography offers the most detailed information for characterizing the colorants in red lakes and dyes.[8-10] However, information on any mordant which might have been used is not usually obtained using this method. X-ray fluorescence spectroscopy can be very useful for analyzing metallic mordants, but it can be difficult to be certain about the mordant when the lakes are in mixtures with or layered over other dyes or pigments, particularly those which contain elements used as mordants, such as aluminum or calcium. It has been demonstrated that determining the mordants on dyes in textiles is complicated by the presence of adventitious material which has accumulated over time.[11] Mass spectrometry may prove useful for identification of both dye and substrate.[12] Raman spectroscopy can be used in many cases, and application of this technique to nonsampling and microsampling analysis continues to be developed.[13, 14]

Nonsampling methods for identification of red dyes and lakes include reflectance and emission spectroscopy. In 1977, Kirby showed that spectrophotometric methods could be used to discriminate among colorants in

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paint cross sections.[15] UV-visible reflectance spectroscopy has been used to identify red lakes in paintings.[16-18] Emission spectroscopy has added greatly to the ability to identify the dyes in situ.[19, 20] Despite a great deal of progress in applying these techniques to the characterization of natural red lakes used for textile dyes and pigments, it remains difficult to discriminate among dyestuffs such as madder, cochineal, and lac using spectrofluorimetry. Several factors complicate the analysis. All three coloring compounds share the same basic anthraquinone molecular structure. The electronic spectra of some dyes are highly dependent on the nature of the metal complexed by them. Interference from a substrate can be considerable; for example, parchment fluoresces brightly.[21] When using in situ methods binding media can contribute to spectra. For example, linseed oil emits at 510 nm[22] and rabbit skin glue at 500 nm as per our measurement. In solution, values of absorption and emission maxima of colorants are strongly dependent on the pH of the system[23-25] and solvent effects can be large. At high concentrations, aggregation of the molecules[26] occurs which affects the emission spectra. These variations mean that there is overlap of the maxima in the spectra of compounds such as carminic acid and laccaic acid and others that are in dyes, making discrimination among species using fluorescence spectroscopy quite difficult. The formation of new chromophores on natural ageing and their effect on the use of electronic spectroscopy to identify the original colorant is still being studied.


Figure 1: A sample card of cochineal-dyed wool prepared using a variety of recipes, photographed by the authors. The sample card was included with the book *Cochineal and the Insect Dyes*.[6]

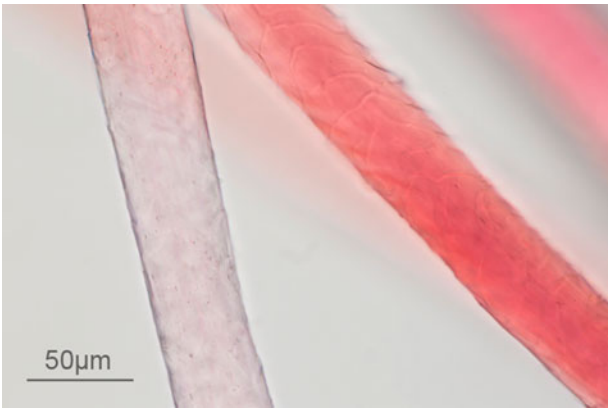


Figure 2: Bright field image of typical fibers studied in these experiments. The sample is mounted in glycerol on a glass microscope slide.

The wide range of colors that can be produced using cochineal, in itself, suggests that the identification of the parent colorant carminic acid using electronic spectroscopy might be challenging. We set out to measure the dependence of wavelength maxima in cochineal-dyed wool on factors including the mordants and pH adjusters used in different recipes designed to give different colors. This paper summarizes our work on the measurement of the absorbance and emission spectra with the goal of determining the variability of the spectra of cochineal prepared according to different recipes, and determining how well various preparations of cochineal can be discriminated. We employed microspectrophotometry which requires an extremely small sample, using only a fiber, generally about 3 mm long, taken from a yarn using forceps, which may be considered minimally destructive (Figure 2).

