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Los Angeles

Photophysical and Chemical Properties at the Solid State of Madder Lake from the Modern
Reproductions of an Ancient Alchemical Recipe

A thesis submitted in partial satisfaction
of the requirements for the degree Master of Science
in Materials Science and Engineering

by

Moupi Mukhopadhyay

2017

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ABSTRACT OF THE THESIS

Photophysical and Chemical Properties at the Solid State of Madder Lake from the Modern
Reproduction of an Ancient Alchemical Recipe

by

Moupi Mukhopadhyay

Master of Science in Materials Science and Engineering

University of California, Los Angeles, 2017

Professor Ioanna Kakoulli, Chair

This research considers the technical knowledge of the Greek-Roman society and investigates operational sequences (*chaîne opératoire*) for the production of madder lake through the application of materials science and engineering principles and methods. In this project, madder lake is re-created based on ancient recipes provided in an alchemical manual and characterized using a multi-scale and multi-analytical approach. Analytical imaging combined with surface-enhanced Raman spectroscopy (SERS) and fiber optics reflectance spectroscopy (FORS) are performed for the characterization of the microstructure, photophysical and chemical properties of the recreated madder lake pigment and how these are influenced by factors such as processing and chromophore ratios in the source, as well as the final product. Probing the production technology of madder lake through the application of materials science and engineering principles and methods, this research further interrogates the extent to which the raw materials, processing and the environment reflect, shape, or modify the properties and functions of this ancient pigment.

The thesis of Moupi Mukhopadhyay is approved.

Jaime Marian

Qibing Pei

Ioanna Kakoulli, Committee chair

University of California, Los Angeles

2017

DEDICATION

I would like to dedicate this to my parents, Kajal Mukhopadhyay and Mousumi Mukhopadhyay, who gave me everything, sacrificed so much for me and put their all into launching me, their much-loved rocket, to a better life. I can never thank these beautiful, wonderful people enough and hope to be worthy of them in this lifetime.

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Acknowledgements

This research was supported by the UCLA Archaeomaterials Group and the UCLA Molecular and Nano Archaeology Laboratory, both a part of the Henry Samueli School of Engineering and Applied Sciences at UCLA, as well as the UCLA/Getty Interdepartmental Program.

I would like to thank Dr. Ioanna Kakoulli, who is the principal investigator of this work, for her continuous support and encouragement not only with the research for this work and classes, but also for being a wonderful, patient and enthusiastic advisor, generous with her time, and providing much needed life and career advice;

Dr. Christian Fischer for all his help with Fiber Optic Reflectance Spectroscopy and Dr. Christina Viano (Director of research at the Centre de recherches sur la pensée antique bibliothèque Léon-Robin (CNRS)) and Dr. Robert Halleux (Director at the Centre d'Histoire des Sciences et des Techniques (CHST)) for their help with the translations and interpretation of the Stockholm Papyrus recipe used in this work;

My classmates Xuanyi Wu, Yuan Lin, Aileen Shin, Lindsey Perry and Krystal Cunningham for helping me with my experiments and supporting me throughout the process of this study, as well as my friends Rashmi Nandakumar, Alethia Shih, Daniel Howell and Philip Lester for keeping my spirits up;

Dr. David Scott, Vanessa Muros and all the conservation students for inspiring me with their love and unending fascination for conservation.

Chapter 1

1.1 Introduction

Madder is the collective name of some of the plants in the *Rubia* genus of the Rubiaceae family with most common species the cultivated *Rubia tinctorum* and the wild grown *Rubia peregrina* [1]. The roots of these plants are used to produce red-colored dyes [1, 2] that have been used since antiquity in dyeing textiles and manufacturing pigments for painting [1, 2] [2]. The two principal coloring compounds in the roots of the madder are alizarin (1, 2, dihydroxy-anthraquinone) and purpurin (1, 2, 4, trihydroxy-anthraquinone). Madder as a dye and a pigment has been found used in a range of different artefacts across the world with varying provenance. It is a mordant dye. A mordant is usually a metallic salt which fixes or sets a dye on fabrics to help the dye better adhere to the fabric; this is accomplished by the metal ion in the salt forming a coordination complex with the dye [3]. The most commonly used mordant is alum, a hydrated potassium aluminum sulfate ($\text{KAl}(\text{SO}_4)_2 \cdot 12\text{H}_2\text{O}$).

Recently many uses and possible applications of this root have been found in fields other than textiles and paintings and this has generated an interest in the properties of the chemical components of this dye, such as its photophysical, acidichromic and pharmacognostic properties, among others [1, 4, 5].

The purpose of this research is to understand production technology and the properties of the madder as a pigment in ancient paintings. Building on previous studies on the properties of its two major anthraquinones, alizarin and purpurin, both as free molecules in solution and when complexed with a metal cation, i.e. aluminum – Al(III) [5] from alum, this research explores the relationships between the composition and properties of madder lake in the solid state, reproduced from ‘recipes’ provided in an ancient alchemical manual of the third century AD known as the Stockholm papyrus, or *Papyrus Graecus Holmiensis* [6]. Since madder lake and

other madder-derivatives fulfilled different functions over millennia, i.e. used as pigment for painting, powder in cosmetics and for medicinal purposes [7], it has been subjected to many changes during its manufacturing history. Research conducted here, is part of a larger project on the use of madder in ancient chemical arts and focuses on its production technology as a pigment during the Graeco-Roman period (roughly between 332 BC and AD 395).

Due to the multidisciplinary nature and focus of this work, great insights are gained on the nature of the materials subjected to scientific investigation. Thus, this chapter contains background information on the various areas of study that have been included to contextualize the perusal of the rest of the work. This leads to considerations taken into the research to further refine the objectives and outcomes. The materials and methods employed for the synthesis and characterization are detailed next, followed by the results obtained from the analysis. Concluding remarks are supplemented by suggestions of the direction of future work.

1.2 Madder Lake Pigment and its History

Madder has been used as a colorant for around four millennia and it has a rich history of high regard, trade, economic status and even secrecy [1]. Mentions of madder have been found in ancient Egyptian hieroglyphics written roughly around 3000 BC [1]. Its continuous exploitation and use continued uninterrupted until the industrial revolution when new synthetic colorants substituted the natural compounds found in madder. ‘Turkey Red’ is a famous hue produced by a dyeing procedure using madder, whose manufacture was a closely guarded secret among the eastern Europeans before it became known in the mid-18th century [1]. During the 40-year boom in the demand for madder dyestuff in the world, the Russians became keenly interested in investing in madder; at one point, in the Russian city of Derbent around 1850, madder had even overtaken the most important local manufacture of arms [1].

Madder lake was also popular among impressionist painters like Van Gogh [8]. This shows the important role madder has played as a colorant since antiquity.

Dyer's madder or *Rubia tinctorum*, has been widely used for dyeing in the past and the present because of its rich dyestuff content, containing mainly alizarin whereas other types of madder such as wild madder, *Rubia peregrina*, and Indian madder, *Rubia cordifolia*, contain mainly purpurin and other coloring compounds [1]. Madder root, when soaked for an extended period, or boiled in distilled water, releases the dyestuff into the water which possess a red hue. The dyestuff contains hydroxy-anthraquinone-based components, mainly the chromophores alizarin and purpurin (Figure 1). These dye components are soluble and not very stable chemically, and therefore they cannot be used as painting materials that require the material to be dispersed (rather than soluble) in a medium and to have a certain photochemical stability. To render these dyes 'insoluble' and to improve their lightfastness it is therefore important to 'fix' them on a substrate through intercalation to form organometallic complexes.

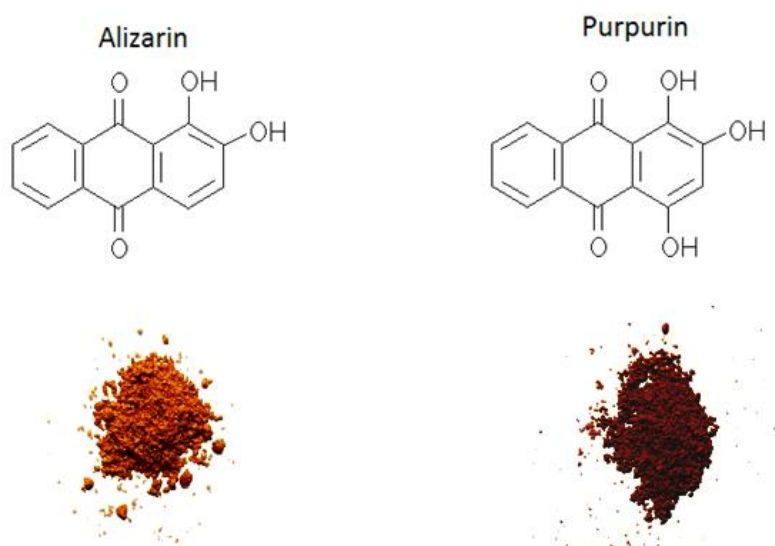


Figure 1. Structural formulas and images of alizarin (1,2-dihydroxy-9,10-anthraquinone) and purpurin alizarin (1,2,4-trihydroxy-9,10-anthraquinone) dyes.

This is a process known as mordanting, used both to fix the dye molecule on a yarn in the dyeing industry and to form the so called 'lake' pigments for painting. A lake is therefore an organometallic complex formed when a dye molecule is chemically bonded to a metal cation. One of the most common metal cations since antiquity is aluminum (Al(III)) added to the dyestuff as alum ($\text{KAl}(\text{SO}_4)_2 \cdot 12\text{H}_2\text{O}$).

There has been a lot of research done regarding the behavior of the chromophores (coloring compounds) under different environments and with different methods of complexation and manufacture [2, 4, 5, 9]. The purpose of this study is to add to this store of knowledge by exploring the behavior of these chromophores through the replication of the processes that may have been utilized in antiquity and use it as a launching pad to better understand the evolution of the manufacture of this noteworthy pigment.

1.3 Alchemy and the Stockholm Papyrus

Ancient alchemical texts have been mined for a deeper understanding of the beginning of science before, and the Leyden and Stockholm papyri [6] are a part of these important texts which provide insight into very early perception of method and reason. These papyri were written in demotic Greek in the third century AD. While the Leyden papyrus [10] focuses mainly on metals and their alloys, the Stockholm papyrus [6], delves into color, specifically the dyeing of rock crystals in imitation of gemstones and to dye textiles. As very little documentation exists in ancient texts on the production of pigments using madder, the recipes in the Stockholm papyrus offer an invaluable resource. Of the 154 recipes in the Stockholm papyrus, three mention madder, two of which are for purple hues (although it appears that the word for purple used at the time of writing could also refer to specific shades of red [11]) and one is to dye textiles a rose color. This recipe is the most detailed and will be referred to in this work frequently (Appendix A).

The reason for replicating the recipe in the Stockholm papyrus to produce a pigment rather than a mordanted dye for textiles is that although archaeological evidence confirms the use of madder lake as a pigment – at least since the seventh century BC [1] – there are no available contemporary documented texts describing its production. The Stockholm papyrus is one of the oldest texts that documents a detailed procedure for dyeing with madder and the only one that comes close to a written ‘chemical’ manual. Recipes for dyeing with madder have therefore been extracted from this manual and experimentally reproduced in the laboratory following modern methods and procedures.

As the recipes were mainly meant to be used as guidelines for alchemists who had already undergone training and had a more nuanced understanding of the proto-science, the procedures described are not always very clear. It is also known that given the “guideline” nature of this work, it is highly possible that code words were used to prevent widespread knowledge of secret recipes and a lot of what is written and translated cannot be taken literally. For example, the word “garlic” was often used by alchemists to refer to human feces; this tells us how differently an alchemical text can be interpreted from its literal meaning [6]. Nevertheless, it gives us valuable information about most of the materials used in the procedure.

To simplify the number of parameters that need to be controlled and observe the effects of isolated changes, this research focused on recipe number 107 described in the Stockholm papyrus [6]. This recipe is quoted and discussed in Appendix A; in order to be able to apply a controlled laboratory approach, the recipe was adapted to a less complicated version as described in section 2.1.1.

The assumption that mordant dyes (for textiles) and pigments of madder were manufactured using similar methods is one that has been made for this preliminary study involving

manufacture of madder lake pigments in antiquity. This is a starting point which can be confirmed or refuted based on consequent testing of archaeological samples of madder pigment that are contemporary to the Stockholm papyrus.

1.4 Photophysical properties of Lake Pigments

One of the identifying characteristics of madder lake pigment is its visible orange-red photoluminescence under ultraviolet (UV) excitation (~365 nm), [12]. Previous studies have shown well-formed trends in the photophysical behavior of alizarin and purpurin, the main coloring compounds of madder lake in different chemical environments and methods of production [2, 4, 5, 13, 14, 15, 16]. Through observations of absorptions and shifts in absorption bands in diffuse reflectance spectra (in the UV and visible regions of the electromagnetic spectrum), and similarly Stokes shifts in Raman spectra, it is possible to identify characteristic changes in chemical activity and bond length [15, 16]. As madder lake is extremely fluorescent, it is often difficult to identify it using Raman spectroscopy, as the spectra obtained for madder lake contains a large fluorescence background. Therefore, the technique of surface enhanced Raman scattering (SERS) can be used to quench the fluorescence and enhance the weak Raman scattering effect [15, 16, 17]. Some important findings relate changes in absorption bands of alizarin and purpurin lakes to the degree of chelation [5]; other research has produced semi-quantitative results which show a direct correlation between the concentration of purpurin to a higher observed emission intensity, in lakes that were produced with exact ratios by weight of the dyestuffs alizarin and purpurin [14].

1.5 Motivations for this research

While a lot of research has already been done on the production technology of historic madder lake [2], very little exists on the production of archaeological madder during the

Graeco-Roman period (332 BC – AD 395). Ancient writers like Vitruvius [18] and Pliny the Elder [19] refer to many pinkish-purple pigments such as *purpurissum*, which have been described to contain anthraquinones that could possibly have come from madder, and Dioscorides [20, 7, 10] emphasizes its medicinal properties. Though these references provide important written evidence on the use of this red chemical substance, information regarding its production remains ambiguous and indirect. The only surviving ancient text describing the production of this material, as a red mordanted dyestuff, is the Stockholm papyrus.

In this research, madder lake is re-created based on ancient recipes provided in this alchemical manual and characterized using a multi-scale and multi-analytical approach. Analytical imaging combined with fiber optics reflectance spectroscopy (FORS) and surface-enhanced Raman scattering (SERS) are performed for the characterization of the microstructure, photophysical and chemical properties of the recreated madder lake pigment. Probing the production technology of madder lake through ancient alchemical texts combined with the application of materials science and engineering principles and methods, this research further interrogates the extent to which the raw materials, processing and the environment reflect, shape, or modify the properties of this ancient material.

Chapter 2

2.1 Materials and Methods

Materials used were Alizarin red dye (1,2-dihydroxy-9,10-anthraquinone), purpurin (1,2,4-trihydroxy-9,10-anthraquinone) and alum ($\text{KAl}(\text{SO}_4)_2 \cdot 12\text{H}_2\text{O}$) from Sigma Aldrich. Calcium hydroxide (or lime water – $\text{Ca}(\text{OH})_2$) was obtained from the supernatant liquid in aged lime putty obtained from Natural Pigments. Its pH was tested each time and was adjusted to pH10 before use. Potassium hydroxide (or lye – KOH) and ethanol ($\text{C}_2\text{H}_6\text{O}$) were obtained from Fisher Scientific. Madder root for the historical reconstruction of the pigment was obtained from the 2016 harvest of *Rubia peregrina* (wild grown) from Cyprus.

2.1.1 Synthesis of Lake Pigments for Analysis

For the synthesis of the lake pigments for analysis, different starting materials were used depending on the desired sample.

Three different methods for the initial synthesis of lake pigments were employed:

- i) Lake pigments produced with alizarin or purpurin (chemical grade) in different environments

Chemical grade alizarin and purpurin used individually were introduced to four media: ethanol ($\text{C}_2\text{H}_6\text{O}$), lime water ($\text{Ca}(\text{OH})_2$), 0.01 M solution of potassium hydroxide (KOH) as the lye and a combination of lime water and lye, to produce complexes or lakes upon the addition of alum solution (Table 1). 10 ml of these materials were added to 1 g of the dyes in clean test tubes. 1 ml of 0.01 M alum solution was initially added, and up to 3 ml of excess alum solution was added to observe precipitate formation. This amount was not enough to form precipitates in samples 4, 6 and 9. Samples 1 through 8

were made 10 days before FORS analysis, whereas sample 9 was made on the day of the FORS analysis due to some observations (see section 3.1.2).

