The chemical profile of *Rubia tinctorum* in wool dyeing and a novel fibre extraction method for compositional analysis

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The candidate confirms that the work submitted is his own and that appropriate credit has been given where reference has been made to the work of others.

Abstract

This report describes the understanding of natural dyes and the work necessary for analysis of historical textiles.

Chapter 1 provides a general introduction into the background of historical dyeing, the methods used to extract these natural dye compounds used and how synthetic dyes overtook natural dyes in the commercial world of dyeing.

Chapter 2 introduces and discusses the research undertaken with one particular natural dyeing plant, *Rubia tinctorum* (common madder). Dyeing studies were all performed on wool, and explore the dyeing properties of the madder plant. Dyeing studies with pre-mordanting and post-mordanting were undertaken.

Chapter 3 explores the extraction of natural dyes from *Rubia tinctorum* and their identification. Extraction and analysis studies were conducted to obtain the ideal method for acquiring glycosidic anthraquinone dye molecules that could be used in the dyeing process.

Chapter 4 focuses on identifying new methods to successfully remove glycosidic anthraquinone dye compounds from wool fibres dyed with *Rubia tinctorum*, whilst keeping their chemical structure intact. Further work in this chapter is undertaken to analyse glycosidic anthraquinone dye compounds successfully removed from photo-degraded wool fibres and the comparison with current literature techniques used for historical sample analysis.

Acknowledgements

Firstly I would like to thank Dr. Richard Blackburn and Professor Chris Rayner for giving me this opportunity to do this PhD. I would also like to thank them for their constant support and guidance throughout my first year.

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Table of Contents

1. Introduction

The overall aim of this research was to successfully analyse glycosidic anthraquinone dye molecules extracted from dyed wool samples. The objectives to achieve this aim were to analyse dyed wool samples with *Rubia tinctorum* and alizarin independently, to successfully extract glycosidic anthraquinone dye molecules from *Rubia tinctorum,* to understand the dyeing mechanism of glycosidic anthraquinone dye molecules compared to the aglycone counterparts and to undertake a study in extracting glycosidic anthraquinone dye molecules from wool samples using literature procedures and newly proposed methods.

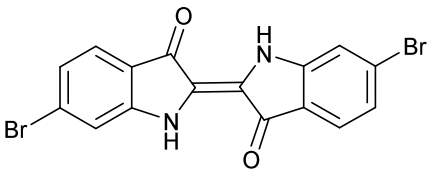
1.1. History of textile dyeing

The history of dyeing fabrics has to be intertwined with the development of several other aspects of making clothes and fabrics, which include such process as rearing of animals and the spinning and weaving of these materials.[1] It will never be known how the first practice of dyeing came into existence, but a plausible explanation is that accidental dyeing happened from coloured berries being dropped onto fibres.[2]

For work to precede on historical dyeing methods the first point of interest has to be the earliest history of mankind.[1] It has been discovered that historical textile dyeing took place as far back as 3000-2000 B.C., but there are few records of these very old dyeing methods available that provide an in depth explanation of how they were produced.[1] Dyeing has always been popular throughout the ages and civilisations have developed on the industry of dyeing; it was, and still is, a very lucrative business.[3] By the time of the Roman Empire, a palette of colours had been established and these natural dyes were being traded all around the world.[3]

Plants are the main source of natural dyes and such plants were grown all over the world, for example the blue dye indigo (C. I. Natural Blue 1) comes from several different species of plants; it is isolated from, Indian indigo (*Indigofera tinctoria*), grown across tropical Asia, woad (*Isatis tinctoria*) grown across Europe, and Japanese indigo (*Polygonum tinctorum*) grown in Japan and South East Asia. The dyes produced by plants (and other sources) are not just for mankind's decorative purposes but are produced for protection or defence purposes against diseases within the plant.[4] Another key historical dye comes from the Madder plant (*Rubia tinctorum*) giving a distinct orange-red colour.[3, 5-6] Madder was mainly grown in Indochina in the early centuries A.D. and continues to be harvested today for use in material dyeing, with a wide variety of species from the *Rubiaceae* family and these are grown in Europe, North and South America, India, and all over Asia.[6]

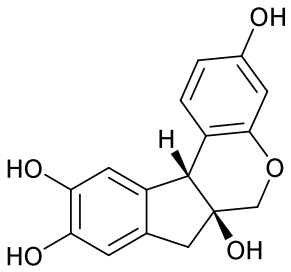
The Romans used dyes for the majority of their clothing, the most famous being, Tyrian purple (6,6'-dibromoindigo; **1.1**).[6] Tyrian purple is not derived from a plant source but from the shellfish *Murex brandaris*.[7] Only the Roman noble elite wore clothes dyed with Tyrian purple, as thousands of shellfish were required to make one ounce of dye.[6] The dye was secreted from the shellfish in a method known as "milking", or could be retrieved by simply crushing the shellfish; the latter method was not cost productive, but at that time it was more about displaying wealth and having the best attire instead of having an efficient and cost-effective production process.[6]



1.1

Not until the 15th Century was the first book on dyeing published in Europe; however, England had to wait until 1583 for the translated version.[5] Leonard Mascall translated this from Dutch to English; it was published in London entitled "A profitable boke declaring dyvers approoved remedies, to take out spotted and staines, in Silkes Velvets, Linnnen and Woollen clothes." [*sic*]

The discovery of the New World led to the unearthing of new natural dyes, examples being brazilin (C. I. Natural Red 24; **1.2**), acquired from the wood of the brazilwood family (*Caesalpinia sp*), and carminic acid (C. I. Natural Red 4; **1.3**), derived from the American cochineal.[3, 5-6] American cochineal is a scale insect, which is a parasite of the cactii plant and the dye is extracted by crushing the animal and collecting the dye.[3,8] Both brazilin and carminic acid are red dyes originating from the North and South Americas, which were subsequently sold all over world.[3]



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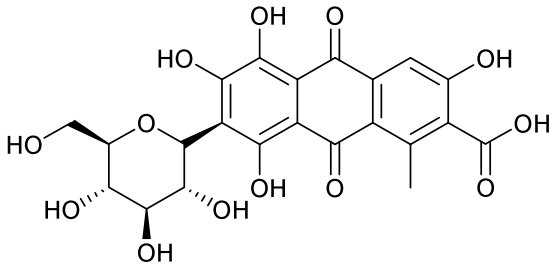


1.3

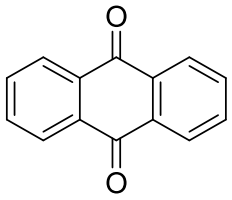
The industrial revolution brought about a rapid development in dyeing and textile manufacturing. In the 19th Century, chemists, as a result of pressure from the textile industries, began investigations into synthetic dyes; this move to cheaper synthetic dyes was the beginning of the end of the use of natural dyes on a commercial scale.[9-11] Indigo was one of the last dyes to be replaced by its chemically manufactured counterpart in 1899.[1] There were also technical dyeing issues with natural dyes: Natural dyes typically have lower affinities to textiles than

synthetic alternatives, often requiring a mordant.[6] A mordant is a metal salt that when in solution dissociates to a metal cation; it is believed that the metal cation co-ordinately binds to the textile first and then binds with the natural dye.[6]

1.1.1. Dyeing methods and natural dyes

There are three main groups of dyes, classified in terms of how they are applied to textiles:

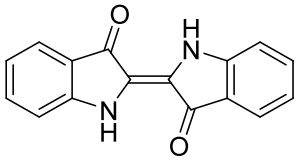
Mordant (or additive) dyes. A high proportion of natural dyes are additive dyes. This means they need a mordant to achieve the required adsorption on to the textile, this procedure is known as pre-mordanting (there is also a post-mordanting technique that helps degradation and fading).[12] This process can change the shade of the dye to give a more vibrant colour or, depending on the shade desired, can make it duller. The process of how a mordant works is still not entirely understood with controversy arising of how the dye-metal-fibre complex is formed.[13] One aspect known is that these mordants are stored as salts but when put into water they become ions, more specifically cations such as Al^{3+} and Fe^{2+}, and it is suggested that the dye can form various structural complexes around the cation.[14] Anthraquinones (**1.4**) are a prominent example of mordant dyes.



1.4

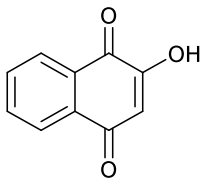
Vat dyes. These dyes are initially insoluble in water, requiring reduction achieved in a vat with the addition of a reducing agent and a base.[15-16] Examples used in the industry are caustic soda and sodium hydrosulfite. As a result of reduction the dye loses its colour, but is adsorbed on to the textile. Subsequent oxidation, most

commonly by oxygen from the air, causes the dye to regain its original colour.[16] Indigo compounds are notable examples of vat dyes; an example is indigotin (**1.5**).



1.5

Direct (or substantive) dyes. There are relatively few natural dyes that can be classed as direct dyes. Cellulose is commonly used as the fibre to dye with when using direct dyes. Salt is used in the process to make the surface of the fibre cationic in nature.[17] The molecular structures of these compounds possess polar functional groups that render the molecule substantive to the cationic chemical moieties in the fibres of the textile.[17] Napthoquinones such as lawsone (**1.6**) from the henna plant (*Lawsonia inermis*) and juglone (**1.7**) from the plant black walnut (*Juglans nigra*) are direct dyes. Other direct dyes include curcuminoids, and an example of this class is curcumin (**1.8**) from turmeric (*Curcuma longa*).



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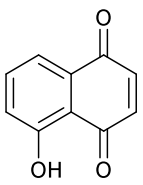

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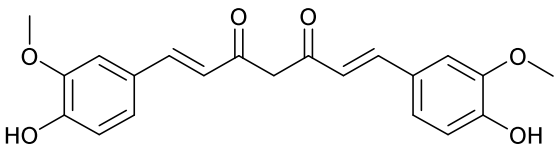

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Natural dyes contain a central chromophore, which is the part of a molecule that has a conjugated π-system. When visible light is shone onto the molecule an electron can be excited from a ground state orbital to an excited state orbital (Figure 1.1).[18-20] It is this absorption of a specific wavelength of energy, an electronic process, in the molecule that produces a colour detected by the human eye.[18-19]

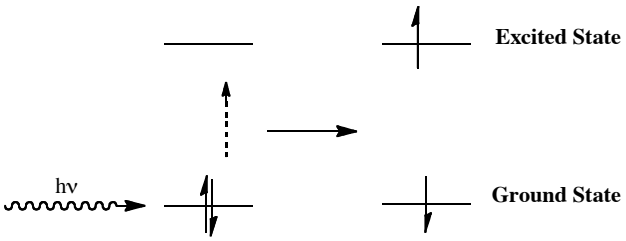


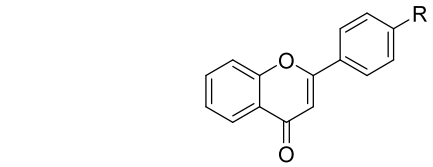
Figure 1.1. Energy level diagram representing the excitation of an electron from a ground state to an excited state through the energy of light**.**

Visible light is electromagnetic radiation emitted from the sun, this radiation can not be seen my the human eye it is the chemistry that happens within certain molecules that is reflected as colour.[18-19] When this light is shone onto compounds with conjugated π-systems, specific wavelengths are absorbed and it is the complementary colour that is observed. The colour observed is not "in" an object but it is the surface of that object that is reflecting the colour we see and absorbing all the other wavelengths of colour.[21-22] A table of the absorbed wavelengths and their complementary colours can be seen below in Table 1.1.

Table 1.1. Wavelengths of visible light broken down into absorbed colours and their respective reflected colours[6]

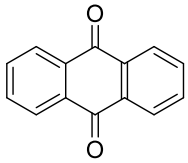
Wavelength (nm)	Colour of absorbed light	Colour reflected
400-420	Violet	Green-yellow
420-450	Violet-blue	Yellow
445-490	Blue	Orange
490-510	Cyan	Red
510-530	Green	Magenta
530-545	Green-yellow	Violet
545-580	Yellow	Violet-blue
580-630	Orange	Blue
630-720	Red	Cyan

The chromophoric molecules of some of the most common natural dyes can be found in Figure 1.2.



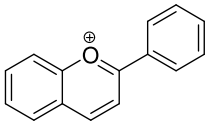
Flavonoids
R = H, Flavone or R = OH, Flavonol

Mostly yellow in colour



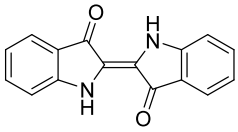
Anthraquinone

Yellow to red in colour



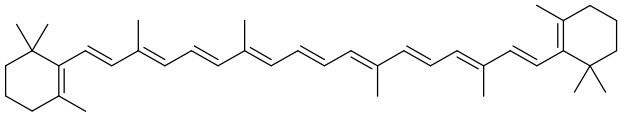
Anthocyanidin

Range of colours seen from orange to blue



Indigo

Mostly blue colours observed



Carotenoids
Example is of β-carotene

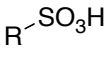
Yellow to red in colour

Figure 1.2. Chromophoric structures of common natural dye pigments.[3, 6]

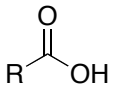
Auxochromes are substituents that can be found on these central chromophores, which can confer different sorption properties to these molecules, render the molecules soluble, and can widen the gamut of colours.[20] These substituents can be wide ranging, including hydroxyl (**1.6**), amino groups (**1.7**), sulfonic acid groups (**1.8**), carboxyl groups (**1.9**), and glycosides (**1.10**).[23]

(**1.6**)

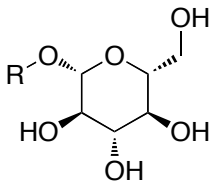
(**1.7**)



(**1.8**)

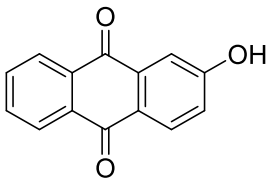


(**1.9**)



(**1.10**)

The nature of the glycoside substituent can also be used to determine to greater accuracy the species of flora or fauna used originally to produce the dye. In the instance of flavanoid dyes there are over 6500 known compounds including glycosidic structures.[24] These glycosidic moieties differ in quantity detected to their aglycone counterparts in different plant sources and their detection can help specifically pinpoint what species of plant was used in the dyeing process.[24] From structures **1.9**-**1.12**, it can be seen that the position of and number of hydroxyl groups can increase the wavelength of maximum absorption (λ_{max}). 2-hydroxyanthraquinone (**1.9**) has a λ_{max} of 366 nm, whereas 1-hydroxyanthraquinone (**1.10**) has a λ_{max} of 399 nm. Furthermore, 1,2-dihydroxyanthraquinone (**1.11**) has a λ_{max} of 426 nm, and 1,2,4-trihydroxyanthraquinone (**1.12**) absorbs at the longest λ_{max} by comparison at 480 nm).[25]



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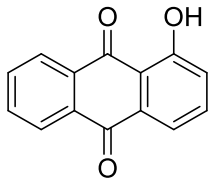

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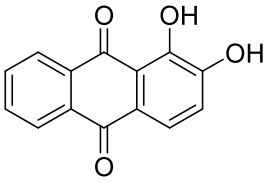

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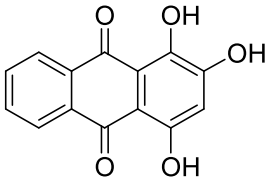
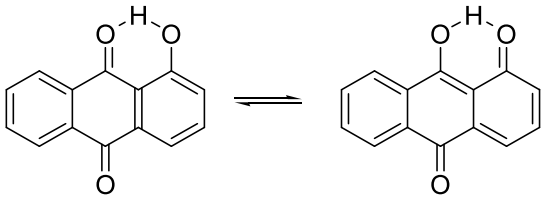


1.12

In the case of adding multiple hydroxyl groups, the conjugated system has become extended. Due to this increase in the conjugated system the molecule can absorb photons of a longer wavelength and the molecule ranges from red to yellow in colour.[26] The difference of 33 nm in the case of 1-hydroxyanthraquinone and 2-hydroxyanthraquinone is due to a phenomena known as, excited state proton transfer (ESPT).[27] Tautomerisation of 1-hydroxyanthraquinone (Scheme 1.1) is made possible by this ability to conduct intramolecular proton transfer, which makes the compound more photochemically stable.[28] The understanding of this phenomenon was very important to the work undertaken in this thesis. Knowing how conjugated molecules produce a colour will help in their detection and analysis especially in different chemical environments.



Scheme 1.1. Tautomerisation of 1-hydroxyanthraquinone through ESPT.[29-30]

1.1.2. Rubia tinctorum and the history of this famous red dye

The main focus of the work undertaken in this PhD was with *Rubia tinctorum*, which is one of the most widely known plants used as a source for natural dyes. *Rubia tinctorum* is a persistent herb and was cultivated all over the world, meaning however much quantity was needed it could be supplied.[1,6] Cochineal on the other hand required a vast quantity of scale insects to supply the world with a red dye.[6] The use of *Rubiaceae* species is littered all over historical literature, especially *Rubia tinctorum*, more commonly known as madder.[31] The name madder comes from the Old English name of *maedere*, which subsequently comes from the Old German word *mantara*, which is believed to originate from the Old Norse word *ma'dra*.[32] Gustav Schaefer wrote, "how closely the madder plant is linked with the idea of red is revealed by the names given to it in various languages."[33] An example is the Greek word erythrodanan for madder whilst the word, erythros means red in Greek.[32] Recipes of dyeing with madder have been found in the Papyrus Graecus Holmiensis dating back to around 300 AD.[34] The oldest ever finding of a textile dyed with madder was a cotton fabric that dates back to 3000 BC and was discovered in Mohenjo-Daro in the Indus valley.[35]

Madder stores the dye compounds within its roots, and these anthraquinonoid compounds are responsible for the chromophore in the natural dye.[6, 36] *Rubia tinctorum* is one of over 13,000 different species of the *Rubiacaece* family currently known.[37] The genus *Rubia* has over 80 different species with all of these containing various amounts of the anthraquinone derivatives within them.[38] Aglycone anthraquinone compounds are present in the *Rubia* genus however there has been the analysis of several glycosidic anthraquinone been reported.[39-41] Additional species include *Rubia peregrina L.* (wild madder), *Rubia cordifolia*

(munjeet), and *Galium verum L.* (ladies' bedstraw).[16] The roots of *Rubia tinctorum* are covered with a rind, beneath which lie the anthraquinone dye compounds, giving a dark red colour.[32] The roots are harvested in the autumn months when the plant is between 15 months and 3 years old. *Rubia tinctorum* does not grow north of 52° longitude and grows only in the modest climate.[32] Extensive work in the extraction and analysis of *Rubia tinctorum* has determined the compounds responsible for the colour produced, leading to the discovery of 36 different anthraquinones in the root.[1,3,5,42-45] The production of madder became hugely important and lucrative and by the 16th Century the Ottoman market was the largest in the world.[32] Around the 17th Century this was mainly due to the vastly sort after Turkish Red dye technique.[32, 46-50] For madder dyeing on plant-derived fibres like cotton, the Turkey red process was undertaken and it was a complex process involving 7 main stages (in some instances up to 20 stages were used) to produce the sort after red colour.[46-50]

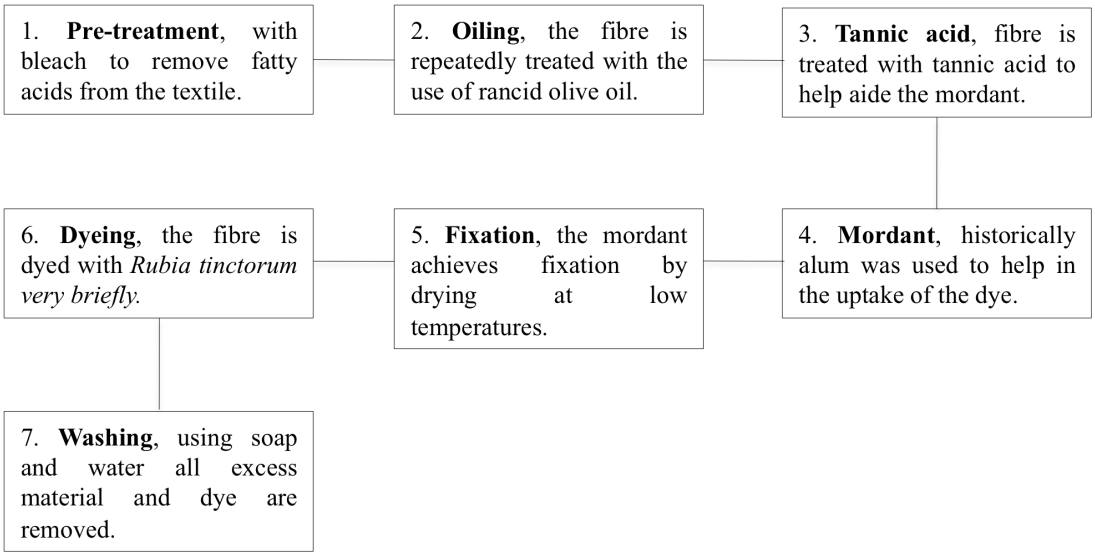


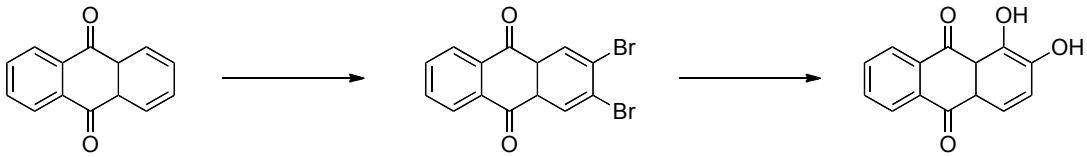
Figure 1.3. Turkey Red dye process[46]

Britain imported the majority of its madder and before the 1860's the cost of importing had reached to £800,000 annually.[32] Britain eventually became the

world's largest consumer of *Rubia tinctorum* and took about a third of the worlds exports and in 1869 it was valued at £1 million annually.[32]

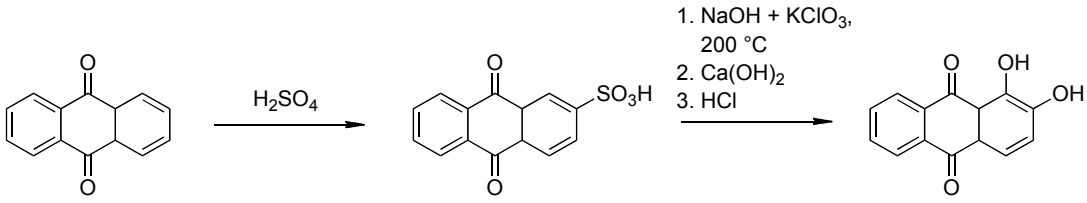
In the 19th Century there was a race between several different groups to understand what yielded madder its dyeing properties.[51] Pierre-Jean Robiquet in 1826 reported the two anthraquinones alizarin (1,2-dihydroxyanthraquinone; **1.11**) and purpurin (1,2,4-trihydroxyanthraquinone; **1.12**) were present in madder.[43]

Alizarin was the first natural dye to be synthetically made. It took over 40 years to make this possible, and was not until 1868 when German chemists Graebe and Liebermann synthetically made alizarin *via* the anthraquinone compound as seen in Scheme 1.2, which was made from anthracene, itself extracted from coal tar.[43]



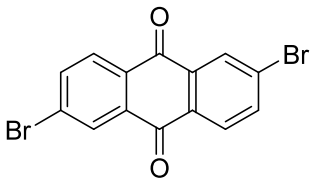
Scheme 1.2. Original synthetic route to produce alizarin.[43]

The process did not actually yield the 1,2-dibromoanthraquinone as they thought, but 2,3-dibromoanthraquinone as shown above in Scheme 1.2; they were very lucky that after boiling off the water and working up with base it resulted in the synthesis of alizarin.[43] This was not a viable option for using on-scale because bromine was very expensive and also the alkaline conditions required the use of vessels that had to resist this dramatic change in pH.[43] This lead to a new route to synthesise alizarin and this exploited an 1867 process of converting a sulfonate group to a phenol group, as shown in Scheme 1.3.[43]



Scheme 1.3. Improved synthetic route for the production of alizarin[43]

However work conducted by W.H. Perkin has confirmed this hypothesis as a myth as this would be a most improbable reaction.[52] Graebe and Liebermann synthesised two dibromoanthraquinone products, one by direct bromination of an anthraquinone and another by oxidation of 2,3,9,10-tetrabromoanthracene and assumed both compounds produced were identical.[53] Perkin discovered that to synthesise 2,3-dibromoanthraquinone the reaction is the oxidation of 2,3,9,10-tetrabromoanthracene.[52] The explanation Perkin gave for this transformation is what we would now deem to be sequential nucleophilic displacement reactions;[53] the product that would be obtained from the method involving direct bromination of an anthraquinone molecule would be a mixture of 2.6-dibromoanthraquinone (**1.13**) and 2,7-dibromoanthraquinone (**1.14**).[53] At the same time in the 19th Century chemists were acquiring modifications in madder extract colours by chemically modifying them.[54] One process led to the production of garacine, the French for madder, by hydrolysis of the compounds extracted.[54] The aim was to increase the dye uptake onto the fibre and produce a more intense red colour, by hydrolysing the glycosidic anthraquinone compounds.[50, 54]



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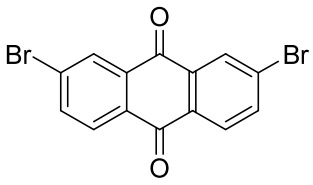


1.14

1.2. Dyeing of Wool

1.2.1. Wool structure

For centuries wool has been used as a fabric to dye upon and in turn many historical textiles are made of wool.[1] Wool is a proteinaceous fibre, classed in a group of proteins known as keratin, along with human skin, hair and nails.[55-56] Primarily derived from sheep and a select few other animals with the overall physical structure shown in Figure 1.4.[57] Before the wool can be used in the dyeing process it has to be cleaned of all the lanolin (wool grease), dirt and suint (sweat salts); once the wool has been thoroughly cleaned it can then be scoured. Historical scouring used soap and wood ash.[2] Until the 1950s, the industrial process of scouring was carried out with soap and soda ash (sodium carbonate).[58]

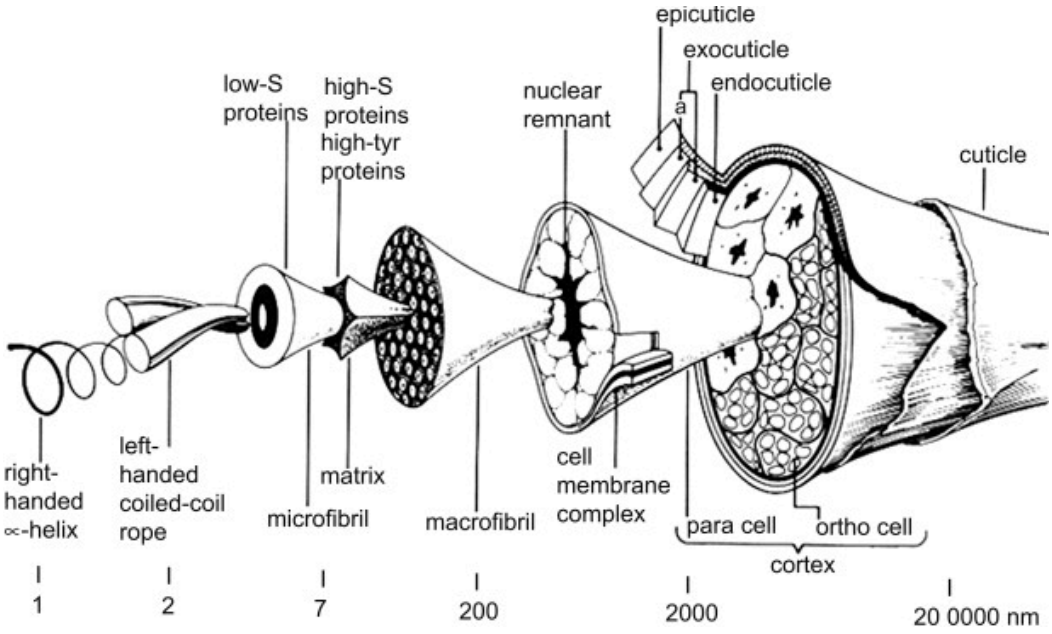


Figure 1.4. CSIRO schematic diagram of wool fibre.[57]

The chemical structure of wool can be seen in Figure 1.5, demonstrating that wool does not have a homogenous structure; it is more complex and random due to thousands of years of evolution.[57] There are several interactions within the chemical structure of the wool ranging from hydrogen bonding to intermolecular disulfide cross-linkages. All these interactions give the wool its strength and are why it has been used in textile manufacturing for thousands of years. These interactions can be used in the dyeing process, such as the ability to form hydrogen bonds with dye compounds, π-π stacking with aromatic compounds and ionic interactions with acid dyes (Figure 1.5).

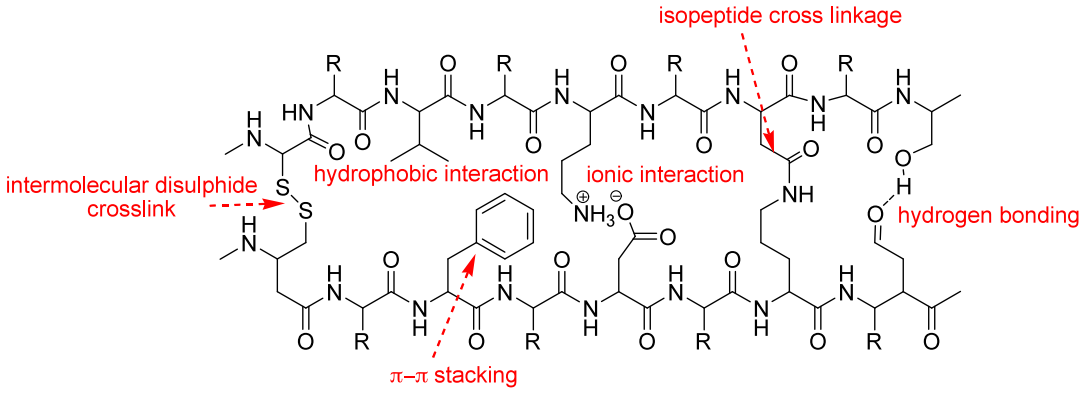
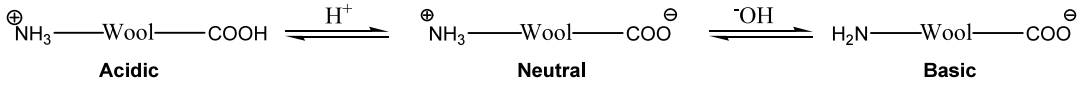


Figure 1.5. Chemical bonds within the structure of wool (R = 18 different amino acid groups).[57]

Wool is a zwitterion at neutral pH, as can be seen in Scheme 1.4.[55] The amine group can become protonated ($–NH_3^+$) and the carboxylic acid group can become deprotonated ($–COO^–$) group.[57] Under acidic conditions the carboxylic acid groups will become protonated again and exist in their neutral form (–COOH), however, under basic conditions it is the NH_3^+ group that becomes deprotonated and exists as a free amine group ($–NH_2$).[57] This ability to change the chemical properties of the wool with ease helps in the dyeing processes, as most of the natural dye compounds used are pH sensitive. The ideal pH for dyeing wool with an acid dye

like alizarin is <7.[17,57] At an acidic pH any amine group on the wool will become protonated (as seen in Scheme 1.4), therefore any carboxylic acid group will exist in their neutral form meaning they can from hydrogen bonds with the polar functional groups (hydroxyl and carbonyl) on the alizarin molecule.[17,57]

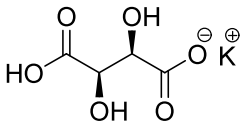


Scheme 1.4. Simplified graphical version of wool in different pH conditions.[57]

1.2.2. Mordanting process

The term mordant comes from the *Latin* "to bite" and is the process when a metal (inorganic salt) is fixed upon the fabric in the dyeing procedure to increase the fixation of the dye.[59] There are two types of mordanting processes, the first is called pre-mordanting and is the method that fixes a metal cation to the fabric before the fabric has been dyed with the dyestuff compound.[60] The second is called post-mordanting and is the method that fixes a metal cation to the fabric after it has been dyed with the dyestuff compound.[60] In both circumstances the use of the mordanting process is to produce better fixation and light/wash fastness properties of the dyestuff compounds.[6,60] However, in the case of the post-mordant it is also used to modify the colour previously dyed upon the fabric.[6,36,60]

In Europe, pre-mordanting was the most commonly used method historically with the most common being the use of alum.[6,36] Alum, also known as potash alum, is chemically known as hydrated potassium aluminium sulfate ($KAl(SO_4)_2.12(H_2O)$).[6] Alum occurs naturally and is found in several minerals such as kalinite, alunite and leucite.[1] Alum was commonly used in conjunction with cream of tartar, chemically known as potassium hydrogen tartrate ($KC_4H_5O_6$; **1.15**).[6]



1.15

This compound is primarily found on the inner sides of wine casks as a salt deposit.[32] Wouters and Verhecken showed that the addition of cream of tartar increases the quantity of the dye adsorbed onto the fabric, in this instance the fabric chosen was wool.[61] Cream of tartar was added to stop the fibre becoming too adhesive by making it softer.[62] There are several other mordants that can be used in the pre-mordanting process as shown in Table 1.2.

Table 1.2. Different mordants used in the pre-mordanting stage, application conditions, and resultant enhancements.[6]

Mordant	Temperature	Time	Enhancement
Alum & cream of tartar $(KAl(SO_4)_2.12(H_2O)$ & $(KC_4H_5O_6)$	90 °C	60 min	Brightens colours
Tin mordant $SnCl_2$	90 °C	45 min	Brightens colours
Chrome mordant $K_2Cr_2O_7$	90 °C	60 min	Darkens warm colours

The post-mordanting method is used to intensify the colour dyed upon the fabric, fix the dye more permanently to the fabric or modify the shade of colour already dyed upon the fabric.[32] The mordant does not increase the quality of the dye at all it only is used to change the colour of the dye and for better fixation.[46] It can be used in combination with the pre-mordanting process to help obtain different shades of the same dyestuff.[32] Several post-mordants are shown in Table 1.3, along with their effects on the dyestuff dyed upon the fabric.

Table 1.3. Different mordants used in the post-mordanting stage, application conditions, and resultant enhancements.[6]

Mordant	Temperature	Time	Enhancement
Iron & cream of tartar $FeSO_4.7H_2O$ & $(KC_4H_5O_6)$	90 °C	15 min	Dull the colour or if tannins are present produce black colours
Tin & cream of tartar $SnCl_2$ & $(KC_4H_5O_6)$	90 °C	15 min	Brighten colours
Chromium & cream of tartar $K_2Cr_2O_7$ & $(KC_4H_5O_6)$	90 °C	15 min	Warm under glow to reds and oranges and darkens all other colours
Copper $CuSO_4.5H_2O$	90 °C	30 min	Greenish or bronze tone to yellow and darkens other colours

As stated before, the exact chemical mechanism involved in mordanting has not been uncovered for the process involving dye-metal-fibre but has been extensively researched involving various dyes and metal interactions.[13,63-69] It is hypothosised that when the metal salt is dissolved into the water the metal will become a cation (*e.g.* Al^{3+}, Sn^{2+}) and can then interact with the fabric.[60] Wool has many different chemical molecules present as can be seen in Figure 1.4 hence; there are many sites for possible chemical interactions to take place between the wool and the metal cation.[70]

The example shown in Figure 1.6 illustrates the complexation of an aluminium ion with wool through the ketone of the amide group and a hydroxyl functional group from an amino acid side chain (serine is the side chain shown). The ligands will be very strained due to the orientation around the relatively large aluminium metal centre. There are 18 different amino acid groups in the chemical composition of wool. However not all the amino acid groups have functional groups that can bind to the aluminium ion so this diminishes the number of sites the aluminium can bind successfully to the wool.

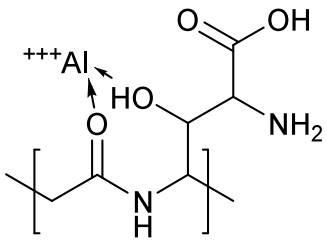


Figure 1.6. Model of a section of wool with a serine group binding to an aluminium cation through a ketone and a hydroxyl group.

In the literature there are many computerised models of the binding and orientation of alizarin towards an aluminium metal; two possible coordination sites have been proposed and are shown in Figure 1.7.