## 2 Experimental

### 2.1 Samples

Samples of cochineal-dyed wool on a sample card, included with the book *Cochineal and the Insect Dyes* by Fredrick H. Gerber (Figure 1), were studied.[6] Gerber prepared the samples using cochineal derived from whole bug *Dactylopius coccus* cultivated in Peru. The wool he used was from the same supplier and batch, and care was taken to ensure that dyeing times, temperatures, and mordanting methods were the same for all samples. Details of the techniques and recipes used to create the samples are described in the book. A general recipe involved 4 oz of potassium aluminum sulphate and 1.5 oz of cochineal per pound of wool. Wool was simmered in the alum solution for one hour to premordant; next it was washed and placed in the cochineal dye bath for one hour. For the studies of madder, samples were prepared by Julia Burke according to recipes provided by Helmut Schweppe.[27]

### 2.2 Microspectrophotometry

Transmittance and luminescence spectra were acquired using a Craic 1000 QDI UV-visible microspectrophotometer (CRAIC Technologies, San Dimas, CA) with a 36x Cassegrain objective that provided an analysis area of 16 $\mu m^2$. Absorbance spectra were obtained in transmitted light mode using a 75 W xenon lamp. For fluorescence microscopy, a 100 W mercury lamp was used. Emission spectra were obtained using a filter cube with a narrow band excitation filter centered at 433 nm and a 476 nm cut-off filter.

With the aid of a stereomicroscope, forceps were used to pull one wool fiber from each wool sample, including the undyed wool Gerber used. Fibers ranged in diameter from 30–50 µm. Each sample was mounted in glycerol (99.5+% spectrophotometric grade: Sigma-Aldrich) on a glass slide with a glass coverslip (Figure 2). Measurements were made with the focal plane of the microscope located within the fiber. Collection of good quality absorbance and fluorescence spectra required approximately 150 ms and 300 ms respectively, and 40 scans were averaged. First and second derivatives were calculated using GRAMS software to determine the positions of maxima.

## 3 Results and Discussion

### 3.1 Absorbance

Wavelength maxima in the visible region of the absorbance and emission spectra of dyed wool fiber samples are presented in Table 1. Most absorbance spectra contain two resolved bands and one higher energy shoulder.

Typical absorbance spectra are shown in Figure 3. The copper-mordanted sample has only one broad absorption band, perhaps due to peaks being unresolved.

Wavelength maxima of the two resolved peaks ($\lambda_{abs2}$ and $\lambda_{abs3}$) in the visible spectrum for all samples are graphed against each other in Figure 4. A fit of all data below 600 nm gives :

$\Delta = \lambda_{abs3} - \lambda_{abs2} = 37$ with $R^2 = 0.9776$

This constancy suggests that the same two electronic transitions occur in all samples, and that the different sample preparations do not change the relative nature of the ground and excited states.

Chromium, copper, iron, and bases cause a bathochromic (red) shift while tin, aluminum, and acids cause a hypsochromic (blue) shift. The acidified sample mordanted with both chromium and aluminum is blue shifted relative to the substantive sample, however an unacidified chromium and aluminum-mordanted sample was not available for comparison. The graph shows that the wavelengths of the absorption bands of tin and aluminum-mordanted samples can be very similar.

Like cochineal, madder absorbance spectra contain two resolved bands and one higher energy shoulder. Figure 4 shows that the two resolved absorption peaks of madder exhibit the same constant relationship as those of cochineal. Some investigators suggest that an identifying characteristic of madder is a blue shift relative to cochineal.[16,17,28] However, Figure 4 shows the absorption maxima of madder are located between unmordanted and tin-mordanted cochineal prepared with acid adjuvants. Figure 5 shows the overlap of madder and cochineal absorbance spectra, particularly unmordanted acidified with sumac.