Sample number	Materials used	State of analyzed sample
1	alizarin+ ethanol+ alum solution	solid
2	purpurin+ ethanol + alum solution	solid
3	purpurin+ lime water+ alum solution	solid
4	purpurin+ lye+ alum solution	liquid/solid mixture
5	alizarin+ lime water + alum solution	solid
6	alizarin+ lye+ alum solution	liquid/solid mixture
7	purpurin + lye+ alum solution + lime water	solid
8	alizarin + lye+ alum solution + lime water	solid
9	purpurin + lye+ alum solution	liquid/solid mixture

Table 1. Samples were synthesized starting with alizarin and purpurin dye and complexing with Al(III) in different media as shown.

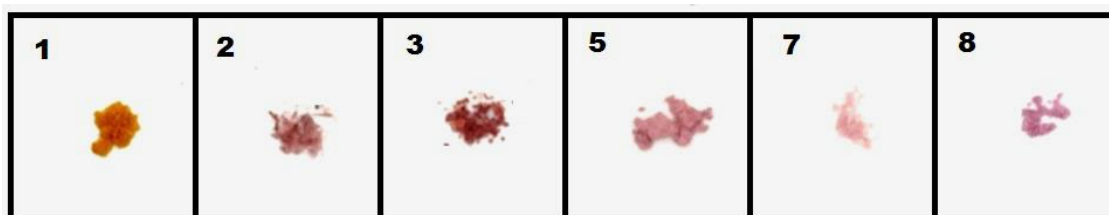


Figure 2. Lake pigments corresponding to the solid-state samples in Table 1.

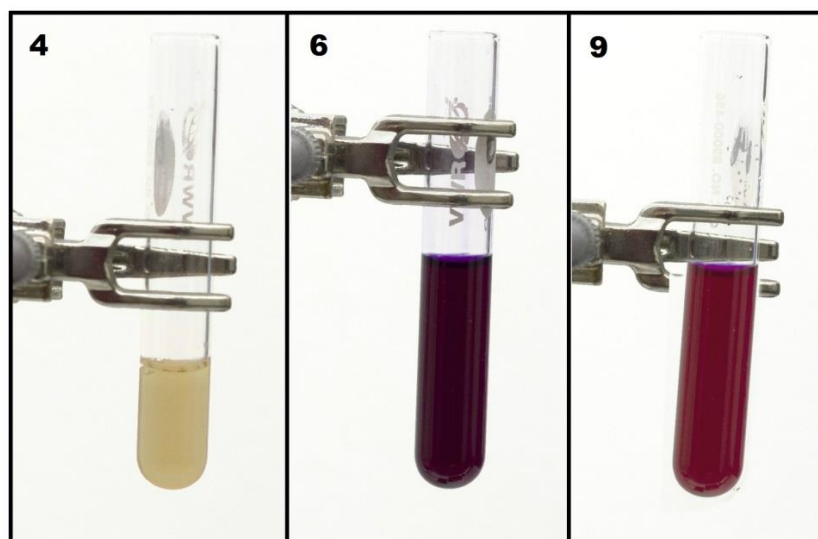


Figure 3. Mixtures of dye, lye and alum solution corresponding to the liquid/solid mixture samples in Table 1.

ii) Lake pigments produced by varying the ratios of alizarin to purpurin with lime water

Different ratios by weight of alizarin and purpurin were measured in separate test tubes, keeping the total weight constant at 0.01 g of the dyestuff as obtained, and 1 ml of the lime water was added. Volume of the addition of excess 5×10^{-5} M alum solution was determined separately as shown in Table 2. The variation in the amount of alum solution needed to produce precipitates with clear supernatant liquid is an interesting trend to be investigated in future studies using the same medium used here as there have been trends observed in the addition of alum solutions in different molar ratios [5].

Sample number	Sample based on dyestuff used	Volume of 5×10^{-5} M alum solution used
S1	Alizarin lake: A-(Al(III))	2 ml
S2	A-(Al(III)):P-(Al(III)) 90:10	2 ml
S3	A-(Al(III)):P-(Al(III)) 80:20	2 ml
S4	A-(Al(III)):P-(Al(III)) 70:30	1 ml
S5	A-(Al(III)):P-(Al(III)) 60:40	3 ml
S6	A-(Al(III)):P-(Al(III)) 50:50	2 ml
S7	A-(Al(III)):P-(Al(III)) 40:60	2 ml
S8	A-(Al(III)):P-(Al(III)) 30:70	1 ml
S9	A-(Al(III)):P-(Al(III)) 20:80	1 ml
S10	A-(Al(III)):P-(Al(III)) 10:90	1 ml
S11	Purpurin lake: P-(Al(III))	1 ml

Table 2. Table showing the ratios of dyestuffs used for the various samples produced to test the dependence of their photophysical properties to the variation in dyestuff content in lakes.

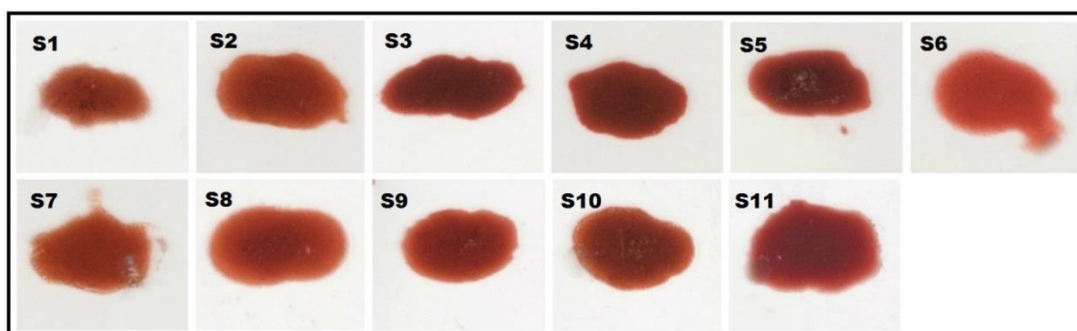


Figure 4. Eleven samples detailed in Table 2 of precipitate collected on glass cover slips.

iii) Lake pigments prepared with madder plant extract

Madder was extracted from wild Cypriot madder (*Rubia peregrina*) roots by heating in deionized water. 8 ml of this heated extract was taken in a test tube and 2 ml of lime water was added to it, followed by 8 ml of 0.01 M alum solution. The ratios were determined by modifying the papyrus recipe.

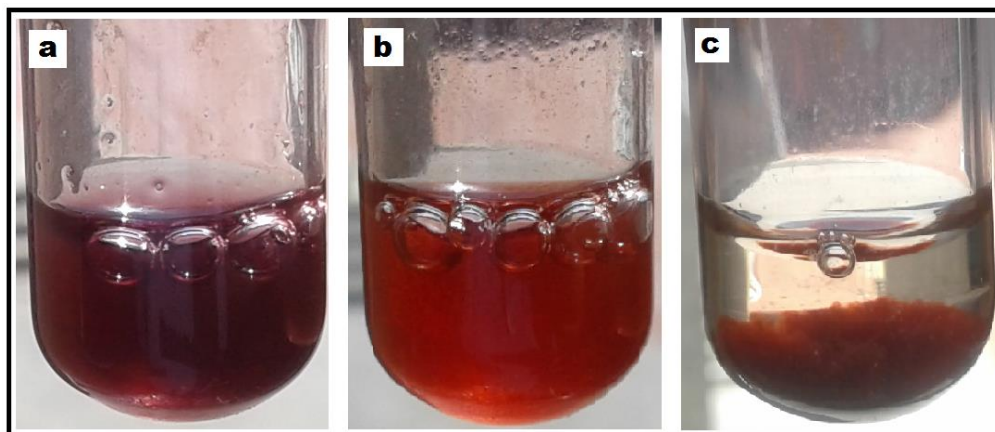


Figure 5. a) Madder root extract; b) madder root extract with lime water and alum solution added; c) same mixture as in (b) after allowing to settle, with observable precipitate and clear supernatant liquid.

Once the precipitates were obtained, they were decanted, washed and dried for analysis. A good amount of precipitate with clear supernatant liquid that indicated that all the dye molecules had been complexed or ‘fixed’, was the endpoint looked for in the preparation of these lake pigments. Upon the addition of lime water to the starting dyestuff the color changed to a dull purple, which turned bright red when enough alum was added. The language used in the recipe from which this procedure has been modified, which includes the word “brighten” to state the effect of adding alum to the dye, is very interesting, as it may well imply that the fabric being dyed was a dull, purple-red color before alum was added. This is a partially afterchrome method of dyeing (Appendix A) as opposed to onchrome or metachrome [3]. This agrees with the recipe followed which indicates brightening with alum where alum has been used in excess to precipitate all the dye in the mixture.

2.1.2 Preparation of samples

Pure dyes (free molecules) were separately mixed with barium sulfate (BaSO_4) to obtain 'dilute' diffuse spectra. One part of alizarin was mixed with four parts by volume (v/v) of BaSO_4 powder to prepare the alizarin test sample. The purpurin dye test sample was prepared using the same method. For the dye mixture, one part each of the 1:4 alizarin to BaSO_4 and 1:4 purpurin to BaSO_4 were mixed thoroughly to ensure the maximum possible homogeneity.

There were also some liquid samples of the lakes made in KOH (lye) and they were collected in a watch glass and set against a Spectralon reference (a standard with Lambertian behavior) as background for FORS.

The solid samples for performing FORS were spread on glass cover slips with some deionized water and left to dry so that a loose cohesion was obtained between the lake pigment and the glass. These were set against reference backgrounds for obtaining the spectra. Glass cover slips were used instead of glass slides as better spectra could be obtained using the thin glass as the loss of signal through the sides of the glass was less compared to glass slides. These pigment depositions on glass cover slips were reused as SERS samples with only the addition of silver colloid nanoparticles, prepared as explained in section 2.2.2.

2.2 Characterization Methods

2.2.1 Fiber Optic Reflectance Spectroscopy(FORS)

Based on availability and sample size, two different instruments were used to perform Fiber Optic Reflectance Spectroscopy (FORS) analysis – the FieldSpec3® spectrophotometer by Analytical Spectral Devices Inc. (ASD) and the Ocean Optics USB 2000+UV-VIS spectrometer. While both are portable and fast, the wavelength ranges, spot sizes and resolutions of these instruments vary. The FieldSpec3® has a range of 350-2500 nm, a spot size of ~1 cm diameter, and a spectral resolution of 3 nm in the VNIR range between 350 and

1000 nm, while the Ocean Optics USB+UV-VIS has a range of 200-850 nm, a spot size of 3 mm diameter and a spectral resolution of ~8 nm in the given wavelength range. When sets of data were collected for the same group of samples using both spectrometers, they were compared to ensure adequate analysis.

2.2.2 Surface Enhanced Raman Spectroscopy (SERS)

SERS was used to analyze the synthesized pigment samples, to obtain an enhanced signal and quench the fluorescence typical of madder Raman spectra. Pigment in solid state was obtained by allowing the synthesized precipitate dry on a glass substrate. For each sample, 0.5 μl of silver nanoparticles (AgNPs) synthesized using the Lee and Miesel method [21] was applied on top with 0.5 μl of KNO_3 solution to induce aggregation. The analysis was conducted with a Renishaw inVia system, which has a spectral resolution of 0.5 cm^{-1} in the visible wavelength range, using a red laser of wavelength 633 nm. Wavelength range was adjusted based on literature and static mode was used for the measurement. To increase signal to noise ratio, the parameters involved in analysis, like laser power, integration time and bleaching time, were adjusted. Once the optimum parameters were found, they were kept constant to enable comparison between data. A 633 nm laser was used at 0.05% power (8.5 kW) for 10 seconds integration time, with 3 accumulations and 3 acquisitions, and 5 seconds bleaching time to reduce fluorescence. Appropriate spots were found on the sample to ensure the correct data would be obtained, by following the protocol used by Prikhodko et al. [22]. Measurements were taken on at least 3 different spots to check for reproducibility and select a good representative. Although SERS using aggregated silver colloids provides good results, it should be taken into account that SERS is extremely distance-dependent and can be difficult to reproduce due to the nature of aggregation of the colloids [15]. Utmost care was taken to ensure that the samples for SERS were made the same way consistently. Furthermore, it has been noted that the methodology using silver nanoparticles (AgNPs) as

the platform for SERS preferentially detect pseudopurpurin (1,3,4-trihydroxy-9,10-dioxoanthracene-2-carboxylic acid) which decarboxylates into purpurin (upon drying) in madder lake pigments, whereas the presence of some other dye components go undetected [23]. Yet, the use of AgNPs in SERS has also been found to be very useful in similar studies involving the characterization of anthraquinone dyes [22, 23, 24, 25].

Chapter 3

3.1 Results and Discussion

3.1.1 Alizarin

Alizarin lakes (complexed with Al(III) ion) were prepared with alum (supply for Al(III)) with the addition of different alkali and solvents including lime water (calcium hydroxide – $\text{Ca}(\text{OH})_2$), lye (potassium hydroxide – KOH) and ethanol, and analyzed using FORS and SERS.

FORS data in the visible range display electronic transitions. Results presented in Figure 6, show that the wavelength corresponding to absorptions in the spectra increase from ethanol (pH 7.3; absorption at ~490 nm) to lime water (pH 10; absorption at ~580 nm) to the mixture of lime water and lye (pH 13; absorption at ~610 nm) to the lye (pH 13.5; absorption at ~620 nm). This agrees with published results, which show red shift of the absorption bands of alizarin with an increase in pH. Miliani et al. [4], ascribe the shift of the absorption to longer wavelength to a change of the dye from neutral, to mono-anion to di-anion form. Their data were obtained by using samples in water-dioxane solutions, yielding isobestic points at 463 nm, separating the neutral and mono-anion form, and at 516 nm, separating the mono-anion form from the di-anion. While alizarin shows an absorption band at 540 nm in its mono-anionic form, an increase in the pH enhances the absorbance and causes the red shift.

The degree of ionization will affect the reaction of the dye with the alum when the lakes are produced. From Figure 6, it is apparent that the preparation of lake in ethanol did not yield effective Al(III) complexes. Visual observation of the preparation revealed that the color of the preparation in ethanol was very similar to the color of the dye itself (Figure 1 and Figure 2), while all the other tests showed markedly different colors, also evidenced by the observed red shift.