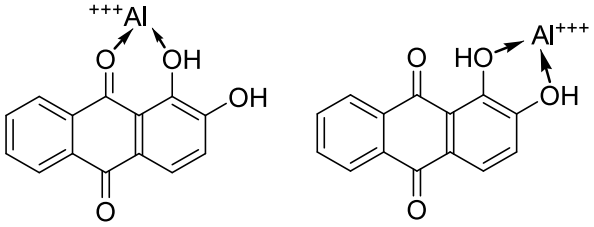


Figure 1.7. Two possible coordination sites formed between alizarin and Al^{3+}: Kiel and Heertjes model[64] (left); and Wunderlich and Bergerhoff model[67] (right).

From these models Sanyova confirmed using electron mass spectrometry (ESM) that two different binding coordinations (Figure 1.8) around an aluminium metal centre do occur, where there are two alizarin molecules for every aluminium metal ion.[71] Hence, if the two suggestions are brought together, the idea of the wool coordinating to the aluminium ion (Figure 1.8)[71] and the demonstration that two alizarin molecules can bind to the aluminium metal centre, a reasonably hypothesis for the chemistry that can occur in the dyeing process can be proposed (Figure 1.9). This model has never been proven in the literature, but it is a proposition to what is actually happening during the dyeing process when natural dyes are dyed with pre-mordants.

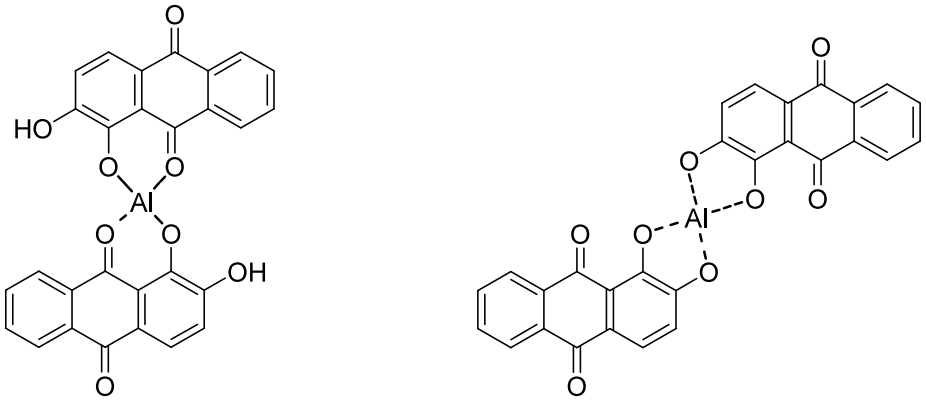


Figure 1.8. Structures of alizarin-aluminium complexes. Ions detected by SIMS, m/z = 505, z = +1 (left) and m/z = 503, z = –1 (right).[71]

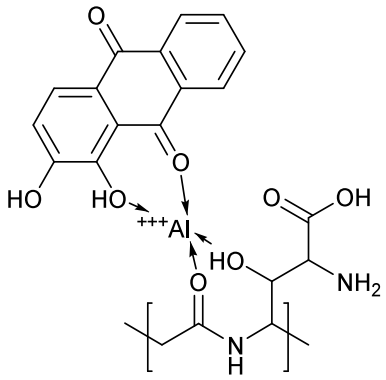


Figure 1.9. Hypothetical structure of an alizarin molecule coordinating to an aluminium metal centre after the pre-dyeing process when the aluminium metal is bound to the internal wool structure.

This hypothetical complexation of aluminium with alizarin and a section of wool has been proposed due to the fact that the addition of aluminium happens separately and before the dyestuff compounds are added.[6,36] Therefore the aluminium metal must bind to the wool through its functional groups, this is a very plausible hypothesis as aluminium has been detected during analysis of wool samples through ICP-MS.[72] Then following on from the work by Sanyova the alizarin molecule can bind to the aluminium metal centre through the carbonyl group and the primary hydroxyl or through the primary and secondary hydroxyl groups.[71] In Figure 1.9 this

complexation is shown with only one alizarin molecule attached to the aluminium metal centre, through the carbonyl and primary hydroxyl group, due to the wool also being complexed to the aluminium. This complexation with the aluminium metal centre could also occur through the primary and secondary hydroxyls of the alizarin molecule.

1.2.3. Dye interactions with wool

As expressed in Figure 1.5 (Section 1.2.1), there are many potential interactions within the wool structure due to an array of functional groups present. All these interactions would add more stability to the fixation of the dyestuff to the wool if they occurred *via* binding to the aluminium metal centre.[60]

π-π stacking. Many of the dye molecules shown in Chapter 1 have at least one aromatic ring and wool can have side chain groups with aromatic rings and it is these aromatic rings that can have attractive, non-covalent interactions between one another (as shown in Figure 1.10). These interactions could occur before or after an interaction with the metal centre imparted and covalently bound to the wool structure during the pre-mordanting stage.

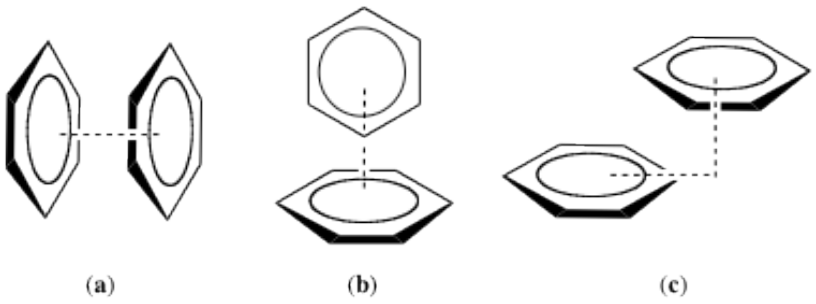
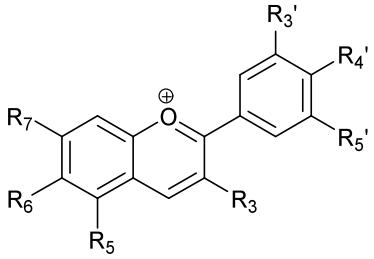


Figure 1.10. Three different p-p stacking interactions between two aromatic rings, sandwich (**a**), t-shaped (**b**) and parallel-displaced (**c**)[73-74]

Ionic bonding. The electrostatic interaction between oppositely charged ions, a cation (positively charged) and an anion (negatively charged).[74] Under

certain pH conditions side chains within the wool structure will exist as ions. The most common functional groups on these side chains are amine groups (can exist as $–NH_3^+$ group in acidic conditions) and carboxylic acid groups (can exist as $–COO^–$ group in basic conditions).[55] Certain natural dyes have an ionic charge associated with them, for example anthocyanins (**1.16**), and it is this ionic bonding that will render the dye substantive to the wool. The central oxonium ion, an oxygen cation with three bonds, is very strongly positive and has the ability to interact with a deprotonated carboxylic acid group on the wool structure.



1.16

Hydrogen bonding. A hydrogen atom in a molecule or a functional group can have an interaction with a strongly electronegative atom such as oxygen or nitrogen (also part of a molecule or functional group) through an electromagnetic interaction.[75] This process is not a covalent bond and is a lot weaker, a hydrogen bond has a typical energy between 4-12 kJ mol^{-1}, compared to a covalent bond of carbon and hydrogen measured at 418 kJ mol^{-1}.[76] In natural dyes there are many auxochromes attached to the chromophore, as explained in Section 1.1.1, primarily these functional groups tend to be hydroxyl groups, carboxylic acid groups, amine groups and glycosidic groups. All of these have the capabilities to form hydrogen bonds with other functional groups within the wool structure. For instance, if the dye molecule had a glycoside group attached to it, following covalent bond formation with the aluminium centre, the hydroxyl groups on the glycoside group could

hydrogen bond to functions in the wool. This addition of hydrogen bonding could give the dyestuff greater substantively towards the wool. Hydrogen bonding could also occur with functional groups on the dye molecule and the wool fibre itself without the need for the presence of a mordant.

1.3. Extraction of natural dyes

Work within this thesis was associated with the extraction of glycosidic anthraquinone compounds to understand the procedures needed to be used in order to extract them from previously dyed wool fibres. Research of historical extraction procedures will help in the understanding of the conditions and methods used and confirm if glycosidic anthraquinone compounds could have been dyed upon textiles.

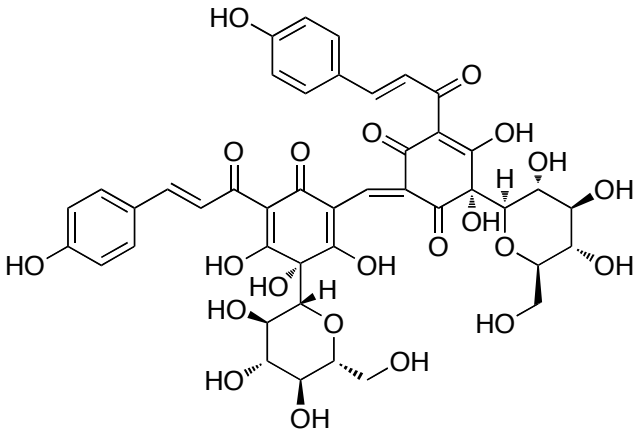
1.3.1. Historical procedures

Examples into how extractions were performed historically can and have been found, but many of the early methods were never recorded or have been lost over time.[1] Another complication is that each plant will be associated with a unique method of dye extraction to yield the maximum quantity for application to the fibre. In the following section, a select few historical methods have been chosen for a number of different plants.

Carthamus tinctorius more commonly known as safflower, is a source of red and pink dyes and has used in many Asian historical textile dyeings.[77] Safflower was once thought to have been used in Egyptian dyeing procedures especially materials that would be used to clothe the mummies in burial chambers.[78] One historical procedure was to put the safflower flowers into a linen bag and to apply force to them with your hands or feet whilst at the same time pouring vast quantities of water over the bags.[79] Macquer in 1763 reported this method of extracting the dye from safflower:

> "This is a long procedure…he will need very strong leather boots…to avoid the skin becoming to tender from the long time spent in the water".[79]

In other countries, the yellow part of the plant was removed and the remaining flower was mixed with washing soda (sodium carbonate) or pearl ash (potassium carbonate). For every 500 g of flower 30-60 g of sodium carbonate was added.[6] Once this was mixed together 7 litres of water was washed over the mixture to produce the required dyebath.[6] A technique known as "turning the bath" was used, where lemon juice was gradually added to precipitate the colour.[6] The main compound that is extracted from the safflower plant is the molecule carthamin (**1.17**), which is responsible for the red colour. Carthamin is not soluble in water so the use of the sodium carbonate in the above procedure is to make the water alkali. In doing this the hydroxl groups on the carthamin molecule will become deprotonated making the molecule soluble in the newly basic water. Addition of lemon juice, which contains a small percentage of citric acid, is to protonate the hydroxyl groups of the carthamin molecule and in doing this the molecule becomes insoluble in the water again and precipitates out.[6]



1.17

Rubia tinctorum more commonly known as madder, was a source of red colours.[32] There has been evidence found that *Rubia tinctorum* was cultivated and used as far back as the Bronze Age.[2]

The climate was not an important factor for the growth of *Rubia tinctorum* as it had been grown in Russia for hundreds of years.[32] Fluctuation of severe temperatures was not ideal, but it could still survive as low as –20 °C and be grown once the temperatures increased; the choice of soil was very important though for the quantity of the dyestuff yield.[46] Once the roots of these plants were harvested they needed to be dried, in the warmer climates around the world this could be done in the open air, but in the colder climates (northern France, Holland and the British Isles), pits were manufactured to steam the roots.[6] The roots would rot if the fields became waterlogged and a natural drainage system was not in place.[32] To yield this precious dyestuff the roots were steamed in underground pits called, *tandirs*.[6] These pits were specially crafted and lined with clay and would glow bright white on the outside, like hot charcoal, when at the right temperature.[2] Before the pits were at the right temperature the roots were bundled together and wetted before being stuffed inside the underground oven.[6] Once in they were wetted again and carpets were placed on top to keep in heat and steam the roots.[80] This process produced a significant reduction in weight of the roots; about a third of the weight was lost.[80] However it did produce a fierce bright purplish-red colour that was sought after all over the world.[81] Miller stated, "the more bitter the taste of the fresh extracted root, the less weight it will lose in drying."[82]

After the drying process the roots were beaten of all dirt and non-essential material by hand with a flail.[83] The roots were then powdered down in pounding mills similar to ones that would be used for wheat and grain.[6] By the 14th Century

this process became very popular and eventually there were several mills for this procedure in Lille and Hesdin.[6] To prevent up to 20% loss of madder during the grinding stage, kerosene was added to keep the product together.[32] This led to a reduction in the quality of the dyestuff reported by M. E. Skorobogatov.[80] Before the discovery of synthetic alizarin in the late 19th Century methods had improved to successfully yield the dyestuff from *Rubia tinctorum*, Schweppe reported a recipe from 1852:

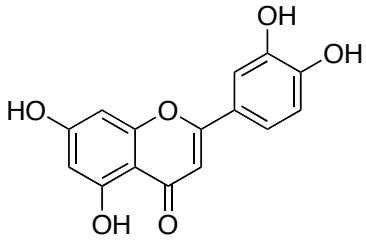
> "Ground madder roots were treated with 12 parts of water containing 0.5% sulphuric acid…allow to stand for a few days until the fermentation started. The madder was then filtered off and rinsed with water until it was neutral. It was then packed in cloth sacks, pressed in a hydraulic press, dried in a hot room at 50 °C to 70 °C and packed in barrels."[32]

Reseda luteola more commonly known as weld or dyer's mignonette is a source of yellow dyes that was grown all around the Mediterranean and even southwest Asia.[6,16] In combination with blue indigo, it was used to generate green colours, and because of these intense yellows and greens weld became synonymous with those colours, much like madder for red colours.[6] The major use of weld in Europe was for the source of different yellows – true yellow, lemon yellow, sulphur yellow, golden yellow and saffron yellow.[83] W. de Nie found and wrote a historical procedure for the extraction of weld in 1937:

> "Weld came on the markets in bundles of dried plants which were boiled in water to which potash and stale urine was added to promote further extraction of the dyestuff from the plant. The solution of the dye was filtered and used for dyeing."[84]

The molecules that are extracted from weld all have the chromophoric backbone of a flavonoid (as seen in Section 1.1.1 - Figure 1.2) with the most prominent being luteolin (**1.18**), which produces a bright yellow dye compound when extracted. These compounds are insoluble in water, however the use of boiling water in the

extraction process is first to break the cells of the plant that harbour these dye compounds and secondly under conditions of 100 °C the dye compounds will be soluble in the water.[32] The addition of potash and urine to the water makes the solution very alkaline meaning that the hydroxyl groups attached to the luteolin molecule become deprotonated.[6] Once deprotonated the molecule becomes more soluble in the water, hence further extraction of the dyestuff from the plant.[32]



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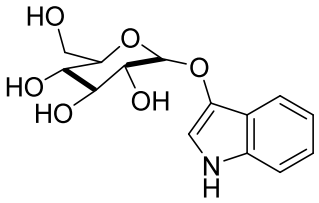
A more in depth extraction and dyeing procedure using weld was discovered in a Dutch dye book by Gerbrandus Nicolai printed in 1648:

> "…take one pound of weld and 4 'loot' of potash crushed into small pieces, first throw your potash into the kettle and put your weld in top of it and press your weld well and add so much rainwater that your work is covered properly and put it in your fire and let it boil for almost an hour. Then take your work out and hang it to cool off. And bring your kettle again on the fire and let it boil again for a quarter of an hour. Then take it from the fire…put your leggings in…take them out and let them cool. Rinse with clear water and this will become beautiful yellow."[85]

Indigofera tinctoria more commonly referred to as the indigo plant, was and still is to this day, possibly the most famous natural dye ever to be cultivated and dyed with.[1] After planting the seeds and 8 to 9 months had gone by, the *Indigofera tinctoria* would start to produce flowers and bloom.[6] The plants were then harvested and cut into smaller pieces and bundled together and placed in large bowls and water was added until the plant was fully immersed.[84] These bowls, or in more extreme circumstances large vats were then heated to high temperatures, to begin the

process of fermentation the hydrolysis of sugar groups from the indigo precursor compounds, namely indican (**1.19**), isatan B (**1.20**), and isatan A (**1.21**).[1,6]



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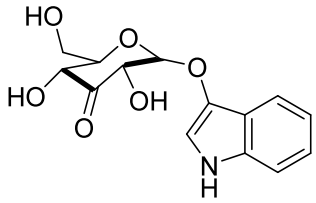

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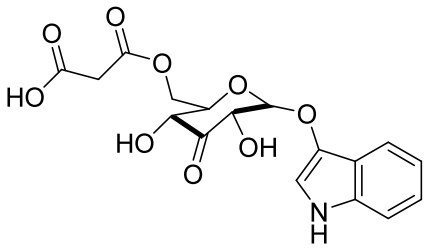
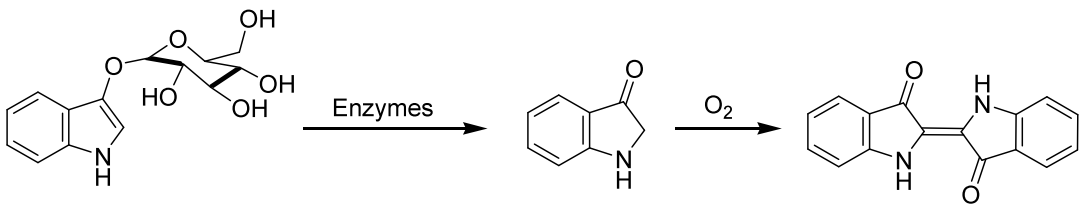


1.21

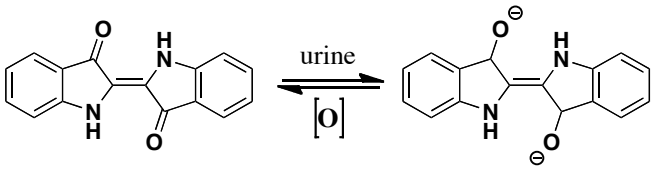
Enzymes within the indigo plant undertake this process of fermentation after the cell walls have collapsed and the contents of the precursors and the enzyme fall into the water.[6] This produced a compound known as indoxyl, shown in Scheme 1.5.[1] Once the liquid was transferred into a clean basin or vat and all the plant material and dirt had been sifted and removed the liquid was beaten with a paddle to get as much oxygen into the water as possible.[84] Indigotin is insoluble in water so once it was formed it precipitated out of solution and then was filtered and dried and has a melting point of 390 °C.[6,86]



indican **indoxyl** **indigotin**

Scheme 1.5. Reaction mechanism of the conversion of indican to indigotin *via* indoxyl.[1]

The historical dyeing process involved the fermenting of the plant and the addition of urine to make the solution alkaline.[87] The solubility of the indigotin is improved in an alkaline solution due to the compound being reduced to the *leuco*-species **1.21**, once in this form the species can penetrate the fibre substrate.[16,88] Once this solution comes into contact with oxygen from the atmosphere, the indigotin species associate together forming insoluble aggregates that can not penetrate from the fibre.[16]

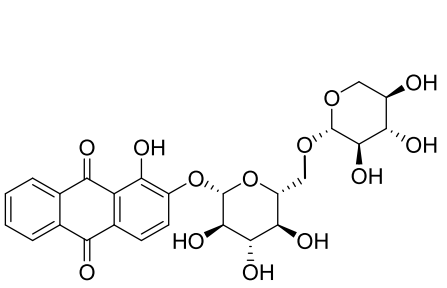


indigo **indigoleuco**

1.21

1.3.2. A modern extraction of *Rubia tinctorum*

An example of this method being utilised to successfully analyse natural dyestuffs from botanical sources has been completed very accurately by Dersken and van Beek on the plant *Rubia tinctorum*.[32] The use of the HPLC (High Performance Liquid Chromatography) instrumentation gave a more accurate account of the molecules present in the crude extract. After completing a solid-liquid extraction of *Rubia tinctorum* in an aqueous ethanol mixture the sample was analysed by HPLC and four compounds were observed, ruberythric acid (**1.23**), lucidin primeveroside (**1.24**), pseudopurpurin (**1.25**) and munjistin (**1.26**).[32]



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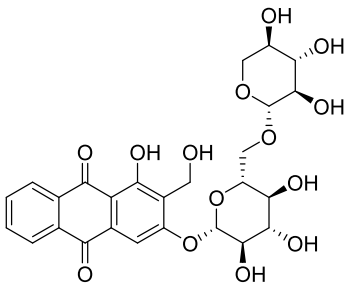

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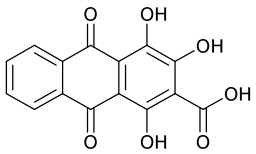

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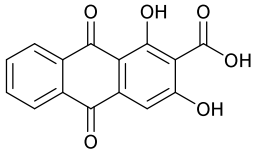


1.26

As previously stated in Section 1.1.2, alizarin is deemed to be the most prevalent dye component in the madder plant.[1,6,25,63] However, Dersken *et al*.[32] have proved that the most abundant dye component in fresh madder root to be lucidin primeveroside and then ruberythric acid.[89-91] There was no detection of alizarin, which was only formed when an acidic aqueous solution was used in the extraction medium that resulted in the hydrolysis of ruberythric acid.[32] Other than the two glycosidic anthraquinone compounds present, there were two more anthraquinone compounds detected, pseudopurpurin (**1.25**) and munjistin (**1.26**).[32] The detection of these minor compounds could only have been achieved with the use of HPLC. The level of accuracy of this technique coupled together with the time and knowledge of Derksen *et al*.[32] has led to very accurate techniques in helping to analyse extracts of *Rubia tinctorum* performed on a large scale.

1.4. Analysis of historical textiles

The analysis of natural dyes in historical textiles and significant cultural heritage objects is vital for conservation of these products *i.e.* treatments and restoration, providing accurate dates and production of these objects, providing information regarding provenance of the natural dye compounds and to understand the international trade routes of these dye compounds.[1,16,92-95]

1.4.1. Methods of analysis

A non-invasive technique has been developed called fibre optic response spectroscopy (FORS) to investigate artworks and textiles with pigments in the

visible region.[96-97] The FORS technique uses the reflectance response from compounds dyed upon the fibre.[96-97] The sample to be analysed is subjected to a spectrophotometer and the parts of the textile, faded and non-faded sections that are of importance are analysed.[96] The major problem with this technique is the overlapping of excited transition states and the weak signals produced.[97] In the work undertaken by Gulmini *et al.*[97] it showed that the use of FORS on historical textiles did analyse the use of indigo in blue and green areas on the samples but could only exclude the use of carotenoids and saffron and inaccurately proved the use of flavonoid compounds.[97] No further detail into what dyes were used could be achieved by this method. One drawback to using the FORS technique is that reference samples have to be produced beforehand; this requires knowledge of what dyestuffs were dyed on the historical textiles.[97] Gulmini *et al.* confirm another limitation is that if the concentration of the dyestuffs on the fibres are too strong or too weak (this can not be altered due to the dyestuffs being attached to the fibre), this prevents the diagnostic reference signal being detected.[97] The technique is very useful in the preliminary analysis of dyestuffs on historical textiles and can highlight if a certain red or blue dyestuff has been used independently from the fibre material itself.[97]

Another technique used in the analysis of historical textiles is Raman spectroscopy or more accurately micro Raman spectroscopy. This process works by something known as Raman scattering, which is when the initial laser from the Raman spectrophotometry machine interacts with excited photons and molecular vibrations from molecules and therefore changes the energy of the laser.[98] The samples are mounted onto the spectrophotometer and measured at various wavelengths.[98] There are several problems associated with using this technique.

Sample degradation by the light source used in the spectrophotometer is a concern with unforeseeable photochemical reactions occurring and also the chance of pyrolysis.[99] Thomas *et al.* reported that this was not seen at all during their investigation of Raman spectroscopy for analysing historical textiles.[99] However the major drawback to using Raman spectroscopy in this type of investigation is that the samples are of an irregular shape, which means the laser struggles to focus the energy produced to the maximum capability.[99] This problem produces results that have a poor signal to noise ratio and very weak signals of the dyestuffs analysed, both of these coupled together result in uncertainty and inaccuracy of the dyestuffs analysed.[99] Additionally Surface-Enhanced Raman Spectroscopy (SERS) can provide even greater improved Raman signal, which in turn provides a higher level of identification of dyestuffs on small sample sizes.[100-104] The procedure used to observe this improved Raman signal is by placing the molecules for detection onto a roughened metal surface, the noble metal substrate quenches fluorescence and enhances the Raman signal.[104-106] Initial work using this technique comprised of analysis of chromophoric molecules for artistic production, however they did not focus on identification of chromophores of two or more dye molecules.[107-109] Whitney *et. al* provided a proof of concept of the identification of two dye molecules in one system but it was not performed on actual historical samples, which are known to provide difficulties to the analysis due to ageing or contamination of the textile fabric.[110] Brosseau *et. al.* showcased that the quality of the spectra from Raman to SERS of natural dye molecules was improved greatly due to the addition of silica gel with silver colloidal substrates.[104] Less quantity of material was needed and a less sophisticated instrument was needed for analysis using this technique, however calculation of relative ratios of dye molecules (known

to help in provenancing of dyestuff compounds) can not be achieved with this method.[104]

In 1934 Pfister published the first example of the technique of stripping/removing the dye compounds from the fibres through a chemical process.[111] For routinely analysing natural dye products from these extractions the method of HPLC (High Performance Liquid Chromatography) is widely used, coupled with DAD (Diode Array Detector).[46] For a more in depth analysis *i.e.* structural elucidation, when it is known what compounds are being analysed HPLC coupled with mass detection is used.[95-112] A very common method used, and is still used today, is the use of 37% HCl/MeOH/H_2O (2:1:1, *v/v/v*) reported by Wouters and Verhecken.[113] The fibre to be analysed was placed in a boiling bath of the solvent mixture and was heated for 10 minutes and then removed.[114] The resulting liquor was subjected to HPLC analysis, and the compounds analysed on a range of different materials gave accurate compound structures, such as alizarin, indigotin, luteolin and many more.[72,113] The method is very destructive and damages the chemistry of the samples analysed.[72] The researchers of this paper and subsequent researches who have used this method have always known that the use of a strong acid, such as HCl, could hydrolyse molecules that are essential for biological provenancing of the samples analysed.[72,104,113-114]

Building on from this technique there has been further research undertaken to develop milder extraction techniques; the use of formic acid and methanol in a 1:19 ratio volume for volume is an example.[115-116] After the sample was refluxed in the mixture for 30 minutes and the sample removed so that the liquor could be analysed, the result of the compounds detected were very similar to the HCl method.[72] Milder hydrolysis methods, such as formic acid shown above, have been

published to avoid the harsh conditions of the HCl method developed by Wouters *et. al*. in 1985.[104] The milder hydrolysis conditions include the use of oxalic acid, trifluoroacetic acid, citric acid and hydrofluoric acid.[107,117-119] The use of 0.1% H_2EDTA (dihydroethylenediaminetetraacetate) in H_2O and Dimethylformamide (DMF) (1:1, *v/v*) by Tiedemann and Yang is another example.[120] After the sample was refluxed in the solvent medium for 30 minutes it was analysed by HPLC.[72] This method has been proved to be the most accurate and most benign undertaken in a review of solvents used to back extract natural dyes from natural fibres.[72] Even more accurate analysis was recorded with this technique, giving rise to compounds with great complexity being analysed, such as compounds with glycosidic groups attached that are not detected using HCl/MeOH/H_2O due to the hydrolysis of the glycoside linkages.[72]

Wouters *et. al.* published a recent investigation involving a two-stage extraction method from historical textiles, an initial mild hydrolysis extraction followed by a harsh hydrolysis extraction.[104] Nine different methods were trialled using this two-stage process, 10 different dye molecules were analysed using the HPLC technique previously discussed, coupled with DAD and mass spectroscopy.[104] It was reported that the use of an oxalic acid method[121] followed by the hydrochloric acid method of Wouters *et. al.* 1985 was the best two-stage extraction technique to perform on silk or wool dyed fibres.[104] Wouters *et. al*. also reported that if the dye molecule being investigated was known an alternative extraction method should be explored.[104] For indigoid molecules on silk or wool the two-stage extraction technique of formic acid hydrolysis followed by hydrochloric acid hydrolysis should be used for better analysis of the dye compounds extracted.[104]

It is stated that the investigation of what extraction technique should be the number one priority otherwise the risk of missing dye molecules is greatly enhanced.[104]

Research into the evaluation of ultrahigh pressure liquid chromatography (UHPLC) compared to HPLC for the analysis of natural dye compounds in cultural heritage artefacts has been undertaken.[122-123] Taujenis *et. al.* developed a method that was successful in shortening the run time of a conventional HPLC method for analysis of a historical object, however it failed to detect the compound indigotin.[123] For this a separate method was produced and was an obvious drawback to the UHPLC technology. However, Serrano *et. al.* developed a method that could precisely characterise a wide gamut of natural dye compounds, to alleviate this problem.[122] From this evaluation of different analytical columns, run times, column oven temperatures and mobile phase compositions they showcased an increase in peak resolutions and peak capacity.[122] This method was shown to be considered as a new method for detecting natural dye compounds more accurately in historical important artefacts.[122]

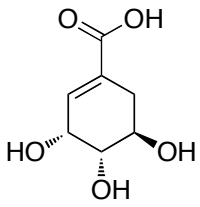
1.4.2. Conclusions of analysis of historical textiles

Of all the methods discussed in Section 1.4.1 the extraction of natural dye compounds from fabrics and analysing the extract by HPLC/UHPLC produces more accurate identification of the natural dye compounds dyed upon the fabrics. This method seems to be the most powerful, accurate and can be used across many different materials and dye compounds used. It is this accurate identification that can help in the provenancing of botanical sources, which is of importance to cultural heritage institutions. The methodology for historical dye analysis is very challenging due to the complex matrices of dye, metal and fibre, the vast number of different dye

classes *i.e.* flavanoids, anthraquinones, indigotins and many other dye classes and the small sample size that is being analysed.

1.5. Extraction of dye compounds by ionic liquids

Ionic liquids are being successfully used in the extraction of natural products.[124-126] The successful extraction of shikimic acid (**1.27**) from *Gingko bilboa* leaves using 1-butyl-3-methylimidazolium chloride (**1.28**) is just one example of were this methodology is being employed.[126] This methodology could be used in the future to extract natural dye compounds from botanical sources.



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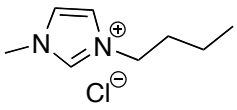
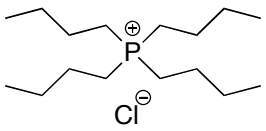


1.28

An example of dye extraction from a dyed textile with the use of ionic liquids has been published.[127] The dyed sample was placed in a vessel containing the ionic liquid, tetrabutylphosphonium chloride (**1.29**, [BuP_4][Cl]) and heated to an appropriate temperature normally above 100 °C.[127]



1.29

Lovejoy *et al*. showcased when the sample was heated to 130 °C and left for 24 hours then the sample fibre was completely destroyed.[127] However, it was reported that after 2 hours the solution had turned the colour of dyed fabric.[127] If this procedure was completed on a single strand of a historical textile it could yield information of minor dye compounds that have previously gone undetected.

1.6. Aims of this research

The main aim of the research in this thesis is to develop a method that can retain more chemical information about the chemical composition of madder relating to its plant source and to the dyeing process by analytical techniques. *Rubia tinctorum* has long been investigated for its chemical dyestuffs and the best methods for extraction of the dyes because it was so widely used as a dyeing source in historical textiles. Herein, *Rubia tinctorum* has been chosen as the dye for the focus of the research in order to attempt to successfully analyse the dye *in planta* and remove the dye from dyed wool fabrics (especially glycosidic anthraquinone compounds), comparing extractions methods and the corresponding botanical extracts. Dyeing studies were also undertaken using *Rubia tinctorum*. The aim herein is to develop an improved method that preserves relevant chemical information of the dye molecule and provides a comparison to the dye molecules found *in planta*.

2. Dyeing with *Rubia tinctorum*

2.1. Background

Rubia tinctorium is one the most scientifically researched plants in the world. There have been hundreds of publications about the natural dyes that this plant produces in its roots.[1-3,5-6,32,44,53,46,63-67] Alizarin is the most famous of all these natural dye compounds.[32] However recent understandings show the most abundant molecules to be the glycosidic compounds ruberythric acid and lucidin primeveroside.[44,46] But further understanding of the techniques used historically for dyeing textiles with madder, and discovery of what compounds would have been responsible for the colours observed for historically dyed artefacts, is desirable to the scientific and conservation fields. If this can be elucidated it will facilitate the analysis of historical textiles. Working back from the colours observed on the historical materials, and applying experimental observations of dyeing studies, the aim is to be able to postulate dyeing protocols used for the production of the fabrics historically.

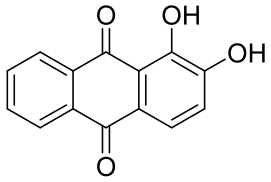
2.2. Dyeing

Initial research focused on the dyeing studies of *Rubia tinctorum* is discussed herein.

2.2.1. Dyeing Studies of Synthetic Alizarin and Madder

Literature suggests that the major dyeing component extracted from madder root during the dyeing process, and hence the effective dyeing species, is alizarin (**2.1**)).[1,6,32] The aim of this research was to understand the main extractable compounds in the madder plant (*Rubia tinctorum*); from this it was hoped to discover if alizarin was the main component responsible for textile dyeing or if other compounds, such as anthraquinone glycosides, were the active dyeing extracts, as

seen in more recent extractions published.[44,46] The materials studied were synthetic alizarin (Sigma-Aldrich), fresh harvested madder root (Aurorasilk), and fine ground madder powder (Aurorasilk).



2.1

A standard madder dyeing protocol was selected from the literature, as it was proposed to be the most historically authentic dyeing procedure.[6] The experimental data and recipes for the work undertaken in this chapter are shown in Section 7.4. Wool was used in the literature as the fibre to be dyed and required scouring prior to the dye application; in the scouring process herein a non-ionic detergent (*Sandozin NIN*) was used. The wool was pre-mordanted with alum (hydrated potassium aluminium sulfate; $KAl(SO_4)_2 \cdot 12H_2O$) and cream of tartar (potassium hydrogen tartrate; $KC_4H_5O_6$).[6] In a separate vessel the dye was extracted from the madder over 3 days at 90 ºC with distilled water (this is not historically relevant, but it was more consistent and reproducible than using tap water).[6] After filtration, the wool was immersed into the extract and the heat maintained at 90 ºC for 3 hours and then allowed to cool down, rinsed with clean water and air-dried.[6] The concentration of the dyebaths was chosen to be 5% and 10% on mass of fibre (omf); this was used not as a historical representation, but as a way to start the understanding of the dyeing process with madder root, and are typical concentrations used when applying dyes compounds.[128-129]

The comparative study of dyeing with alizarin, madder root and madder powder, using the above dyeing procedure, gave the following results: The K/S (a

colour strength measurement calculated from reflectance of the sample at λ_{max} as shown in Section 7.7 in Equation 7.3) result for alizarin was 11.6 over a range of 410-430 nm, whereas for the madder (both powdered and from the root) it was between 3.2-3.6 over a range of 410-510 nm. There was a similarity in the results of alizarin and both madder samples implying the madder samples have dye compounds present that have comparable UV-vis reflectances (Figure 2.1).