| Dyestuff | Sample Group | Mordants and/or Adjuvants | Absorbance Maxima (nm) | | | Fluorescence Maxima (nm) | | |
|---|---|---|---|---|---|---|---|---|
| | | | $\lambda_1$ | $\lambda_2$ | $\lambda_3$ | $\lambda_1$ | $\lambda_2$ | $\lambda_3$ |
| Cochineal | Substantive | none | 498 | 532 | 575 | 547 | 607 | 646 |
| | Alum and Chrome | alum only | 492 | 527 | 566 | 571 | 607 | 654 |
| | | chrome only | 501 | 539 | 588 | n/f | n/f | n/f |
| | | alum tartar | 483 | 521 | 562 | 575 | 610 | 648 |
| | | chrome tartar | 498 | 534 | 576 | n/f | n/f | n/f |
| | | alum chrome tartar | 496 | 529 | 570 | 554 | 606 | 650 |
| | Acid Only | oxalic acid | 479 | 504 | 538 | 545 | 582 | 637 |
| | | tartar | 479 | 505 | 538 | 545 | 581 | 641 |
| | | sumac (tannic acid) | 479 | 502 | 538 | 544 | 581 | 638 |
| | | vinegar | 479 | 501 | 535 | 545 | 580 | 644 |
| | Tin | tin only | 485 | 521 | 562 | 578 | 606 | 646 |
| | | tin tartar | 479 | 515 | 556 | 580 | 610 | 648 |
| | | tin lime juice | 480 | 513 | 552 | 571 | 608 | 649 |
| | | tin oxalic acid | 480 | 514 | 552 | 576 | 609 | 650 |
| | | tin vinegar | 480 | 519 | 557 | 572 | 610 | 649 |
| | | tin sumac (tannic acid) | 481 | 515 | 552 | 574 | 608 | 649 |
| | Copper | copper only | 541 | 569 | 600 | n/f | n/f | n/f |
| | | copper tartar | 497 | 532 | 582 | vwf | vwf | vwf |
| | Iron | iron only | vwa | vwa | vwa | n/f | n/f | n/f |
| | | iron tartar | 532 | 582 | 604 | n/f | n/f | n/f |
| | | iron oxalic acid | 496 | 532 | 578 | 544 | 580 | 645 |
| | Ammonia | alum only ($NH_3$) | 500 | 539 | 582 | 574 | 607 | 653 |
| | | tin oxalic acid ($NH_3$) | 501 | 529 | 572 | 573 | 609 | 646 |
| Madder | Alum and Tin | alum tartar | 479 | 510 | 549 | 564 | 605 | 648 |
| | | alum tin tartar | 478 | 508 | 548 | 561 | 605 | 649 |

Table 1: Wavelength maxima in the visible region of the absorbance and emission spectra of dyed wool sample fibers. Emission from the wool protein at c. 516 nm, present in many spectra, is not reported in the table.
**vwa** = very weak absorption; **n/f** = no fluorescence; **vwf** = very weak fluorescence

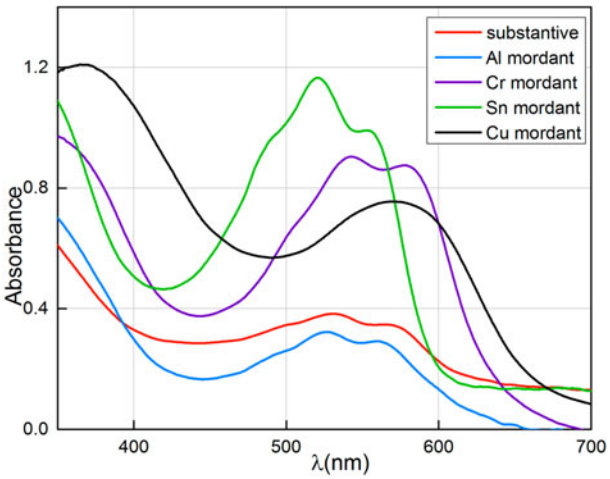


Figure 3: Absorbance spectra of fibers of wool dyed using cochineal and various mordants.

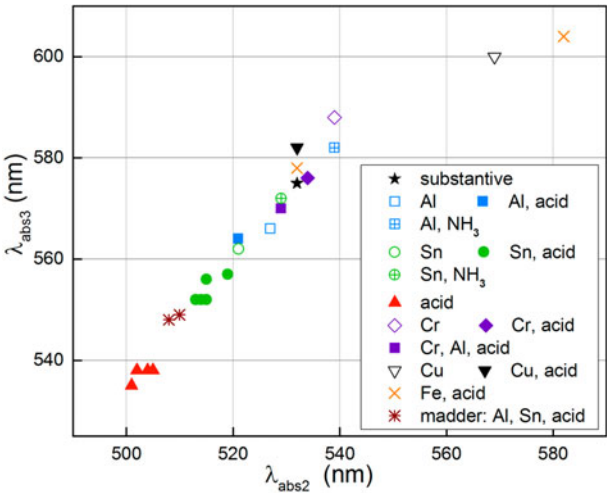


Figure 4: Plot of absorbance peaks $\lambda_{abs2}$ against $\lambda_{abs3}$ for all samples listed in Table 1.