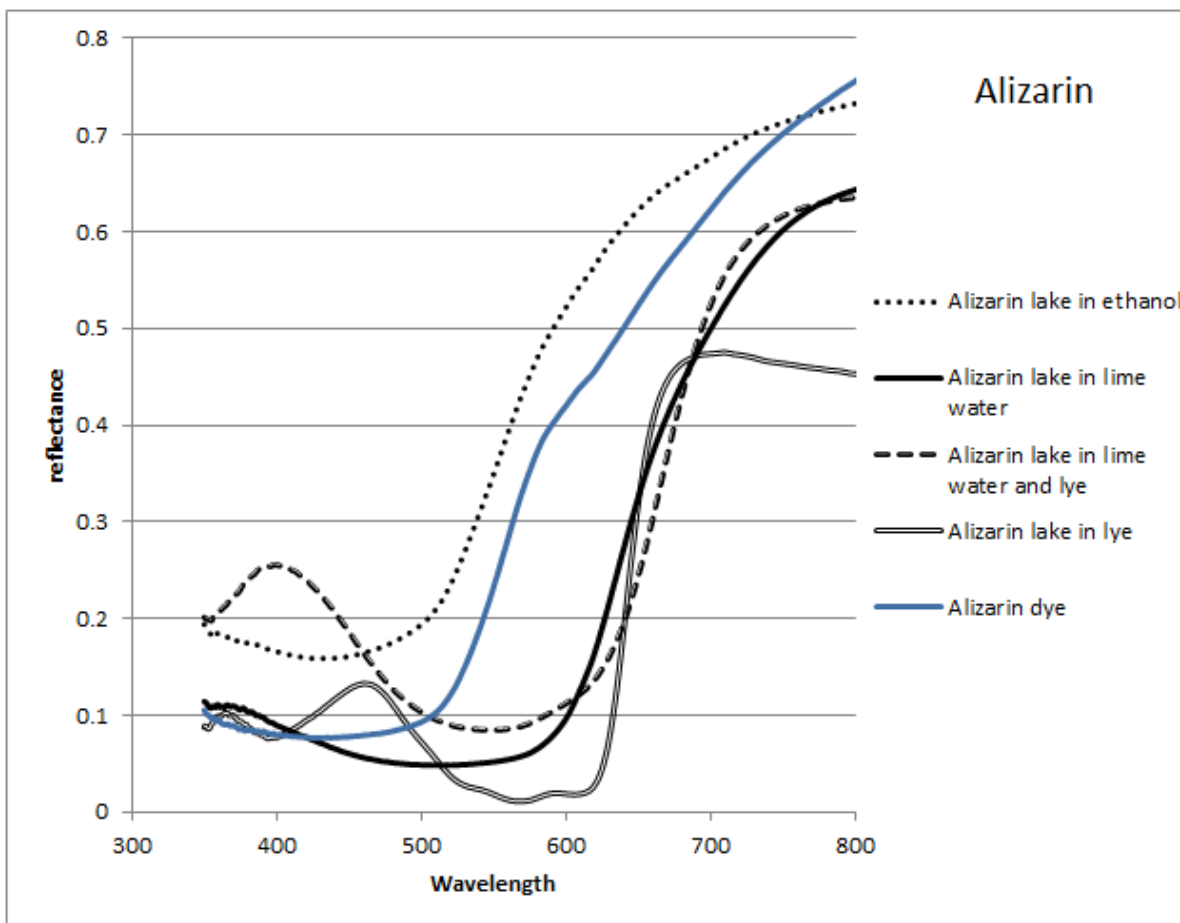


Figure 6. FORS spectra of alizarin lakes (complexed with Al(III)) in different media and alizarin dye (free molecule) obtained with the FieldSpec3® spectrophotometer.

Figure 7 shows the spectrum and second derivative of alizarin lake prepared in $\text{Ca}(\text{OH})_2$. Second derivative data were smoothed using the Savitzky-Golay filter. It is observed that the relatively prominent and more significant positive peaks in the second derivative graph correspond to the main absorptions that are identifiable in the main reflectance graph, but evident to a lesser degree. The absorptions for alizarin lake can be observed at ~ 462 nm and ~ 587 nm. The distinctive quality of the second derivative peaks are helpful in analyzing shifts.

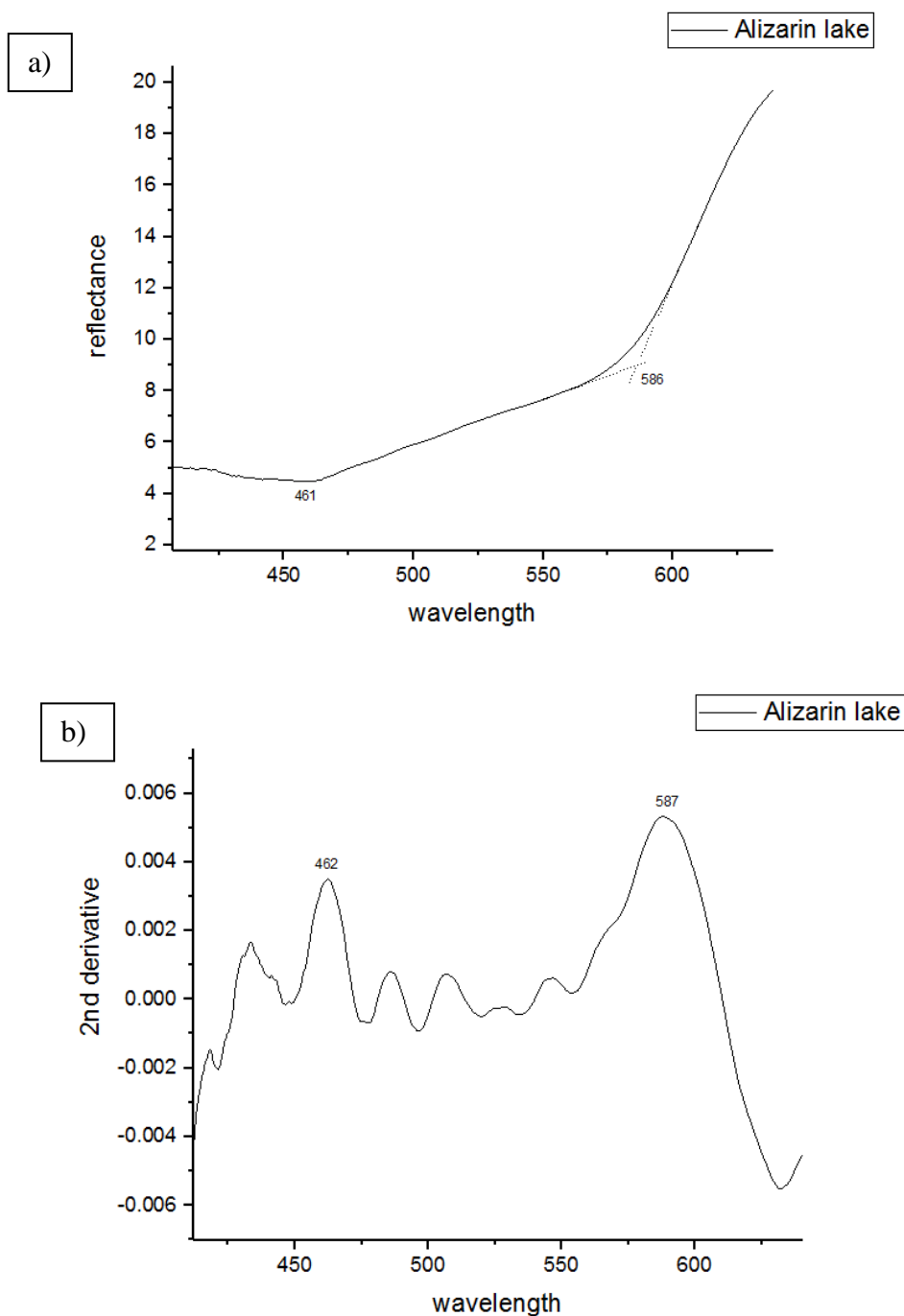


Figure 7. A comparison of the (a) reflectance spectrum of alizarin lake and (b) its second derivative. The reflectance spectrum was obtained using the Ocean Optics USB2000+UV-VIS spectrometer. The positive peaks in the second derivative represent the absorptions in the reflectance spectrum.

SERS was also performed on samples of alizarin (complexed with Al(III)) and alizarin dye (free molecule). As diagnostic marker bands in Raman spectra for the unambiguous identification of alizarin dye (free molecule) were previously identified as 473, 900 and 1157

cm⁻¹ [15], these regions have been further investigated (Figure 8). For the dye molecule, peaks are observed at 895 and 1159 cm⁻¹ as the peaks close to 473 cm⁻¹ are out of the range of the collected data. The alizarin lake showed peaks at 477, 898 and 1154 cm⁻¹. These values are very close to those suggested as fingerprint markers. By observing the peaks near 895 and 1159 cm⁻¹, we can see that the spectrum of the lake has peaks that are crowded into a slightly more restricted range of wavenumbers, when compared to the dye spectrum, so that the peak of the lake at 1154 cm⁻¹ has a red shift compared to the dye (1159 cm⁻¹) while peak of the lake at 898 cm⁻¹ is blue-shifted compared to the dye (895 cm⁻¹). While FORS data in the visible wavelength shows a distinct red shift from the dye free molecule to the lake (complexed molecule), the SERS data are not as conclusive.

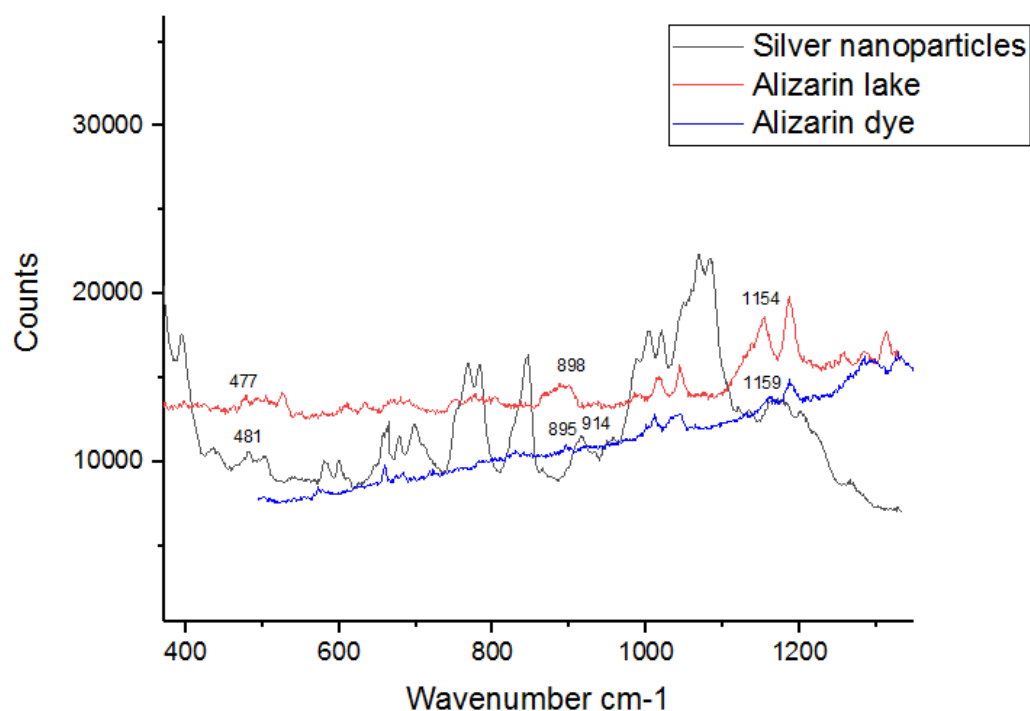


Figure 8. SERS spectra of alizarin lake (complexed with Al(III)) and dye (free molecule), compared to the spectrum of the AgNPs used for SERS.

3.1.2 Purpurin

Purpurin lakes (metal complexes with Al(III)) created in various media were also analyzed using FORS and SERS. In the visible range, purpurin lake (after complexation with Al(III)) usually displays two characteristic sub-bands at 505-515 nm and 540-550 nm [26] (Figure 9) regardless of the solvent in which it was prepared. It is interesting to note that the purpurin lake preparation in ethanol does not show a marked difference compared to the other lakes, as was the case in alizarin. Red shifts occur as the pH of the solution increases; however, the spectra are confined to a more restricted wavelength range [4], which is why the differences are not as readily observable as they are in alizarin lakes.

Another interesting observation was made while obtaining the FORS data for these lake preparations. Samples were created around ten days before FORS measurements could be taken. Within these ten days, the color of the preparation of purpurin lake in lye had drastically changed from what was documented as a deep pink color to a pale-yellow color. This is seen also in the FORS data (Figure 10) as the graph for 'Purpurin lake in lye (old)' and shows a much broader reflectance curve than the other lake. Further investigation into this phenomenon is a part of a comprehensive research of these lakes.

Absorption bands characteristic of purpurin lake prepared in lime water can be observed at ~470 nm, ~502 nm, and ~548 nm (Figure 11).

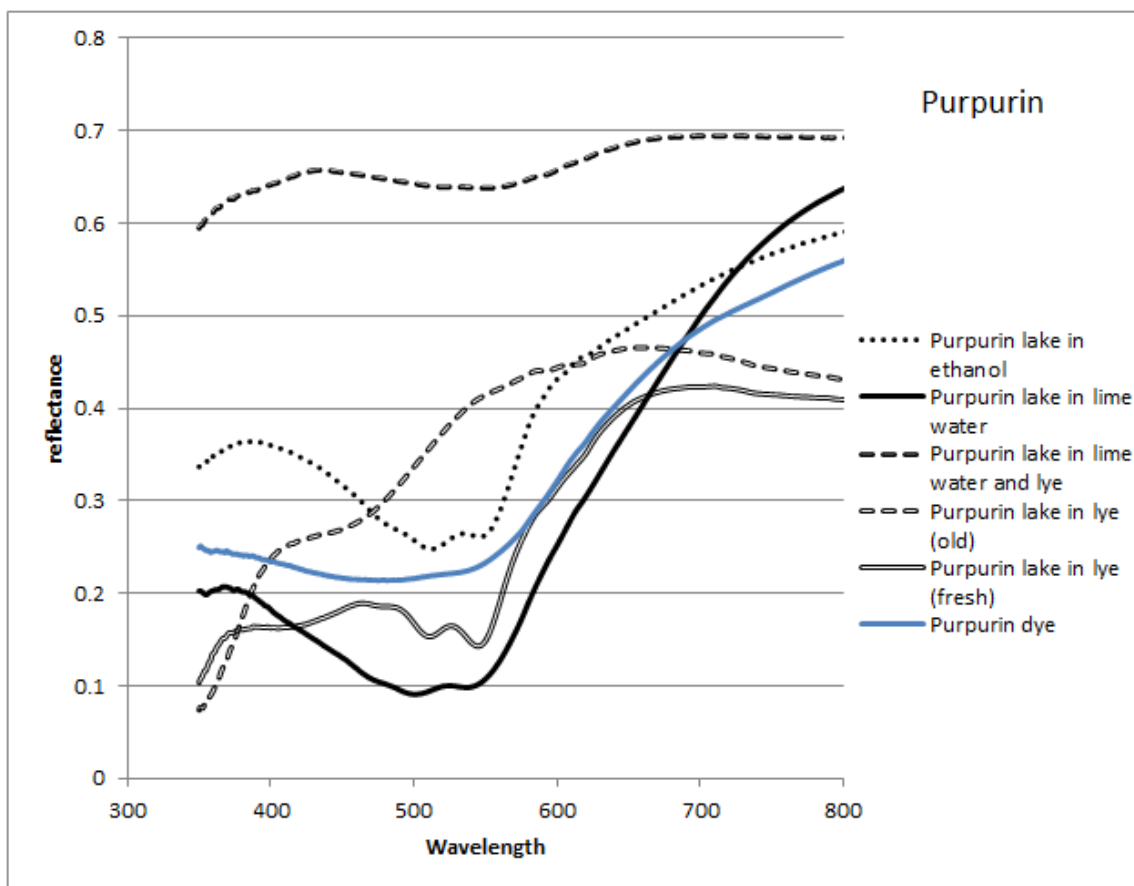


Figure 9. FORS spectra of purpurin lakes in different media and purpurin dye obtained with the FieldSpec3® spectrophotometer.

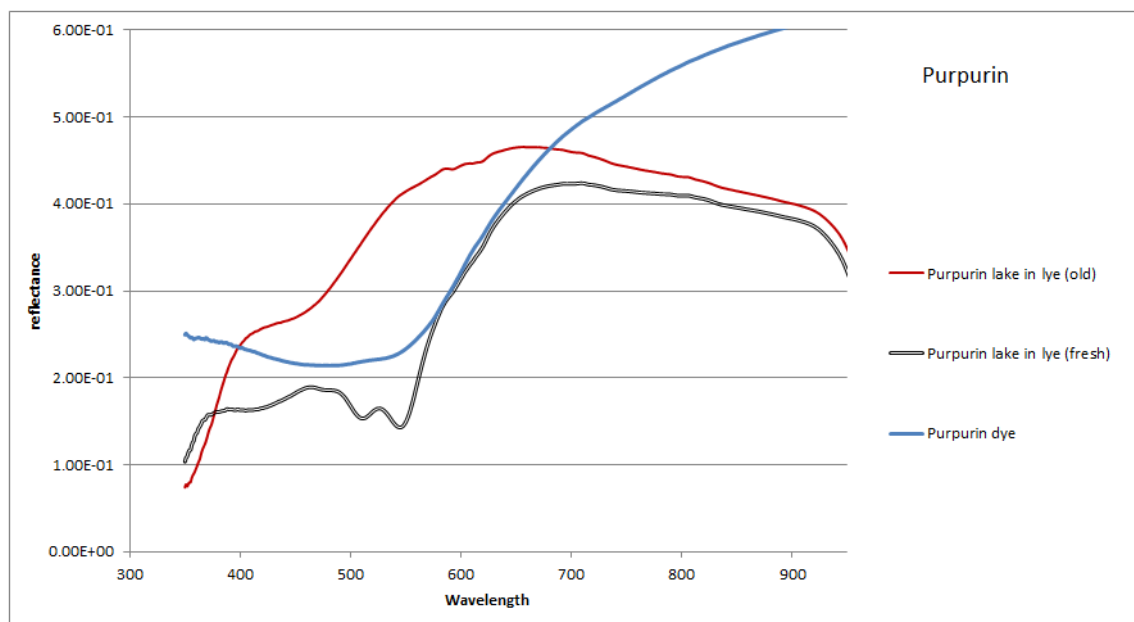


Figure 10. FORS spectra of "old" and freshly prepared purpurin lakes in lye medium, along with the spectrum of the dye (free molecule). The spectrum of the "old" preparation is much broader and stretched out.