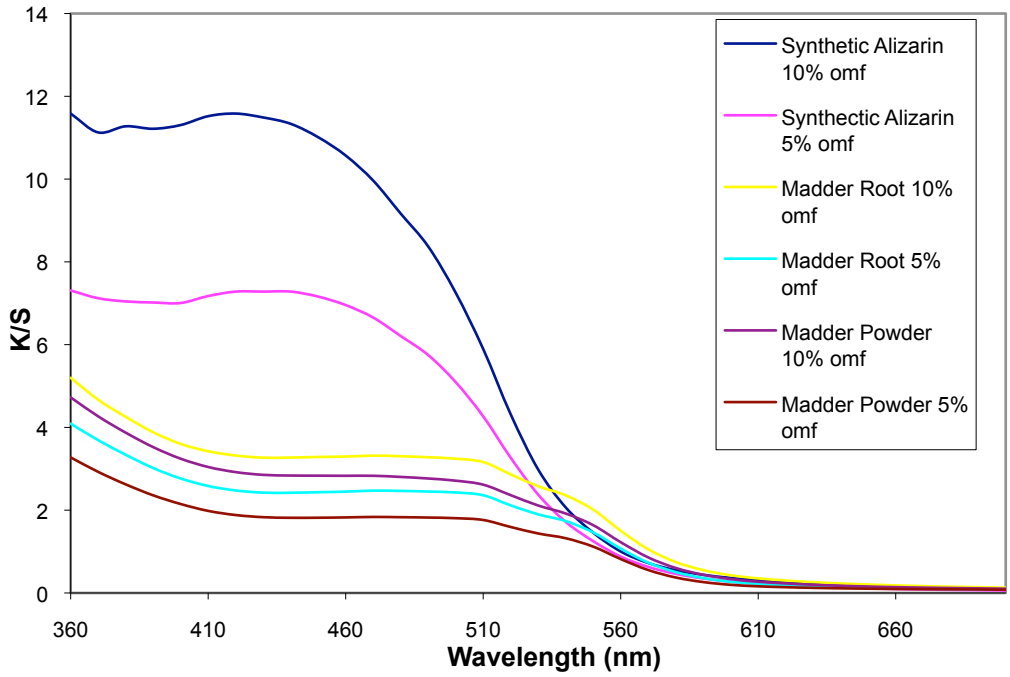


Figure 2.1. Colour Measurements of Wool Dyed with Alizarin, Madder Powder and Madder Root.

Figure 2.1 illustrates that alizarin could be present in both madder extracts and be responsible for dyeing the wool fibre. However the result of both madder samples is over a longer reflectance range, 410-510nm, this could be due to the amount of dye present in the extracts or there could be other compounds present that provide a reflectance in the region beyond 430nm to 510nm. Discussions within some areas of the literature imply that other components, such as anthraquinone

glycosides, tannins and other polyphenolic compounds are also responsible for contributing to the colour observed when dyeing with the madder root extract.[130]

The difference in observed colour of these dyed materials was not assessed purely by K/S, but also by visual inspection; it was observed that wool dyed with alizarin was an orange colour (Figure 2.2), whilst wool dyed with madder was a reddish-pink (Figure 2.3).

Figure 2.2. Wool dyed with 10% omf of synthetic alizarin.

Figure 2.3. Wool dyed with 10% omf of madder root.

If glycoside compounds are predominantly present in the madder root extract, over aglycone compounds, a reason for this difference in colour could be

due to arrangement around the aluminium metal cation used in the pre-mordanting process. In the literature there is an example of a computerised model crystal structure where four alizarin molecules complex with two aluminium (III) ions, as seen in Figure 2.5.[6,44]

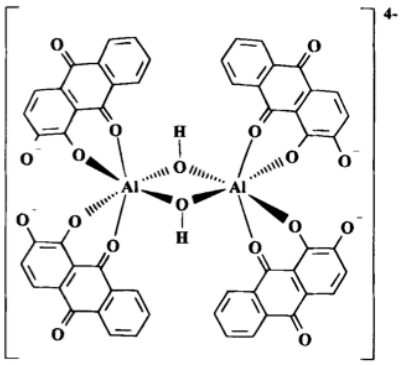
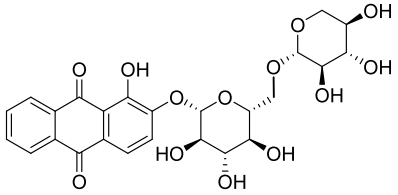


Figure 2.5. Binuclear complex of alizarin and aluminium.[44]

Alizarin (**2.1**) is a smaller compound compared to the glycosides ruberythric acid (**2.2**) and lucidin primeveroside (**2.3**), as the glycosides each have two sugar moieties attached to the anthraquinone structure. Increased steric bulk from the disaccharides could therefore limit the number of molecules that are able to arrange around the aluminium ion, thus yielding an alternative on-fibre colour to that observed for the aglycone alizarin. Also the sugar moiety has several more hydroxyl groups, which are possible coordination sites for complexation to the aluminium metal instead of an additional compound, potentially limiting the number of molecules of able to co-ordinate per complex. In conclusion, it was suspected that alizarin was not the sole component in the madder root extract responsible for wool dyeing, and that other compounds, possibly ruberythric acid and lucidin primeveroside, could be present.[44,46,130]



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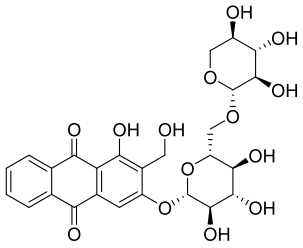


2.3

From the same experiment above, exhaustion of the dyebaths were calculated by measuring absorbance of dyebath solutions before and after dyeing using UV-vis absorption (experimental procedures can be found in Section 7.4.4 and Section 7.6). UV-vis spectra further support the conclusion that alizarin was not the sole component in the madder root extract, with the extract giving a broader signal and a different λ_{max} (Figure 2.6). λ_{max} for synthetic alizarin was 434 nm, with a defined peak; after dyeing there was still an absorption maximum at 434 nm suggesting alizarin was still present in the dyebath liquor. If there was any alizarin in the madder root extract or powder dyebaths it was a miniscule amount and was dwarfed by the amount of other compounds in the madder root extract. Dyebaths for the madder powder and the root extract gave a UV-vis absorption over a wide range of 400-510 nm. It is this broadening and shifting of the UV-vis absorption, caused by compounds other than alizarin, that were responsible for the different on-fibre colour observed when the wool was dyed with synthetic alizarin (orange), madder powder (reddish-pink) and madder root extract (reddish-pink).

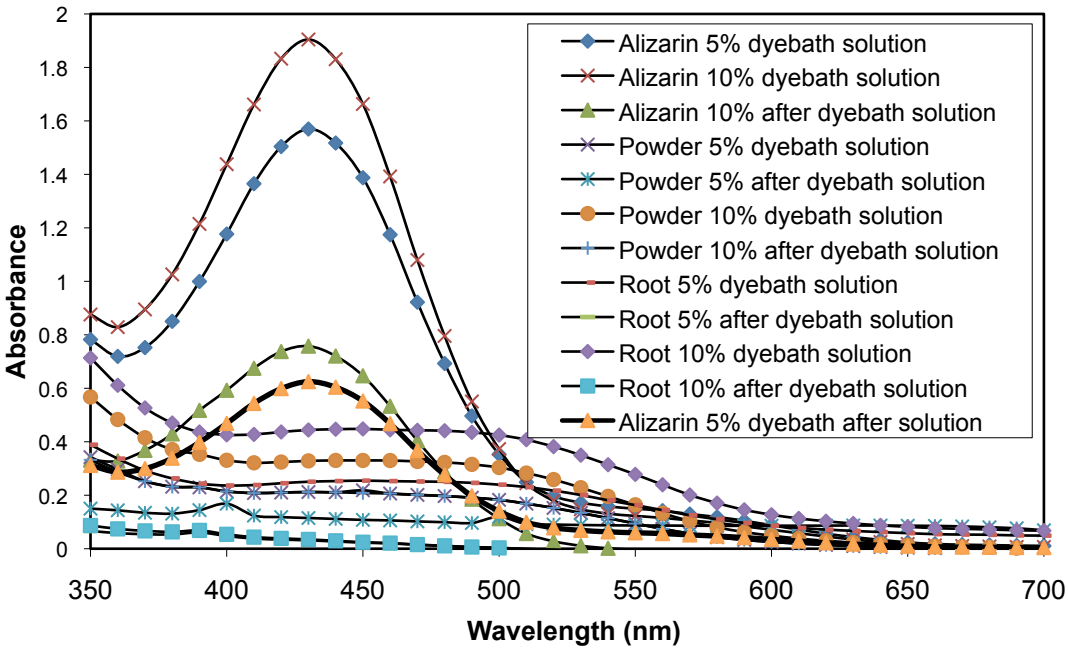


Figure 2.6. UV-vis spectra of dyebaths before and after dyeing with synthetic alizarin and madder.

Analysis of the dyebaths also indicated that dyeing with the madder root extract was more efficient then dyeing with synthetic alizarin. A higher exhaustion was observed for dyebaths using the madder root extract, implying that less dye remained within the dyebath, and thus more dye was adsorbed onto the surface of the wool (Figure 2.7). A reason that this result was observed could be due to there being less anthraquinone compounds in the madder root extract and madder powder leading to a greater exhaustion. Whereas the alizarin solution was purely alizarin and if the solution was over saturated then a lower exhaustion would be recorded.

Previous experiments have shown so far that alizarin is not the only component of the madder root extract, be it powder or root. However, literature states that both these glycoside compounds, ruberythric acid and lucidin primeveroside, are yellow in colour when synthesised and purified, therefore it can be concluded they are also not the sole compounds responsible for this difference in colour when compared with alizarin.[89-91] However there is no literature on the

change of colour of the compounds ruberythric acid and lucidin primeveroside might have when complexed to a metal centre, such as aluminium. This complexation could result in a change from the observed yellow colour, which arises due to the absorption of a short wavelength of visible light, to a dark perceived colour *i.e.* red, which is a result of the absorption of a longer wavelength of visible light. Results so far show whatever substances are present in the madder root extract, even though in less quantity, have an affinity to the pre-mordanted wool like alizarin. The proposed occurrence for the anomaly of the madder powder could be due to the fact this has been exposed to the air for longer and not had the protection of the rind on the madder root bark. This exposure to the air, most probably oxygen, could yield in the destruction of certain dye molecules within the extracts.

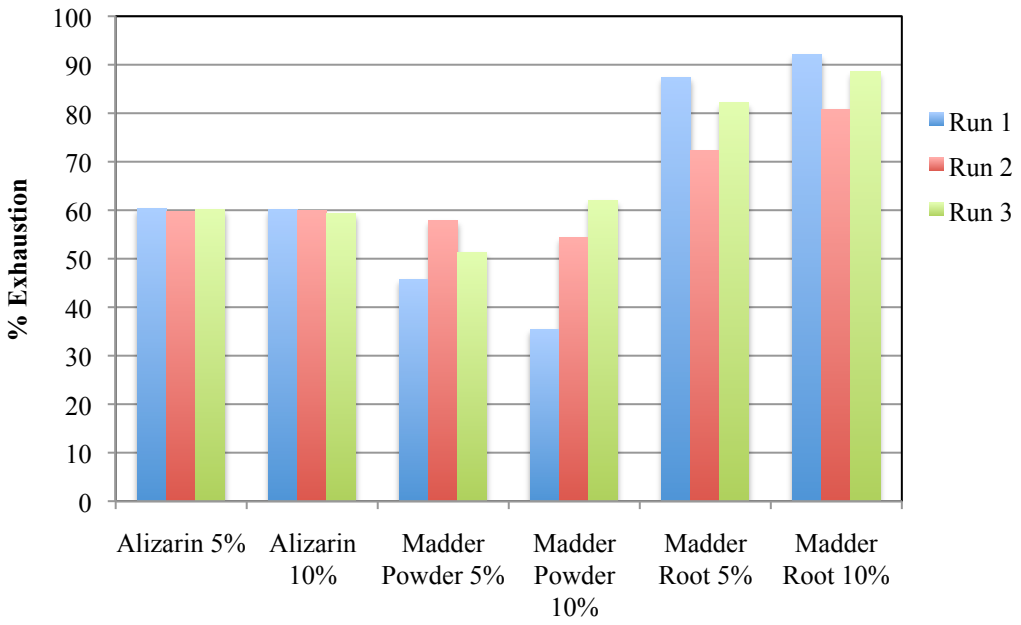


Figure 2.7. % Exhaustion of all dyed wool fibres.

Overall it was concluded that alizarin was not the only component of *Rubia tinctorum* extract responsible for dyeing, and that the glycosides ruberythric acid and lucidin primeveroside were more likely to be involved as stated in some literature sources.[44-131] However, the main reason for this famous red colour that has

been attributed to madder dyeing for centuries is most probably down to tannins, polyphenolics compounds and polysaccharide and free sugars.[32] Tannins are well known to be very dark compounds and are very water-soluble.[132] So with the fact that the extraction procedure involved only water as the liquor it is most likely that the tannins and other impurities are the reason behind this darkening of the colour prominent by the anthraquinone molecules.

2.2.2. Dyeing Studies of Fresh Ground Madder Root

Further investigations about the role of mordants (pre and post) were undertaken using freshly ground madder root (FGMR). This process was trialled with and without pre-mordants (alum) and post-mordants (typically salts of Fe, Cu, and Sn), using a range of fresh ground madder root (FGMR) concentrations (5%, 10% and 50% omf). Details of the experimental data of the following procedures are found in Sections 7.4, 7.6 and 7.7. The aim was to investigate the different colours that are achieved using different mordants and dye concentrations; as above, the procedure for dyeing was based on that from the literature.[6] Again wool was chosen as the fibre to be dyed, this being an accurate representation of native historical textiles. Wool was scoured, then air-dried and used after the dyebath was prepared. The dye-bath was prepared by grinding up the madder root and boiling in water 90 ºC for 3 hours.[6] Wool was pre-mordanted in four cases with alum and cream of tartar; one wool sample was not pre-mordanted. Scoured and pre/non-mordanted wool samples were then placed into the dyebaths, and heated to 90 ºC for 3 hours.[6] 15 minutes before the end of the dyeing post-mordant metal salts (iron, tin and copper) were added to three of the pre-mordanted wool sample dyebaths.[6] Once the dyebaths had cooled the wool fibres were removed, washed and air-dried.[6] Samples of the

dyebaths before and after dyeing were taken and analysed by UV-vis spectroscopy (Figure 2.8).

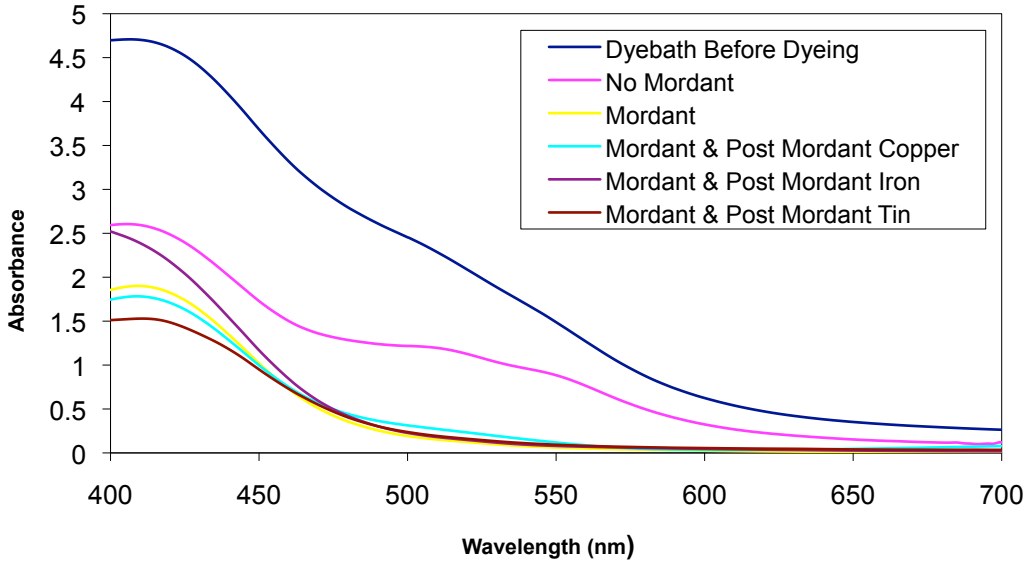


Figure 2.8. An example of UV-vis spectra of dyebaths when dyed with madder root (50% omf) and various mordants.

When no pre-mordant was used, uptake of the dye was lower onto the wool, especially over the wavelength range of 470-570 nm, and to a smaller extent over the wavelength of 400-430 nm. This is exactly what was expected as pre-mordants have been used for centuries with the knowledge that they improve the uptake of dyes onto fibres.[6] When a pre-mordant was used the molecules responsible for the absorbance between 470 nm to 570 nm were completely absorbed onto the wool. The absorbance of the compound responsible for the wavelength around 470-570 nm would be a seen as a reddish colour and from the previous work before it is unknown that any of the compounds mentioned in the literature would be responsible for this absorbance at this wavelength range. As explained in the previous section, tannins and polyphenols present in the madder root could be responsible for this absorbance as they will be very prominent in a water

extraction.[132] As discussed previously, ruberythric acid (**2.2**) and lucidin primeveroside (**2.3**) are compounds that are likely also present within the madder root, and the literature states that these compounds are yellow in colour when purified with a wavelength of 415 nm and 406 nm respectfully.[130] The highest point on all the traces is around 400-420 nm and this could be an indication that this was due to the compounds ruberythric acid and lucidin primeveroside; this range around 400-420 nm also drops in intensity owing to the fact that the compound(s) responsible for the absorbance was/were being absorbed onto the wool fibres. If this was due to the two disaccharides, then the sugar groups attached to these molecules could be assisting the absorption of these compounds onto the wool, as previously stated. This hypothesis that other compounds are present in the madder extract and could be responsible for the dyeing of the wool fibre alongside alizarin agrees with the literature; if alizarin is present; it is known to be in lower concentration in comparison with the two disaccharide compounds.[44] An orange colour is seen in the final dyed wool fibres this could be due to a mixture of the compounds at 400-420 nm (yellow in appearance) and the compounds at 470-570 nm (reddish-brown in appearance). As the literature states alizarin is present but in conjunction with other anthraquinone dye compounds, tannins and polyphenols and this works showcases it is this mixture of compounds and dyes that give the madder dyed fabrics their distinct orange-red colour.[1,3,32] The use of iron as a post mordant produced a different UV-vis spectrum compared to that of using a pre-mordant and the use of copper and tin as post-mordants. A measurable λ_{max} of the iron mordant was measured at 394 nm before it sharply increased into the UV region. An explanation for this shift could be due to the complexation of the iron metal and the dye

molecules adsorbed upon the wool fibre absorbing a shorter wavelength of light in the visible spectrum.

From the results in Table 2.1 it can be seen that with an increase in concentration of dye in the case of the no mordant and mordant procedures, there is a greater adsorption of dye onto the wool. For the difference in 5% and 10% concentrations this could be due to the number of sites available for adsorption on the wool. More dye molecules means more sites become occupied on the wool. The increase of the dye uptake in the 50% concentration could be due to the dye molecules that are present in the madder extract.

Table 2.1. % Exhaustion of All Dyebaths

	% Exhaustion of Dyebaths		
	5% omf	**10% omf**	**50% omf**
No Mordant	23.2 ± 8.4	29.6 ± 9.1	44.8 ± 10.2
Mordant	31.8 ± 5.7	39.7 ± 5.9	59.6 ± 8.4
Copper	66.2 ± 2.2	61.8 ± 4.2	62.1 ± 5.4
Iron	33.1 ± 3.0	36.9 ± 4.8	48.9 ± 7.3
Tin	68.4 ± 3.0	71.6 ± 4.9	67.5 ± 6.1

From the literature we know these molecules have an anthraquinone chromophore (i.e. **2.2** and **2.3**).[44] Molecules of this nature have the ability to interact with themselves through π-π interactions or hydrogen bonding and because of these interactions an increase in the uptake of dye from the dyebath could be observed.

Another result seen in Figure 2.8 and Table 2.1, was the difference between the uptake of dye onto the fibre between the use of a mordant and no mordant in the dyeing process. Having a pre-mordant greatly helps in the adsorption of the dye

compounds present in the liquor onto the wool fibres, giving a better exhaustion than when no pre-mordant is used. This could be attributed to the fact that the dye compounds have a better affinity to the metal than they do to the wool. When no mordant is used the dye is most probably adsorbed onto the wool through hydrogen-bonding forces. The use of a metal ion pre-absorbed onto the wool suggests that ligand-metal bonds would now dominate the bonding complexes upon the wool fibre. This type of complex would not be seen when no mordant is used, in this instance it would be primarily hydrogen bonding of the dye molecule to the fibre. This does not preclude hydrogen bonding of dye compounds to the wool, when a mordant is used. However an increase in dye exhaustion during the use of a mordant showcases that the dye molecules have a greater affinity for the metal then the wool fibre itself and once attached through this metal-dye complex the dye compound does not seem to be leached back into the solvent. This again is evident in the literature, where it is stated that the use of a mordant improved the wash-fastness of the dyes.[133]

Of the three post-mordants used only the procedure of using the tin compound increased the dye uptake onto the wool fibre significantly compared to using a pre-mordant or no mordant at all. In fact, what has occurred is that the metals have enhanced the λ_{max} of the dye, making it increase the absorbance as observed in the UV spectra, *i.e.* more dye being adsorbed on the wool fibres. This is seen the most readily in the case of the tin post-mordant with a greater exhaustion observed. In the case of the iron mordant the results shows a lower absorbance measured, which could be interpreted as a loss of dye from the fibre, but this is not what has actually happened. The iron mordant has changed the colour observed upon the wool and has shift the λ_{max} to a shorter wavelength. The λ_{max} is higher in

absorbance compared to the other procedures undertaken meaning the complexation between the iron metal and dye molecules results in a higher absorbance of light in this region of the visible spectrum.

The results shown in Figure 2.9 demonstrated that using a pre-mordant gave a stronger reflectance value when compared to using no pre-mordant. The K/S result when no mordant was used was measured at 7.1 at a λ_{max} of 400 nm but when a pre-mordant was used the K/S result was 12.1 at a λ_{max} of 400 nm. This suggests that the colour was stronger and more vibrant confirming that the result is due to more dye being adsorbed onto the fibre, hence a greater exhaustion of the dyebath. The copper and iron post-mordants gave a reflectance measurement similar to that achieved when only a pre-mordant was used. The use of the copper and iron post-mordants therefore do not enhance the colour already adsorbed onto the fibre in this method. The tin post-mordant yielded a result of λ_{max} = 460 nm and a K/S result of 12.5.

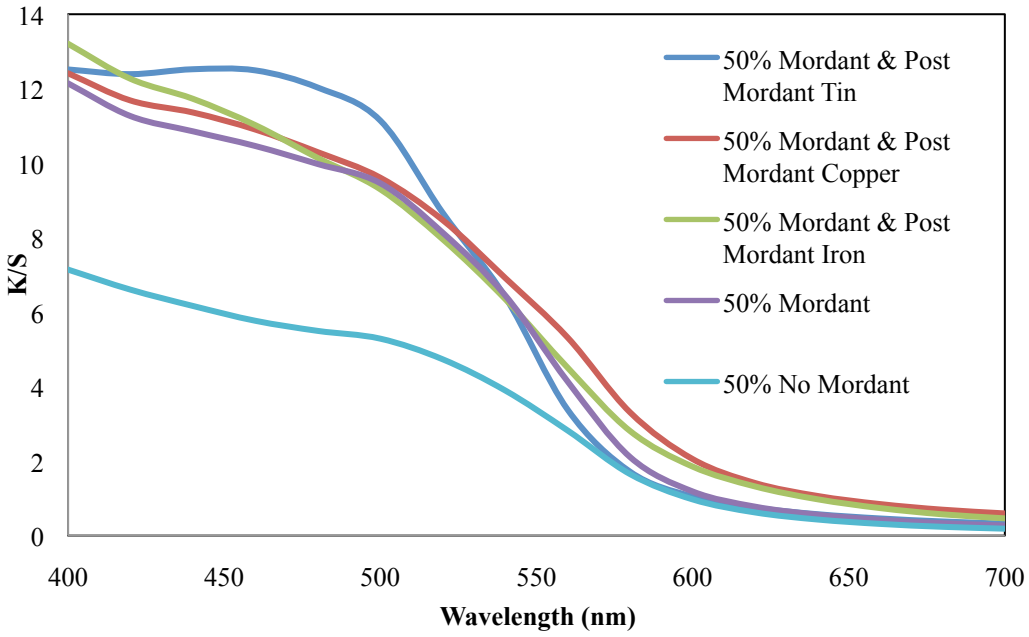


Figure 2.9. K/S for Wool Dyed with 50% omf of Madder Root.

As can be seen in Figure 2.10 and 2.11, both dyed wool fibres were the same colour. The only difference was that the pre-mordanted sample was darker or more vibrant

in appearance, suggesting more dye had adsorbed onto the fibres. K/S results suggested that there was an increase in colour intensity from the no mordant to the pre-mordant.

Figure 2.10. 50% omf No mordant Madder Root.

Figure 2.11. 50% omf Pre-Mordant Madder Root.

When examined visually this conclusion could also be observed. The use of the post-mordants produced different shades of colours for all three metals used: for iron a subtle brightening of the colour was observed with just a pre-mordant used (Figure 2.12); copper also gave a subtle visual effect, but with a dulling of the colour compared to just pre-mordanted material (Figure 2.13); tin gave the most drastic of

colour changes when inspected visually, with a significant darkening of the on fibre colour (Figure 2.14). These visual interpretations supported the K/S result, which showed a shift in λ_{max}, confirming the different colours. These results also supported the outcome of post-mordanting by Cardon *et. al.* with natural dyes and fibres.[6]

Figure 2.12. 50% omf Iron Post-Mordant Madder Root.

Figure 2.13. 50% omf Copper Post-Mordant Madder Root.

Figure 2.14. 50% omf Tin Post-Mordant Madder Root.

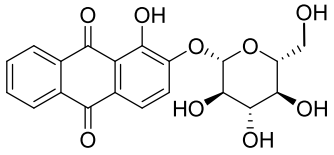
To conclude, the resulting information of the use of pre/post-mordants could be used in future work in the understanding of historically dyed fabrics. These results can help with the identification of pre/post-mordants being used as different shades of the same colour might be seen. More work is needed to be able to do this though, such as, investigating the effect of mordants on solubility of the dye and the nature of the interaction of mordants with the wool and dyestuff.

2.2.3. Dyeing Study of Alizarin vs. Alizarin Glucoside

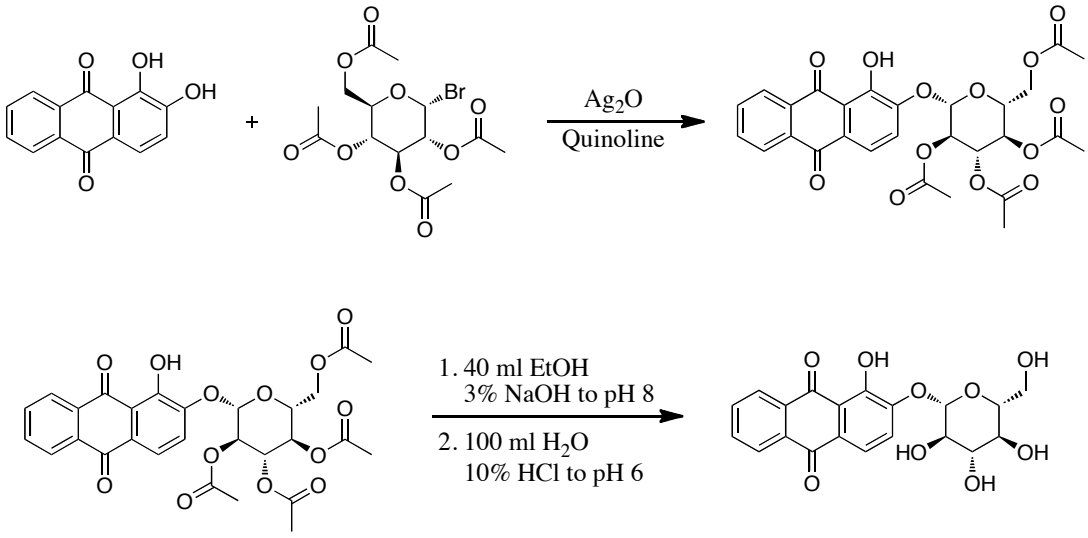
At the beginning of this project it was believed that dye compounds with glycosidic groups attached would provide superior dyeing properties, in comparison with the equivalent dye compound with no sugar moiety attached (aglycone). It was proposed to test this hypothesis, and because the work undertaken herein was primarily with anthraquinone compounds, they would be the compounds chosen for this evaluation. Experimental data for this section can be found in Section 7.5.

Alizarin (**2.1**) is one of the most famous dyeing compounds to exist and was known to successfully dye wool (see Section 2.2.1), hence it was chosen as the aglycone in this experiment. The corresponding compound with a sugar group attached is alizarin monoglucoside (**2.4**), which is not a naturally occurring

compound and cannot be extracted straight from a botanical source. The only option to obtain this compound was to chemically synthesise it from alizarin. The synthetic route undertaken to produce this compound was the Koenigs-Knorr type reaction involving the substitution of a glycosyl halide with an alcohol to give the glycoside compound as stated by Frackowiak *et al*.; a schematic of the reaction is shown in Scheme 2.1.[134]



2.4



Scheme 2.1. Synthetic route of alizarin monoglucoside proposed by Frackowiak *et al*.[134]

The final step of this reaction did not yield the desired compound due to the similarity in the resulting ^{1}H NMR spectra of the acetylated alizarin glucoside and this final unknown compound (results of this can be seen Section 7.5). The problem was due to the solubility of the acetylated alizarin glucoside in ethanol, with the acetylated alizarin glucoside not being dissolved in the solution it was not being deprotonated to further take in the reaction. Even though Frackowiak *et al*. stated

alizarin glucoside was produced it was never observed herein. However, an alternative reaction (Section 7.5) was undertaken using tetrahydrofuran (THF) and methanol as the solvent mixture and with the addition of sodium methoxide resulted in the reduction of the acetyl groups to hydroxyl groups. After 16 hours, the reaction did proceed to completion and yielded one compound that was yellow in appearance. After several recrystallations and by using ^{1}H and ^{13}C NMR spectra the compound was analysed as alizarin glucoside and with a purity of 100%. This analysis was confirmed to be structurally correct by comparing the ^{1}H and ^{13}C NMR spectra with the literature results.[134]

The concentration of the dyebaths used were 10% omf, 5% omf, 2% omf, 1% omf, 0.5% omf and 0.05% omf. These concentrations were chosen so that an adsorption isotherm of the dyebaths could be achieved to accurately show which compound, alizarin or alizarin glucoside, dyed better onto the wool substrate. Synthetic alizarin was purchased from Sigma Aldrich however analysis by ^{1}H NMR highlighted several impurities were present. Recrystallation using boiling ethyl acetate produced a 100% pure alizarin compound analysed by ^{1}H NMR spectroscopy. An adsorption isotherm can enable assessment of which functional groups on the sorbate have the greatest affinity to the sorbent (substrate). The need for a range of concentrations used in the dyeing process is because the adsorption isotherms measure the affinity at all concentrations. The most prevalent dyeing isotherms correspond to three well-known isothermic equations, Nernst, Langmuir and Freundlich.[135]

2.2.3.1. Nernst isotherm

The Nernst isotherm is acquired through the Nernst equation as shown below in Equation 2.1. The meaning of this refers to the adsorption of the dye molecule(s) *via* hydrophobic interactions at non-specific sites upon the substrate; in all the cases in this work the substrate was wool. In Equation 2.1, q_e is used to express the concentration of dye in the fibre (mg g^{-1}), and C_e the concentration of the dye in solution (mg dm^{-3}).

$$q_e = K_N \times C_e$$

Equation 2.1. The Nernst isotherm

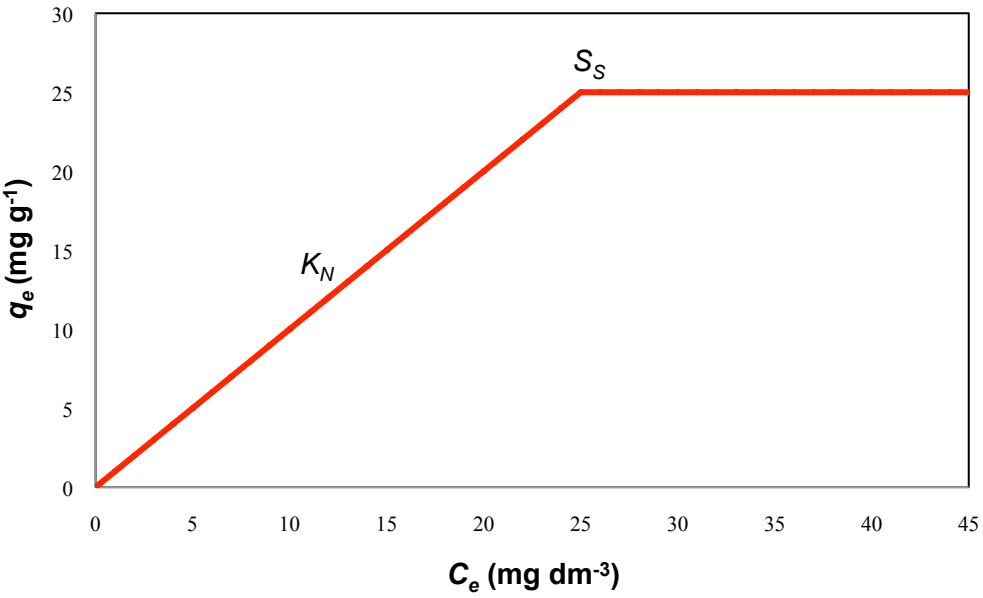


Figure 2.15. Data plot displaying typical Nernst characteristics, the plateau signifying the saturated solution point (S_S) and the gradient representing the Nernst constant K_N.[135]

As can been seen above in Figure 2.15, when the concentration of the dye molecule in solution (C_e) is plotted against the concentration of the dye molecule in the fibre (q_e) a straight line is observed if the Nernst isotherm is agreed.[135] The expression K_N refers to the partition coefficient between of the dye molecule between the substrate and the solution.[135] The plateau (S_s) is achieved when the dye

is absorbed onto the substrate no more and the substrate is fully saturated.[135] Typically, dyeing of polyesters with disperse dyes follows a Nernst isotherm.[136]

2.2.3.2. Langmuir Isotherm

The Langmuir isotherm is acquired through the Langmuir equation (equation 2.2).[137-138] This isotherm assumes that sorption of the dye molecule occurs at specific homogeneous sites upon the substrate.[135] Once the dye molecule occupies this site, it becomes fully saturated and no further binding can occur at this position.[135] Theory states that a monolayer exists upon the substrate and no further adsorption takes place.[137-138] In equation 2.2, q_e is used to express the concentration of dye in the fibre (mg g^{-1}), and C_e the concentration of the dye in solution (mg dm^{-3}).[135] Data of these different concentrations against one another produce a hyperbola as shown in Figure 2.16.[136] The point at which maximum adsorption occurs is shown on the graph as S_f.

$$q_e = \frac{K_L \times C_e}{1 + a_L \times C_e}$$

Equation 2.2. The Langmuir isotherm

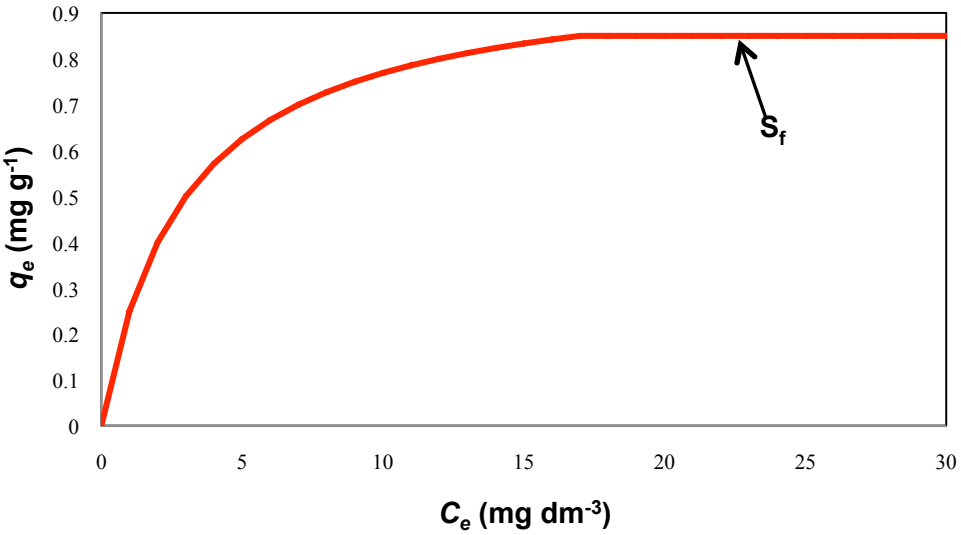


Figure 2.16. Langmuir plot of dye adsorption at equilibrium (q_e) against dye concentration at equilibrium (C_e). The term S_f, corresponding to the saturation value of the dye in the fibre.[137-138]

The term K_L (dm^{-3} mg^{-1}) is the constant that reflects the level of adsorption energy and the term a_L (mg g^{-1}) is the adsorption capacity.[135] These two Langmuir constants calculate the affinity the dye molecule has for the substrate. The plot of q_e/C_e against C_e gives a straight-line graph (Figure 2.17), where a_L/K_L corresponds to the gradient of the graph and $1/K_L$ relates to the intercept of the graph.[136] An example of where the Langmuir isotherm operates is in the dyeing of nylon and wool with anionic dyes (electrostatic interaction).