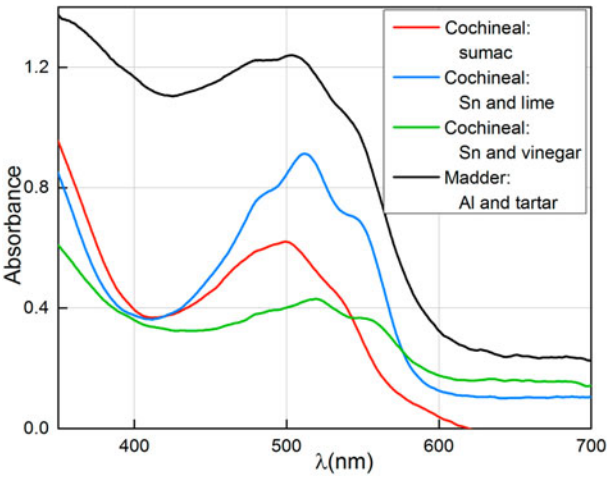


Figure 5: Absorbance spectra of cochineal and madder dyed wool.

## 3.2 Fluorescence

Emission spectra of cochineal-dyed wool have three bands in the visible region. They generally have one relatively intense band with two shoulders-one at higher and one at lower energy than the most intense peak. The exception was the fluorescence spectrum of the substantive sample in which the most intense peak is at the highest energy as shown in Figure 6.

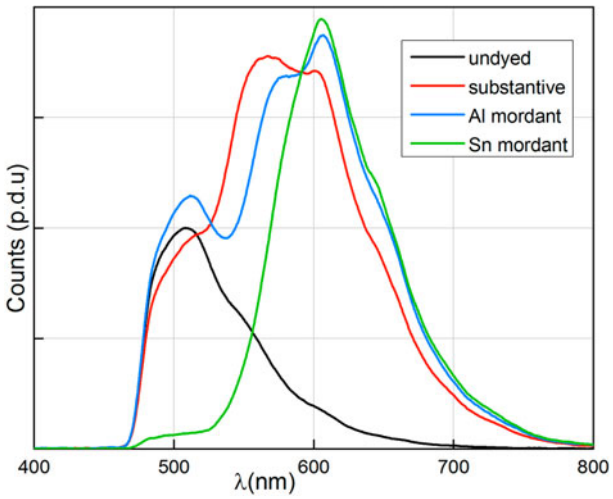


Figure 6: Fluorescence spectra of fibers of undyed wool and cochineal dyed wool. The undyed wool spectrum shows the fluorescence contribution of wool, $\lambda_{ex}$ = 433 nm.

Stapelfeldt et al. have suggested that the lowest energy band is related to the formation of aggregates.[26] The highest energy band is sometimes obscured by self-absorption due to high concentration of the dye, and in these cases a correction for self-absorption may be applied.[20] However, since this emission is clearly visible in our spectra, we assumed that self-absorption can be ignored, and the spectra are presented uncorrected. Additionally, this correction is intended for opaque samples and is therefore not suitable for these translucent samples.

Six samples had no measureable emission attributable to cochineal or carminic acid. These were the chromium-mordanted dyes with and without tartar as an adjuvant, the copper-mordanted dye with and without tartar, and the iron-mordanted samples with and without tartar. Other researchers have observed fluorescence quenching of carminic acid by a metal ion mordant. For example, Ni(II) complexes of carminic acid are nonfluorescent.[29] The iron-mordanted sample prepared with oxalic acid is fluorescent, but the emission wavelengths of the three maxima are so close to those of acidified, unmordanted carminic acid that we propose this species is present in the wool fiber and provides the emission observed.

For the samples that do fluoresce, the wavelength maxima of the two higher energy emission peaks in the visible region ($\lambda_{em1}$ and $\lambda_{em2}$) are plotted against each other in Figure 7. Three groups emerge from this plot: a) the substantive; b) the set dyed using tin or aluminum mordants; c) the set acid-treated but not mordanted. The aluminum and tin-mordanted samples all have $\lambda_{em1}$ in the range 570 to 582 nm, which is more than 20 nm longer than the substantive sample $\lambda_{em1}$ of 547 nm. The acid-treated, unmordanted samples have the same $\lambda_{em1}$ as the substantive sample, however the range of $\lambda_{em2}$ is 580 to 582 nm, which is more than 20 nm shorter than the substantive sample $\lambda_{em2}$ of 607 nm. From these observations three groups can be discriminated using fluorescence spectroscopy. The $\lambda_{em1}$ of the acid-treated sample mordanted with both chromium and aluminum falls between the substantive and aluminum and tin-mordanted samples and is somewhat similar to madder.