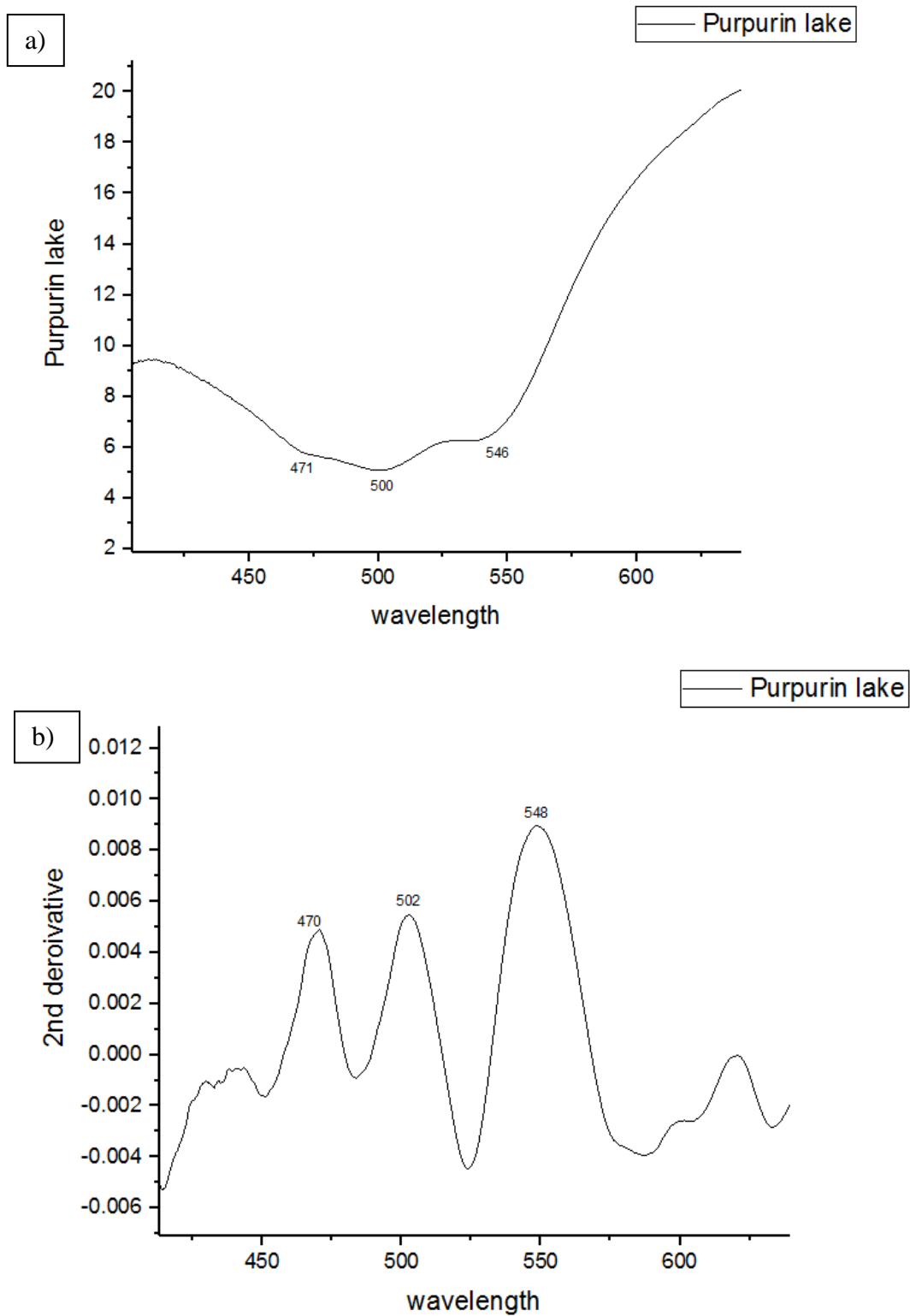


Figure 11. A comparison of the (a) reflectance spectrum of purpurin lake and (b) its second derivative. The reflectance spectrum was obtained using the Ocean Optics USB2000+UV-VIS spectrometer.

SERS data of purpurin lake (complexed with Al(III)) and purpurin dye (free molecule) were normalized along with the AgNPs spectrum (Figure 12). Suggested marker bands for unambiguous identification of purpurin dye (free molecule) are: 366, 606, 970, 1020 and 1401 cm^{-1} [15], of which regions around 606, 970 and 1020 cm^{-1} can be investigated in the overlapping data of the lake and the dye. SERS data for purpurin lakes present some challenges due to the high fluorescence and the difficulty of the settling of the AgNPs on needle-like structure of purpurin that can be observed at 50x magnifications. Peaks are observed at 609, 963 and 1019 cm^{-1} for purpurin dye (free molecule) and 970 and 1018 cm^{-1} for purpurin lake (complexed molecule); there is no noticeable peak at $\sim 606 \text{ cm}^{-1}$ for the lake. Here also, the lake shows similarities to the dye (free molecule).

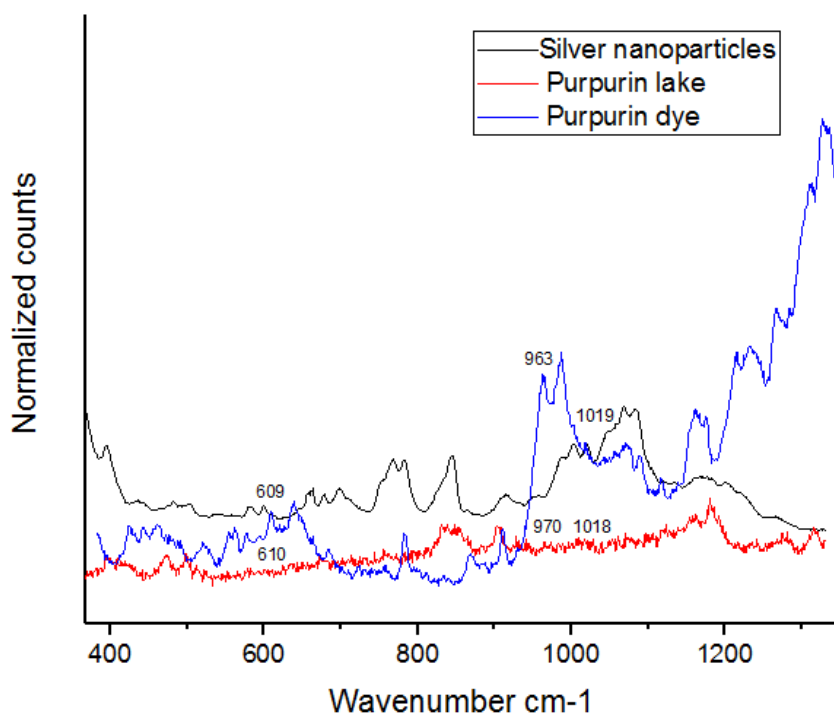


Figure 12. SERS spectra of purpurin lake (complexed with Al(III)) and dye (free molecule), as compared to the SERS spectrum of AgNPs used in the procedure.

3.1.3 Combination lakes of alizarin and purpurin

Samples were created as described in section 2.1.1 and analyzed using FORS. Reflectance spectra of the individual lakes (alizarin and purpurin respectively) and their mixtures, as well as the second derivative can be seen in Figure 13, Figure 14 and Figure 15. Sets of data were collected from both the OceanOptics USB2000+UV-VIS spectrometer and the FieldSpec3® spectrophotometer which have different spectra resolution (~8 nm and 3nm respectively) and were found to agree on the major absorption trends discussed here. Therefore, only data collected from the OceanOptics USB2000+UV-VIS are presented.

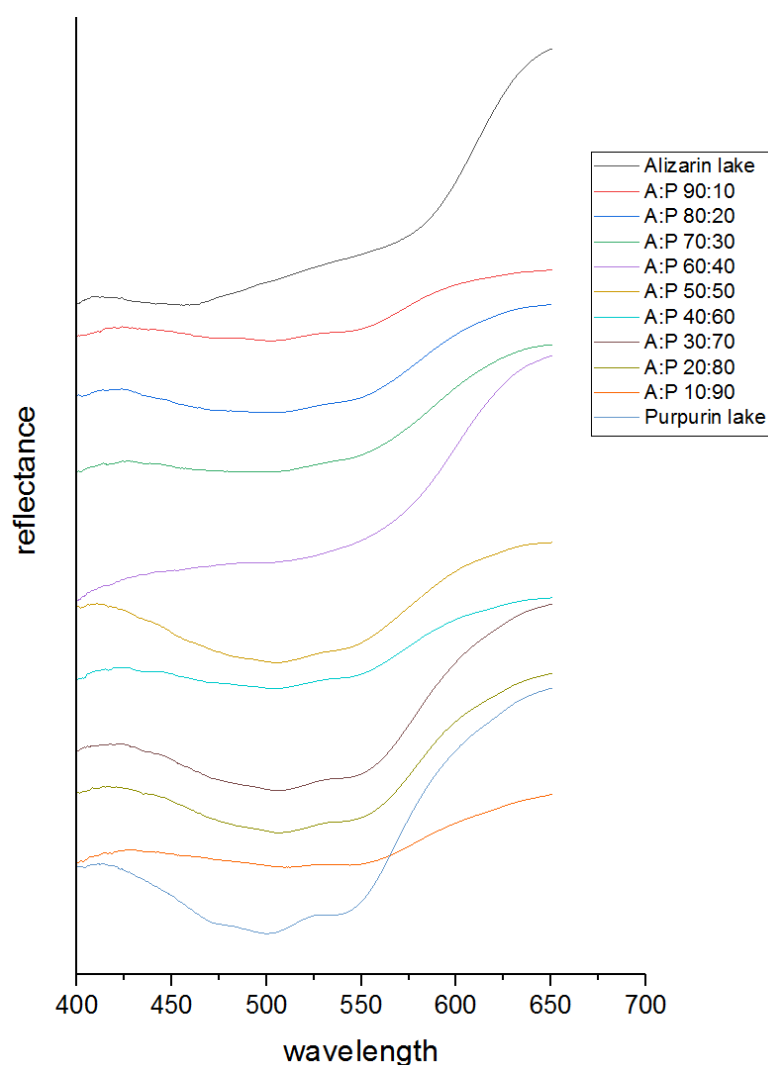


Figure 13. Stacked reflectance spectra of the combination lakes of alizarin and purpurin, with the relevant reflectance spectra of alizarin and purpurin lakes for comparison. Reflectance spectra were obtained using the Ocean Optics USB2000+UV-VIS spectrometer.

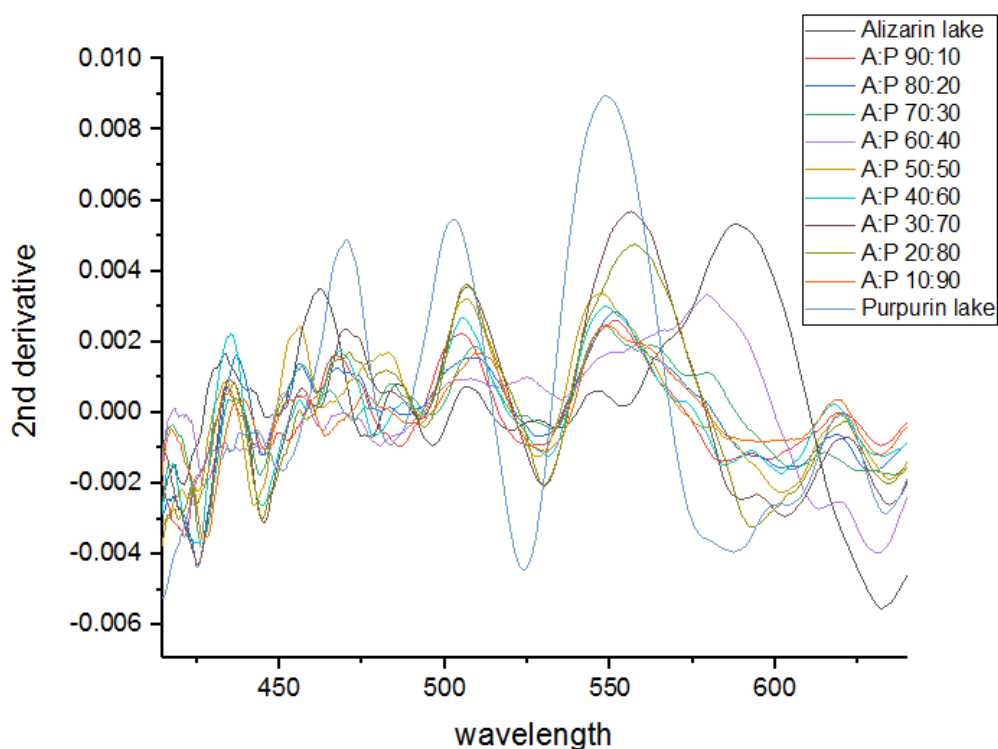


Figure 14. Second derivative of the combination and individual lakes of alizarin and purpurin.

Figure 15 compares the absorption bands of alizarin-(Al(III)) lake, purpurin-(Al(III)) lake and the A-(Al(III)):P-(Al(III)) 50:50 lake (see Table 2). Absorption bands can be observed at ~463 nm, ~487 nm, ~506 nm, ~545 nm and predominantly at ~587 nm for alizarin lake. Purpurin on the other hand shows a broad absorption at ~470 nm and two structural sub-bands at ~502 nm, and ~548 nm. The spectrum of sample A-(Al(III)):P-(Al(III)) 50:50 has some absorption bands in common with both alizarin lake and purpurin lake: at ~506 nm and ~546 nm, the sample matches the two predominant absorption bands of purpurin lake. It does not match with the predominant absorption band of alizarin lake at ~587 nm, but shows one at ~483 nm which is close to the ~487 nm absorption band of alizarin lake. A blue shift seems to have occurred at its ~456 nm peak, which has a lower wavelength than both the alizarin and purpurin lakes.