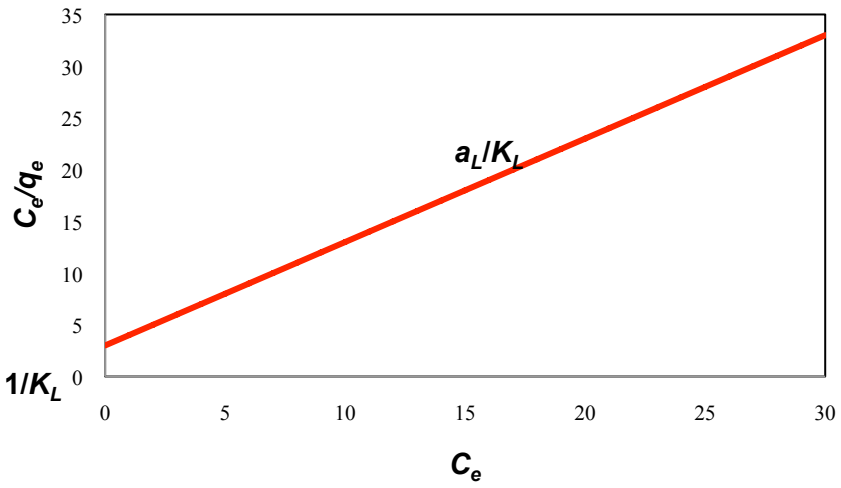


Figure 2.17. Langmuir plot of q_e/C_e against C_e.

2.2.3.3. Freundlich Isotherm

The Freundlich isotherm is acquired through the following (equation 2.3):

$$q_e = K_F \times C_e^{\frac{1}{n_F}}$$

Equation 2.3. The Freundlich isotherm equation

The way in which this isotherm works is it assumes that the sorption process is a heterogeneous system.[139] More than one type of mechanism can occur in the dyeing process. A different interaction not discussed herein is that of the dye molecule interacting with itself (aggregation) and forming multi-layers of adsorbate on top of one another. Due to these different mechanisms the adsorption of dye upon the fibre is unlimited as concentration increases. q_e is used to express the concentration of dye in the fibre (mg g^{-1}), and C_e the concentration of the dye in solution (mg dm^{-3}).[139] Data of these different concentrations against one another produce a parabola as shown in Figure 2.18.

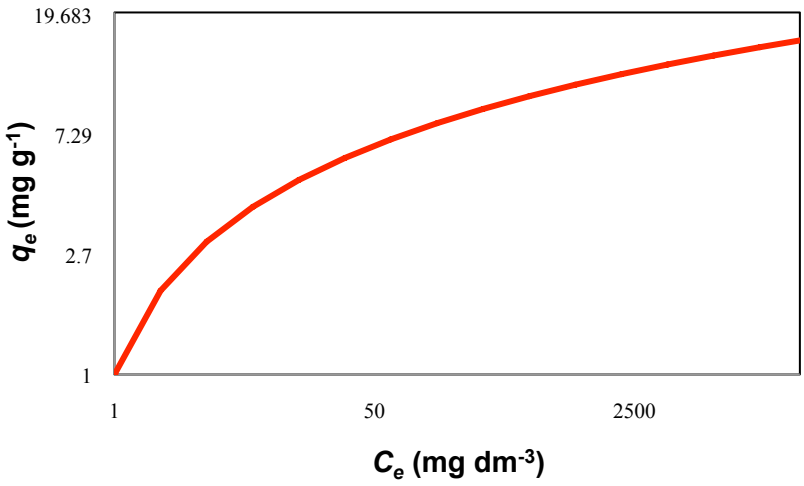


Figure 2.18. Plot of concentration in the fibre (q_e) against dye concentration in the solution (C_e), which yields a Freundlich isotherm.

The term K_F is the Freundlich constant that is the adsorption capacity and $1/n$ is the heterogeneity factor and is the affinity constant. When equation 2.3 is expressed in the logarithmic form, data from the Freundlich isotherm are plot in a linearised graph (Figure 2.19). The constants, which are now measured on the logarithmic scale, $\ln K_F$ and $1/n_F$ now become the intercept and gradient respectively.[135] These isotherms are generally observed when dyeing has taken place on cellulosic fibres with all dye molecule. The major interactions usually observed are van der Waals forces and hydrogen bonding.[139]

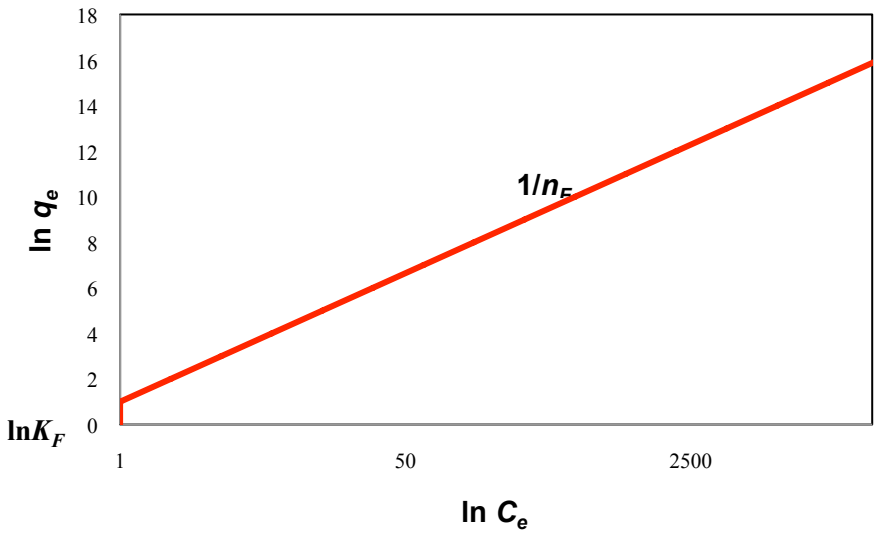


Figure 2.19. Freundlich plot of lnq_e against lnC_e

2.2.4. Isotherm Results of Dyeing with Alizarin and Alizarin Glucoside onto Wool With and Without a Pre-Mordant

Sorption isotherms were calculated for all four dyeing studies undertaken, alizarin with the use of a pre-mordant, alizarin without the use of a pre-mordant, alizarin glucoside with the use of a pre-mordant and alizarin glucoside without the use of a pre-mordant (Table 2.2 and Table 2.3).

Table 2.2. Adsorption isothermic data (Freundlich) for the dye molecules alizarin and alizarin glucoside when dyeing has occurred on wool with and without a pre-mordant.

Freundlich	Mr dye (g mol^{-1})	R_2	K_F (dm^3 g^{-1})	K_F (dm^3 mol^{-1})	n_F	$-\Delta\mu$ (kJ mol^{-1})
Alizarin (No Mordant)	240.21	0.7171	0.155	37.23	1.49	-10.92
Alizarin (Mordant)	240.21	0.9137	0.328	78.79	1.55	-13.18
Alizarin Glucoside (No Mordant)	402.35	0.9112	0.03	12.07	0.873	-7.52
Alizarin Glucoside (Mordant)	402.35	0.8537	0.023	9.25	0.889	-6.72

Table 2.3. Adsorption isothermic data (Langmuir) for the dye molecules alizarin and alizarin glucoside when dyeing has occurred on wool with and without a pre-mordant.

Langmuir	R_2	K_L (dm^3 g^{-1})	K_L (dm^3 mol^{-1})	a_L	q_0 (mg g^{-1})	$-\Delta\mu$ (kJ mol^{-1})
Alizarin (No Mordant)	0.203	--	--	--	--	--
Alizarin (Mordant)	0.9894	0.179	43.00	0.00821	21.80	-11.35
Alizarin Glucoside (No Mordant)	0.1317	--	--	--	--	--
Alizarin Glucoside (Mordant)	0.1759	--	--	--	--	--

Standard affinity ($\Delta\mu^0$) is used to determine whether a spontaneous process has occurred. The process is represented by Equation 2.4, where T is temperature in Kelvin, R is the gas constant, and K is the partition coefficient (kJ mol^{-1}), and shows the difference between the chemical potential of the dye in its standard state in the fibre and the corresponding chemical potential in the dyebath. If $\Delta\mu^0$ is negative then a spontaneous process has occurred; if the process is in equilibrium then $\Delta\mu^0 = 0$; if a non-spontaneous process has taken place then a positive value for $\Delta\mu^0$ will be observed.

$$-\Delta\mu^0 = RT\ln K$$

Equation 2.4. Standard affinity equation

When the results from Table 2.2 and Table 2.3 are compared it shows that alizarin (**2.1**) has a greater affinity for the substrate in comparison with alizarin glucoside (**2.4**). However, this is not the whole story; it has been demonstrated that alizarin molecules, when a mordant is used, adsorb by potentially different mechanism. Alizarin (when a mordant was used) adsorbs preferentially *via* a Langmuir isotherm, meaning that one dye molecule will interact with one site on the

substrate and once this site is occupied no more interactions will occur. This implies that the alizarin molecules will not interact with themselves and π-π stack on top of one another. Conversely, the other three sorbates adsorbs *via* a Freundlich process. In this process more than one mechanism can occur in the dyeing procedure especially the molecule interacting with itself *via* π-π stacking. Due to these different types of mechanisms, in theory, the adsorption of the dye upon the substrate can be unlimited, to an extent, as concentration increases.

Therefore, all the sorbates will be measured against the Freundlich isotherm in order for them to be directly compared. In the instance of dyeing with no mordant, alizarin displays $\Delta\mu^0 = -10.92$ kJ mol^{-1}, meaning that there is a very good affinity from the dye molecule to the wool substrate; for alizarin glucoside, $\Delta\mu^0 = -7.52$ kJ mol^{-1}, meaning that it has lower affinity to the wool substrate when compared to alizarin. As alizarin glucoside has a larger molecular size than alizarin, it is proposed that the overall affinity onto the substrate, at least in a monolayer was limited by steric effects.

When the same dye compounds were dyed in the presence of pre-mordanted wool alizarin has greater affinity for the aluminium metal present (that is covalently bound to the wool in the pre-mordanting stage) than it does with the wool structure itself ($\Delta\mu^0 = -13.18$ kJ mol^{-1}). The Langmuir sorption process was observed to be have a greater correlation (Table 2.3) but to compare the two molecules the Freundlich process results were used. This interaction does agree with the literature of the crystal structure of alizarin; Guilhem states both carbonyl groups have intermolecular hydrogen bonding between molecules to form a 'triple molecule complex'.[140-142] In this triple state an alizarin molecule bound to an aluminium ion can interact with another alizarin molecule *via* π-π stacking.[140-142]

When dyeing with alizarin glucoside onto pre-mordanted wool $\Delta\mu^0 = -6.72$ kJ mol^{-1}, which was slightly lower than when no pre-mordant was used, indicating that alizarin glucoside has greater affinity for the wool structure itself than, rather than the aluminium metal, in contrast to the alizarin molecule. This could be due to the size of the molecule as alizarin glucoside is a lot larger than alizarin as an individual molecule. As shown in Figure 2.5 in this chapter two alizarin molecules can interact with the aluminium metal in a binuclear complex. Due to the size of the alizarin glucoside molecule it is proposed there could be just one molecule attached to the aluminium metal in a similar mononuclear complex (Figure 2.20). Therefore the drop in affinity may not be attributed to the interaction of the alizarin glucoside molecule with the aluminium metal centre, but the alternatively space the alizarin glucoside can occupy around the aluminium metal.

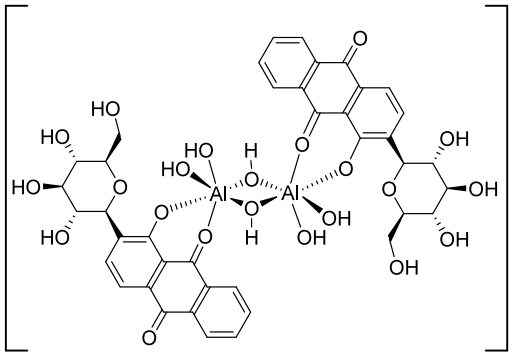


Figure 2.20. Hypothesised mononuclear complex of alizarin glucoside and aluminium.

To conclude, it has been demonstrated herein that alizarin and alizarin glucoside both display a good correlation to the Freundlich sorption isotherm. Alizarin also exhibits good correlation with the Langmuir sorption isotherm when a pre-mordant was used. Alizarin shows to have a greater affinity to the wool fibre but it is almost twice as small as the alizarin glucoside molecule. It is proposed that it is the size of the molecule (alizarin compared to alizarin glucoside) that attributes to the dyeing potential of the compound.

2.3. Conclusions

The aim of this chapter was to see from dyeing studies if alizarin was indeed the main dyeing component of *Rubia tinctorum* and to understand the dyeing of anthraquinone molecules in more detail. It has been demonstrated that alizarin is not the only dyeing component in *Rubia tinctorum* because the substrate, in this circumstance it was wool, does not dye the same colour as when the substrate is dyed with alizarin alone. Therefore it is proposed are other compounds present that are responsible for this famous red colour in historical dyeings. It is believed that these compounds are a mixture of anthraquinone compounds, tannins and other compounds found in plant matter. More work is to be completed in this area to fully discover what the main components in the dyeing with the *Rubia tinctorum* actually are.

The use of pre and post mordants have been shown to drastically change and enhance the dyestuff compounds on the substrates. The use of aluminium in the pre-mordanting procedure helps bind the alizarin molecule to the substrate better, but when alizarin glucoside was used on the same pre-mordanted wool it slightly hindered the dyeing process. This finding could prove useful in later work if glycosylated anthraquinone molecules are found to exist within *Rubia tinctorum*.

It has also been discovered that dyeing with an aglycone and the alternative glycosylated compound resulted in the compounds being adsorbed *via* a Freundlich process. This process showcased that the alizarin and alizarin monoglucoside molecules do dye preferentially to the wool (alizarin has greater affinity) when no mordant was used. They adsorb in a process were more than one mechanism can occur especially the molecule interacting with itself *via* π-π stacking. Due to these different types of mechanisms, in theory, the adsorption of the dye upon the

substrate can be unlimited, to an extent, as concentration increases. However it has been proposed that the size of the glycosylated anthraquinone molecule plays a major role in how much of the dye molecules in the solution adsorb onto the wool fibre. This result becomes even more evident when a mordant is used with a reduction in the affinity the molecule possesses for the wool substrate. In the case of the alizarin molecule the opposite effect was observed, the molecule has a greater affinity for the wool substrate when a mordant was used. This was hypothesised to the strong fixation bonds formed, notably covalent bonds, between the metal and dye molecule. Once formed these bonds would require a significant amount of energy to break, owing to a greater affinity being measured compared to when no mordant was used and hydrogen bonds were preferentially the chemical processes being undertaken.

3. Extraction of *Rubia tinctorum*

3.1. Extraction and Analysis

Following on from the dyeing studies undertaken in Chapter 2, the focus shifted to understanding what dye compounds were being adsorbed onto the wool fibres. This work was fundamental in going forward with the overall goal of this thesis, which was to devise a new technique for successfully analysing glycosidic anthraquinone dye compounds extracted from naturally dyed textile fibres. To be able to know what chemical compounds should be present, to gauge if it was a success or not was key to this analysis. Knowing what chemical compounds are present on the textile fibre in the first place was a major part of this process. Herein, an investigation into the extraction from *Rubia tinctorum* root under various conditions, and subsequent analysis of the isolated extracts was conducted. The extraction and analysis of glycosidic anthraquinone compounds was very important in this section so that future work could be undertaken to dye with these compounds upon wool and to try and successfully extract them from dyed wool samples with new and previously documented techniques. All experimental data for this chapter can be found in Chapter 7, Section 7.8.

3.1.1. Solvent Studies of *Rubia tinctorum*

It was anticipated that varying the solvent would alter the extract compound profile, *i.e.* the relative ratios of the dyeing compounds (*e.g.* alizarin, ruberythric acid and lucidin primeveroside *etc.*) present within the fresh madder root extract. This is very important for the provenancing of botanical sources as the relative ratios of anthraquinone compounds changes from different species, to where it was grown

and what conditions it has been grown from.[1-3,6,16,46] The following solvents were used for the initial extractions from fresh *Rubia tinctorum* root: water (HPLC grade); ethanol; and a 1M aqueous NaOH solution. A water extraction was used in conjunction with the literature reference undertaken in the dyeings in Chapter 2.[6] Ethanol was chosen as a solvent of interest as there are literature precedents that perform ethanolic and water/ethanolic extractions of fresh *Rubia tinctorum* and in the majority of cases, glycosidic anthraquinone compounds are detected and analysed.[3,44,46,53,143-149] The use of NaOH in the extraction process was trailed to see the effects of an alkaline solution have on compounds extracted, it was proposed that the alkaline conditions would preferentially extracted anthraquinone compounds by deprotonating the hydroxyl groups readily associated with them.[150] As stated in the aims of this chapter the extraction of glycosidic anthraquinone compounds was essential for future work, with this being the case, an acidic solution was not used in this instance due to the fact it would hydrolyse any anthraquinone glycosides extracted.[44,46] For all extractions the root was finely ground before immersion in the solvents. All extractions were performed at 60 ˚C for 24 hours, with 120 ml of solvent used per 1 g of ground madder root; this was performed to observe the changes of concentrations of the dye molecules in solution over time. UV-vis spectroscopy was chosen as the analytical technique to follow this reaction as the results that were being measured were the absorbance range of the dyebath solutions produced and the time needed to reach the highest absorbance achievable. Shown below in Figure 3.1 are the overlaid UV-vis spectra for the three solvent systems investigated, at their highest absorbance measured (results were performed on diluted samples so the absorbance range was between 0.2 and 0.8 to satisfy Beer Lambert's Law, then multiplied to show the correct absorbance of the solutions).

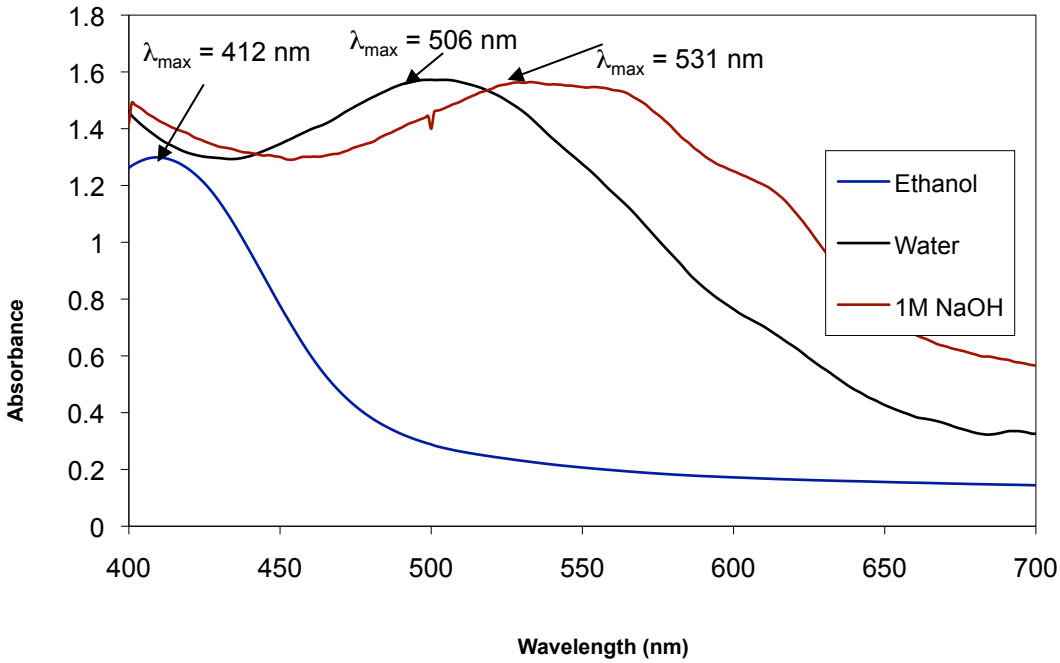


Figure 3.1. Preliminary solvent studies for the extraction of *Rubia tinctorum* root.

From the results shown above, the water extraction has a λ_{max} of 506 nm and the colour of the dyebath was observed as a reddish-orange solution. This result agrees with the colour of the wool samples dyed with *Rubia tinctorum* from a water extraction seen in Chapter 2. Anthraquinone compounds such as alizarin, lucidin primeveroside, ruberythric acid and several other known compounds have λ_{max} absorbance's between 400 – 480 nm.[44,46,151-152] A proposed reason for this λ_{max} of 506 nm being analysed has been attributed to the extraction of other compounds from *Rubia tinctorum* other than anthraquinone molecules, such as polyphenolics and tannins, which are often very dark in colour. The use of 1M NaOH solution in the extraction procedure produced a UV-vis profile with a λ_{max} of 531 nm, this profile was proposed to be due to the deprotonation of the compounds extracted, shifting the λ_{max} to a longer wavelength than that observed in the water extraction. For the case of the ethanol extraction the λ_{max} is substantially different at 412 nm and so in fact is the entire UV-vis profile. Known anthraquinone compounds with a

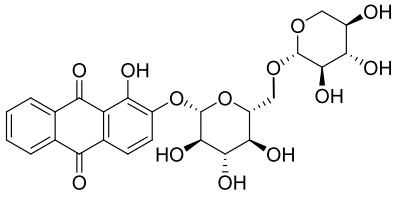
λ_{max} similar to this figure include ruberythric acid (406 nm), lucidin primeveroside (415 nm) and alizarin (424 nm).[44,46,151-152] The use of ethanol as a solvent seemed to preferentially extract glycosidic anthraquinone compounds, which was the aim of this chapter. Following on from this preliminary work, analysis by ^{1}H NMR spectroscopy followed to get a more detailed analysis of the compounds present in the three extraction procedures (details from the spectroscopy results can be observed in Section 7.8). The ^{1}H NMR spectrum data of the 1M NaOH solution extract composed of mainly polysaccharides (δ 3.0 – 5.5) and no aromatic protons were analysed by this technique. The ^{1}H NMR spectrum data of the water extraction yielded a result of aromatic protons (δ 8.3 – 7.4) and polysaccharides (δ 3.0 – 5.5). The ratio of polysaccahrides to anthraquinones was measured at 98:2. The ^{1}H NMR spectrum data of the ethanol extraction yielded a result of aromatic protons (δ 8.3 – 7.4) and polysaccharides (δ 3.0 – 5.5). However the ratio of polysaccahrides to anthraquinones was measured at 90:10.

The ethanol extraction process produced a UV-vis spectrum that was very distinctive to well-known anthraquinone compounds especially ruberythric acid and lucidin primeveroside. Coupled with the results from the ^{1}H NMR spectroscopy, a greater proportion of aromatic protons were extracted in the ethanol extraction procedure than the water extraction procedure. It was understood that the use of ethanol in the extraction process would be used in future extractions to provide a dyebath liquor with more glycosidic anthraquinone compounds present.

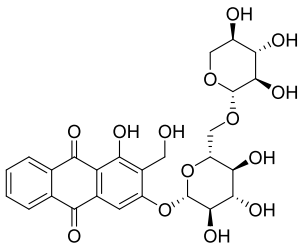
3.1.2. Water and Ethanol Used for the Extraction of *Rubia tinctorum*

Following on from the conclusion of the previous section (3.1.1), the next logical step was to investigate the effects on extraction from madder root using an aqueous ethanolic mixture. Some literature sources state that the glycosides ruberythric acid

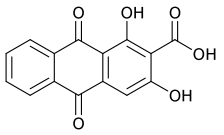
and lucidin primeveroside are present in the madder root, but that there are up to 36 other anthraquinone compounds reported in *Rubia tinctorum*.[1-4,44,46] Derksen *et al.* stated they used a water/ethanol solvent system, which was refluxed for the extraction of *Rubia tinctorum*.[44,46] This procedure claimed to have extracted four compounds using this technique, which were ruberythric acid (**3.1**), lucidin primeveroside (**3.2**), munjistin (**3.3**) and pseudopurpurin (**3.4**).[44,46]



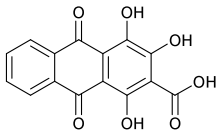
3.1



3.2

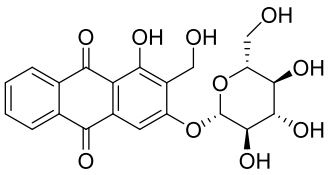


3.3

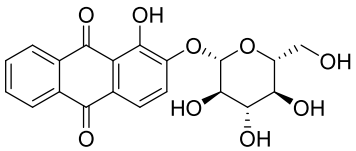


3.4

The quantity of these compounds detected is very important in the provenancing of madder sources, especially in historical samples. Derksen *et al*. undertook a second extraction in 2% H_2SO_4 after the filtrate was evaporated to dryness under reduced pressure.[44,46] The result was the detection of 4 different compounds, lucidin glucoside (**3.5**), alizarin glucoside (**3.6**), alizarin and purpurin.



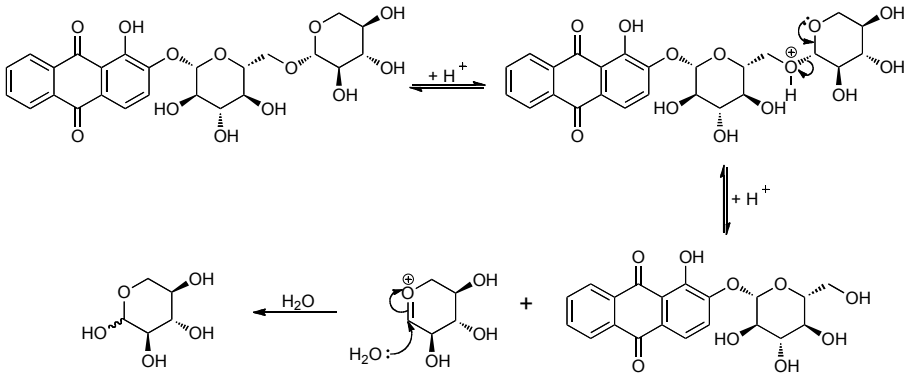
3.5



3.6

Acidic conditions can have a detrimental effect to glycosylated compounds, one reason why different compounds were detected in this second extraction could be due to hydrolysis of sugar moieties, as shown in Scheme 3.2. Under acidic

conditions, the ether oxygen can become protonated. Once this step has occurred the lone pair of the oxygen in the ring can push its electrons into the neighbouring carbon bond resulting in the loss of the oxonium sugar moiety. The oxonium ion can be attacked by water resulting in the formation of a single sugar group (xylose). These resulting compounds can be misleading in the identification of anthraquinone compounds as they are not naturally occurring in *Rubia tinctorum*, but chemically hydrolysed when acidic conditions are used.



Scheme 3.2. Hydrolysis of ruberythric acid.

This final procedure by Derksen *et al.* was not taken forward as a way of extracting anthraquinone compounds in this work due to hydrolysis of the glycosidic anthraquinone compounds, most notably ruberythric acid and lucidin primeveroside. The aim of the extraction studies herein was to fully understand and analyse what the components of the madder root were and if certain compounds could be preferentially extracted using a water/ethanol solvent *i.e.* glycosidic anthraquinone compounds.

The materials used for the extractions were madder root from Aurorasilk (material was sold as *Rubia tinctorum* L. but was never botanically analysed if this was correct), HPLC water, methanol and ethanol from Fisher Scientific UK. The madder root was ground up into a fine powder using a Wahl ZX595 Mini Grinder, more experimental details can be found in Section 7.8. As can be seen in Table 3.1 the

conditions are different for each entry just the volume of liquid used and the length of the extraction time are constant. The temperatures were initially chosen for A1 and A2 to have the liquid in the process at a refluxing temperature. For entries A3 and A4 it was proposed that the use of no heat might preferentially extract undesirable compounds such as polysaccharides and tannins. The example of how the % of polysaccharide present in the sample was calculated can be observed in Section 7.3.

Table 3.1. Experiments for extraction of anthraquinones from madder root.

Entry	Conditions (500 ml of solvent & 3 hours)	Madder Used (g)	Mass Extracted (g)	Average Yield (%)	Polysaccharide (%)
A1	Water/Ethanol 80 ºC	17	3.30	19.4	>96%
A2	Water 100 ºC	8	1.10	13.8	100%
B2	Ethanol Room Temperature	7	<0.05	<1.0	n/a
A3	Ethanol Room Temperature	8	0.90	11.3	>99%
A4	Ethanol 78 ºC	17	2.50	14.7	90%

The literature states that when a 50:50 mixture of water and ethanol are used as the reagents in the extraction procedure and refluxed along with finely ground madder root, four compounds were extracted.[44,46] When this extraction procedure (entry A1) was repeated and analysed by HPLC and LC-MS (Figure 3.2 and Figure 3.3), only two compounds were observed: ruberythric acid and lucidin primeveroside. In negative ion mode the LC-MS peaks detected were, compound (1) over a range of 1.32-1.40 minutes with a maximum mass (m/z) of 533, which corresponded to ruberythric acid and compound (2) over a range of 1.40-1.50 minutes with a maximum mass (m/z) of 563, which corresponded to lucidin

primeveroside. The HPLC method is given in detail in Section 7.1.1 and peaks were detected at a wavelength of 254 nm; peak (1) retention time = 5.84 minutes (λ_{max} = 406, $[M-H]^-$; 563), lucidin primeveroside and peak (2) retention time = 6.86 minutes (λ_{max} = 415, $[M-H]^-$; 533), ruberythric acid.

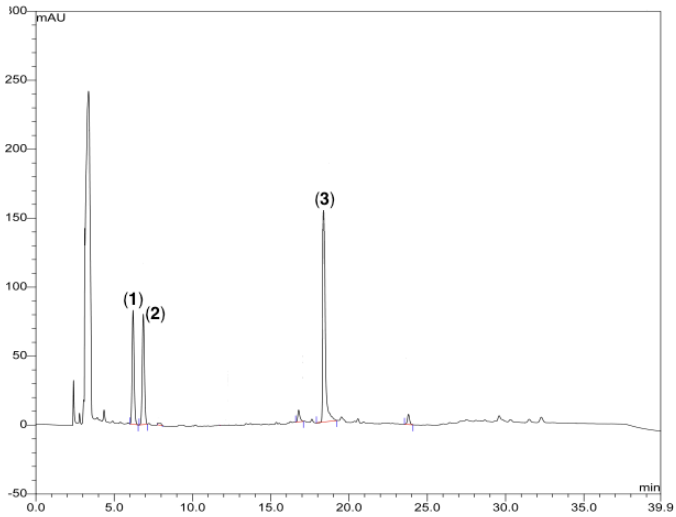


Figure 3.2. HPLC chromatogram of the madder extract showing three compounds present (**1**) lucidin primeveroside (**2**) ruberythric acid (**3**) alizarin.

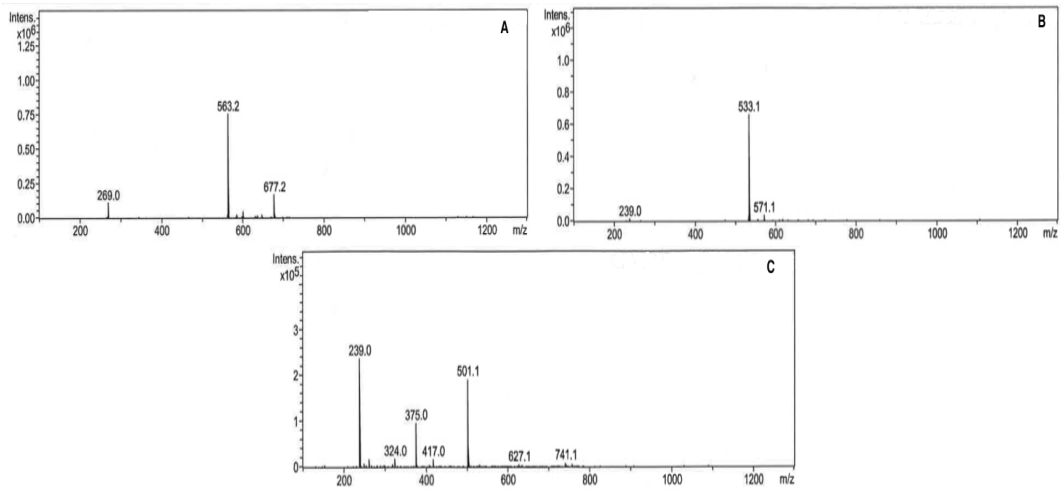


Figure 3.3. Mass spectra data of the madder extract showing three compounds present (**1**) lucidin primeveroside (**2**) ruberythric acid (**3**) alizarin.

The liquid extract was dried to a solid using a rotary evaporator and produced a reddish black solid that was very sticky and difficult to handle; from experience this suggested the presence of free sugars and mainly non-anthraquinonoid compounds.

When analysed by ^{1}H NMR spectroscopy it became clear that not only were there two anthraquinone glycosides present (δ 8.3 ppm – 7.4 ppm), but a significant amount of polysaccharide material and non-anthraquinone compounds (δ 3.0 ppm – 5.5 ppm). Removing these polysaccharides and non-anthraquinone compounds proved difficult, and was not achieved for this entry.

With a high proportion of the extract liquor being polysaccharides/free sugars when analysed by ^{1}H NMR spectroscopy, efforts were made to try and extract these materials in an initial extraction and then extract the anthraquinone compounds in a subsequent extraction procedure. If this could be achieved it would mean that only anthraquinone dye compounds (especially glycosidic anthraquinone compounds) would be present in dyebath when dyeing resulting in the maximum number of anthraquinone compounds adsorbed onto the wool fibres. The process proposed was an initial boiling water treatment of the madder root, directly followed by an ethanol extraction of the residual matter. Entry A2 was the initial extraction using refluxing water; when the residual liquor from this extraction was analysed by LC-MS in negative ion mode, a peak was detected over a range of 2.06-2.20 minutes with a maximum mass (m/z) of 785. This peak had no UV-vis response over the range 390-650 nm. The liquor was dried (*in vaccuo*) and a black sticky gel was obtained that when analysed by ^{1}H NMR spectroscopy the extract consisted of polysaccharides and other non-anthraquinone compounds (δ 3.0 – 5.5). Thus it was theorised that a water extraction could be used as a method of preferentially removing polysaccharides and non-anthraquinone compounds from the madder root. However, the room temperature ethanol extraction of the residual root from B2 only isolated a negligible amount of product. This was seen by the solvent changing colour, from colourless to orange, during the extraction process but the product was too low to

analyse effectively by either LC-MS or ^{1}H NMR spectroscopy (entry B2); this would suggest that the presence of polysaccharides and/or sugars might assist the extraction of anthraquinones from the madder root.

The use of ethanol as a solvent in the above procedures has been proven to aid in the extraction of more anthraquinone compounds in the resulting liquor. However extraction of the polysaccharides in the procedure has been emphasised as vitally important in acquiring these anthraquinone compounds. The use of ethanol as the sole solvent in the extraction procedure was the next logical step in understanding if a greater amount of anthraquinone compounds could be extracted. Entry A3 was an extraction with ethanol, but only at room temperature. It was believed that using no heat would also reduce the amount of polysaccharide in the final product. The result was not one that was expected; the amount of anthraquinones extracted was significantly lower than when the reaction was heated (entry A4), and about 99% of the product was polysaccharide (analysed by ^{1}H NMR spectroscopy, δ 8.3 ppm – 7.4 ppm represented the aromatic protons present in the anthraquinone compounds and δ 3.0 ppm – 5.5 ppm represented the polysaccharide/free sugars) whereas in entry A1 >96% of the product was polysaccharide. However, the observation of anthraquinones in the extract from entry A3 supports the conclusion that polysaccharides and non-anthraquinone compounds assist the extraction of anthraquinone compounds, as highlighted by the low anthraquinone yield from entry B2.