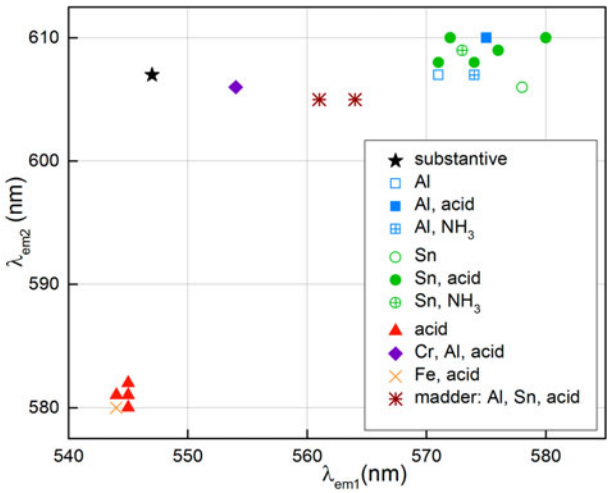


Figure 7: Plot of fluorescence peaks $\lambda_{em1}$ against $\lambda_{em2}$ for all samples listed in Table 1.

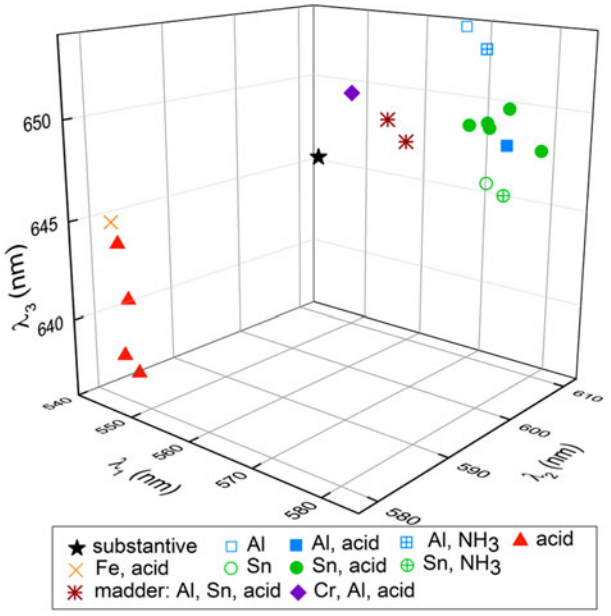


Figure 8: Three-dimensional plot of three emission maxima ($\lambda_{ex}$ = 433 nm) occurring between 540 and 700 nm.

When all three emission peak maxima are plotted (Figure 8) the aluminum and tin-mordanted samples are less tightly grouped than in Figure 7. The aluminum without adjuvant and aluminum/ammonia samples are red shifted along the z-axis ($\lambda_{em3}$) relative to the group formed by the acid-treated tin and acid-treated aluminum-mordanted samples. The tin without adjuvant and tin/ammonia samples are blue shifted along the z-axis ($\lambda_{em3}$) relative to this group. The graph of all three emission peaks shows that it is possible to separate the aluminum and tin-mordanted samples from each other.

## 4 Conclusions

This work, focused on dyed wool-an important historical textile material-demonstrated that minimally destructive microspectrophotometry can be useful for the characterization of cochineal with certain caveats. The use of reflectance spectroscopy alone is problematic since, as shown here, there is a wide range in the wavelengths of the maxima in the absorbance spectra of different preparations of cochineal. The values of unmordanted samples prepared with acids have considerable overlap with those of madder. Some preparations of cochineal fluoresce, yet some do not due to quenching by metal ion mordants. Just as for the absorption spectra, there is considerable variation in the wavelength of the maxima in emission spectra. However, a 3D plot of the maxima of all three emission peaks shows that groups of cochineal prepared according to different recipes can be segregated. This approach discriminates among substantive, mordanted, and acid-treated unmordanted cochineal. It also provides some separation of aluminum from tin-mordanted samples. This offers information that is useful for preliminary categorization of colorants based on anthraquinones, and is an improvement on using only 2D plots. But for complete characterization of the dyestuff, complementary techniques are still required.

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