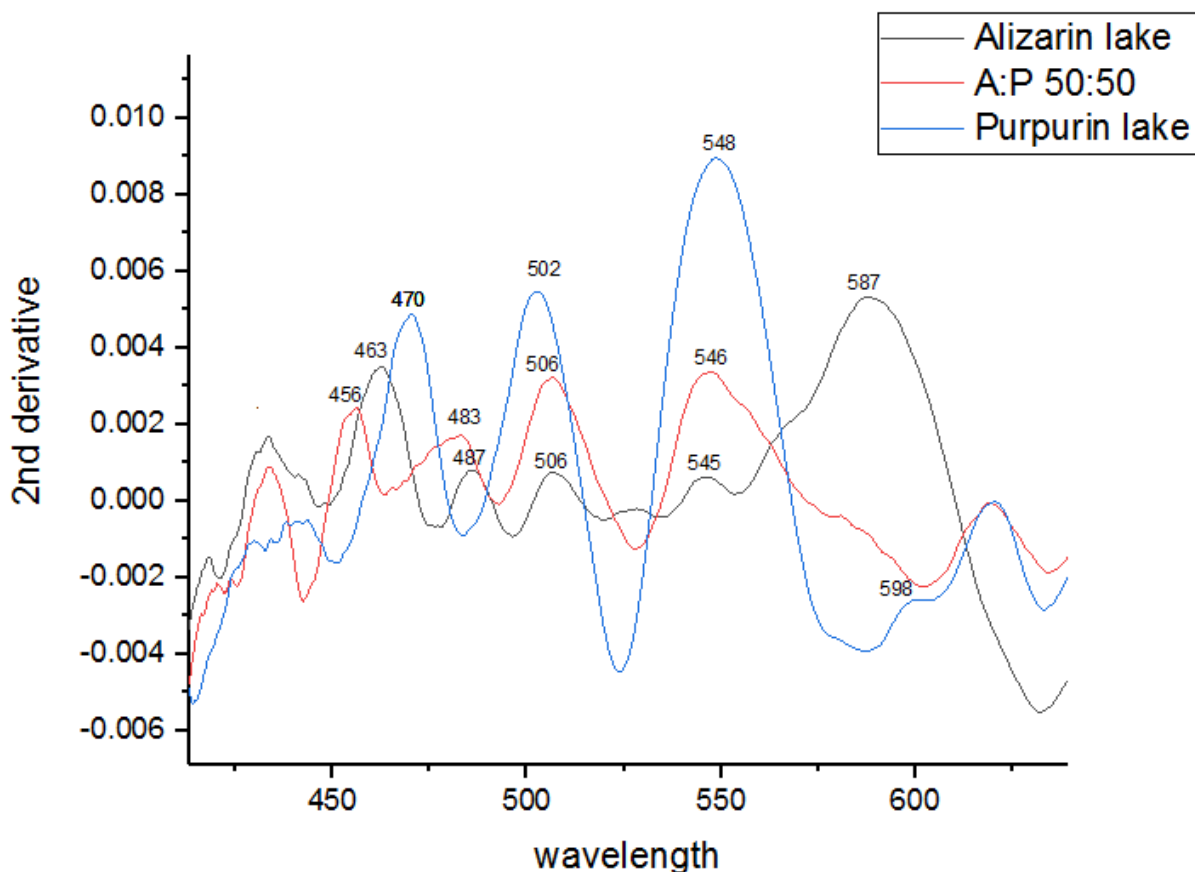


Figure 15. The second derivative FORS spectra of individual lakes of alizarin and purpurin, with a combination lake prepared by using 50% by weight each of alizarin and purpurin dyestuff as starting material. Absorption bands in the reflectance spectra can be clearly identified as positive peaks in the second derivative.

Similar analyses are discussed in section 3.1.5 comparing the spectra and examining the trends in the data.

SERS analyses of these mixtures were performed as well, using the spectrum of a A-(Al(III)):P-(Al(III)) 30:70 along with those of the individual alizarin and purpurin lakes and the AgNPs. Major peaks for the A-(Al(III)):P-(Al(III)) 30:70 mixture were recorded at 467, 483, 612, 916, 999, 1024, 1149 (could be an artefact) and 1172 cm^{-1} (Figure 16). The peak of this mixture at 483 cm^{-1} corresponds to the 477 cm^{-1} peak of the alizarin lake, which means that the Raman peak of the mixture is blue-shifted as compared to the peaks in the spectra of alizarin and purpurin lakes.

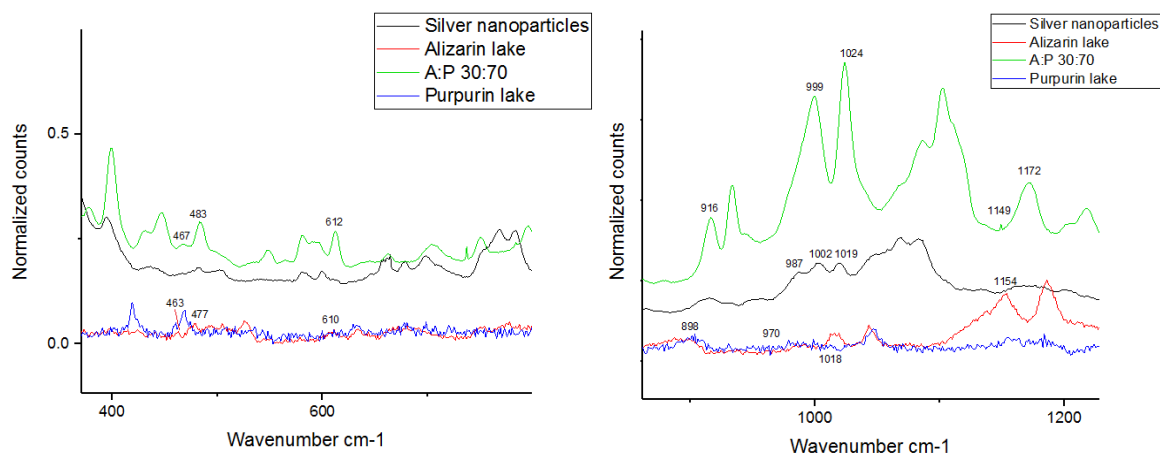


Figure 16. SERS spectra of alizarin lake, purpurin lake, silver nanoparticles and the A-(Al(III)):P-(Al(III)) 30:70 to compare peaks. Two ranges are magnified and inspected to find peaks near the suggested marker band wavenumber values.

3.1.4 Madder lake

Madder lake was prepared as described in section 2.1.1. Lime water medium was used as the medium per the recipe from the Stockholm papyrus and other published literature indicating that calcium carbonate was used in the preparation of ancient madder lakes in ancient paintings [27]. Figure 17 shows the FORS spectra obtained for the recreated madder lake and its second derivative spectrum.

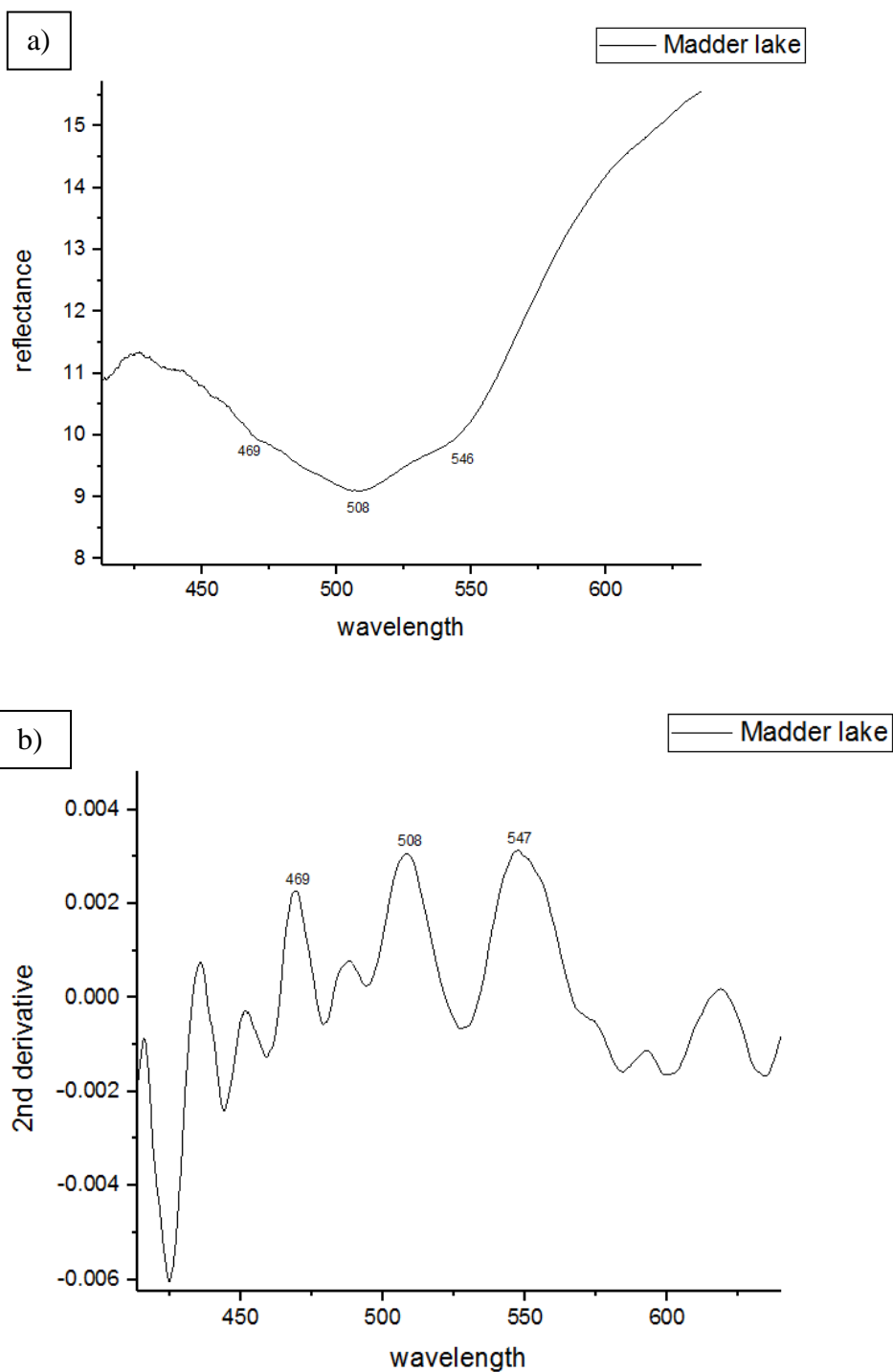


Figure 17. A comparison of the (a) reflectance spectrum of madder lake and (b) its second derivative. Reflectance spectra were obtained using Ocean Optics USB2000+UV-VIS spectrometer.

The absorption bands in the second derivative of the madder lake are observed at 469 nm, 508 nm, and 547 nm. Of these, the absorption bands at 508 nm and 547 nm are the most eminent in the reflectance spectrum. These spectra are compared to the other lakes of alizarin,

purpurin and their mixtures in the next section. Similarly, the SERS spectrum of the recreated madder lake (Figure 18) shows peaks at 488, 623, 880, 1001, 1024 and 1150 cm^{-1} , selected due to their proximity to the suggested marker bands, which will be used for comparison in the following section.

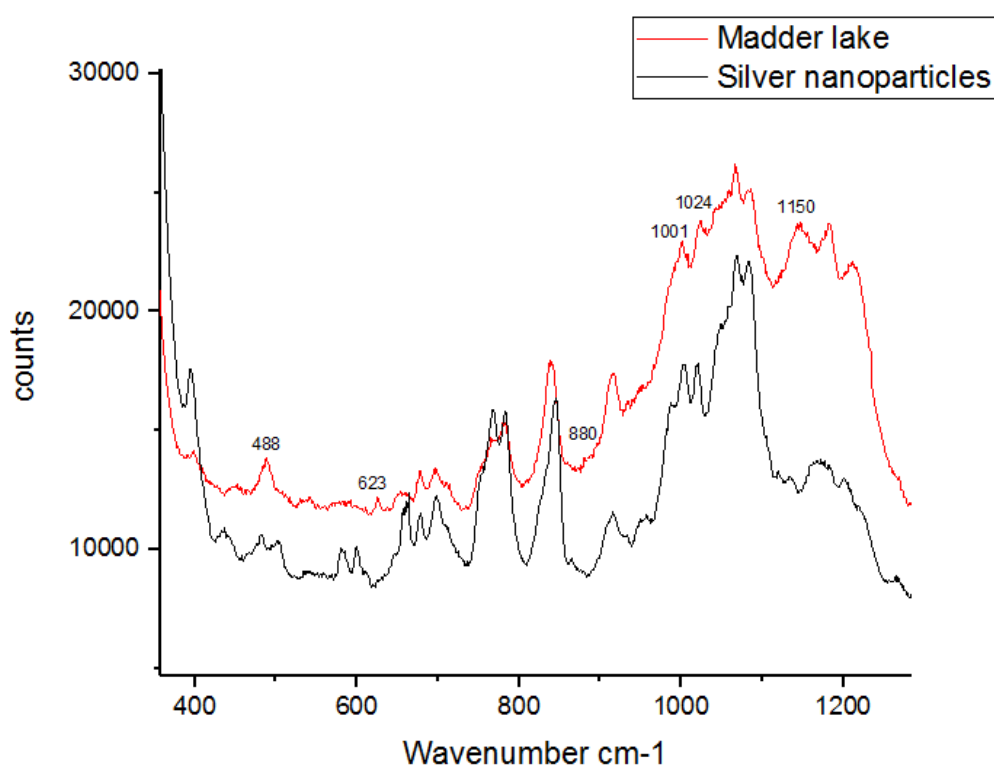


Figure 18. SERS spectra of the recreated madder lake and the AgNPs.

3.1.5 Comparisons and parallels

In this section, FORS data of the alizarin and purpurin dyes and the various recreated alizarin and purpurin lakes and their mixtures, are compared to the madder lake pigment made from the *Rubia peregrina* plant from Cyprus based on the ancient recipe in the alchemical papyrus.

Each dye was first individually compared to a 50:50 mixture of the dyes. As it can be seen in Figure 19, the reflectance spectra and the second derivative of the 50:50 mixture corresponds more closely to the spectra of alizarin rather than the purpurin dye. The absorption of the

purpurin dye, in comparison, is red shifted when compared to the alizarin dye and the mixture. This is also visually apparent, as purpurin dye is a dark, brick red color compared to alizarin dye which has an orange-red color. When the mixture was made as described in section 2.1.2, it was found to look more orange-red than brick red. Each dye has been compared to its lake earlier in the discussion.

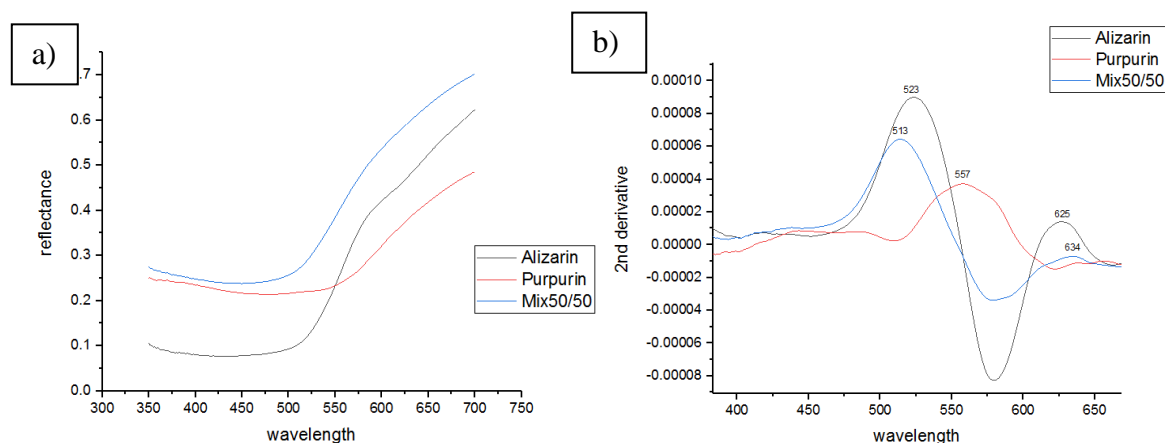


Figure 19. A comparison of the (a) reflectance spectra of alizarin and purpurin dyes and a mixture of the two dyes and (b) second derivative. Reflectance spectra were obtained using the Ocean Optics USB2000+UV-VIS spectrometer.

Figure 20 shows the FORS reflectance spectra and second derivative of the madder lake along with the lakes of alizarin, purpurin and the combination lake A-(Al(III)):P-(Al(III)) 50:50. The spectrum and second derivative of madder lake finds close affinities with both alizarin and purpurin and their mixture. At ~ 506 and ~ 546 nm, its predominant absorption bands match with those of the A-(Al(III)):P-(Al(III)) 50:50, similar to the bands of purpurin lake. Unlike the A-(Al(III)):P-(Al(III)) 50:50 sample, however, the madder lake also shows a weak absorption at ~ 593 nm, which is similar to the predominant peak of alizarin lake. This data should be looked at with caution as the different solubilities in water of alizarin and purpurin from the madder extract affect the FORS spectrum obtained. This caveat has been discussed further at the end of this section.