Using refluxing ethanol as the solvent of choice, corresponding to entry 4, produced a bright orange solution after 3 hours. When this solution was analysed by LC-MS in negative ion mode 4 peaks were detected; (1) retention time = 1.39-1.51 mins ($[M-H]^{-}$; 677), (2) retention time = 1.74-1.78 mins ($[M-H]^{-}$; 269) corresponded

to lucidin, (3) retention time = 1.78-1.87 mins ([M-H]$^-$; 239) corresponded to alizarin, (4) retention time = 1.96-2.15 mins ([M-H]$^-$; 297) corresponded to lucidin-ω-ethyl ether.[115] Further investigation revealed that peak (1) was in fact two peaks very close together comprising of values, [M-H]$^-$; 563 and 533, these two peaks being lucidin primeveroside and ruberythric acid respectively. As can be seen in Figure 3.4 the result of the new analysis of entry 4 yielded four compounds; lucidin primeveroside (**1**) and ruberythric acid (**2**) were present but also two new compounds were observed, alizarin (**3**) and lucidin-ω-ethyl ether (**4**).

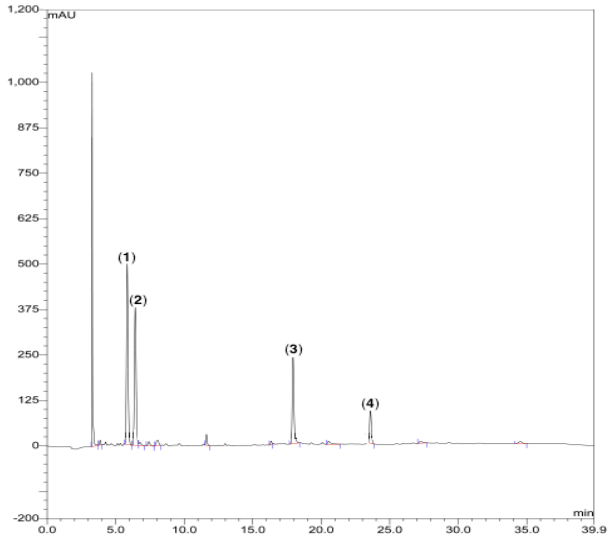


Figure 3.4. HPLC chromatogram of the madder extract showing four compounds present (**1**) lucidin primeveroside (**2**) ruberythric acid (**3**) alizarin (**4**) lucdin-w-ethyl ether

UV-vis chromatograms were obtained along with mass spectrometry (Figure 3.5 and Figure 3.6) to clearly identify the signals seen. The results were as followed, lucidin primeveroside (λ_{max} = 406, [M-H]$^-$; m/z = 563) ruberythric acid (λ_{max} = 415, [M-H]$^-$; m/z = 533), alizarin (λ_{max} = 430, ([M-H]$^-$, m/z = 239) and lucidin-ω-ethyl ether (λ_{max} = 412, [M-H]$^-$; m/z = 298).

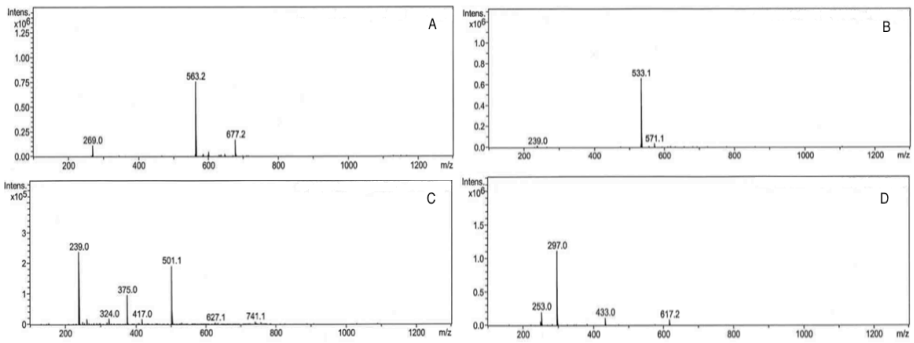


Figure 3.5. Mass spectra data of the madder extract of the four compounds present (**A**) lucidin primeveroside (**B**) ruberythric acid (**C**) alizarin (**D**) lucidin-w-ethyl ether

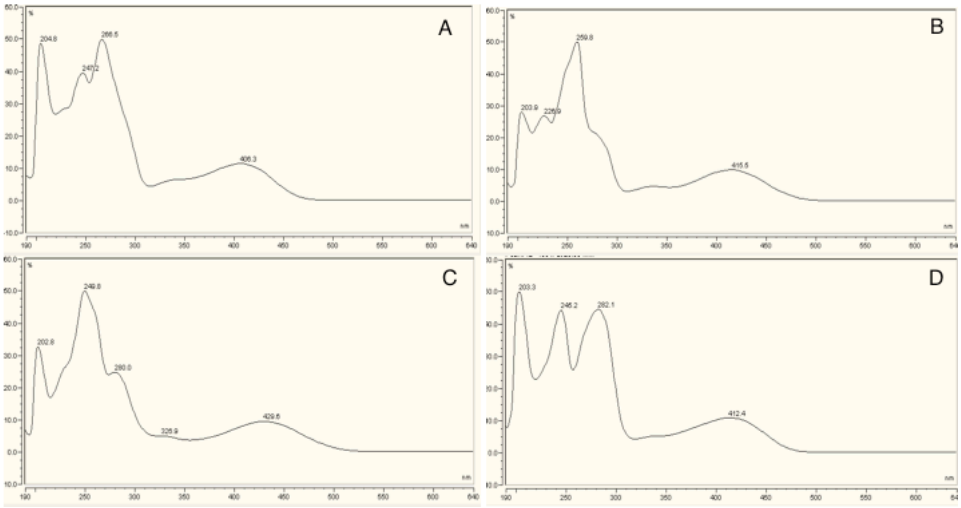
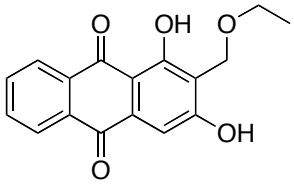


Figure 3.6. UV-vis spectra, from HPLC data of the madder extract before dyeing at 10% omf, of the four compounds present (A) lucidin primeveroside (B) ruberythric acid (C) alizarin (D) lucidin-w-ethyl ether.

Lucidin-ω-ethyl ether had not been previously observed during these studies and after looking in the literature it became apparent that this compound was not one that was very abundant in other extraction profiles of *Rubia tinctorum*. Nevertheless Orban *et al.* have reported detecting this compound by their HPLC methods.[46,143,149] Many attempts were made to successfully isolate and fully analyse the lucidin-ω-ethyl ether (**3.7**) with the techniques available, but it proved unsuccessful.



3.7

^{1}H NMR spectroscopy of this material also showed that a mixture of lucidin and alizarin compounds were present over a range of δ 8.3 ppm – 7.4 ppm; it was also shown by the ^{1}H NMR spectrum that there was significantly less polysaccharide, δ 3.0 ppm – 5.5 ppm, in this orange solid then in any other entry analysed. The mixture of alizarin and lucidin by ^{1}H NMR spectroscopy was measured as 1:1. The extract solid was also the only one out of all the extractions undertaken to not be hygroscopic and stored without becoming an insoluble black solid after time. This was the most promising result out of all the extractions thus far, and was used as the main extraction procedure to obtain pure samples of the glycosides so that they could be analysed further, as discussed later.

To conclude, the use of ethanol as the solvent for extraction of anthraquinone dye molecules from madder root proved to be the most effective for extractions of the glycosides ruberythric acid and lucidin primeveroside. This work showcased that when extracting with water, glycosidic anthraquinone compounds were also extracted, though not as efficiently as when ethanol is used. Knowing that ruberythric acid and lucidin primeveroside are extracted by water verifies the results shown in the dyeing experiments that alizarin is not the main component in madder root responsible for the coloration of the wool. However throughout all the extractions undertaken no munjistin or pseudopurpurin was observed by analytical methods. This could be due to them not being present in the madder root that was used in these extractions or they are not present to the same level of quantity of

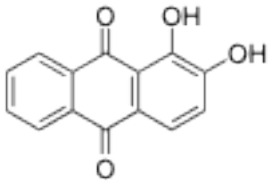
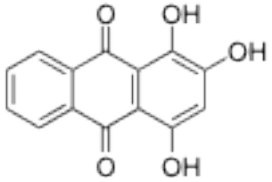
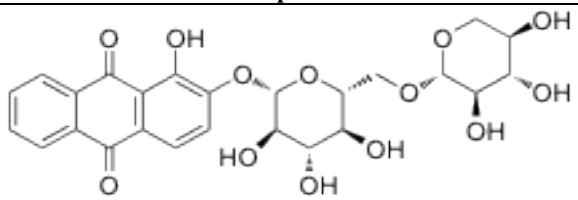
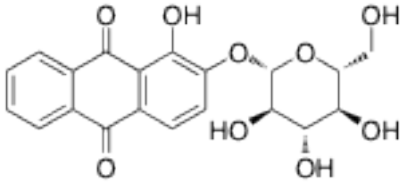
ruberythric acid and lucidin primeveroside especially by analysis using ^{1}H NMR spectroscopy. Due to the successful extraction of glycosidic anthraquinone molecules the use of ethanol as the extracting solvent will be continued and used in future work within this research.

3.1.3. pH Studies of Known Samples

There is very little information in the literature regarding how the anthraquinone chromophores respond to pH variation.[153] A better understanding of the anthraquinone pH response would give a clearer understanding of the conditions needed to extract certain anthraquinones from the madder or even potential alterations to anthraquinones once they have been extracted. Alizarin (Sigma Aldrich [97% purity], 100% purified in-house), purpurin (Sigma Aldrich, 90% purity) ruberythric acid (APIN Chemicals, 92% purity), alizarin glucoside (synthesised from alizarin, 100% purity) and madder root extract (Aurorasilk, ethanol extraction process) were analysed during the following pH study. These were the compounds analysed along with the madder root extract in Table 3.2. Entries A5-A9 were all dissolved into a 50/50 mixture of acetone and water to obtain a spectrum on the UV-vis spectrophotometer. Acetone was used, as the commercially bought ruberythric acid would not dissolve into a 50/50 mixture of water and ethanol and consistency was required to analyse any differences between UV-vis spectra. A Thermo Electron Orion 310 pH/T meter was used to calculate the pH values of the solvents in this section. Using 1M HCl and 1M NaOH solutions, the pH values of entries 8-11 were altered from pH 2 to pH 13, with pH increments of 1. A 20 μL P20 Gilson PipetMan was used to add drop wise additions of the acid and base solutions to get the desired pH value, on average this equated to 3 μL of solution. All 12 different pH solutions for each entry were analysed by UV-vis

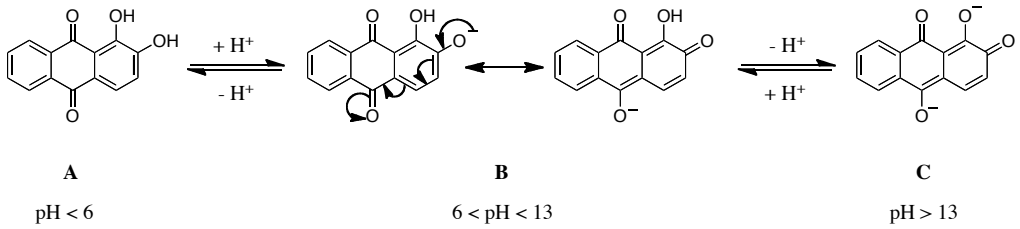
absorbance spectroscopy, and graphs of the results were obtained (Figure 3.7 – Figure 3.11).

Table 3.2. Compounds used in pH study.

Entry	Compound	Solvent
A5	 **Alizarin**	50/50 acetone water mixture
A6	 **Purpurin**	50/50 acetone water mixture
A7	 **Ruberythric Acid**	50/50 acetone water mixture
A8	 **Alizarin Glucoside**	50/50 acetone water mixture
A9	**Madder Root** Mixture of Compounds	50/50 acetone water mixture

Under acidic conditions alizarin (entry A5) was fully protonated and retained its original structure (Scheme 3.3, A). When the pH was between 6 and 13 (Scheme 3.3, B) the phenol group in the secondary position becomes deprotonated, this being stabilised by resonance. Above pH 13 the phenol group in the primary position also becomes deprotonated (Scheme 3.3, C), this can then resonate and stabilise over the four oxygen atoms. Evidence for this was found in the literature, claiming that the pK_a (β phenol = 12.0, γ phenol = 8.2) for the phenol group in the second position is the lower of the two phenol groups.[153] This can be seen in Figure 3.3 with the

spectrum trace for pH 13.04, the trace has one distinctive λ_{max} but there are signs of other absorbance bands and this was due to the fact that the compound was distributing the charge over a range of atoms and rearranging from conformation to conformation in the solution.



Scheme 3.3. pH affect on alizarin.[153]

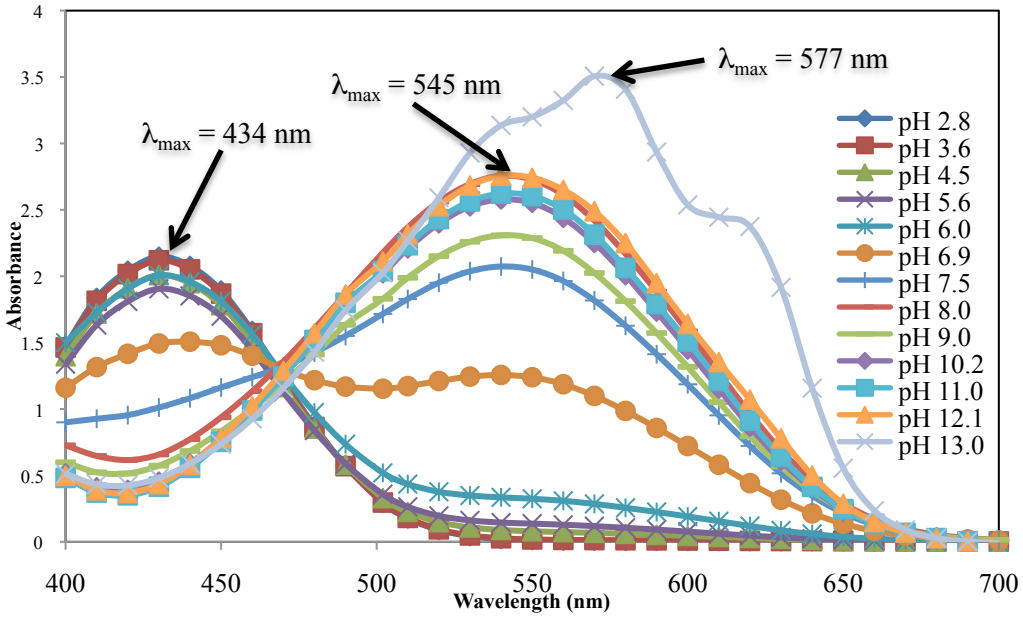


Figure 3.7. Alizarin chromophoric responses to change in pH.

The purpurin molecule (entry A6) has an extra phenol group compared to alizarin; again it was analysed that under acidic conditions, up to pH 6, the molecule was fully protonated, this was seen at a λ_{max} of 482 nm (Figure 3.8). When the pH was increased above pH 6 the phenol group in the second position becomes deprotonated and this is seen by a change, λ_{max} shifts to 513 nm (Scheme 3.4). As the pH was taken above 10 the phenol group in the fourth position is deprotonated and this is the

λ_{max} at 548 nm. This deprotonation of the phenol group in the primary position can be seen in the UV-vis spectra at pH 13; there is a shoulder appearing at 610 nm.

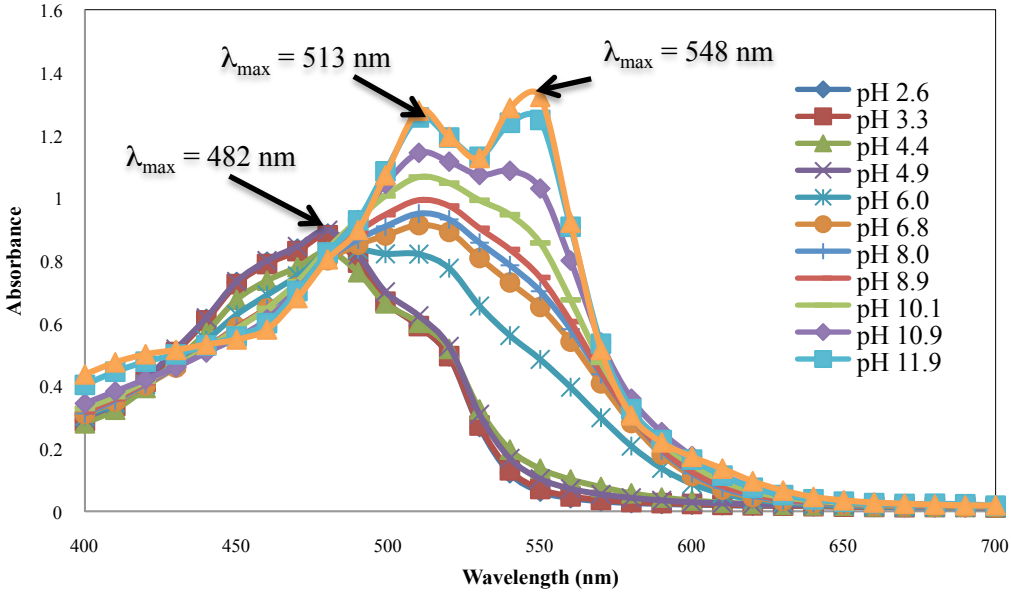
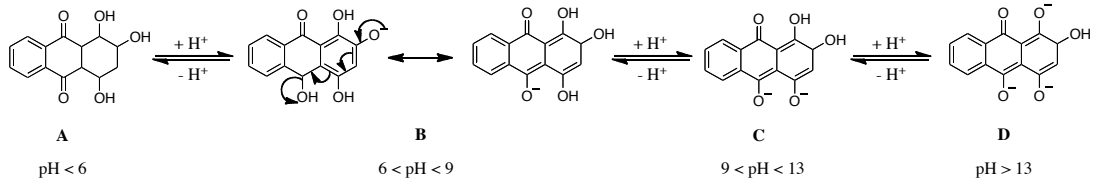


Figure 3.8. Purpurin chromophoric responses to change in pH**.**



Scheme 3.4. pH affect on purpurin.

The ruberythric acid molecule (entry A7, Figure 3.9) gave a similar result as alizarin with just the phenol group in the second position substituted for a glycoside group consisting of two sugars (glucose + xylose). The absorbance with a λ_{max} of 410 nm was in acidic conditions; this is when the molecule is fully protonated (Scheme 3.3, A). It is not until pH 9 and 10 that a second absorbance was seen at a λ_{max} of 506 nm, due to the deprotonation of the hydroxyl group in the primary position of ruberythric acid. It is proposed that it was not the same base induced absorption as alizarin, suggesting that it was not the oxygen group on the secondary position with the sugar group attached to it that was deprotonated, as this would have given a

similar absorbance as alizarin (545 nm). Because the deprotonation occurred at such a high pH, this suggests it was the phenol group in the primary position that is deprotonated; deprotonation of this phenol group was also suggested to be around pH 9 when using a pK_a predictor.[154] The reason why these two pHs differ in ruberythric acid and alizarin is because in the alizarin molecule there are two phenolic protons, while in ruberythric acid there is only one. Once the phenol group in the secondary position of alizarin has been deprotonated it is subsequently harder to deprotonate then the phenol group in the primary position and this is seen when the two graphs are compared.[153]

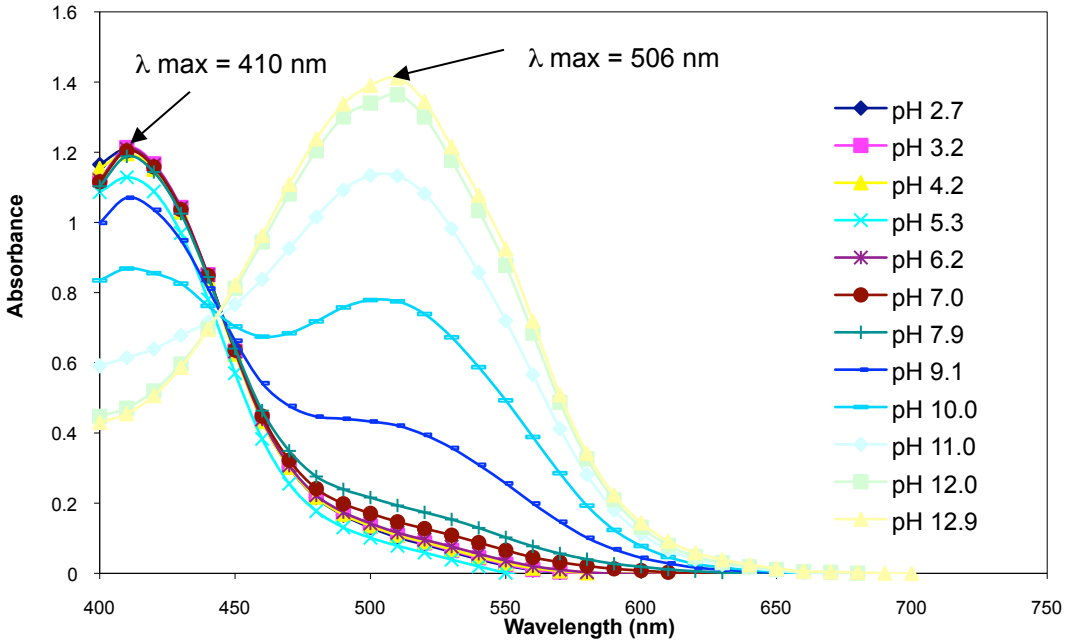


Figure 3.9. Ruberythric acid chromophoric responses to change in pH.

Alizarin glucoside was used in the pH study to see if there was indeed a difference in the chromophoric response between an anthraquinone aglycone compound (alizarin), an anthraquinone monosaccharide compound (alizarin glucoside) and an anthraquinone disaccharide compound (ruberythric acid). Under acidic conditions, = for alizarin glucoside (Figure 3.10) has a λ_{max} at a shorter wavelength, λ_{max} = 415

nm, compared with the λ_{max} of 434 nm for alizarin (Figure 3.2) in the same conditions. This indicates that the addition of a sugar group does affect the chromophore and ultimately the colour of the compound produced. In these acidic conditions the hydroxyl group in the primary position will be protonated (Entry A8 in Table 3.2).

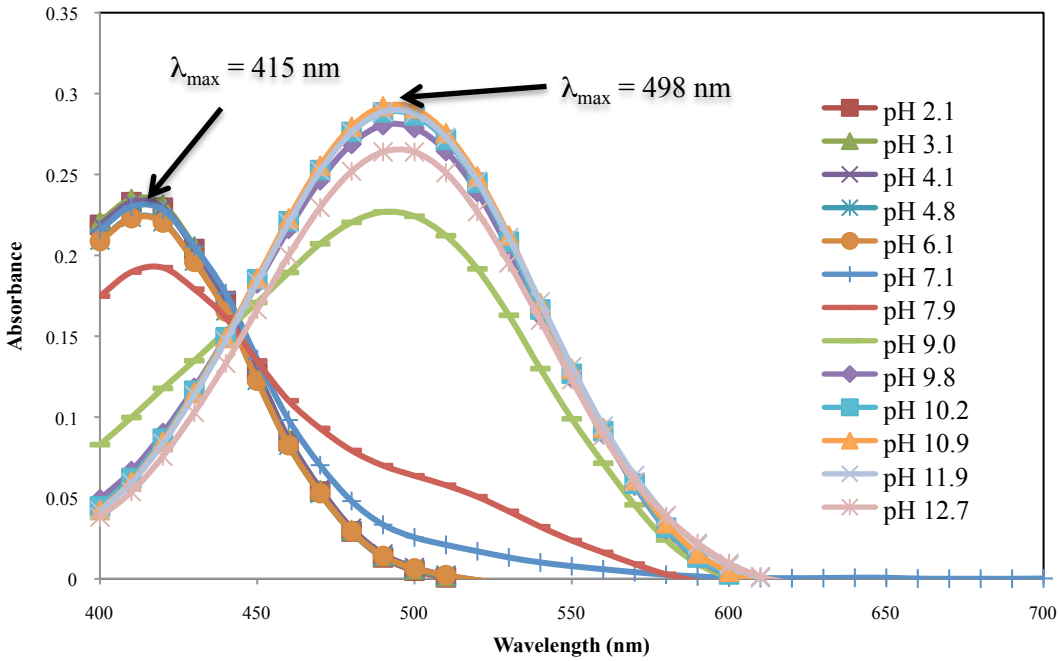


Figure 3.10. Alizarin glucoside chromophoric response to change in pH.

It is at pH 8 that the primary hydroxyl group becomes partially deprotonated and this can be seen with a second absorbance peak at 498 nm; as the solution becomes more basic this absorbance peak at 498 nm is increased and the peak at 415 nm is diminished above pH 9, which is when the alizarin glucoside compound is fully deprotonated at the hydroxyl position. This observation is characteristic of an anthraquinone compound with a hydroxyl group in the first position;[153] however, there is a clear distinction between alizarin glucoside and alizarin. The pH at which the deprotonation of the hydroxyl group in the primary position occurs was significantly less basic during the pH study of alizarin glucoside (pH 8) than the same study with alizarin (pH 13). The proposal for this result is due to the nature of

the functional group present in the secondary position. Alizarin has a hydroxyl group present in position two that has already been shown to make the hydroxyl in position one harder to deprotonate, whilst alizarin glucoside has a glucose group linked to the anthraquinone compound through an ether linkage. This glucose group can only be hydrolysed under acidic conditions and under basic conditions the oxygen in the ether bond does not feed electrons into the benzene ring of the anthraquinone.

The final pH study was acquired on the madder root extract (Figure 3.11) to determine if there were any similarities in the studies already obtained for the pure compounds. Again at an acidic pH (pH < 6) the chromophoric compounds appear to be fully protonated, as a similar result is seen for alizarin and ruberythric acid. The most interesting observation was that around pH 7.94 a deprotonation looks to have taken place, with a λ_{max} of 515 nm. A subsequent deprotonation at pH 12.8 seems to have been induced, with a λ_{max} of 544 nm.

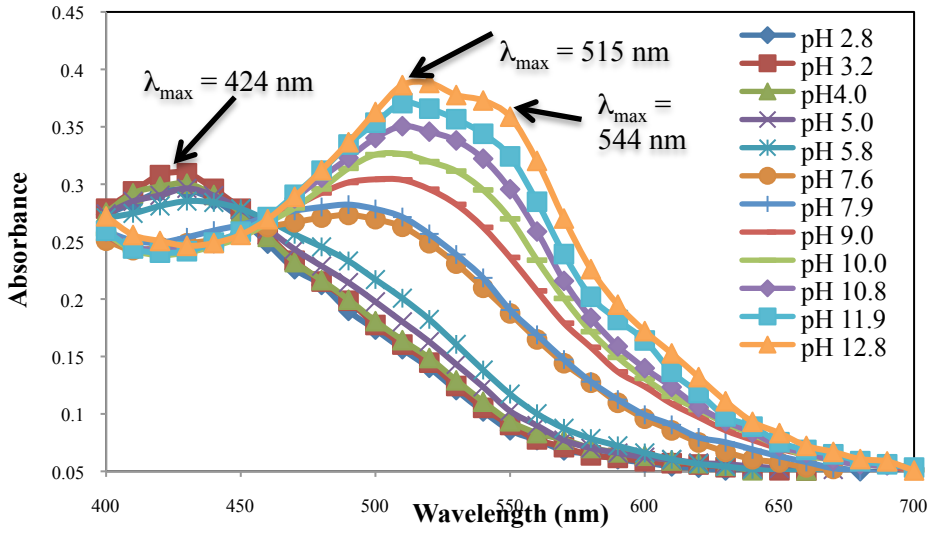


Figure 3.11. Madder root chromophoric responses to change in pH.

To conclude, the pH study results have demonstrated that under acidic conditions the structures of the anthraquinones are fully protonated, and it was not until a pH

above 8 that deprotonation of hydroxyl groups are induced. This was useful information as it could assist experimental planning in the future, especially to predict solubilities of these compounds for subsequent purifications. Responses to pH change may also be useful knowledge for future investigations when the glycosidic compounds are to be hydrolysed under acidic conditions forming the aglycones. The results are concordant with the literature in stating the pK_a of the phenol in the secondary position in alizarin is more acidic then the phenol in the primary position.[153] However it has been hypothesised that the phenol at the primary position can be deprotonated at a lower pH when there is a sugar group attached to the phenol at the secondary position.

3.1.4. Studies on Ruberythric Acid

To gain a better understanding of the madder root, which was a very important objective in this work, further analysis of the compounds within the madder root was needed. The analysis included ^{1}H NMR spectroscopy, LC-MS, HPLC and UV-vis spectroscopy. After initial analysis of ruberythric acid (90% purity) bought from Apin Chemicals, shown by the UV-vis spectra in Section 3.1.3 that ruberythric acid was present (λ_{max} = 415), further analytical evidence was sought.[44,46,145-146] The first study completed was a ^{1}H NMR spectrum of the sample, which produced an unexpected result (Figure 3.12).

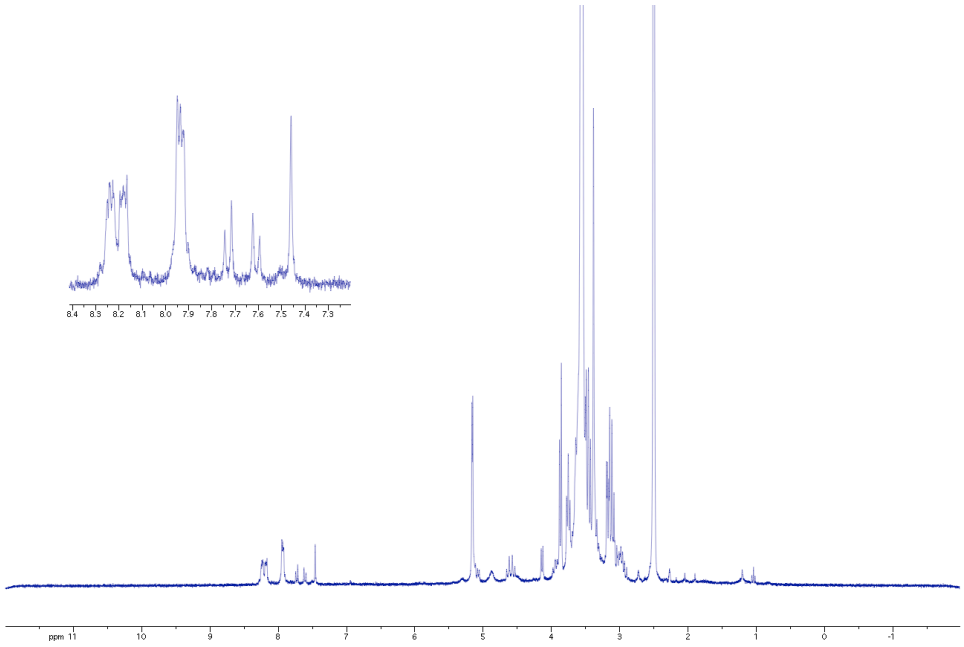


Figure 3.12. ^{1}H NMR spectrum of ruberythric acid.

Closer inspection of Figure 3.12 indicates that there is a significant amount (>90%, Section 7.3) of polysaccharide/free sugar in this sample, a greater percentage (10%) than would be expected for a sample of ruberythric acid with a stated purity of 90%. Also, the aromatic region is far too complex (δ 8.29 - 8.12, 2H, m; δ 8.00 - 7.91, 2H, m; δ 7.74, 1H, d; δ 7.62, 1H, d; δ 7.48, 1H, s) for there to be only ruberythric acid in this sample;[46,143-145,147,149] in this region there an extra hydrogen atom for a sample of ruberythric acid (90% purity), but it could be a mixture of two compounds ruberythric acid and lucidin primeveroside.[144] This ^{1}H NMR spectrum obtained from the ruberythric acid sample appears identical to the ^{1}H NMR spectra obtained from a water/ethanol and ethanol extracts undertaken in the previous work stated. This was an unexpected result, as explained above was an extra proton signal at δ 7.48, which demonstrated the extraction of two glycosidic anthraquinones, ruberythric acid and lucidin primeveroside are formed as this is the process by which APIN Chemicals would have obtained this mixture. In negative ion mode the LC-MS peaks (Figure 3.13) detected were, compound (**A**) over a range of 1.32-1.40 minutes with a

maximum mass (m/z) of 533, which corresponded to ruberythric acid and compound (**B**) over a range of 1.40-1.50 minutes with a maximum mass (m/z) of 563, which corresponded to lucidin primeveroside.[44,46,145-146]

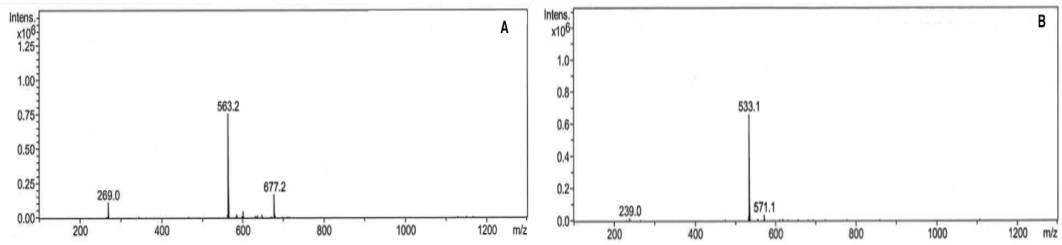


Figure 3.13. Mass spectra data of the two compounds present in ruberythric acid APIN (**A**) lucidin primeveroside (**B**) ruberythric acid

This suggested was has been discussed previously in other results that the main composition of madder when extracted with ethanol were the glycosides and not the aglycones alizarin and lucidin.

3.1.5. Recrystallisation studies of entry A4

In the literature it is claimed that ruberythric acid and lucidin primeveroside have been purified from extractions, and X-ray crystal structures for both have been obtained.[89-93] Reading these literature precedents, it was believed that full characterisation of ruberythric acid and lucidin primeveroside were incomplete. Further work was undertaken to try and achieve pure samples of both ruberythric acid and lucidin primeveroside. Analysis of entry A4 (Table 3.1) by ^{1}H NMR spectroscopy (δ 8.29 - 8.12, 2H, m; δ 8.00 - 7.91, 2H, m; δ 7.74, 1H, d; δ 7.62, 1H, d; δ 7.48, 1H, s) gave a similar result to that of Figure 3.12 that was determined to be a mixture of ruberythric acid, lucidin primeveroside and polysaccharides. These works included dissolving the madder extract from entry A4 into different solvents examples of some were ethanol, water/ethanol, ethyl acetate and propan-1-ol. However no separation of the anthraquinone glycosides were seen using these

solvents. When this sample was dissolved into a solution of methanol at room temperature, and a sonicator bath was used to agitate the solution to disrupt the intermolecular interactions of the larger aggregates until all the material dissolved into the solution. Once the madder extract was fully dissolved in the methanol it was placed in a refrigerator and after for 24 hours several crystals had formed in the solution, these crystals were collected and analysed by X-ray crystal spectroscopy (Figure 3.15). From the highlighted area seen in Figure 3.14, 5 hydrogen signals are detected in the aromatic region, δH; 8.19 – 8.17 (m, 1H, [red area]), 8.14 – 8.12 (m, 1H, [red area]), 7.92 – 7.86 (m, 2H, [green area]), 7.40 (s, 1H, [purple area]). From the literature this analysis was confirmed to be lucidin primeveroside.[147-148]

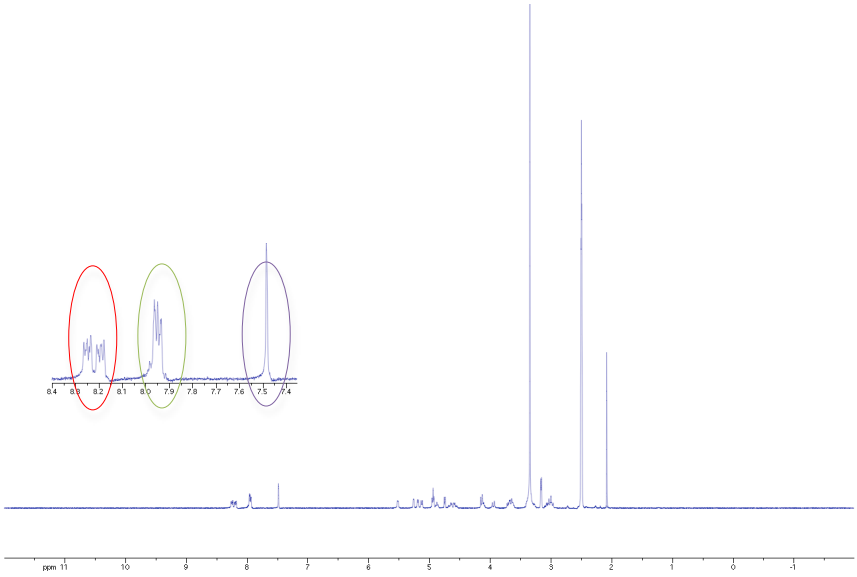


Figure 3.14. ^{1}H NMR spectrum of lucidin primeveroside.