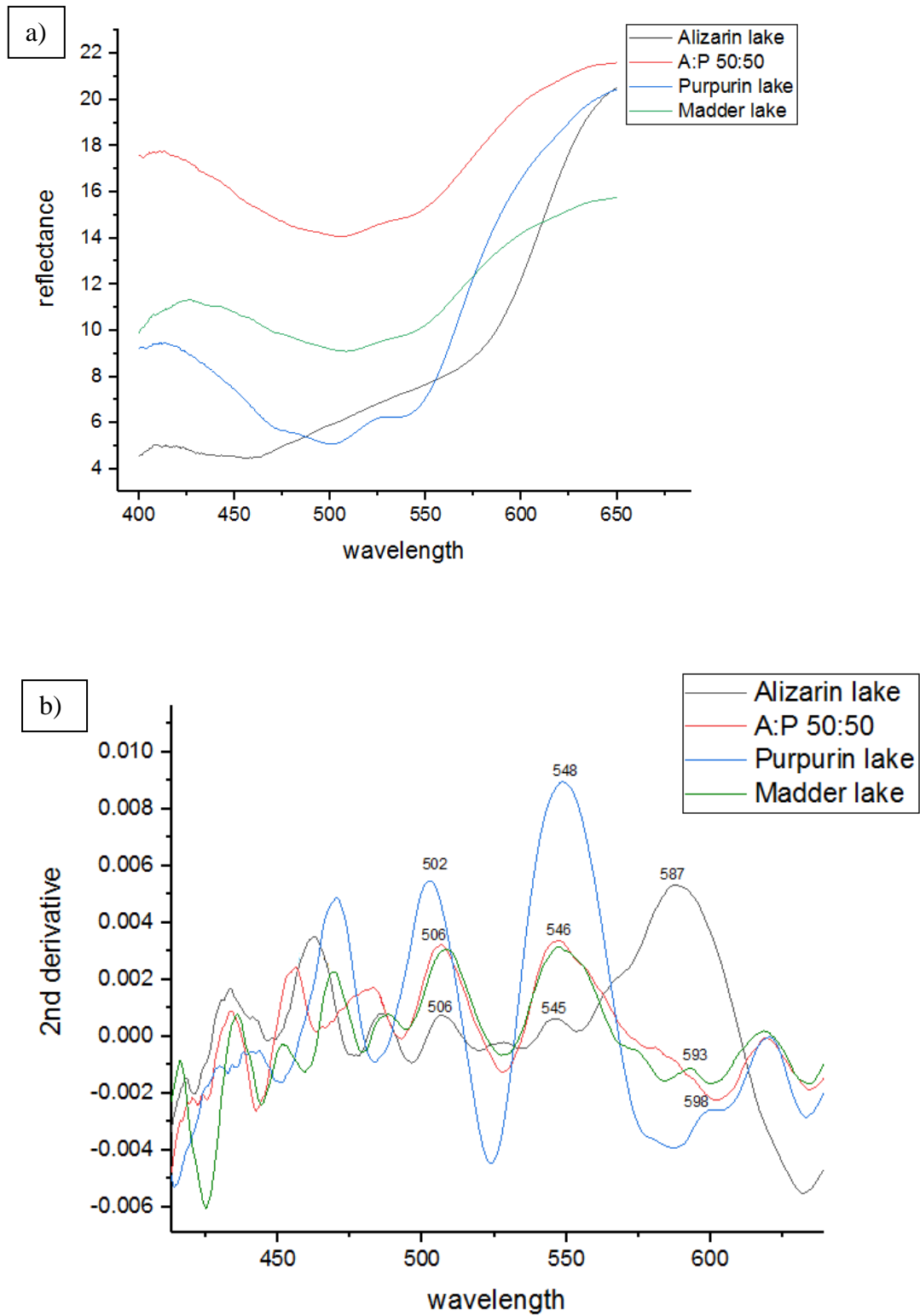


Figure 20. A comparison of the (a) reflectance spectra of alizarin and purpurin lakes, a 50:50 combination lake and recreated madder lake, and (b) second derivative. Reflectance spectra were obtained using the Ocean Optics USB2000+UV-VIS spectrometer.

In Figure 21, it is observed that in the 587-599 nm range, the A-(Al(III)):P-(Al(III)) 90:10 combination lake displays an absorption at 593 nm, which is red-shifted compared to the absorption of alizarin lake at 587 nm. In the range 462-470 nm, it is observed that the combination lake's absorption band at 462 nm is blue-shifted compared to the absorption of both individual lakes, with purpurin lake displaying an absorption at 470 nm and alizarin lake, at 467 nm.

In the 545-552 nm range, however, the A-(Al(III)):P-(Al(III)) 90:10 combination lake is red-shifted compared to both individual lakes, showing an absorption maximum at 552 nm. This could be due to the influence of the absorption of alizarin lake at 587 nm, causing the red-shift of the mixture due to the predominant presence of alizarin complex compared to the purpurin complexes in the mixture. A shoulder at around 560 nm also seems like it could be the effect of the absorption of alizarin. More in-depth research would be required to better understand these features.

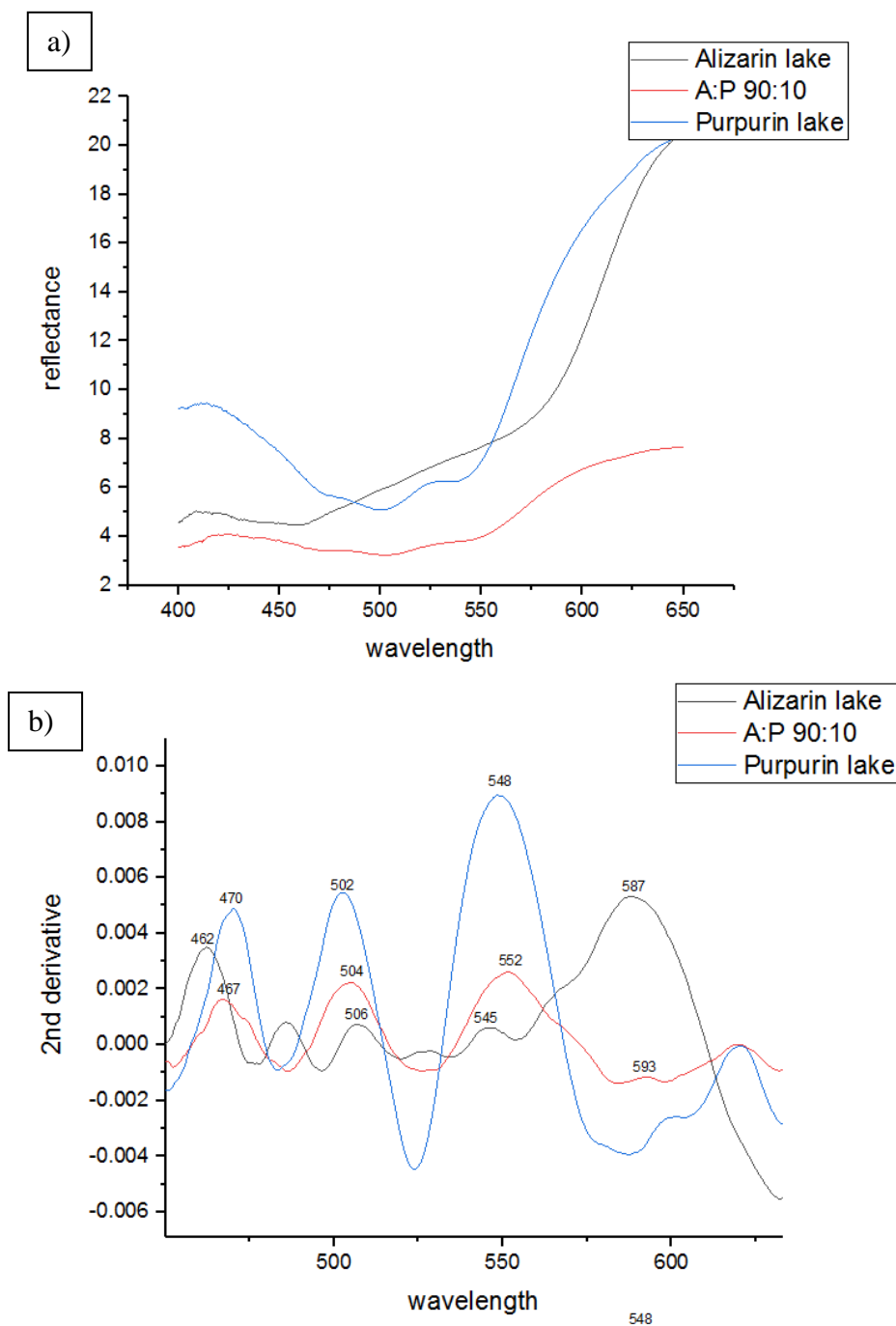


Figure 21. A comparison of the (a) reflectance spectra of alizarin and purpurin lakes and the 90:10 (alizarin/purpurin ratio by weight) combination lake (mixture), and (b) second derivative spectra. Reflectance spectra were obtained using the Ocean Optics USB2000+UV-VIS spectrometer.

To examine the contributions of the alizarin and purpurin components in the combination lakes (mixtures), the A-(Al(III)):P-(Al(III)) 70:30 and A-(Al(III)):P-(Al(III)) 30:70 samples were selected for further analysis (Figure 22). Here there seems to be a trend in the shifts

observed in the absorption bands. In the A-(Al(III)):P-(Al(III)) 70:30 mixture, which contains a higher amount by weight of alizarin, a red shift is noticed (with absorption maximum at ~548 nm compared to the absorption maximum of alizarin lake at ~545 nm). The mixture also shows three absorption bands at ~548, ~562 and ~580 nm which seem to be contributions from both the alizarin and purpurin lakes.

At ~500 nm (Figure 22(b)), a recognizable trait is noticed starting from the A-(Al(III)):P-(Al(III)) 70:30 combination lake showing an absorption band at ~509 nm, moving to the A-(Al(III)):P-(Al(III)) 30:70 combination lake which has a blue shifted absorption band at ~506 nm (compared to the alizarin/purpurin lake at 70:30 ratio), culminating in the absorption band of purpurin lake, at ~502 nm wavelength. While it is observed that slight addition of purpurin complexes cause a red-shift to the combination lake compared to the individual purpurin lake, the same occurs with a slight addition of alizarin complexes to a purpurin complex composition of lake (mixture).

The overall shifts at the two main absorption ranges seen in the FORS spectra of all the lakes (alizarin lake, purpurin lake and mixtures) can be seen in Figure 23(c) and there is the slight beginning of a non-linear trend observed of the narrowing and widening of the gap between the absorption bands obtained in the wavelength ranges 545-558 nm and 502-509 nm, which is almost sinusoidal. A greater set of reliable data points could be used to explain this behavior. Some of the aberrations in the data could also be due to the presence of un-complexed dye in the obtained precipitate. Insufficient addition of alum to complex all the dye molecules may have resulted in leftover dyestuff in the mixture and even though the precipitate was washed and filtered, it cannot be guaranteed that it completely removed all the dye that may have remained in the precipitate because the dyes are not completely soluble in water.

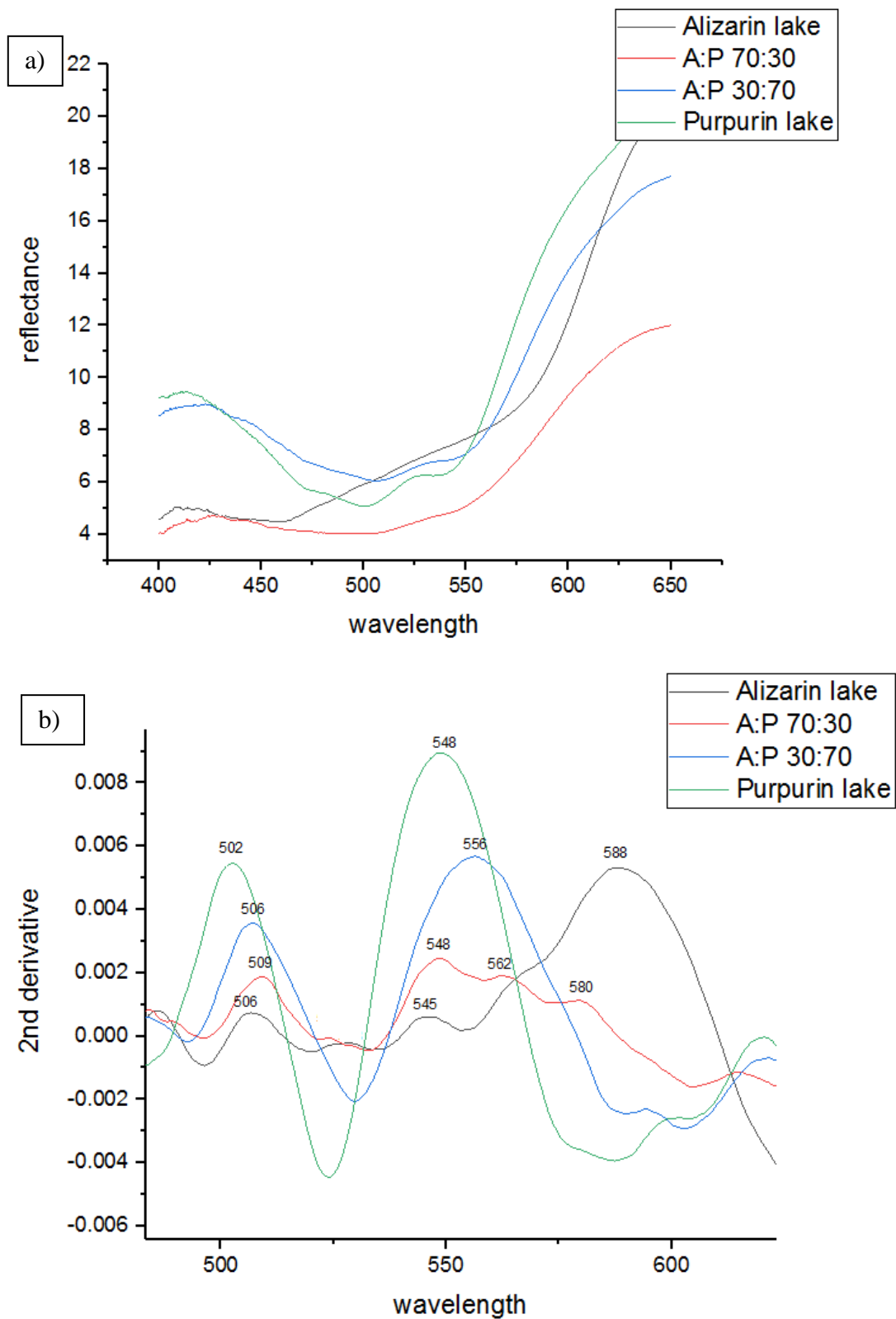


Figure 22. A comparison of the (a) reflectance spectra of alizarin and purpurin lakes, the 70:30 and 30:70 (alizarin/purpurin ratio by weight) combination lake, and (b) second derivative spectra. Reflectance spectra were obtained using the Ocean Optics USB2000+UV-VIS spectrometer.