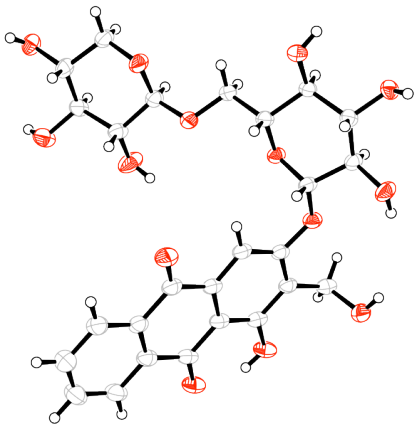


Figure 3.15. Crystal structure of lucidin primeveroside.

Ruberythric acid was excluded as being the compound because 6 signals would have had to be observed in the aromatic region. With this not the case it can clearly be stated it was not ruberythric acid.[144] Compared to the ^{1}H NMR spectrum of the purchased ruberythric acid, it can clearly be seen that the sample is a lot purer as there is considerably less polysaccharide or free sugars in the sample (Figure 3.14, 5.5-3.0 ppm). The interpretation of the isolated compound being lucidin primeveroside was further justified by LC-MS analysis; in negative ion mode the LC-MS peak detected was, compound (1) over a range of 1.35-1.38 minutes with a maximum mass (m/z) of 533. Further validation of the sample being lucidin primeveroside was acquired when a crystal structure was obtained (Figure 3.15, Section 3.1.6). With all the data acquired it has been proven that one of the main compounds extracted from *Rubia tinctorum* by an ethanol extraction medium was lucidin primeveroside.

After removing the crystals from the solution, this solution was still orange in colour and was left in the refrigerator for a further 24 hours. After this time a brown precipitate had formed in the solution and was filtered through a Buchner funnel; ^{1}H NMR spectroscopy demonstrated this compound to be ruberythric acid

(Figure 3.16). Further analytical methods (HPLC, LC-MS) were unsuccessful, due to a large quantity of polysaccharides/free sugars (>97%) extracted with this compound, which hindered in the dissolving of this precipitate into solution or detection of the compound in the analytical methods. Thus, only one compound was successfully extracted from the madder root and analysed robustly, that being lucidin primeveroside.

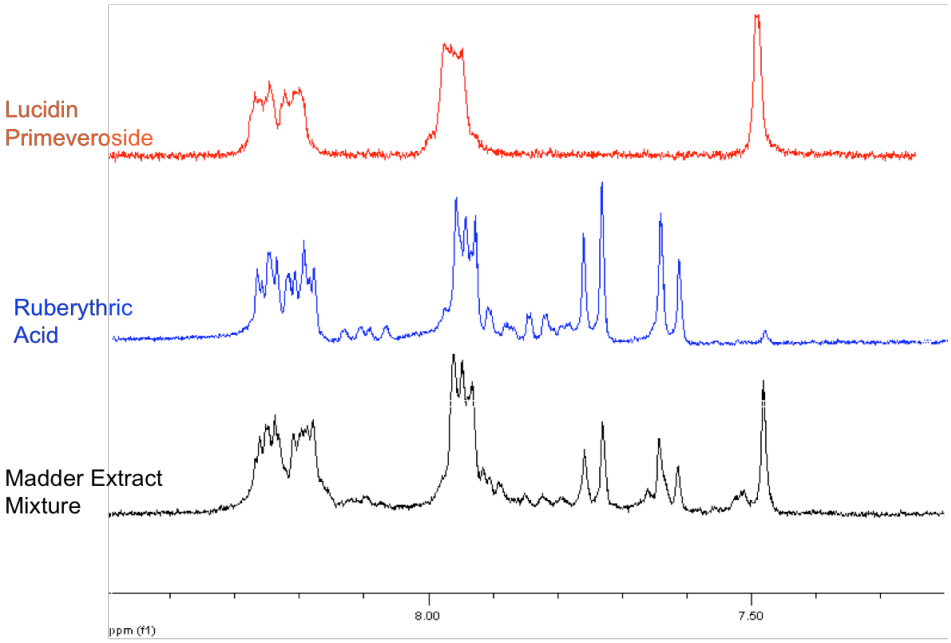


Figure 3.16. Aromatic region of ^{1}H NMR spectra of isolated products from madder extract.

From this experiment the conclusive result was that the main chromophoric components of madder are not alizarin, but the glycosides lucidin primeveroside and ruberythric acid.[16,44,46,53,144] This also explains why different results were seen in all the dyeing studies when madder was used along with alizarin. It also suggests that if alizarin is determined as a component in the madder plant then the extraction process must have caused hydrolysis of ruberythric acid to form alizarin.

3.1.6. X-ray Crystallography Data of Lucidin Primeveroside

A crystal structure of lucidin primeveroside was obtained by X-ray crystallography (Figure 3.17); this is the first known example of an anthraquinone glycoside crystal structure in literature. The structure has up to 10 hydrogen bonding interactions (Table 3.3), and to form the crystal lattice several are intermolecular hydrogen bonds, which occur *via* the methanol solvent molecule and one is an intramolecular hydrogen bonding interaction between O10 and H12. This is in keeping with the results from previous work (Section 3.1.3), as it requires a pH greater than 13 to deprotonate the hydroxyl group in the b-position to the carbonyl in alizarin as it has a pK_a of 12. This interaction does contradict the crystal structure of alizarin proposed by Guilhem who states both carbonyl groups will have intermolecular hydrogen bonding between molecules to form a 'triple molecule complex'.[142] If the carbonyl group was hydrogen bonding with the phenol group in the primary position as proven in Figure 3.17 then it cannot hydrogen bond with the phenol group of another anthraquinone molecule as Guilhem states.[142]

Table 3.3 Hydrogen Bonding interactions and lengths of the lucidin primeveroside crystal structure.

Donor Atom	Hydrogen Atom	Acceptor Atom	Hydrogen-Acceptor Distance (Å)	Donor - Acceptor Distance (Å)	Donor-Hydrogen-Acceptor angle (°)
O12	H12	O10	1.86	2.605(3)	146.3
O14	H14	O32	1.97	2.804(3)	175.6
O18	H18	O32	1.99	2.815(3)	165.7
O19	H19	O27	1.88	2.721(3)	175.0
O20	H20	O31	1.99	2.823(3)	170.2
O25	H25	O3	2.16	2.719(3)	131.8
O26	H26	O18	1.88	2.700(3)	164.6
O27	H27	O19	1.95	2.755(3)	161.4
O31	H31	O14	2.08	2.899(3)	165.4
O32	H32	O26	1.88	2.686(3)	159.3

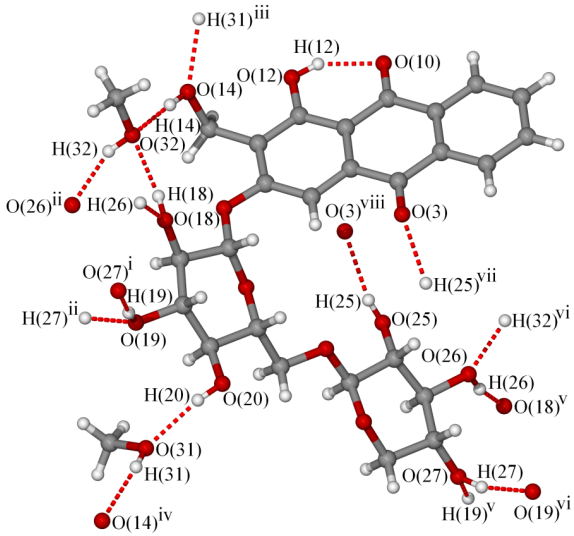


Figure 3.17. Hydrogen bonding interactions observed in the solid state in the single crystal structure of lucidin primeveroside. Symmetry operations for symmetry generated atoms i: 1+x, y, 1+z; ii: x, y, 1+z; iii: 1-x, ½+y, 1-z; iv: 1-x, y-1/2, 1-z; v: x-1, y, z-1; vi: x, y, z-1; vii: 1+x, y, z; viii: x-1, y, z.

Each lucidin primeveroside molecule hydrogen bonds to two additional lucidin primeveroside molecules *via* three intermolecular hydrogen bonding interactions (O19-H19-O27, O25-H25-O3, O26-H26-O18). Hydrogen bond O25-H25-O3 connects lucidin primeveroside molecules stacked directly on top of one another. Hydrogen bonds O19-H19-O27 and O26-H26-O18 connects lucidin primeveroside stacked directly above a neighbouring lucidin primeveroside molecule, as viewed in Figure 3.18, Figure 3.19 and Figure 3.20.

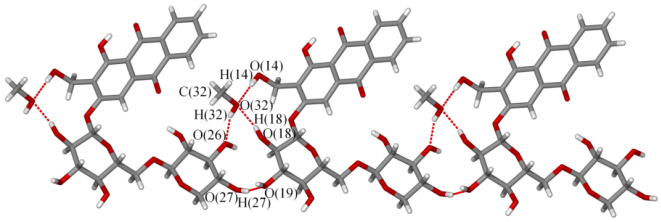


Figure 3.18. 1D chain propagated through three intermolecular hydrogen bonds via MeOH solvent molecule (C32 O32) and one intermolecular hydrogen bond O27-H27-O19. MeOH solvent molecule (C32 O32) shown as ball and stick model. Hydrogen bonding interactions shown as dotted lines.

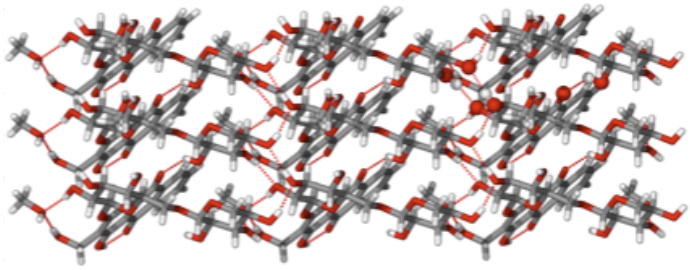


Figure 3.19. Stacks of hydrogen bonded lucidin primeveroside. Donor, hydrogen and acceptor atoms involved in hydrogen bonding the lucidin primeveroside layers are shown as spheres. Hydrogen bonding interactions shown as dotted lines.

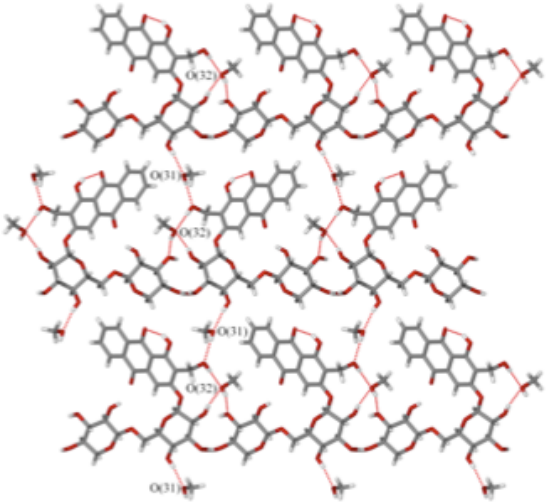


Figure 3.20. 2D hydrogen bonded sheet propagated through intermolecular hydrogen bonding interactions via methanol solvent molecule O31-C31.

Observations of Figure 3.18 show that a phenol group from the xylose group of one lucidin primeveroside molecule forms a hydrogen bond with the phenol group form the glucose group from another lucidin primeveroside molecule. This interaction is proof that the sugar moieties of glycosidic anthraquinone molecules have the functionality to form hydrogen bonds with each other. The lucidin primeveroside molecules have the ability to stack one on top of another, there is clear hydrogen bonding from sugar moieties of lucidin primeveroside molecules above and below one another in Figure 3.19 and Figure 3.20. The information gained and the knowledge of how lucidin primeveroside interacts with itself and packs around other

molecules will be useful when determining how this molecule binds towards the aluminium metal, the mordant in the wool dyeing process. All the results gained from the x-ray crystallography data point to the evidence that the oxygen carbonyl on the anthraquinone backbone could interact with the aluminium metal and the disaccharide group could also interact by hydrogen bonding with the amine groups and carbonyl groups in the wool structure. This could be one reason why there is evidence that the anthraquinone glycosides appear to bind better to the wool fibre than the aglycone alizarin.

3.2. Conclusions

The aim of this chapter was to successfully extract and analyse glycosidic anthraquinone compounds from the *Rubia tinctorum.* To reduce the amount of free sugars and polysaccharides extracted the use of ethanol as the only solvent in the extraction process was undertaken. The results proved successful in that analysis of the glycosidic anthraquinone compounds present in *Rubia tinctorum* was achieved. It was shown that the major compounds present in the madder root were lucidin primeveroside and ruberythric acid when an ethanol extraction was undertaken. A crystal structure of the compound lucidin primeveroside was obtained using methanol as the solvent, after the initial extraction with ethanol. This was the first known example of a glycosidic anthraquinone crystal structure in the literature and the finding could prove to be very useful, as it will help in the understanding of the binding properties of lucidin primeveroside in the dyeing process.

4. Analysis of Dyed Wool Samples

4.1. Background

The main aim of this project was to devise a new method for successfully back extracting (removing dye molecules from previously dyed fabrics) glycosidic anthraquinone molecules from wool fibres.

Extraction of dyes using this back extraction technique and analysis of this liquor by HPLC-DAD (diode array detection), coupled with mass spectrometry (due to the great number of different dyestuffs back extracted) is vastly applied to analysis of historical textiles.[7,16,72,95,112,115,119,151,155-171] The initial approach that was undertaken for back extracting dyestuffs from textiles was using strong acids *i.e.* hydrochloric acid (HCl).[61,72,113,119,157,162,166] Further work in this area has led to the undertaking of softer extraction methods *e.g.* trifluoroacetic acid (TFA), dimethylformamide (DMF) and boron trifluoride in methanol.[115,119-120,170,173] By using these milder techniques more accurate information about the dyestuffs can be analysed as glycosidic moieties can be preserved.[77,157] A key reason this is useful is to help in understanding the provenance of the samples analysed.

4.2. Back Extractions

The aims of the initial investigation into analysing dyed wool samples were split into two key areas:

1. Back extractions from previously dyed samples of wool with madder root using a range of techniques, some of which have been used in the literature, some novel. Experimental data can be observed from Section 7.9 to Section 7.9.6.

2. Back extractions of photodegraded dyed wool samples with madder root using a range of techniques previously used in the literature and a novel, well balanced, non-invasive in-house technique. Experimental data can be observed in Section 7.9.7.

Initial research focused on understanding the back extraction procedure and what compounds extracted through an ethanol extraction of from *Rubia tinctorum* (Section 3.1.2) were adsorbed upon the wool fabric.

4.2.1.1. Analysis of the Madder Extract Before and After Dyeing

The same concentration of sample (10% omf, which equates to 500 mg per 200 ml of water) was prepared to get an accurate account on what anthraquinone compounds were present in the initial dyebath (Chapter 3). As can be seen in Figure 4.1 the result of the HPLC analysis of the madder extract has yielded what was already known; lucidin primeveroside (**1**) and ruberythric acid (**2**) were present but also two new compounds were observed, alizarin (**3**) and an unknown compound, lucidin-ω-ethyl ether (**4**). UV-vis chromatograms (Figure 4.2) were obtained along with mass spectrometry (Figure 4.3) to clearly identify the signals seen. The results were as followed, lucidin primeveroside (λ_{max} = 406, $[M-H]^-$; m/z = 563) ruberythric acid (λ_{max} = 415, $[M-H]^-$; m/z = 533), alizarin (λ_{max} = 430, ($[M-H]^-$, m/z = 239) and lucidin-ω-ethyl ether (λ_{max} = 412, $[M-H]^-$; m/z = 298). This final compound had not been previously observed during these studies and after looking in the literature it became apparent that this compound was not one that was very abundant in other extraction profiles of *Rubia tinctorum*.[143,149] Many attempts were made to successfully isolate and fully analyse the lucidin-ω-ethyl ether (**3.7**) with the techniques available, but it proved unsuccessful.

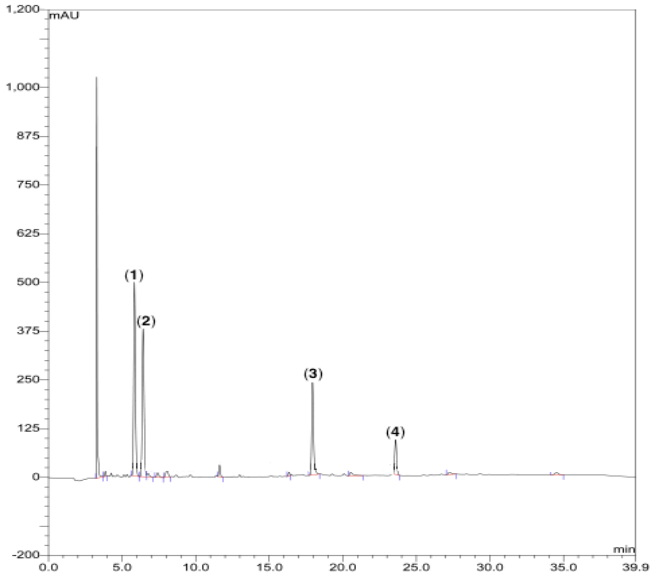


Figure 4.1. HPLC chromatogram (Relative Areas %) of the madder extract before dyeing at 10% omf showing four compounds present (**1**) lucidin primeveroside (29.95 %) (**2**) ruberythric acid (24.69 %) (**3**) alizarin (15.26 %) (**4**) lucidin-ω-ethyl ether (6.03 %).

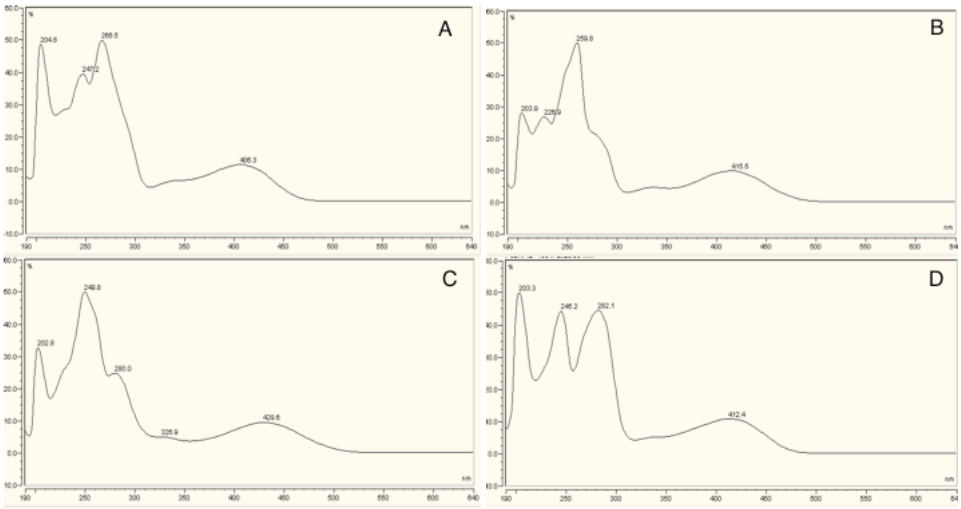


Figure 4.2. UV-vis spectra, from HPLC data of the madder extract before dyeing at 10% omf, of the four compounds present (**A**) lucidin primeveroside (**B**) ruberythric acid (**C**) alizarin (**D**) lucidin-w-ethyl ether.

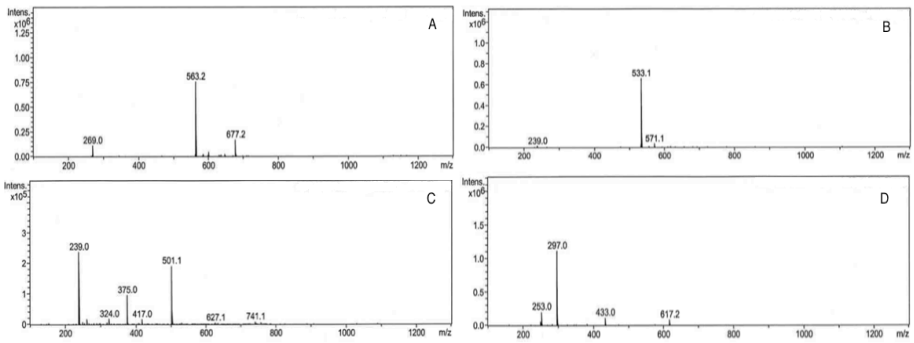


Figure 4.3. Mass spectra data of the madder extract before dyeing at 10% omf, of the four compounds present (**A**) lucidin primeveroside (**B**) ruberythric acid (**C**) alizarin (**D**) lucidin-w-ethyl ether.

The next sample that was subjected to the analysis of the HPLC method devised to help identify the compounds present in the back extraction liquors was a sample of the dyebath (10% omf) after the dyeing of the wool had taken place. The result is shown in Figure 4.4, and it is observed that only lucidin primeveroside (**1**), ruberythric acid (**2**) and alizarin (**3**) remain in the dyebath. The relative heights of these compounds are very interesting compared to the dyebath before dyeing. Lucidin primeveroside (**1**) and ruberythric acid (**2**) only decrease by around 50+ mAU (micro absorbance units) each, whilst alizarin (**3**) decreases by over 150 mAU and lucidin-ω-ethyl ether (**4**) decreases by 125 mAU and is not present in this mixture at all. What can be deduced from this it that alizarin is being absorbed onto the wool fibres more than any other compound with the two glycosidic anthraquinone molecules not reducing in any real quantity. A proposal for why this is occurring could be due to the fact that lucidin primeveroside and ruberythric acid are larger molecules than alizarin, 564 g/mol and 534 g/mol in molar mass compared to 240 g/mol respectively, so only a small amount of the glycosides can bind to the wool fibres when all four compounds are competing for adsorption sites.

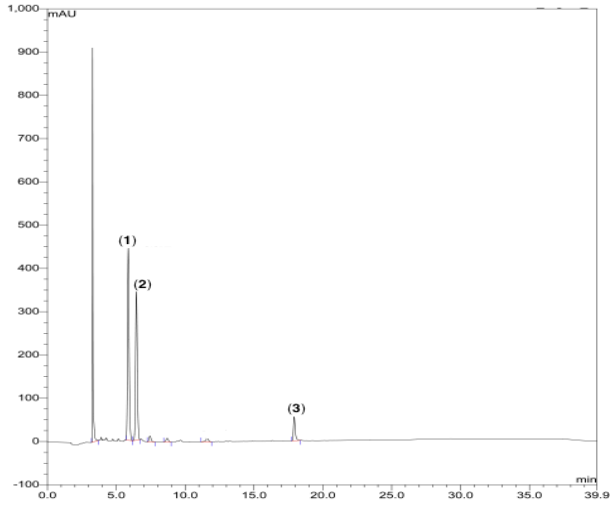


Figure 4.4. HPLC chromatogram (Relative Areas %) of the madder extract after dyeing at 10% omf showing 3 compounds present (**1**) lucidin primeveroside (38.23 %) (**2**) ruberythric acid (31.99 %) (**3**) alizarin (5.29 %)

To conclude, the compounds present in the dyeing process are lucidin primeveroside, ruberythric acid, alizarin and lucidin-ω-ethyl ether. Even though the two glycosidic anthraquinone are present in a higher concentration than alizarin, it is the latter that is predominately absorbed onto the wool during the dyeing process. It is proposed that the fact alizarin is a smaller molecule in mass that it has the ability to adsorb to the wool more successfully than the two larger glycosidic anthraquinone compounds. Another compound was detected, lucidin-ω-ethyl ether but could not be successfully isolated and fully characterised by the techniques at hand.

4.2.2. Back Extraction of Wool Samples Dyed with Madder

4.2.2.1. Initial Back Extractions Using Known Methods

Previously published work primarily uses historical samples to complete back extractions.[151-152] The work undertaken in this chapter was carried out on samples of wool weighing 5 g (after scouring). This sample size was chosen as a proof of

concept that the proposed methods worked. The intention was that once it was confirmed to work, future work could be undertaken on smaller sample sizes to replicate historical analysis of textiles.

Manhita et al. and Valianou *et al.* both published reviews on back extractions of natural dyes from wool so these were believed to be a good initial reference point.[72,119] Four extraction methods from the reviews were chosen to encapsulate the range of techniques used, two strong acids known to disrupt the metal-dye/metal-fibre bond, a ligand to displace the dye form the metal ion by ligand substitution and a weak acid also known to disrupt the dye-metal bond. All back extractions were subjected to replication on the in-house samples dyed using the technique stated in Section 7.2.1. These methods are tabulated in Table 4.1.

Table 4.1. Four methods used for initial back extraction analysis.

Name	Extraction Procedure	Time Scale (min)	References
Formic Acid Method	5 g of dyed wool immersed in 200 ml of HCOOH/MeOH (1:19, *v/v*) and heated at 40 °C[113]	30	152
HCl Method	5 g of dyed wool immersed in 200 ml of 37% HCl/MeOH/H_2O (2:1:1, *v/v/v*) and heated at 100 °C[114]	10	61 and 113
EDTA Method	5 g of dyed wool immersed in 0.1% Na_2EDTA in 200 ml of H_2O/DMF (1:1, *v/v*) and heated at 100 °C[91]	30	120
2M TFA Method	5g of dyed wool immersed in 170.2 ml of H_2O and 29.8 ml Trifluorocetic acid (TFA) added and the mixture heated to reflux	10	119

Initially UV-vis spectra for the three methods were recorded which gave the following results shown in Figure 4.5.

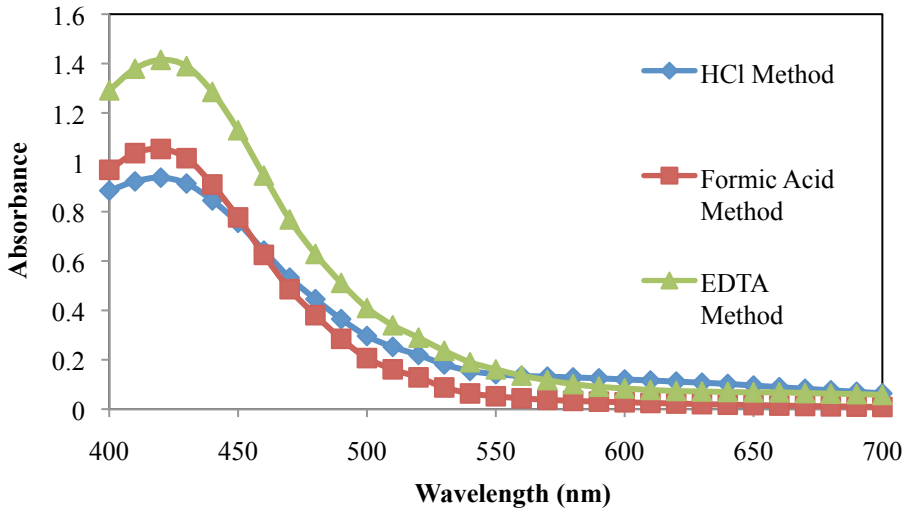


Figure 4.5. UV-vis spectra of three back extraction methods.

As can be seen from this analysis, the EDTA method was the most effective method, in terms of the extraction system that gave the highest absorbance peak recorded, and followed by the formic acid method then the HCl method. All three profiles had the same λ_{max} at 424 nm, exactly the same λ_{max} was achieved in the extractions of madder in Chapter 2 and 3. This result is conclusive evidence that the compounds being back extracted are the same as the compounds that were dyed upon the wool fibre.

The HPLC results for the formic acid method are shown in Figure 4.6, were it was observed that three compounds present in the sample liquor were separated and resolved. The first two compounds, peaks **1** and **2**, were analysed from their UV-vis spectra (Figure 4.7) as being lucidin primeveroside (λ_{max} = 406) and ruberythric acid (λ_{max} = 415), respectively. Knowing that the madder extract used to dye the wool had been analysed before and only lucidin primeveroside and ruberythric acid were detected, seeing both appear on this analysis demonstrated that the use of formic acid and methanol to remove the dye from the dyed sample was successful. However those two compounds were clearly not the most abundant in

the liquor, with a third peak (**3**) which when analysed by UV-vis (λ_{max} = 430) was found to be alizarin.

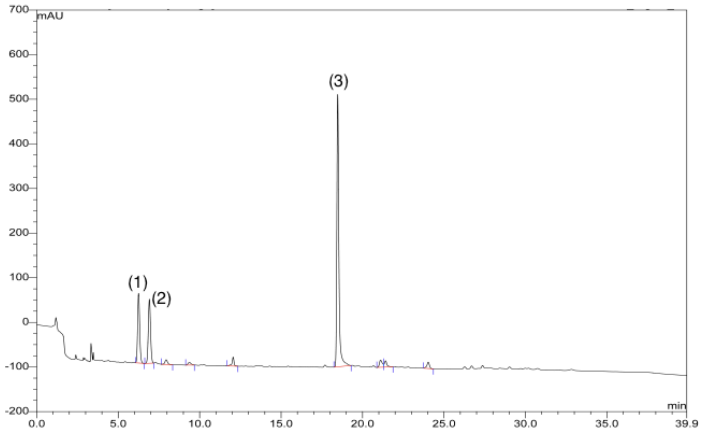


Figure 4.6. HPLC chromatogram (Relative Areas %) of the formic acid method back extraction showing three compounds present (**1**) lucidin primeveroside (14.42 %) (**2**) ruberythric acid (14.38 %) (**3**) alizarin (55.17 %).

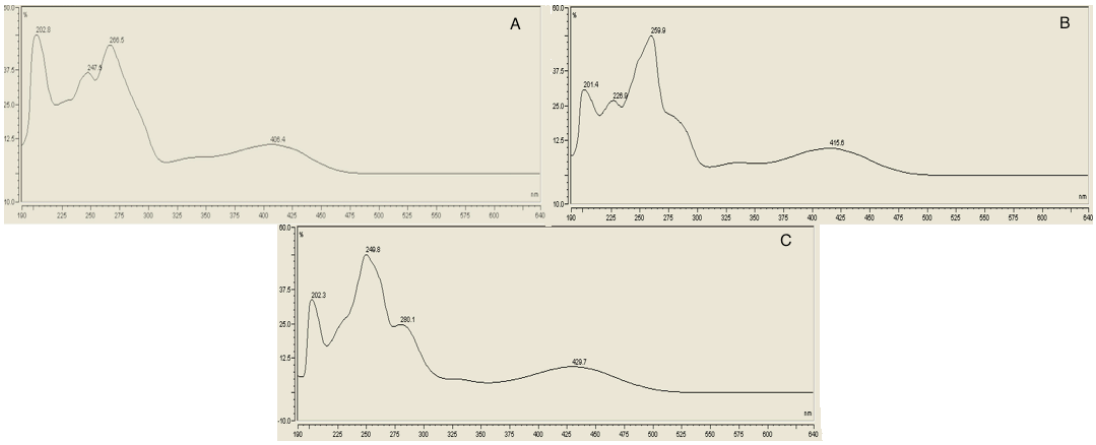


Figure 4.7. UV-vis spectra, from HPLC data of the formic acid method back extraction showing three compounds present (**A**) lucidin primeveroside (**B**) ruberythric acid and (**C**) alizarin.

The increase in the absorbance detected for alizarin has increased by over 500 mAU and is greatly amplified to the absorbance detected in the extract liquor before dyeing (Section 4.2.1.1). This observation could not be solved in this body of work but was believed to be an area of work that could be undertaken during future work of this thesis. The results from the formic acid method showcase that anthraquinone

dye molecules are back extracted from the wool fibre, including glycosidic anthraquinone molecules (however in low amounts), and that alizarin was detected in greater abundance than all other anthraquinone compounds as seen when historical samples are analysed using the same technique.[72,119,152]

The result for the HCl method used in the back extraction can be seen below in Figure 4.8.

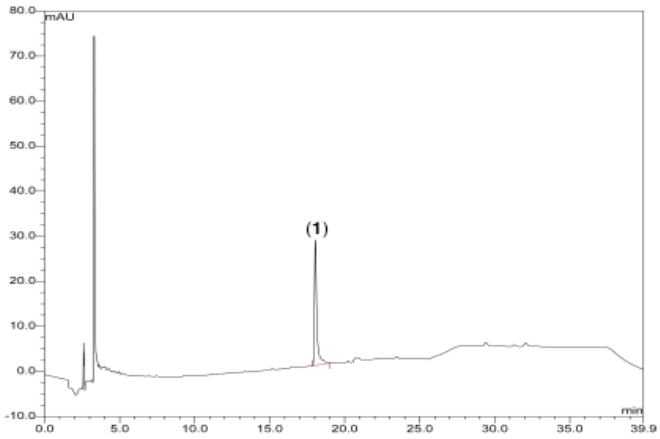
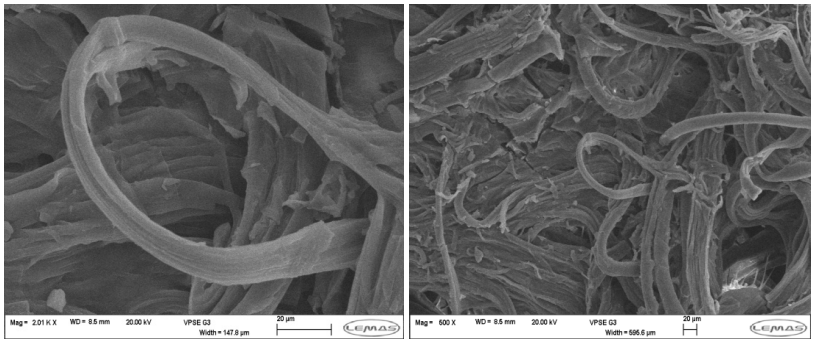


Figure 4.8. HPLC chromatogram (Relative Areas %) of the HCl method back extraction showing one compound present, (**1**) alizarin (52.50 %).

Using the HCl method, only one compound was observed, alizarin, which was confirmed when analysed by UV-vis spectrophotometry (λ_{max} = 430 nm) and mass spectrometry ($[M-H]^-$, m/z = 239). This method showed that using harsh conditions, such as 6M HCl led to the hydrolysis of the glycoside anthraquione molecules and their omission from the final analysis. As previously discussed, it is believed that the aluminium used in the pre-mordanting process binds to the wool and during the dyeing process the anthraquinone molecules covalently bind to the aluminium ion through the carbonyl groups. Therefore in order to remove the dye molecules either the metal–wool bond or the metal–dye bond must be broken. As can be seen, there are no distinguishable cuticle marks on the wool (Figure 4.9 (A)), which shows that the structure of the individual wool strands are starting to be compromised in the

process. It can be seen in Figure 4.9 (B) the wool fibre was starting to lose its overall structure. From these images it can be hypothesised that the use of such a strong acid at pH <1 was hydrolysing the protein backbone of the wool. Further work using the technique ICP-MS on the back extraction liquor would give the results needed to prove this proposed hypothesis by measuring the aluminium metal ions present in the liquor.



(A) **(B)**

Figure 4.9. Images of the wool fibre after the HCl method using an Scanning Electron Microscope (SEM): (**A**) magnification 2,000 times; (**B**) magnification 500 times.

This method was repeated again to see what would happen if left for longer than the 10 minutes and after 45 minutes the wool sample began to break apart (unfortunately no image of this could be obtained due to the poor state of the fibre) and after one hour there was no sign of the fibre in the solution, which had now become a blackish yellow colour. The conclusion that can be made is that the use of this HCl method only yields alizarin from the dyed wool in this experiment. It is known that alizarin is not the major component of the madder extract used in these dyeing processes and that the two glycosidic compounds, lucidin primeveroside and ruberythric acid are the most prevalent compounds. This method is too destructive to be used for their detection as it destroys the glycosidic compounds and in turn was unsuccessful at fully justifying the anthraquinone compounds present on dyed wool

fibres. A better method, for the indentification of glycosidic anthraquinone molecules, was the use of the formic acid and methanol method (1:19, *v/v*) as it was more benign and gives the indication that lucidin primeveroside and ruberythric acid are present.