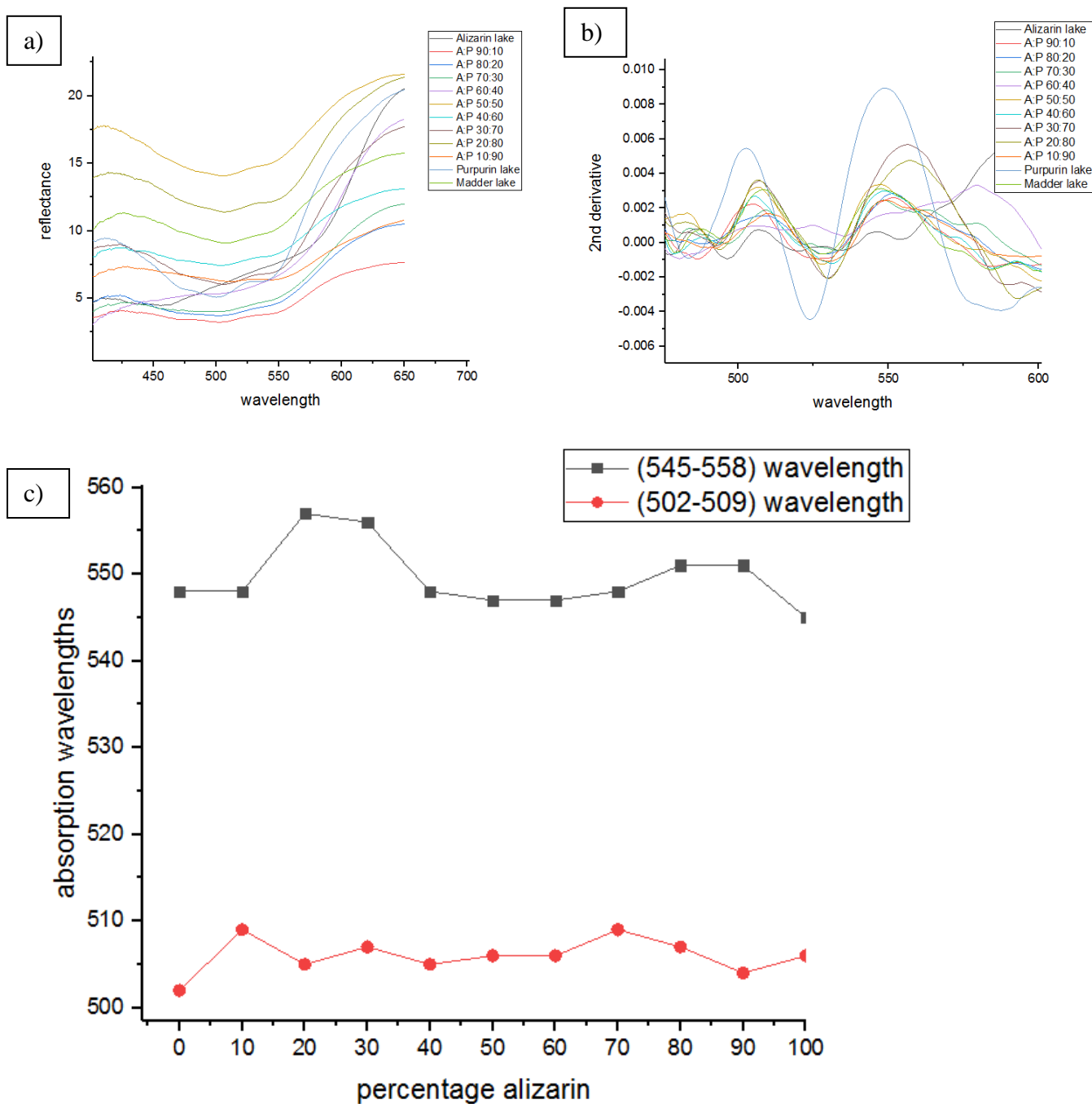


Figure 23. (a) Reflectance spectra of alizarin and purpurin lakes, all their combination lakes, and (b) second derivative. Reflectance spectra were obtained using the Ocean Optics USB2000+UV-VIS spectrometer. (c) Comparison of the absorption maxima (wavelength) of the lakes based on the percentage of alizarin in the initial dyestuff showing the relative red and blue shifts in the wavelength regions 545-558 nm and 502-509 nm.

Comparing the FORS data of the different ratios of alizarin/purpurin with the madder lake produced using the Cypriot plant, the closest match was to the A-(Al(III)):P-(Al(III)) 40:60 combination lake, with excess of purpurin complexes (Figure 24).

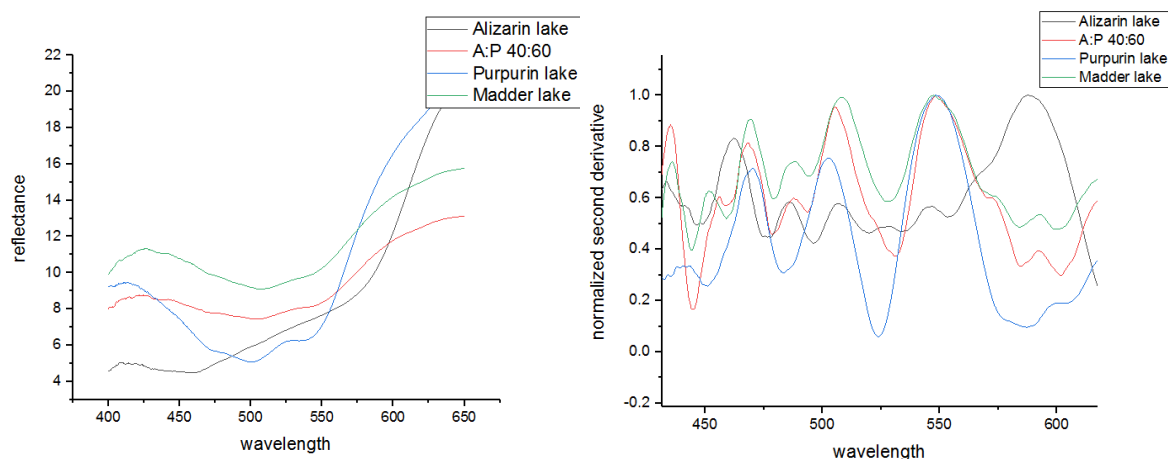


Figure 24. A comparison of the (a) reflectance spectra of alizarin and purpurin lakes, the 40:60 (alizarin/purpurin ratio by weight) combination lake, and the recreated madder lake; and (b) second derivative. Reflectance spectra were obtained using the Ocean Optics USB2000+UV-VIS spectrometer.

SERS data obtained from all samples are shown in Figure 25. Peaks were observed in the proximity of the suggested marker bands in the spectra (Figure 26).

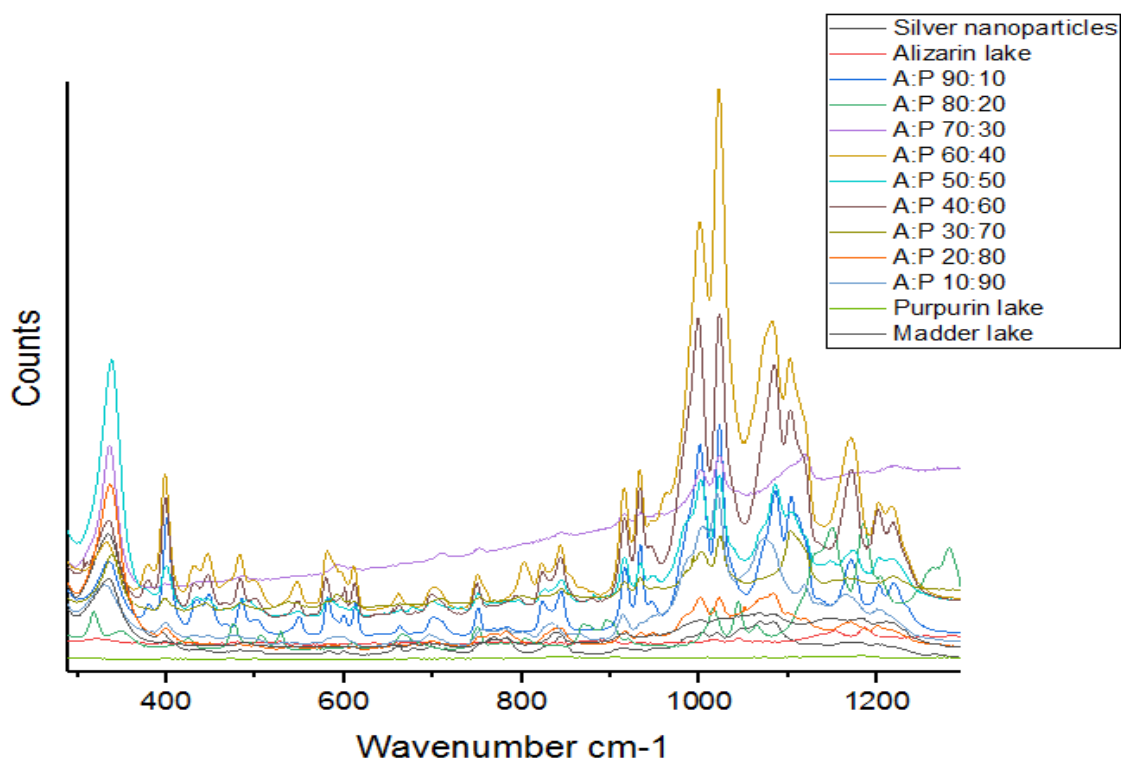


Figure 25. SERS spectra of all combination lakes, alizarin and purpurin lakes, recreated madder lake from the Cypriot plant and the AgNPs used as platform for SERS.

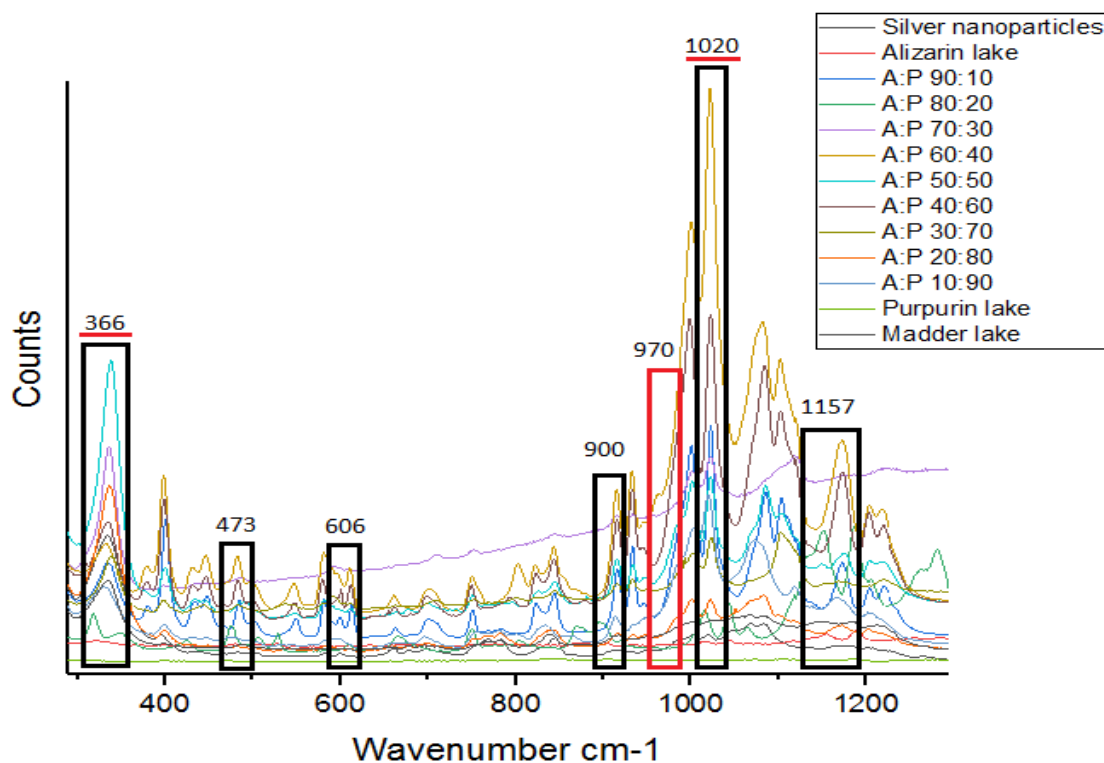


Figure 26. SERS spectra of all combination lakes, alizarin and purpurin lakes, recreated madder lake and AgNPs used as platform for SERS. Peaks in the proximity of marker bands for alizarin and purpurin dyes were observed and enclosed in borders labelled with the wavenumber of the assigned peaks.

Around 473 cm^{-1} , peaks are mostly observed between 475 and 484 cm^{-1} , with the majority of them being at a higher wavenumber than the marker band for alizarin dye. A similar trend can be observed for the shifts near 606 and 900 cm^{-1} , with the majority of the combination lakes showing shifts at higher wavenumbers when compared to the marker bands for the dye. At $\sim 970\text{ cm}^{-1}$, there were no recognizable peaks from any of the complexes except for that of the purpurin lake at 971 cm^{-1} . At 366 and 1020 cm^{-1} , there are strong peaks, which agree with the recommended peak value for purpurin, but this could be strongly influenced by the AgNPs. The peaks observed near wavenumber 1157 cm^{-1} , are quite small, and found to be mostly at lower wavenumbers.

Though the size of the data set for this study poses limitations to the interpretation, an attempt was made at consolidating the data obtained from SERS (Figure 27). Some data points are

missing because no peaks could be detected within the regions of interest (of characteristic marker bands). The curves have been normalized to facilitate comparisons, which have been made between the individual lakes, shown in the graphs at the positions of 0 and 100 (according to percentage of alizarin concentration by weight), while the remaining positions in between them correspond to the combination lakes of varying chromophore concentrations. Most peaks of the combination lakes are blue-shifted when compared to the individual lakes, in the surroundings of lower wavenumbers from 470 cm^{-1} to 1020 cm^{-1} , while near the higher wavenumber of 1157 cm^{-1} , red shifts are observed in combination lakes containing a lower percentage of alizarin by weight. However, the A-(Al(III)):P-(Al(III)) 80:20 seems to be consistently shifted to a lower wavenumber, which doesn't result in a good fit for the trend. This means that the spectra collected for this sample was inconsistent with rest of the spectra, and a reiteration of the characterization of this sample is necessary for future research.

Comparing all sets of SERS data, it is found that the recreated madder lake is very similar to A-(Al(III)):P-(Al(III)) 40:60, which is in agreement with the results from FORS as well. Figure 28 shows the spectra of A-(Al(III)):P-(Al(III)) 50:50, A-(Al(III)):P-(Al(III)) 40:60 and the madder lake, showing the close fit and the similar peaks. However, this result should be examined with caution as madder roots have a complex chemistry which could undergo chemical reactions to change the chromophore ratios, depending on the processing method used. Furthermore, the solubilities of alizarin and purpurin in water are different [5], and can be influenced by the complex chemistry of the madder extract, which could possibly change the pH of the solution. This means the madder extracted through this process cannot be said to clearly contain the ratio stated (A-(Al(III)):P-(Al(III)) 40:60), although it can be a good approximation for the composition.

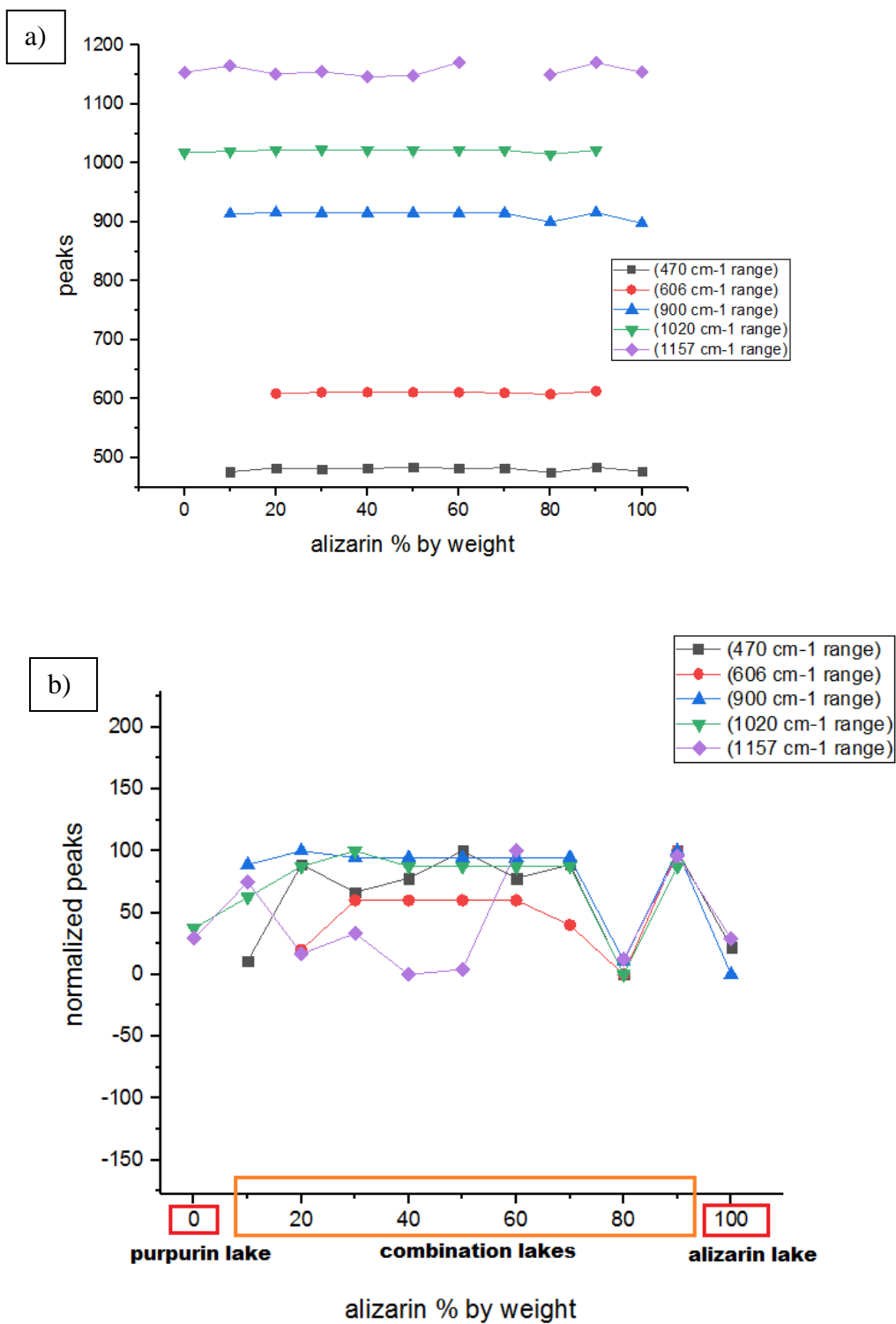


Figure 27. (a) Data of peaks (wavenumber cm^{-1}) versus the percentage (by weight) of alizarin in the lake observed in the vicinity of different marker bands of SERS spectra; (b) normalized form of the same data.