The final method used in this initial study was the EDTA method. The result of the liquor was analysed using the same method as the previous two extraction methods and can be seen in Figure 4.10.

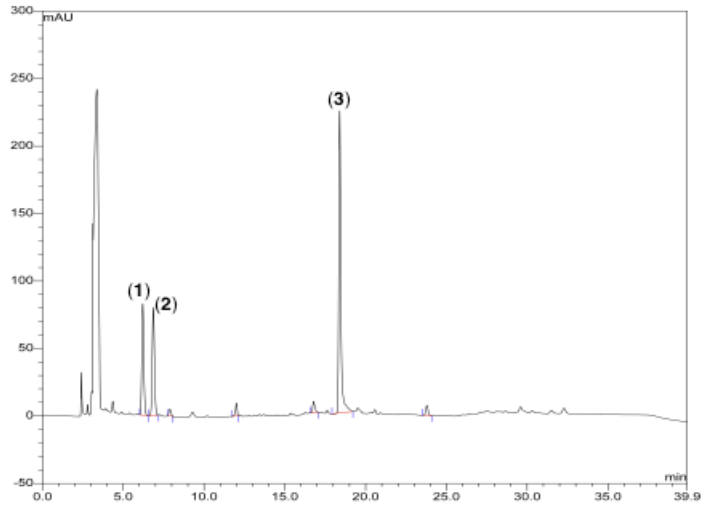
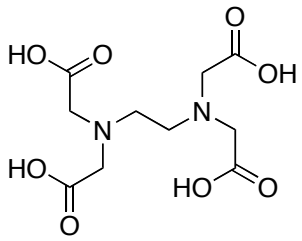


Figure 4.10. HPLC chromatogram (Relative Areas %) of the EDTA method back extraction showing three compounds present (**1**) lucidin primeveroside (8.60 %) (**2**) ruberythric acid (9.02 %) (**3**) alizarin (24.33 %).

The EDTA method gave a similar outcome to the formic acid method used, three compounds were present, lucidin primeveroside (λ_{max} = 406) ruberythric acid (λ_{max} = 415) and alizarin (λ_{max} = 430) all subsequently identified by UV-vis spectrophotometry compared with retention times from the analysis in Section 4.2.1.1. The only difference being the EDTA method gives a lower peak for the amount of alizarin present in the back extraction liquor. This analysis was still incongruous with the original analysis of the madder extract, but it has been demonstrated to be the best of the three methods and falls in line with the conclusion

by Manhita *et al.* in their summary of efficiency of analysis of historical textiles.[72] The reason behind the use of EDTA was first described by Tiedemann *et al*., who believed that the best approach was not to use acid to break the metal to dye bond but to use a ligand, in this case EDTA (**4.2**), which would bind to the aluminium metal with more affinity than the anthraquinone molecules.[120] The reason for this being EDTA was a hexadentate ligand, meaning it can bind through six non-continuous donor sites. This outcome was observed as can be seen in Figure 4.10, the anthraquinone dye molecules have been successfully removed from the dyed wool with the use of EDTA.



4.2

Valianou *et al*. had shown in their work in this area of expertise that the use of a 2M TFA solution for 10 minutes was the most successful when working with anthraquinones.[119] This method was attempted with the dyed wool samples that had been prepared as previously stated in Section 7.4. The result of the 2M TFA method are shown in Figure 4.11, which indicates that five compounds are present.

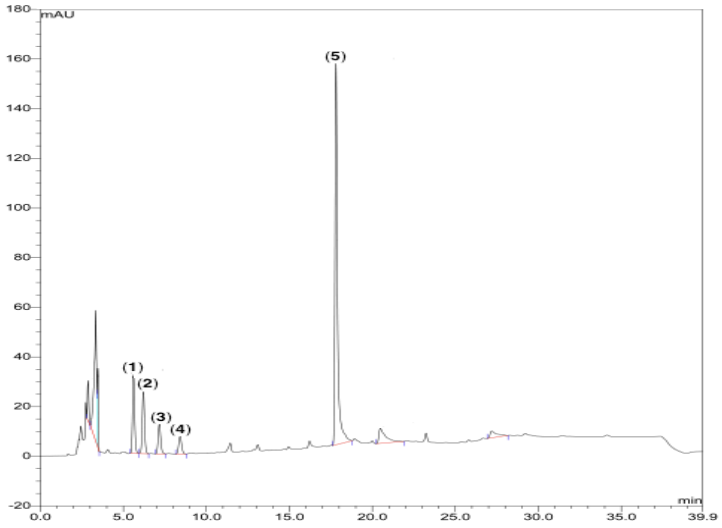
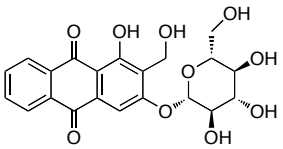
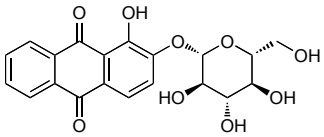


Figure 4.11. HPLC chromatogram (Relative Areas %) of the 2M TFA, back extraction showing five compounds present (**1**) lucidin primeveroside (8.33 %) (**2**) ruberythric acid (7.18 %) (**3**) lucidin monoglucoside (3.85 %)(**4**) alizarin monoglucoside (2.63 %)(**5**) alizarin (47.85 %).

These compounds were lucidin primeveroside, ruberythric acid, lucidin monoglucoside [λ_{max} = 406, $[M-H]^-$; m/z = 431] (**4.3**), alizarin monoglucoside [λ_{max} = 412, $[M-H]^-$; m/z = 401] (**4.4**) and alizarin. At first inspection and without thinking about the chemicals used in this process it appears that along with the standard compounds lucidin primeveroside, ruberythric acid and alizarin a further two compounds have been newly detected. These compounds when analysed by UV-vis spectrophotometry and mass spectrometry were identified as lucidin monoglucoside (**4.3**) and alizarin monoglucoside (**4.4**).



4.3



4.4

However, these two compounds are not observed in the initial madder extract before dyeing. A 2M TFA solution is very acidic (pH 1.7). The reason why these two compounds are being observed is due to the fact that lucidin primeveroside and ruberythric acid are being hydrolysed from the parent

disaccharide into their monoglucoside counterparts. Acidic conditions can have a detrimental effect on glycosylated compounds, one reason why different compounds were detected in this second extraction could be due to hydrolysis of the sugar moiety, as shown in Scheme 3.2. Under acidic conditions, the ether oxygen can become protonated. Once this step has occurred the lone pair of the oxygen in the ring can push its electrons into the neighbouring carbon bond resulting in the loss of the oxonium sugar moiety; the oxonium ion can be attacked by water resulting in the formation of a single sugar group (xylose). These resulting compounds can be misleading, as is shown in Figure 4.11, in the identification of anthraquinone compounds that are not found occurring naturally in *Rubia tinctorum*, but chemically hydrolysed when acidic extraction conditions are used.

The absorbance peaks of lucidin primeveroside and ruberythric acid are significantly smaller than the alizarin peak, which is due to the fact that the two compounds have been hydrolysed so have diminished in yield. A further hydrolysis reaction could also take place resulting in the two aglycone compounds lucidin and alizarin; no lucidin was seen in the chromatogram meaning that this reaction must not take place. A proposed reason this did not take place was due to the nucleophilicity of the oxygens that need to be protonated: in the hydrolysis of ruberythric acid the oxygen being protonated was an ether oxygen, as was the oxygen needed to be protonated in the hydrolysis reaction of alizarin monoglucoside to alizarin, but in this case it was adjacent to a benzene ring; the latter will have a lower pK_a due to the benzene ring removing electron density from the oxygen. This results in the oxygen's electron density being tied up in the bond, which was a proposed explanation for why the aglycone compounds are not observed in this back

extraction. Alizarin was only detected because it was present in the madder extract that was dyed upon the wool fibre.

So to conclude the use of 2M TFA solution as the solvent medium in trying to remove natural dyestuff compounds from dyed wool fibres has been demonstrated not to be a successful method. The reason for this is that the analysis detected five compounds, two of these (lucidin monoglucoside and alizarin monoglucoside) were chemically modified from natural compounds (lucidin primeveroside and ruberythric acid found in *Rubia tinctorum*) through hydrolysis. In this step the yield of lucidin primeveroside and ruberythric acid reduced from the observed levels in other back extractions, which is a misrepresentation of what molecules are present on the dyed wool fibres. This being the case, if this method was used to analysis historical samples then the wrong species could be identified as the sample used. This means that a 2M TFA solution should not be used to remove dye from dyed samples, be they historical or not historical.

To conclude, this preliminary study into extracting natural dyestuff from dyed wool fibres has yielded some interesting results. It has been demonstrated that the use of a HCl yields a result of alizarin only, as observed in the back extraction liquor, hence providing a limited indication of what dye molecules are present dyed upon the wool samples. The application of this technique, as observed in literature precedent, did not maximise the glycosidic information known to be present on the wool fibre. The use of 2M TFA solution as used in the literature was shown to be a poor method to use as several of the compounds detected are not naturally occurring compounds but chemically modified compounds because such a strong acid solution has been used. The use of formic acid/methanol (1:19, *v/v*) and 0.1% EDTA in H_2O/DMF (1:1, *v/v*) are better at removing glycosidic anthraquinone dye molecules from the dyed wool

more efficiently than the strong acid method. But the anomaly in the amount of alizarin present has to be a discerning factor and keeps the door open for another method to be found in this quest to accurately remove the dye from dyed fibres.

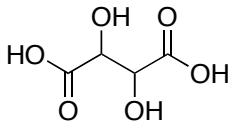
4.2.2.2. A Further Study into the Back Extractions of Dyed Wool with Madder Extract

The glycosidic information observed from back extractions in Section 4.2.2.1 could be improved by back extracting a greater quantity of glycosidic anthraquinone dye molecules in the process. The use of a ligand in the EDTA method led to the consideration that a study performed using different ligands would result in different levels of glycosidic anthraquinone detected. As can be seen in Table 4.2 several new methods were undertaken to try and expand on the knowledge already gained in this chapter. Samples were prepared using the dyeing methods described in Chapter 7, Section 7.4.

Table 4.2. Methods used for new studies into back extractions of dyed wool samples.

Name	Extraction Procedure	Time Scale (min)
Tartaric Acid Method A	5g of dyed wool immersed in 200 ml of EtOH and 5g of tartaric acid added and the mixture heated to reflux	30
Tartaric Acid Method B	5g of dyed wool immersed in 200 ml of H_2O and 5g of tartaric acid added and the mixture heated to reflux	30
Sodium Citrate Method A	5g of dyed wool immersed in 200 ml of EtOH and 5g of sodium citrate added and the mixture heated to reflux	30
Sodium Citrate Method B	5g of dyed wool immersed in 200 ml of H_2O and 5g of sodium citrate added and the mixture heated to reflux	30
2M TFA Method	5g of dyed wool immersed in 170.2 ml of H_2O and 29.8 ml Trifluorocetic acid (TFA) added and the mixture heated to reflux	10
Glucose Method	5g of dyed wool immersed in 200 ml of H_2O and 5g of glucose added and the mixture heated to reflux	180

Initially trailed was the use of tartaric acid (**4.5**) as an additive to the reaction mixture similar to the method undertaken by using EDTA. The logic behind this was to not use acidity to break the aluminium-dye bond, which was believed to covalently bind the anthraquinones to the wool fibres, but to use a ligand that wanted to be bound to the aluminium with more affinity than the anthraquinone compounds. This was the understanding of the use of EDTA method in the previous section (4.2.2.1) and was believed to be a factor in this method achieving the best results compared with the other two methods used.



4.5

Tartaric acid is a tridentate ligand so would have less affinity than EDTA if they were both put into the same system and competing for the space binding space. Tartaric acid was chosen on the basis it could be implemented into a simpler solvent system than that used for the EDTA method (H_2O and DMF) and one that has been demonstrated the anthraquinone molecules will be extracted into. Using tartaric acid in the back extraction solvent mixture did yield a chromatogram with the results shown in Figure 4.12.

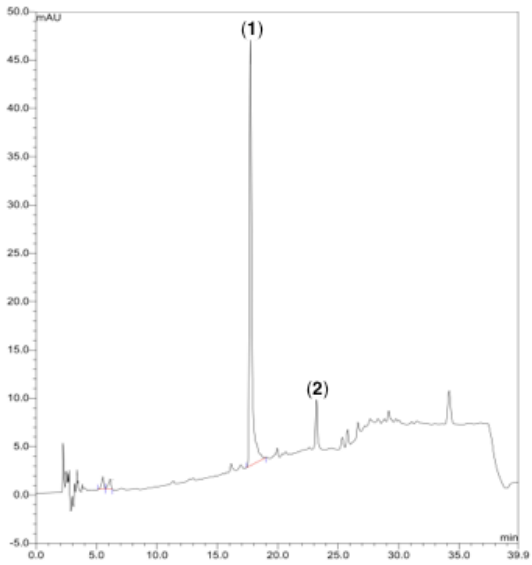


Figure 4.12. HPLC chromatogram (Relative Areas %) of the Tartaric Acid Method A, back extraction showing two compounds present, (**1**) alizarin (87.43 %)(**2**) lucidin-ω-ethyl ether (9.77 %).

Only two compounds were identified. Analysing by UV-vis spectrophotometry (Figure 4.12) resulted in these two compounds being identified as alizarin (**1**), and lucidin-ω-ethyl ether (**2**). Two peaks in lower intensity were detected around 5 minutes believed to be lucidin primeveroside and ruberythric acid but their chromatograms were overlapping and could not be purely analysed. The retention time of these two peaks/compounds were lucidin primeveroside and ruberythric acid when observed on other chromatograms. The relatively low quantity of mass meant that neither compound was observed through mass spectrometry analysis either. It is the first time that lucidin-ω-ethyl ether has been observed after a back extraction has taken place on dyed wool. The proposed reason for this is due to the solvent used in this back extraction. Ethanol was the solvent used in the extraction procedure in Chapter 3 and it is a reaction between ethanol and lucidin that produces the compound, lucidin-ω-ethyl ether.[143,149] An obvious indication was that lucidin-ω-ethyl ether has a great affinity for ethanol. This was a clear result that the lucidin-ω-ethyl ether was part of the dyeing procedure and can be removed successfully from

the wool fibre. There was one drawback to this result; the analysis of lucidin primeveroside and ruberythric acid was non-conclusive even though they are most probably back extracted in very low quantities. This was not the case for the compound alizarin that has been detected with a decrease in absorbance. This phenomenon was the first time it has been seen. In all the previous cases (except the use of HCl in the solvent liquor) the amount of alizarin detected has far exceeded the reported amount absorbed onto the wool fibres in Section 4.2.1.1. An explanation for this was unknown but was proposed to be attributed to the reactivity of the solvent mixture used in these back extractions. Ethanol is less polar than any of the solvents used in Section 4.2.1.1 (water, methanol or dimethylformamide). This decrease in polarity could be affecting the reactivity the solvent has with the wool fibre or dye-metal bond. To conclude, the use of tartaric acid in ethanol as a solvent medium for the back extraction of glycosidic anthraquinone dye molecules from dyed wool fibres was not an improvement on the reported back extractions used in the literature.

The next method that was undertaken was using tartaric acid again but this time the solvent chosen was water. As the last method had yielded the identification of only two compounds is was unsure if the use of tartaric acid was a good ligand to use in the back extraction. Changing the solvent was proposed as a way to eradicate this problem. So after running the back extraction the liquor was collected and analysed by HPLC and the following results were achieved (Figure 4.13).

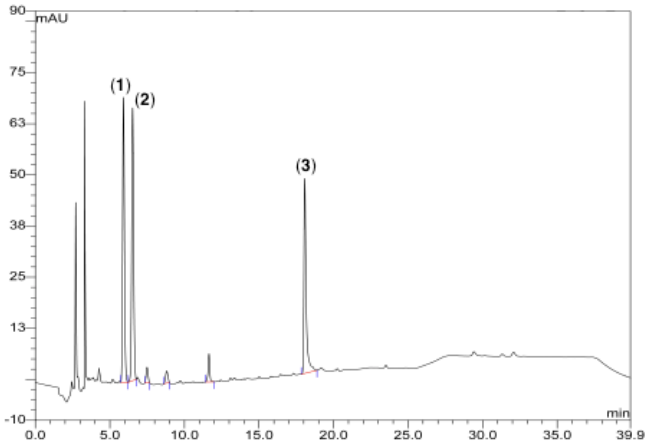


Figure 4.13. HPLC chromatogram (Relative Areas %) of the Tartaric Acid Method B, back extraction showing three compounds present (**1**) lucidin primeveroside (27.45 %) (**2**) ruberythric acid (28.60 %) (**3**) alizarin (23.43 %).

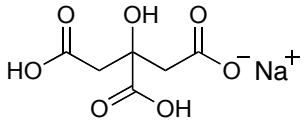
A surprising change had occurred between the outcomes of the two methods by only changing the solvent. Water is more polar than ethanol so it was hypothesised that this change in polarity results in the ligand having a better interation with the fibre and dye-metal bond. In the new method using water as the solvent the compounds observed were lucidin primeveroside (1), ruberythric acid (2) and alizarin (3). There was no detection of lucidin-ω-ethyl ether, which had clearly been identified using Tartaric Acid method A. The amount of alizarin detected was significantly less than the two glycosidic anthraquinones. This was a major breakthrough as for the first time it showed that the amount of alizarin removed from dyed fibres could be less than glycosidic anthraquinones. This result, barring the unsuccessful identification of lucidin-ω-ethyl ether, was more in line with identification of the madder extract used in the dyeing procedure showing that the major components are the glycosidic anthraquinones lucidin primeveroside and ruberythric acid. With this information at hand a successful analysis of dyed wool fibres could result in a more specific analysis of what species of madder this came from and possibly on which continent

this plant was grown, as the ratios of anthraquinone glycosides to alizarin are more in line with the initial analysis.

To conclude, the use of tartaric acid as a ligand to displace the anthraquinone dye molecules from the aluminium bound to the wool fibres has been demonstrated to work. However, the choice of solvent is very important also: with the use of ethanol only two compounds are analysed; the use of water as the solvent elucidates the problem of the ratios of anthraquinone molecules detected, but loses the identification of a minor compound in the analysis. A reason for this could be due to the temperature used in the process. In the case of ethanol the temperature at reflux was 78 °C whereas with water the temperature was 100 °C. This increase of 22 °C could be helping penetrate the wool deeper and expanding the fibres to access more sites to back extract the anthraquinone dye molecules. The reason of the loss of the minor compound could be due to the fact that lucidin-ω-ethyl ether (**3.7**) is more hydrophobic than the other three compounds so does not get extracted into the water. So far it has been demonstrated that using tartaric acid in water is a better solvent mixture than EDTA in H_2O/DMF (1:1, *v/v*), for back extraction of glycosidic anthraquinone molecules.

The next solvent mixture that was trialled as a potential new way of removing natural dye molecules from dyed wool fibres was that of monosodium citrate (**4.6**) in ethanol. This compound again was chosen for its ability to behave as a polydendate ligand much in the same way as tartaric acid and EDTA and to have more affinity to the aluminium ion than the anthraquinone molecules and displace them. The results for this process are shown in Figure 4.14, where it was observed that four compounds were analysed, those being the four compounds present in the

madder extract before dyeing: lucidin primeveroside (**1**), ruberythric acid (**2**), alizarin (**3**) and lucidin-ω-ethyl ether (**4**).



4.6

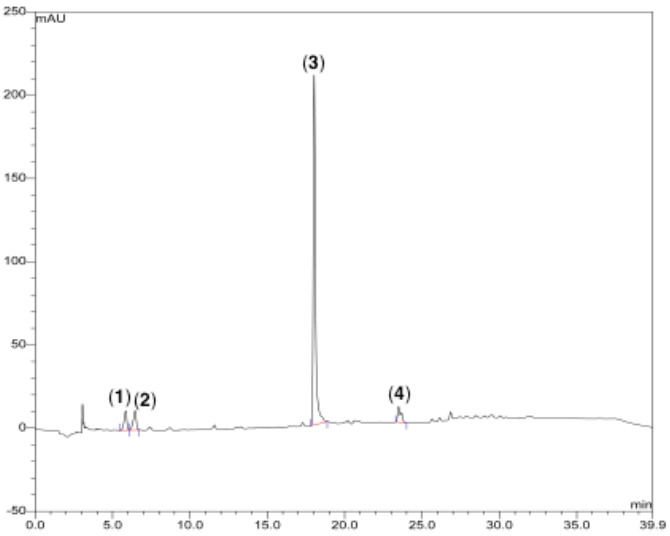


Figure 4.14. HPLC chromatogram (Relative Areas %) of the Sodium Citrate Method A, back extraction showing four compounds present (**1**) lucidin primeveroside (4.31 %) (**2**) ruberythric acid (5.11 %) (**3**) alizarin (86.49 %) (**4**) lucidin-ω-ethyl ether (4.09 %).

Sodium citrate has been demonstrated to be a successful ligand at displacing these four natural anthraquinone dye molecules from the wool fibres and the associated aluminium metal that is believed to be a linkage between the wool and dye. The relative ratios of the peaks analysed are inconsistent with initial ratios, with an abnormally large amount of alizarin detected. As previously seen with some of these back extraction techniques the amount of alizarin detected is greater than the amount of alizarin that has been removed from the dyebath. The reason for this anomaly is unknown, but as shown in the previous example using tartaric acid it seemed to be attributed to the use of certain solvent mixtures. What this means is that the chemical compounds used in these back extractions with certain solvents produce

different levels of results of alizarin and the other anthraquinone compounds detected. This could be due to the degrading of the compounds at high temperatures in certain solvent mixtures, but if this was the case then it should be that lucidin, the aglycone of lucidin primeveroside, should also be detected by the HPLC technique. As this does not occur in any of the back extractions analysed, it can be stated this is not reason for this anomaly. Another explanation is that in certain solvent mixtures only the top layer of the wool fibres is removed (as in the case of the HCl method in section 4.2.2.1) and further penetration into the wool structure cannot be successfully obtained. With this only alizarin is extracted into the back extraction liquor with great amount, meaning that it has a greater molar coefficient then all the other molecules present resulting in a misinterpreted signal. Thus, in the method using sodium citrate in ethanol the outcome is that this technique is flawed and does not yield the correct products in the correct ratios. Again it has been demonstrated that another technique can yield four compounds in the resulting back extraction liquor meaning a more detailed analytical process than the EDTA method has been established.

The next method investigated was the use of sodium citrate with the solvent being water to determine if any difference was observed compared to using ethanol as solvent, as was observed when this was undertaken in the tartaric acid methods. As can be seen in Figure 4.15 there are only three compounds observed, lucidin primeveroside (**1**), ruberythric acid (**2**) and alizarin (**3**). Again the changing of the solvent has resulted in the unsuccessful detection of lucidin-ω-ethyl ether. As previously stated, this is due to the fact that it is more hydrophobic than the other three compounds and is not extracted into the solvent during the back extraction process. The other result in the use of this method is that the peak of alizarin has

dropped in height and is more in line with the two glycosidic anthraquinone molecules lucidin primeveroside and ruberythric acid.

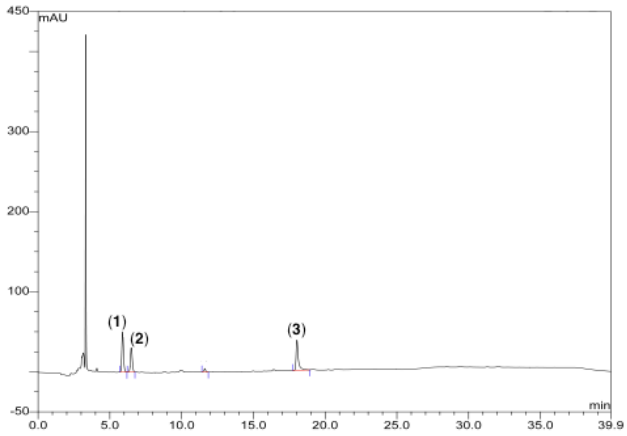


Figure 4.15. HPLC chromatogram (Relative Areas %) of the Sodium Citrate Method A, back extraction showing three compounds present (**1**) lucidin primeveroside (18.02 %) (**2**) ruberythric acid (12.17 %) (**3**) alizarin (18.13 %).

To conclude, the phenomenon that the use of different solvents, especially ethanol and water, results in different levels of the anthraquinones being extracted as observed previously in the use of tartaric acid has been demonstrated to be correct with the use of sodium citrate. The choice of solvent system and chemicals to use for the back extraction procedure has to be very precise or an exaggeration of the differing amounts on the compounds observed, or in some cases is not observed at all, can and will occur.

When thinking about the use of ligands to aid the back extraction process and observing the results form those techniques, a lot of consideration went into other compounds that could interact with and disrupt the metal dye bond. The use of sugar molecules, such as glucose, had never been seen in any of literature and with an array of hydroxyl groups around these molecules they quickly became of interest to this line of work. It was believed they could bind with the aluminium metal and free the anthraquinone dye molecules into the solvent, allowing for detection and

analysis. The next method that was undertaken was using glucose dissolved in water and heated to reflux for 180 minutes; this was significantly more time than the other methods, but after 30 minutes the liquor had not changed colour and was left for a further 150 minutes. By this time the colour of the liquor had intensified to an intense yellow solution and was then analysed. This technique has not been previously reported in the literature and is believed to be the first example of this being used to remove natural dye compounds from dyed wool. The result yielded three compounds: lucidin primeveroside (**1**), ruberythric acid (**2**) and alizarin (**3**), as shown in Figure 4.16.

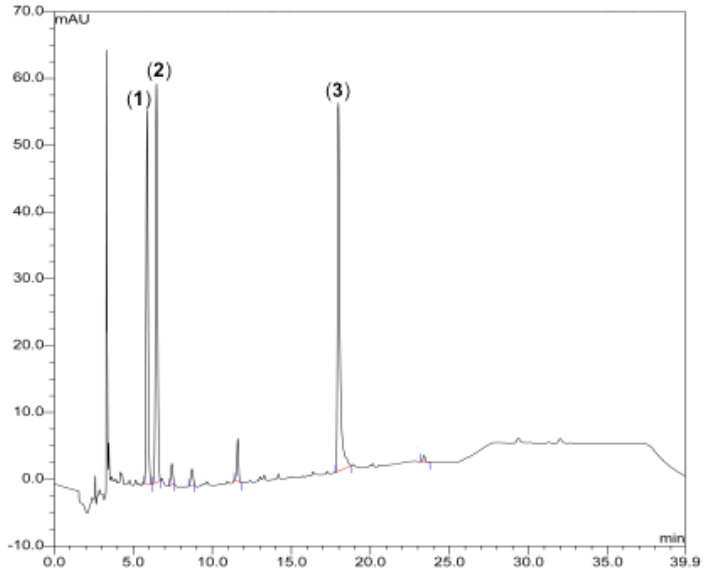


Figure 4.16. HPLC chromatogram (Relative Areas %) of the, Glucose method, back extraction showing three compounds present (**1**) lucidin primeveroside (26.14 %) (**2**) ruberythric acid (29.97 %) (**3**) alizarin (32.99 %).

The ratio levels of the three compounds are in line with the madder extract analysis, which means that lucidin primeveroside, ruberythric acid and alizarin are all detected around the same absorbance maxima, which demonstrates that all three compounds are being removed from the dyed wool samples in exactly the same amount. Lucidin-ω-ethyl ether was not detected and that could be due to the use of

water as the solvent. This is the best chromatogram that has been achieved from all the back extraction methods. As well as being the best chromatogram it is also the most benign method used as the pH of the solution before the back extraction was pH 6.89 and after had altered slightly to pH 6.65. At this pH no hydrolysis reactions can take place, so any chemically modified anthraquinone glycosides will not be synthesised.

As can be seen in Figure 4.16 there was minimal difference in the appearance of an original wool sample dyed with madder extract and the dyed wool sample extracted with glucose in water. The reason why glucose works in this method as a way of removing dye from dyed wool samples could be due to the ability of the glucose to chelate to the aluminium metal centre, which could indicate that the glucose may have better affinity to the metal than the anthraquinone molecules. The reason for this could be due to the size of the molecules around the aluminium metal centre. The anthraquinone molecules are larger in size than glucose molecules, are also planar in orientation owing to a very rigid geometry around the aluminium metal. Whereas the glucose molecules may arrange themselves in a more compact form around the aluminium metal. Another advantage the glucose molecule has is several hydroxyl groups meaning that it can hydrogen bond to the wool fibres for more stability. The use of glucose in this context has demonstrated to be a viable option for removing natural dye molecules from wool fibres giving a chromatogram very much in accordance with the chromatogram of the madder extract before the dyeing had taken place. This method could be used in conjunction with other back extracting techniques as a means to initially analyse for glycosidic anthraquinone molecules.

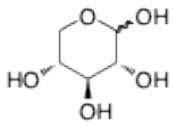
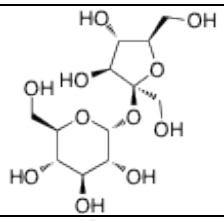
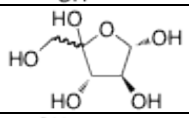
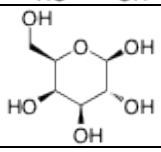
The work carried out in this section has produced some new and interesting results. It has been shown that the choice of different solvents in back extraction methods does have a profound effect. Certain compounds are not detected and the ratios of the amount of compounds detected are widely off the mark off what is actually on the dyed wool samples. The use of tartaric acid and sodium citrate in water respectively gave the same if not better results than the methods from the literature as shown in Section 4.2.1.2.

The most promising method that was undertaken was that using a glucose dissolved in water solvent mixture. This gave the most detailed chromatogram with all the levels of the three compounds detected at ratios that were proportionate to the initial madder extraction chromatogram. This method using glucose was believed to be first time that this has ever been used and shows that a benign method using a natural molecule is all that is needed to remove the anthraquinone molecules from dyed wool samples.

4.2.2.3. Back Extractions Using Different Natural Sugar Compounds

In the previous section it was demonstrated that using glucose dissolved in water was a promising solvent mixture to remove glycosidic anthraquinone dyestuff compounds from dyed wool fibres; it resulted in the best analysis for detection of glycosidic anthraquinone molecules that has been undertaken in this project. As the use of glucose gave such promising results it was envisaged that a study of back extractions using different sugar moieties might yield an even better method for back extracting glycosidic anthraquinone dye molecules from dyed wool. The compounds are shown in Table 4.3 and the extraction procedures are described in Section 7.9.5.

Table 4.3. Back extraction procedures undertaken using different sugar moieties.

Method	Sugar	Structure
A	xylose	
B	sucrose	
C	fructose	
D	galactose	

All four of these methods were again run in triplicate to see how reproducible the results obtained were. In Figure 4.17 only one of each chromatogram for each method was chosen but the difference in the repeated runs were negligable and in each circumstance gave the same result.

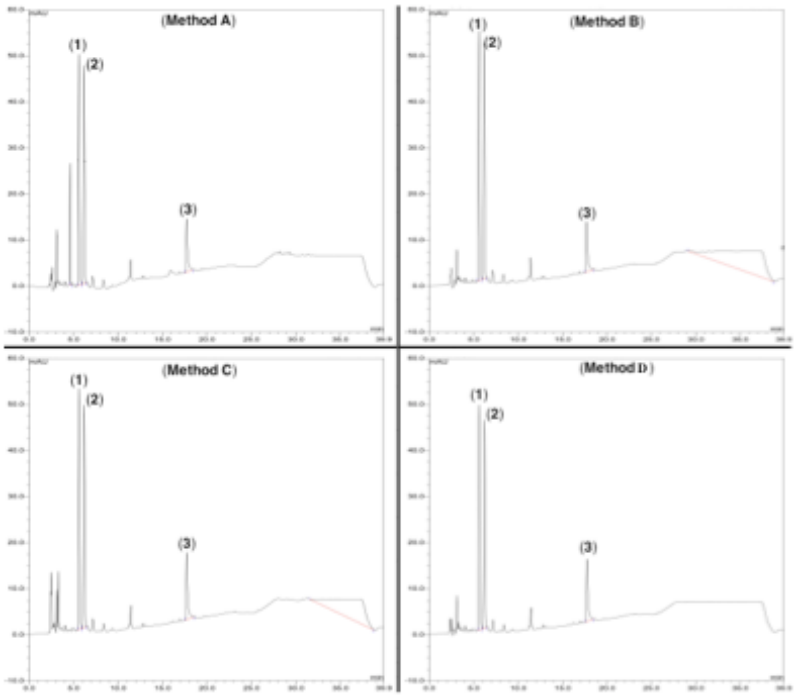


Figure 4.17. HPLC chromatograms (Relative Areas %) for **Method A**: xylose dissolved in water, **Method B**: sucrose dissolved in water, **Method C**: fructose dissolved in water and **Method D**: galactose dissolved in water all detecting 3 compounds that are (**1**) lucidin primeveroside (37.80 %) (**2**) ruberythric acid (37.53 %) and (**3**) alizarin (12.58 %).

As described in the previous sections the compounds detected by HPLC were subjected to further analysis by UV-vis spectrophotometry and mass spectrometry to fully determine the identity of these compounds analysed. In all four instances using different sugar compounds the same analytical result was obtained. The only compounds analysed were lucidin primeveroside (**1**), ruberythric acid (**2**) and alizarin (**3**); the ratio of the amounts of each compound analysed are the same for each method used. There is a drop in the amount of alizarin detected in all these cases, compared with the original glucose method from Section 4.2.2.2. The difference between these 4 compounds (xylose, fructose, sucrose and galactose) and glucose is the hydroxyl group in the primary position. In two of the four compounds tested (fructose and sucrose) there is no hydroxyl group in the primary position. In the other two compounds the hydroxyl group in the primary position is the

alternative orientation compared to glucose. This non-existence of the hydroxyl group or alternative orientation of the hydroxyl group in the primary position could have an effect on how well the molecule binds to the aluminium metal. In the case of glucose the molecule could have greater affinity to the aluminium than the other four compounds and that is reason why more alizarin is detected when the glucose is used. There is obvious evidence that lucidin-ω-ethyl ether is present in the madder extract before dyeing and is removed after dyeing owing to the fact that it is absorbed upon the wool. This compound is not seen in any of the methods used in this work performed, nor is it seen in the majority of the back extractions performed, which could be due to the solvents used or it could be due to the amount of this compound present in every dyeing process. The more redeeming analytical evidence found in using different sugar compounds are that all of the results for each method produce the same chromatogram. This along with the use of glucose in the previous section has demonstrated that the use of sugar compounds to remove anthraquinone compounds from wool fibres is a very successful and benign method. To get more evidence that this is an accurate technique to use in the analysis of historical samples it would have to be trialled first using other dyed wool fibres, other dyed fabrics and finally the use of historical samples. If this project was undertaken the results could be compared to methods already used especially in the analysis of historical samples to see if the use of sugar compounds in water is a better alternative technique.

To conclude, the use of other sugar compounds to remove the anthraquinone dye molecules from wool samples works and a successful analysis has been achieved in every instance. The use of glucose is still a better alternative as more detail is seen in the analytic measurements but the concept of using sugar

compounds dissolved in water is a viable option when wanting to complete this detailed analysis work on dyed fibres

4.2.2.4. Back Extractions Using Different Molarities of HCl in H_2O/MeOH

The use of 37% HCl in H_2O/MeOH (2:1:1, *v/v*) is a widely used technique for removing natural dyestuff molecules from historical wool and silk fibres. Some work has already been undertaken in this project proving that the use of 37% HCl in H_2O/MeOH (2:1:1, *v/v*) (6M HCl), is a technique that should not be used to analyse historical samples. This is due to the degradation of vital compounds, especially glycosidic compounds (in this project, anthraquinone glycosides) but also due to the destruction of the fabric itself

Table 4.4. Methods used in the analysis of different concentrations of HCl in H_2O/MeOH (2:1:1, *v/v*) in removing madder extract dyed on wool fibres.