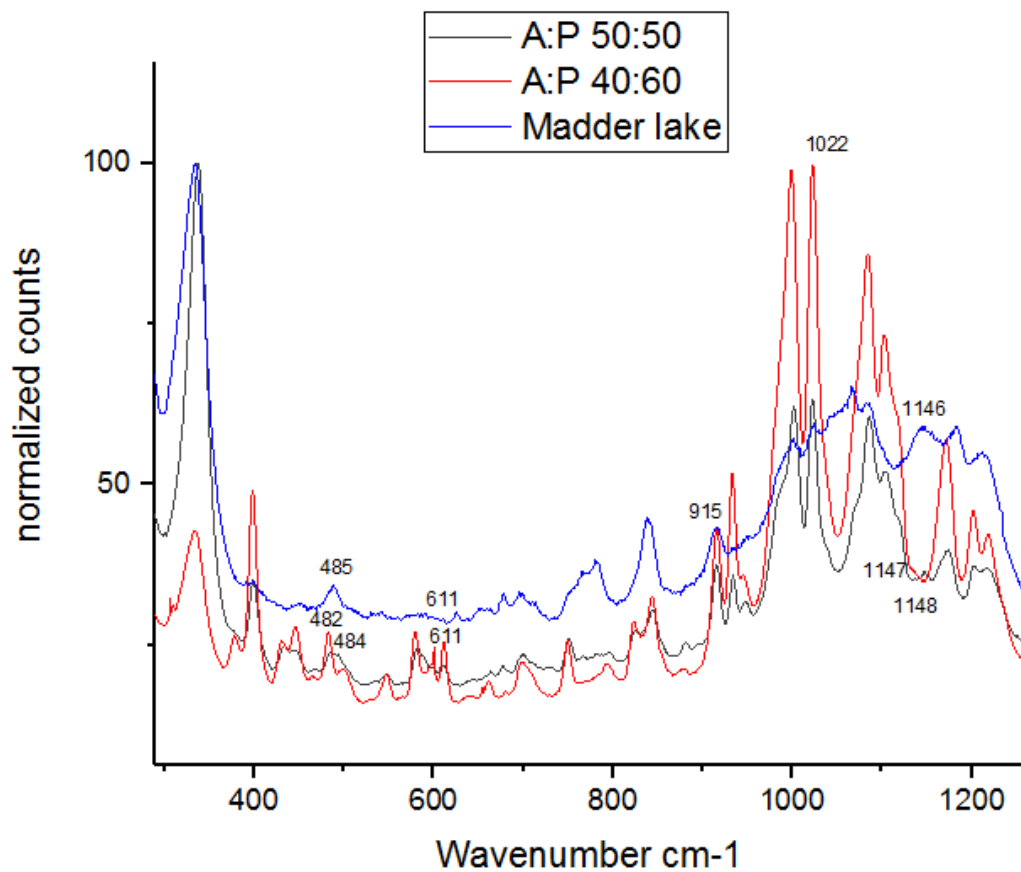


Figure 28. Comparison of SERS spectra of A-(Al(III)):P-(Al(III)) 50:50, A-(Al(III)):P-(Al(III)) 40:60 and madder lake shows similarity of the madder lake spectrum to both the complexes.

Chapter 4

4.1 Conclusions

The design of the experiments performed in this research aimed to examine the relationships between the photophysical and chemical properties of lakes containing different chromophores to their composition and relating the observed trends to the recreated madder lake based on the ancient alchemical recipe from the Stockholm papyrus.

Results provided an insight into the processes used to manufacture madder lake pigments in antiquity and how their chemistry and production technology influence their color and properties. Analysis was mainly based on fiber optics reflectance spectroscopy (FORS) and surface enhanced Raman scattering (SERS) which provided data that could be linked directly to raw materials selection and manufacturing processes.

FORS and SERS preliminary data suggested non-linear trends as the ratios between the alizarin and purpurin varied in the synthesized lakes. For example, in Figure 22(b), if the second derivatives of the FORS spectra of A-(Al(III)):P-(Al(III)) 70:30, A-(Al(III)):P-(Al(III)) 30:70 and purpurin lake are observed in that order, in the proximity of wavenumber 500 cm^{-1} , then a linear trend is observed in the shifts of the absorption band. However, when the absorption bands and Raman shifts of the full range of chromophore concentrations are observed, a more complicated trait emerges, as observed in the graphs in Figure 23(c) and Figure 27. As only the selected ratios (mixtures of the two chromophores) were produced and analyzed, these results may not be representative of the full spectrum. More data points – by varying the ratios of the chromophores – would provide an extensive dataset which can be used to draw further conclusions, and reiterated to eliminate the possibility of measurement error.

The recreated madder lake pigment displayed chemical and photophysical properties similar to A-(Al(III)):P-(Al(III)) 40:60 according to the FORS and SERS spectra, indicating that the Cypriot plant used for the production of the lake contained higher concentration of purpurin. This was expected given that the *Rubia peregrina* plant used in the recreation of the madder lake recipe, is known to contain more purpurin dyestuff than alizarin, often containing almost no alizarin [2]. The high concentration of alizarin to purpurin of ~40% in the madder lake produced from the Cypriot plant, could be linked to the process of preparation of the pigment, involving heat extraction of the dyestuff from the madder roots in a neutral pH environment. This process may have been more favorable for the extraction of alizarin compared to purpurin dyestuffs present in the roots, owing to the different solubilities of the coloring compounds. As modern industrially developed madder lakes commercially available today only contain synthetic alizarin, the presence of purpurin (even at very low concentrations) may be indicative of a natural dye as the source of the raw material. This could be useful not only in understanding production technology, but also in cases of disputed artifacts for authentication purposes.

4.2 Future research

Many questions have been raised through this research which require further detailed investigation. The observation of the fading caused in the purpurin lake preparation in lye (Table 1, Sample number 4) from a deep pink to a dull yellow, can be further investigated to check if it is caused due to a change in pH or a different reason. Previous research include fading studies on pigment reconstructions [28], titrations done to observe effect of varying chromophore ratios on chemical and photophysical properties [13, 24] and analysis done using varying molar concentrations of added alum solution to observe the effect on their photophysical properties [5], but not under the conditions of ancient pigment making, which is worth looking into.

Due to the complex chemistry of the madder root, detailed research on the components of the products at every step of the process of the production of madder lake is necessary to understand the chemical changes that take place throughout. Starting with the composition of the root itself, to the heated extract and its introduction to different media, an exhaustive analysis would help determine the variation in the chromophore ratios all through the process.

Further research on the entirety of the Stockholm papyrus recipes would further help understand the functions of the various materials used, as well as the operational sequences and how raw materials and processes can affect the chemical structure and properties of the lakes produced. There have been speculations about the reason behind the materials and procedures [29, 30], and experimental study on these specific topics will help confirm or deny them and provide much insight on the proto-scientific methods used in alchemy as to how much of it was based on rigorous experimentation and observation and how much was guesswork or embellishment. This would also help understand the evolution of a small part of the scientific method.

Appendix

Appendix A : The Stockholm papyrus recipe and its interpretation

The recipe used for this purpose of this study was taken from the Stockholm Papyrus. In the English translation by Caley [6], recipe number 107, “Dyeing in Rose Color” is the only recipe that uses madder exclusively to obtain a colorant. The process recorded in this recipe was used to dye wool, and had to be modified to obtain a lake pigment, by considering how much of the procedure affected the wool without affecting the pigment and not including those parts of the procedure. The recipe as found in Caley’s translation is as below:

107. Dyeing in Rose Color

Rose color is dyed in the following way. Smear the rolls of wool with ashes, untie them, end wash the wool in the liquid from potter's clay. Rinse it out and mordant it as previously described. Rinse it out in salt water after mordanting and use rain water (which is so) warm that you cannot put your hand in it. Then take for each mina of wool e quarter of a mina of roasted and finely pulverized madder end a quarter of a choenix of bean meal. Mix these together by the addition of white oil, pour it into the kettle and stir up. Put the wool in the kettle and again stir incessantly so that it becomes uniform. When it appears to you to have absorbed the dye liquor, however, brighten it by means of alum, rinse it out again in salt water, and dry it in the shade with protection from smoke.

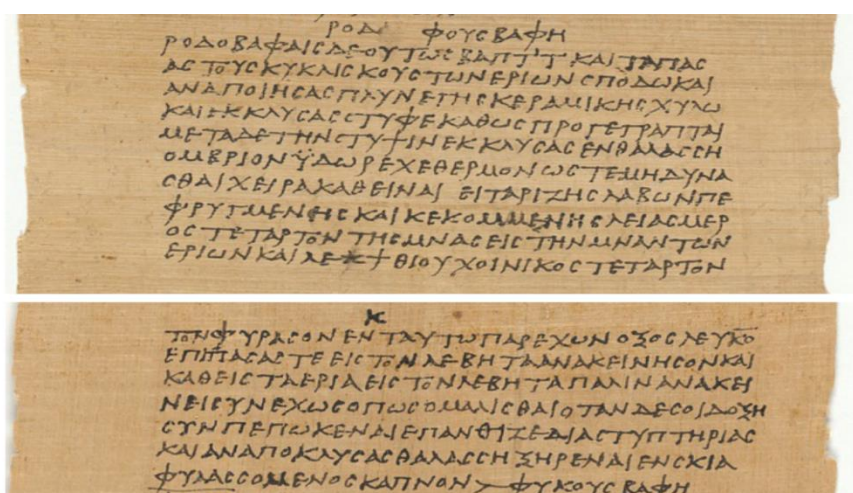


Figure 29. Image of the recipe number 107 in the original document of Stockholm papyrus.

Caley's translation of the ancient recipe provides much insight into some of the materials used in dyeing wool with madder, but Robert Halleux's translation of the same recipes offer some more clarification with his French translation of the same text. The English translation of Robert Halleux's French text, courtesy of Google Translate, Dr. Christina Viano* and Dr. Christian Fischer† is as below:

Dye it in pink as follows: sprinkle the wool balls with ashes, make penetrate, wash with the potter's juice, rinse, mordant as described above. After mordanting, rinse with sea water. Have warm rain water so that you cannot plunge your hand. Next, take the dried and finely minced root, a quarter of a woollen mine, and a quarter of bean flour chenice. Mix together by pouring white vinegar. Stir in cauldron, stir, put the wool in the cauldron and stir again constantly to homogenize. When they appear to have absorbed you, give the shine by alum, rinse with sea-water, dry in the shade, taking care of the smoke.

This version of the translation offers some clarification of the recipe, particularly about the “white oil” mentioned in the earlier recipe, which turns out to be vinegar. There are several steps to this wool-dyeing procedure and it is not apparent which parts of it lend towards making pigments, but careful consideration has been taken to understand this recipe. To make better sense of the recipe, it has been broken down into three parts: treatment of wool, introduction of madder and introduction of alum.

While the introduction of madder and alum must both go into the pigment-making in order to fix the dye to the substrate, the treatment of wool is a complicated part because it has many steps and it is not clear which steps lead only to the roughening of the surface of the wool to increase surface area, and which steps introduce compounds to the wool that bond to it and further help the dye adhere to it. The process notes the use of ashes, which is an important

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ingredient in making lye, or potassium hydroxide, but it is not treated in a way that results in traditional lye being made [31]. The potter's juice might contain kaolin clay which has been used to make pigments before [7], but it is immediately succeeded by rinsing and mordanting. According to the recipe, mordanting must be done as described in a previous recipe in the same papyrus, which states the following:

87. Mordanting

Boil chalcantum and skorpiurus and employ for any desired color. These substances, however, also mordant all kinds of stones and skins.

Caley explains in his translation that *chalcantum* referred to either copper or iron sulfates or a mixture of these salts and *skorpiurus* could refer to one type of sapindaceous plant [6]. This treatment is meant to roughen the fabric to promote good adsorption, as well as for general cleaning of the fabric to ensure dust particles do not prevent the adherence of the dye. These steps are left out of the modified procedure used in this research.

It is then rinsed in sea water, followed by hot rainwater. This component has been introduced to the modified procedure as hot distilled water. It is better to extract dye from madder roots at a temperature that is below the boiling point [32], and the recipe itself does not say to boil this rainwater, the temperature of the water in the procedure used in this research was kept between 60 and 75 degrees Celsius.

The addition of dried and minced madder root was done as is. *Rubia peregrina* or wild madder was used in this recipe due to the probable abundance of wild madder compared to the cultivated version. The next steps in the procedure involves adding wool and bean flour. As the wool is the fabric being dyed, it was omitted. The function of the bean flour was not completely clear. While it has been speculated that it was added to prevent the action of lime compounds [29], it could have served different purposes too. Lime is usually used to degrease wool and remove wool fat [11] and further investigation is required to understand why this action is not needed in this recipe. Beans usually constitute of 40% starch [33] and could

have been used to stiffen the wool for later use, but it also contains significant amounts of phosphates and magnesium which may contribute to other chemical changes. As more research needs to be done on the function of bean flour on dyeing, it was omitted from the pigment making procedure. The recipe then moves on to adding white vinegar to the mixture, the translation provided by Halleux [30] here is more accurate as the word *όξος* translates to vinegar [34] rather than oil, as mentioned in Caley's translation. It is known that at the low pH the anthraquinones in madder are not ionized and hence remain in the neutral state [4], so an acid like vinegar with pH 3 would not readily allow for the formation of complexes. Supplemental research on this topic would help in determining the function of vinegar in the research and determine whether this was a reasonable addition to the recipe or not. For the purposes of this research, vinegar was not included. After this step the wool is added to absorb the colorant.

The use of milky lime water as a medium was investigated in this research due to the fact that calcium carbonate was used as a substrate to make pigments for wall paintings in antiquity [7, 27] and both lime water and burnt lime was used in dyeing in antiquity, with plenty of mentions in the Stockholm papyrus as well [6, 11]. The introduction of alum comprises the third set of steps in the process. The recipe directs the alchemist particularly to 'brighten' or 'add shine' to the dyes fabric with the use of alum. This is a form of post-mordanting, or afterchrome, which can be used for stable dyes [3]. It is in this step that the dye is fixed by complexation. In the modified procedure this is the last step, and it interesting to see that the phenomenon reflects the terminology used; indeed, the heated madder extract which turns reddish purple upon the addition of lime water is 'brightened' to a bright red color once the alum solution is added to it. Thus the final pigment making procedure modified from the aforementioned recipe is simpler than the papyrus recipe to observe isolated effects of the involved materials on the resulting pigment.

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