Method	Volume 37% HCl (ml)	Volume H2O/MeOH (1:1, *v/v*) (ml)
1M HCl	16.7	183.3
3M HCl	51.5	158.5
5M HCl	83.5	126.5
~6M HCl*	100.0	100.0
10M HCl	167.0	33.0

***Typical literature method: 200 ml of 37% HCl/MeOH/H_2O (2:1:1, *v/v/v*)**.[114]

The aim of the work performed in this section was to follow the reactions involved in the degradation of anthraquinone products and destruction of the wool samples through differing molarities of HCl as can be seen in Table 4.4, in each case 5 g of wool was extracted at reflux (100 °C) for 10 minutes. In the UV-vis spectroscopy results (Figure 4.17), it can be seen that the absorbance maxima measure for each concentration of HCl solution is at λ_{max} 424 nm, which is the λ_{max} also reported of

the madder extract before dyeing. This gives an indication that nothing is changing during the process of using the HCl back extractions other than the anthraquinone dye molecules being removed from the wool fibres. But as previously seen this is not a justifiable analysis of the liquor and gives no clear indication of the chemistry that is taking place within the reaction vessel. The UV-vis spectroscopy results as indicated in Figure 4.18 do give a misleading result of what compounds are being back extracted.

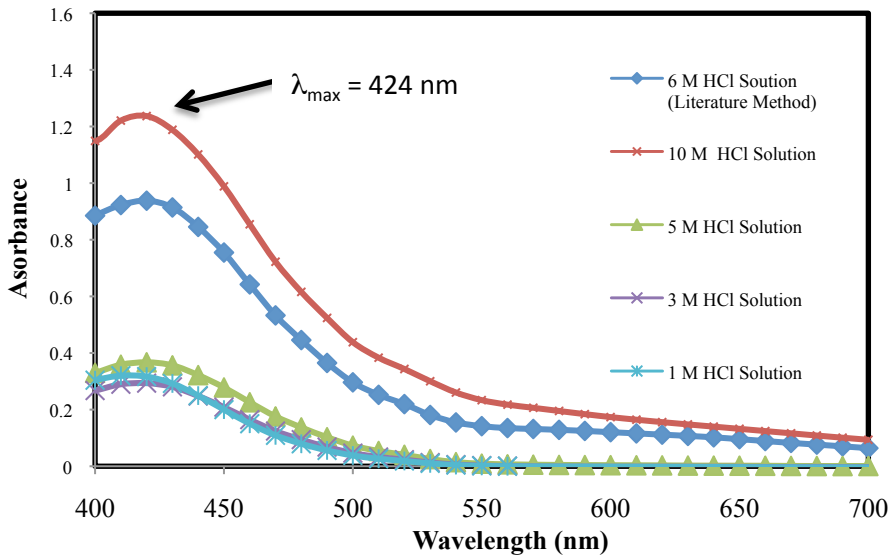


Figure 4.18. Back extraction methods using different molarities of HCl and H_2O/MeOH solutions analysed by UV-vis spectrophotometry

This can be seen in Figure 4.19 where in three of the methods there is more than one compound observed in the extraction liquor by HPLC.

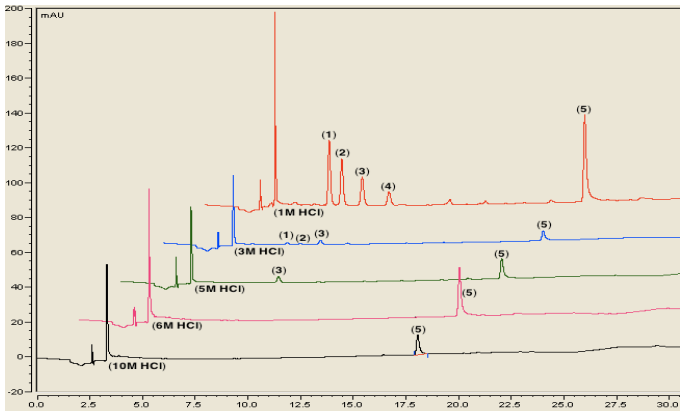


Figure 4.19. Stacked HPLC chromatograms of differing HCl concentrations in H_2O/MeOH (1:1, *v/v*) used in the back extraction of madder dyed wool fibres cut at 30 minutes (run was 40 minutes long), detailing a maximum of five compounds analysed (**1**) lucidin primeveroside, (**2**) ruberythric acid, (**3**) lucidin monoglucoside, (**4**) alizarin monoglucoside and (**5**) alizarin.

The first example to be subjected to analysis was the 1M HCl method. Using a much less acidic solution, a 1M HCl solution (pH 4.72), than the literature standard, (~6M HCl, pH <1), it can be clearly seen has a profound effect. There are five compounds analysed in this extract, lucidin primeveroside (**1**), ruberythric acid (**2**), lucidin monoglucoside (**3**), alizarin monoglucoside (**4**) and alizarin (**5**). This analysis is almost identical to the 2M TFA back extraction analysis from Section 4.2.2.2, and as reported in that instance this method has yielded two new compounds (compared to the initial madder extract before dyeing see Section 4.2.1.1), but they are a result of chemical modification, which are the hydrolysed products of lucidin primeveroside and ruberythric acid, respectively. As such, this would not be a suitable method for analysis of dyes on historical textiles or any textiles of importance to analyse the properties dyes upon them.

The aim of this section though was to try and show the mechanism of the solvent in extracting anthraquinone molecules from dyed wool fibres. It has been shown that using a strong acid solution does extract the anthraquinone molecules from the wool fibres, but the glycosidic anthraquinones are then hydrolysed to their

monoglycosidic equivalent. The height of the alizarin peak, when compared in height to the two disaccharide anthraquinone molecules (**1**) and (**2**), is not much taller. This is an indication that the acidity of the 1M HCl solution is strong enough to disrupt the metal-dye complex and displace the dye into the solution. However the 1M HCl solution is strong enough to hydroylse ludicin primeveroside and ruberythric acid to their relative monoglucosides. The amount of lucidin primeveroside and ruberythric acid in the 1M HCl solution extract are less than expected (results from the glucose extraction in Section 4.2.2.2), but this is because some of these molecules have been hydrolysed to their monoglucoside counterparts.

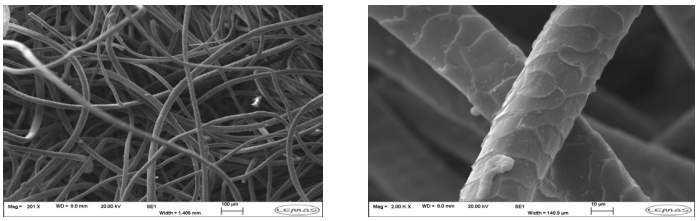
When a 3M HCl solution was used in the back extraction process only four compounds were observed by the HPLC technique. These compounds were lucidin primeveroside (**1**), ruberythric acid (**2**), lucidin monoglucoside (**3**) and alizarin (**5**). In this back extraction a stronger acid solution was used (pH 2.91) and this has an extreme effect on the products analysed. The amount of lucidin primeveroside and ruberythric acid has been reduced to a very low amount, meaning that they have been totally degraded from their original chemical structure. There is more lucidin monoglucoside than lucidin primeveroside meaning that hydrolysis of the former has taken place to the latter, but compared to the 1M HCl method the amount of lucidin monoglucoside has been reduced significantly. The most considerable outcome is that the lucidin monoglucoside has not been hydrolysed into its aglycone compound lucidin. The only other explanation is that the compound does not become hydrolysed in strong acid solutions but is degraded into compounds that are not detected by HPLC or compounds that do not have a chromophoric response. The compound alizarin monoglucoside is also not detected, and the same reasoning as for the lucidin monoglucoside can be attributed to its non-detection in the use of

HPLC, UV-vis spectrophotometry and mass spectrometry. The compound alizarin monoglucoside is not hydrolysed into its aglycone alizarin either as the detected peak for alizarin has not increased in height meaning more of the compound is in the liquor, but it has decreased in height meaning it also has been subjected to degradation and is no longer in the yield it was when a 1M HCl solution was used.

This trend was followed when stronger acidic solutions are used in back extractions as can be seen in Figure 4.19 and the outcome was more compounds are undetected meaning that they have been degraded by the strength of the solution. There is one anomaly observed and that is using a 5M HCl solution exhibited more alizarin than a 3M HCl solution this was continued to be seen up to when a 10M HCl solution was used and it exhibited signs that less alizarin was present in the back extracted mixture.

4.2.2.5. Observations of wool dyeings with SEM

The next analytical technique that was used was a scanning electron microscope (SEM) with an energy-dispersive X-ray spectroscopy (EDX) analysis technique in order to try and analyse the amount of aluminium were present on the wool samples. The EDX technique proved unsuccessful because the samples were too thin, so the X-ray beams penetrated all the way through them. Another technique to measure elemental composition, X-ray photoelectron spectroscopy (XPS) was also undertaken but again proved unsuccessful because the samples were too thin. But at the same time as using the SEM, images were obtained of the wool samples at close quarters that gave some indication of why the results obtained by HPLC were observed. As can be seen by examining the original sample in Figure 4.20 and the wool sample after being subjected to a 1M HCl solution back extraction in Figure 4.21 there is no difference in the overall structure of the wool fibres.



200 times magnification **2000 times magnification**

Figure 4.20. SEM images of originally dyed wool samples before any back extraction magnified to 200 times on the left and 2000 times on the right.

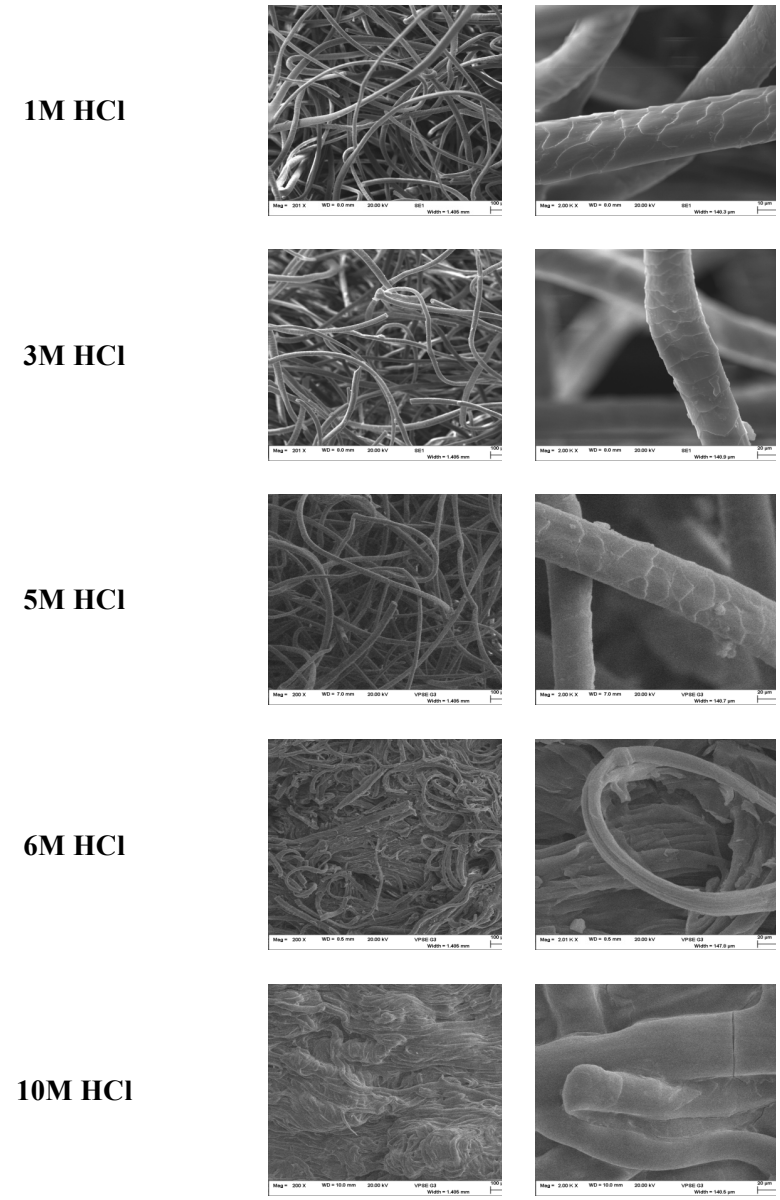


Figure 4.21. SEM images of wool sample magnified to 200 times on the left and 2000 times on the right, at different HCl concentrations for the back extractions.

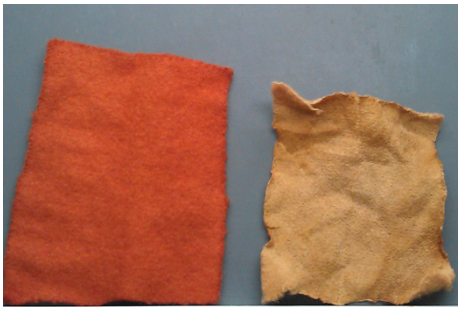
In both cases there are identifiable cuticles on individual fibres meaning that the acidity of the solution used in the back extraction is not strong enough to erode this away and alter the integral structure of the wool fibre. This agrees with the finding of the HPLC results in Figure 4.19 where five compounds are analysed, owing to the fact that the acid used is only breaking the aluminium dye bond or the aluminium wool bond and not attacking the structure of the wool itself.

The use of stronger acid in the instances of 3M HCl and 5M HCl there is no significant deterioration of the wool fibres as seen in Figure 4.21. They both have a similar appearance of the tangled wool fibres in the 200 times magnification compared with the originally dyed wool sample and they both have the detail of the cuticle layer in the 2000 times magnification picture. This again is due to the fact that the acid is not strong enough in this time (10 minute back extraction) to degrade the wool fibre and integral structure of the sample. This is shown to be the case with a reduction in all molecules analysed by the HPLC.

When a stronger acid was used as in the instance of the literature method of 37% HCl in H_2O/MeOH (2:1:1, *v/v/v*) with a molarity of 6M this has been demonstrated to have a detrimental effect on the wool sample. It has been hypothesised in previous sections of this work that at such higher acidity it is the wool fibre that gets hydrolysed and it was the metal-fibre bond that was broken. This has demonstrated to be the case with images of individual wool fibres as shown in Figure 4.21. In the 2000 times magnification picture there is no identifiable top cuticle layer, all the morphology of the wool fibre has been lost. This was made even more apparent in the 200 times magnification picture were the overall structure of the wool sample has started to deteriorate. In the original wool sample clear wool fibres can be seen in a random pattern overlapping one another but this is not the

case when a 6M HCl solution is used. This indicates that the acidity of the solution has started to attack the wool fibre itself and it is quickly degrading the sample and if left for much longer in this solution would cease to exist at all a solid piece of wool. This was shown to happen in the example of using a 10M HCl solution by looking at the pictures in Figure 4.21. This time it was harder to make out individual fibre strands as they have been degraded that much they are merging together.

This was shown to be more apparent in Figure 4.22. In the instance of the dyed wool samples subjected to a 6M HCl solution the sample became brittle and pale in appearance and had lost most of its original orange colour. This sample does appear to have been stripped of the colour what was absorbed onto it but it has been demonstrated that is the wrong approach to take when you want to clearly identify the dye compounds on the wool. In the case of the dyed wool sample subjected to a 10M HCl solution the sample became very brittle that it could be broken by just gently pulling at it.



Original dyed madder sample (left); dyed wool sample subjected to 6M HCl solution (right)

Original dyed madder sample (left); dyed wool sample subjected to 10M HCl solution (right)

Figure 4.22. Images taken of originally dyed wool samples next to dyed wool sample subjected to 6M HCl solution back extraction (left); dyed wool sample subjected to 10M HCl solution back extraction (right).

The colour of the sample has completed changed from the original vibrant orange colour to a brown/black colour. This again is why strong acids should not be

used to clearly identify glycosidic anthraquinone dye molecules that have been dyed upon wool fibres.

4.2.3. Back Extraction of Photodegraded Wool Samples Dyed with Madder Extract

The work undertaken using the technique of removing natural dyestuff molecules, especially glycosidic anthraquinone dye molecules, from wool fibres has all been on freshly dyed samples. It has been shown that the use of some literature techniques give a misrepresentation of the actual molecules involved in the dyeing process and most techniques give a misrepresentation of the different amounts of molecules present or in some cases compounds that were not even present in the dyeing process. The main aim of this whole project was to provide a new method to retain dye information *i.e.* successfully back extract glycosidic antrhaquinone dye molecules. Obtaining historical wool samples proved unsuccessful and without subjecting the newly developed method (*i.e.* the methods using natural sugar compounds especially glucose) to wool samples that had been subjected to sunlight and especially UV light over a few years it was unknown if this method could be used to analyse glycosidic anthraquinone dye molecules in historical samples.[174] The next best thing than having historical samples was subjecting dyed wool samples that had been made in-house to a supply of UV light, from a xenon arc lamp and artificially degrading the fibre samples.[175] An apparatus was set up to photo-degrade wool samples that had been dyed with madder extract (Figure 4.23).

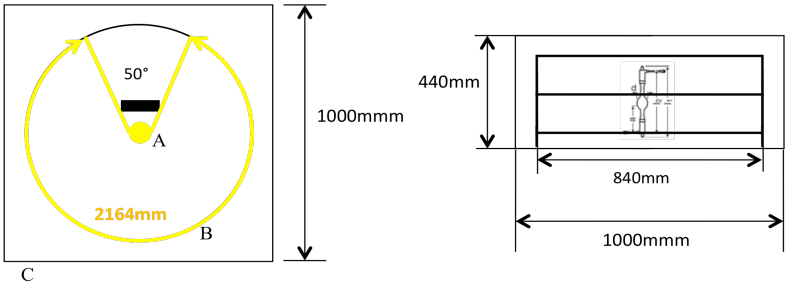


Figure 4.23. Schematic diagram of the light box. (**A**) the Orsam XBO 450 W/4 xenon arc lamp (unfiltered), (**B**) wire frame to hold the samples in place and (**C**) wooden box to support the whole structure.[175]

The wool samples were positioned 30 cm away from the bulb in a 310° arc so that each sample had the same amount of light irradiation from the xenon arc lamp shone on them. A reference of blue wool samples ranging from blue wool 1 to blue wool 8 were placed in the machine to showcase the amount of degradation the dyed wool fibres had received. Once the samples were all secured in place around the xenon arc lamp, the lid of the box was locked shut and the extractor fan turned on. The xenon arc lamp was then turned on continuously for 400 hours. 400 hours was chosen as the time frame to expose the dyed wool samples to, in order to get the most degradation possible if any was to occur. Literature precedent regarding this subject matter continually analyses the wool sample at intervals such as 4, 8, 12, 24, 36, 96 hours continually up to 400 hours and in some cases beyond.[72,119,176-182] Work has also been undertaken on the photodegradation of samples dyed with mordant and metal salts and it has been suggested that over 1500 hours was needed to overcome the protective element the mordant has over the dye molecules.[179] This timeframe was unrealistic in this body of work and it was believed that 400 hours would do some damage to the dye molecules and wool sample and future work after this PhD would focus on different time frames of exposure to photodegration all the way above and beyond 1500 hours. In Figure 4.24 the similarities in the spectral analysis

of natural sunlight and a filtered xenon arc lamp can be seen to nearly match up especially between the range of 300 – 500 nm.

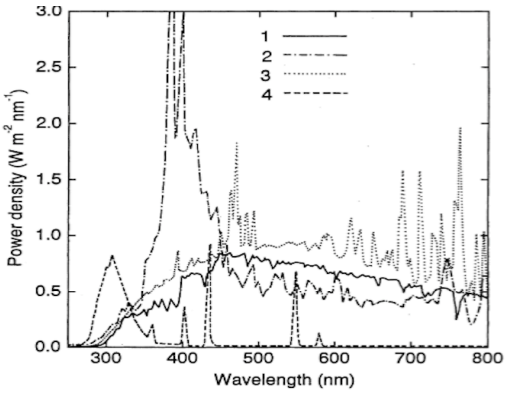


Figure 4.24. Spectral energy distribution pattern of various sources (**1**) Miami sunlight (**2**) filtered carbon arc lamp (**3**) filtered xenon arc lamp (**4**) FS-40 florescent sun lamp.[180]

A filter was not attached to the apparatus at hand, however as can be seen in Figure 4.25 the power density (acquired from manufacturer when asked for more information) from the xenon arc lamp follows a similar pattern just at greater intensity. It can clearly be seen there was power density ranging from 400 nm to 700 nm and seeing as the anthraquinone molecules present on the wool samples absorb energy around 400 nm to 430 nm this bulb should give the required energy to begin the process of photodegradation of these molecules.

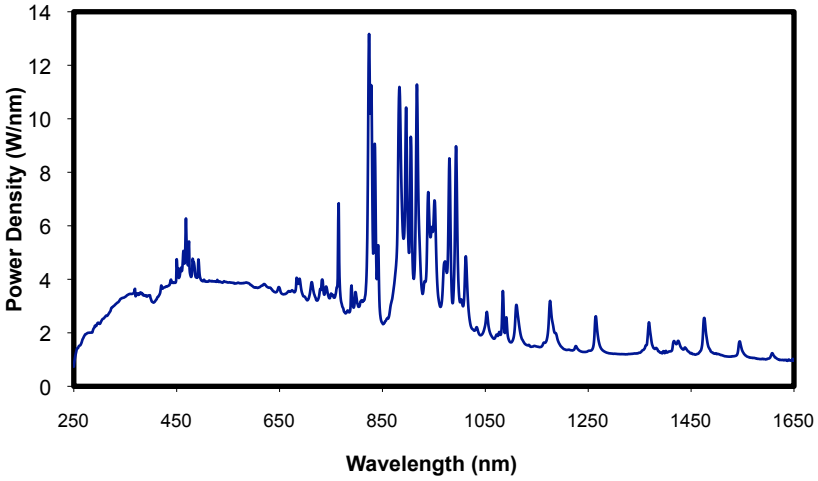
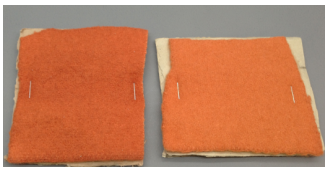


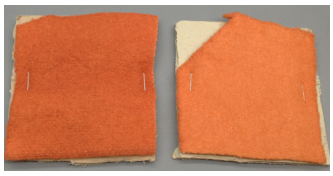
Figure 4.25. Power density of the xenon arc lamp ranging from 250 – 1650 nm used in the light box experiment.[183]

After the samples had incurred the 400 hours of light box treatment they were removed and the apparatus shut down. As can be seen below in Figure 4.26 the samples had been visually altered.

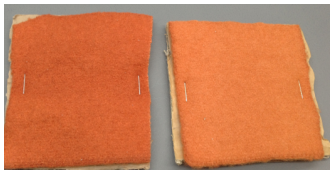
Sample AB



Sample AC



Sample AD

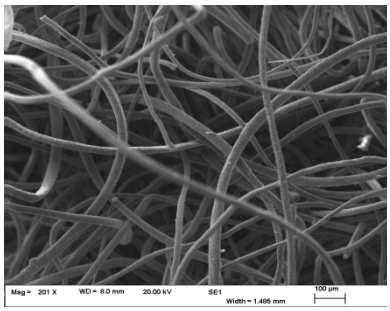


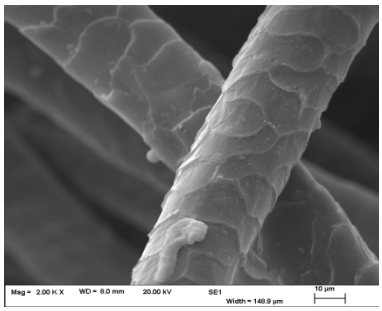
Sample AE

Figure 4.26. Images taken of wool samples subjected to 400 hours of light box exposure. On the left is the original wool sample before and on the right is the wool sample after the 400 hours of light box exposure.

It was surprising that the colour of the wool samples were not impaired even more but this shows that the use of the alum in the pre-mordanting stage does help in the light fastness of the anthraquinone molecules.[179] But there was evidence that some chemical change had happened to the wool samples but more analytical work was needed to demonstrate what this was. The wool samples were then analysed by SEM and can be seen in Figure 4.27.

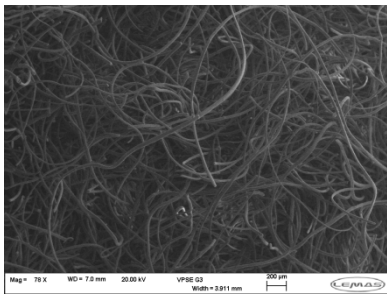
Originally Dyed Wool Sample

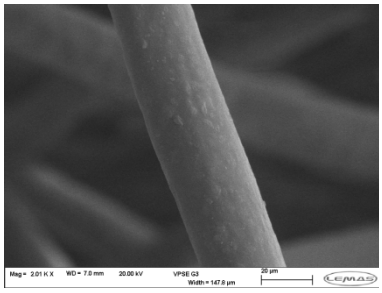




200 times magnification **2000 times magnification**

Dyed Wool Sample After 400 hours of light box exposure





78 times magnification **2000 times magnification**

Figure 4.27. SEM images of an originally dyed wool sample and a dyed wool sample after 400 hours of light box exposure. On the left is a small magnification image and on the right a more magnified image showing an individual strand.

The samples when viewed at the lower magnification look very similar owing to the fact that the overall structure of the wool fibre has not be destroyed or modified when subjected to 400 hours of light box exposure. The more interesting observation is seen when the sample is magnified 2000 times greater than the original image and you can clearly see the loss of the top cuticle layer in the sample that has been subjected to 400 hours of light box exposure. This was very interesting as it shows that the wool fibre has indeed been destroyed but only to a minimal extent.

After this initial analysis of the samples they were all subjected to different back extraction techniques. The techniques that were implored were those that had given the best results in the previous sections and the use of 37% HCl in H_2O/MeOH (2:1:1, *v/v/v*) to identify if any degradation products could be analysed.

Table 4.5. Methods used in the analysis of different back extraction techniques concentrations in the removal of madder extract dyed on wool fibres after being subjected to 400 hours of light box exposure.

Method	Extraction Procedure	Time (min)
Glucose	5g of dyed wool immersed in 200 ml of H_2O and 5g of glucose added and the mixture heated to reflux	180
Fructose	5g of dyed wool immersed in 200 ml of H_2O and 5g of fructose added and the mixture heated to reflux	180
EDTA	5g of dyed wool immersed in 0.1% Na_2EDTA in 200 ml of H_2O/DMF (1:1, *v/v*) and heated at 100 °C	30
1M HCl	5g of dyed wool immersed in 200 ml containing 16.7 ml of 37% HCl and 183.3 ml of H_2O/MeOH (1:1, *v/v*) and heated to reflux	10
~6M HCl	5g of dyed wool immersed in 200 ml of 37% HCl/MeOH/H_2O (2:1:1, *v/v/v*) and heated to reflux	10

After the samples had been back extracted using the procedures as described in Table 4.5 they were analysed by the HPLC method used in Section 4.2.1.1 and any peaks observed analysed by UV-vis spectrophotometry and mass spectrometry to get characterisation of the molecules detected. In the first example the glucose method that had been demonstrated to be the most informative, in terms of molecules detected and amounts of these molecules detected, and the most benign method used gave the following results (Figure 4.28).

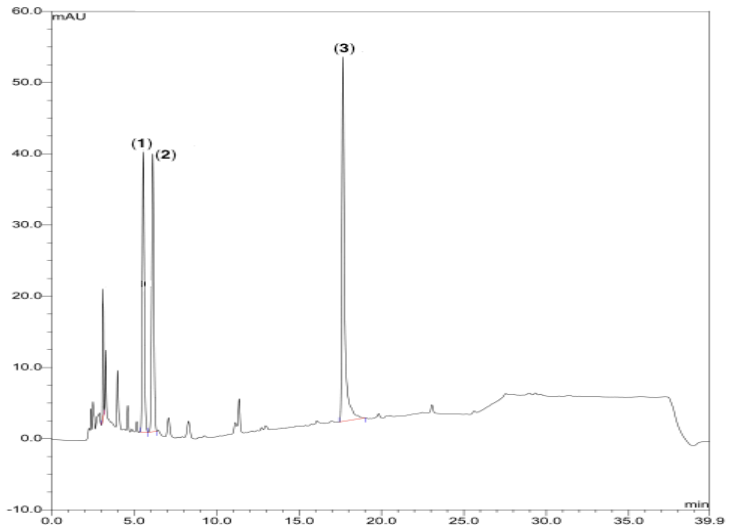


Figure 4.28. HPLC chromatogram (Relative Areas %) of the glucose back extraction showing three compounds present (**1**) lucidin primeveroside (30.75 %) (**2**) ruberythric acid (30.24 %) (**3**) alizarin (39.01 %).

Three compounds were analysed using the glucose back extraction technique and the amount detected of the compounds lucidin primeveroside and ruberythric acid had reduced compared to the original back extraction on freshly dyed wool, but the compounds lucidin primeveroside and ruberythric acid were still detected which has rarely been reported in the literature. This was a clear indication that the use of glucose and water as a medium for back extracting natural dyestuff molecules, especially anthraquinone compounds, was a technique that could be used to successfully detect glycosidic compounds that are destroyed or chemically modified in other techniques. The unsuccessful detection of lucidin-ω-ethyl ether in this extract does not mean the technique was flawed but could be an indication that the compound was photodegraded by exposure to the xenon arc lamp and was not present on the wool fibre anymore. These results were run in triplicate and lucidin-ω-ethyl ether was never detected in any of the HPLC runs. However unsuccessful detection of lucidin-ω-ethyl ether in the original glucose method on freshly dyed madder is likely the reason why lucidin-ω-ethyl ether was detected in this method.

To demonstrate that the use of glucose was not a one off result the method was re-run with fructose as the sugar of choice used this time. Again three compounds were detected when the use of fructose in water was undertaken for the back extraction (Figure 4.29).

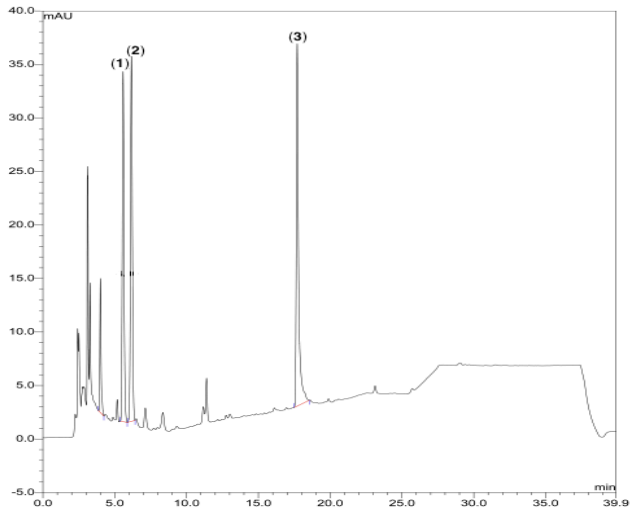


Figure 4.29. HPLC chromatogram of the fructose back extraction showing 3 compounds present (**1**) lucidin primeveroside (31.03 %) (**2**) ruberythric acid (32.37 %) (**3**) alizarin (36.60 %).

These compounds were lucidin primeveroside, ruberythric acid and alizarin and they were all at similar ratios of the samples recorded before the photodegradation had taken place. In the fructose method the alizarin had reduced in the overall amount detected and this could have been due to the amount of light it was exposed to, even though all the samples were evenly spaced around the xenon arc lamp, more so than the fructose method not extracting as much dye off the wool samples. Again there were no signs of the compound lucidin-ω-ethyl ether and as described previously this could be due to the degradation of the compound. Another explanation could be the use of water as the solvent. It has been shown that lucidin-ω-ethyl ether is more hydrophobic than the other three compounds and is seen when more hydrophobic solvents are used.

To conclude the use of glucose and fructose dissolved in water can be a useful technique to successfully analyse the dye compounds present on wool fibres. They have been shown to work on freshly dyed wool samples and now have been shown to also successfully work on degraded dyed wool samples. This technique is believed to be useful in the correct and successful analysis of historical samples.

The next method undertaken was the use of 0.1% EDTA dissolved in H_2O/DMF (1:1. *v/v*) that had been demonstrated to work just as successfully as the methods using glucose and fructose but was not as benign a method as the latter two methods. The result of this method was quite surprising, as can be seen above in Figure 4.30, there was only one compound successfully detected which was alizarin.

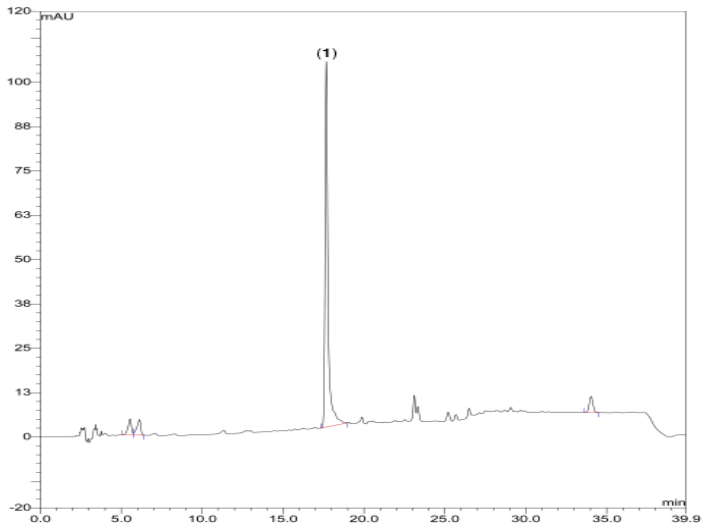


Figure 4.30. HPLC chromatogram (Relative Areas %) of the EDTA method back extraction showing 1 compound present (**1**) alizarin (93.47 %).

This result was so different to the original chromatogram of the result from the freshly dyed wool sample. This demonstrates that using 0.1% EDTA dissolved in H_2O/DMF (1:1. *v/v*) on photodegraded samples gives a misrepresented result that being only alizarin is detected. This is known to be incorrect as the use of glucose and fructose as the ligand in water produced a chromatogram that identified 3 compounds and two of those being lucidin primeveroside and ruberythric acid. The

use of 0.1% EDTA dissolved in H_2O/DMF (1:1. *v/v*) does not detect these compounds so the explanation is the method is too harsh, when compared to the use of glucose or other sugar molecules, and destroys these compounds or as the wool sample as been degraded the EDTA cannot penetrate passed the degraded top cuticle layer and extract any more dye molecules. The method of using 0.1% EDTA dissolved in H_2O/DMF (1:1. *v/v*) as a way of analysing historical samples has been shown not to be a good representation for analysing glycosidic anthraquinone compounds that are dyed on wool samples. This is because the only compound analysed was alizarin and as that has been shown that was not the case when glucose or fructose was used.

The next methods that were used in the back extraction of photodegraded dyed wool samples were the use of 1M HCl in H_2O/MeOH (1:1, *v/v*) and 37% HCl solution in H_2O/MeOH (2:1:1, *v/v/v*) shown in Figure 4.31.

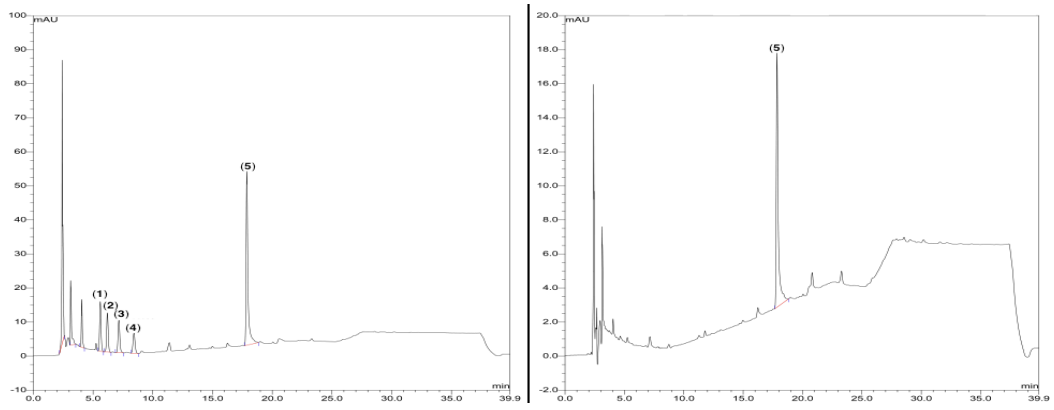


Figure 4.31. HPLC chromatogram (Relative Areas %) of the 1M HCl method back extraction on the left showing 5 compound present (**1**) lucidin primeveroside (18.81 %), (**2**) ruberythric acid (14.42 %), (**3**) lucidin monoglucoside (10.12 %), (**4**) alizarin monoglucoside (5.60 %) and (**5**) alizarin (31.90 %); and the 37% HCl solution in H_2O/MeOH (2:1:1, *v/v/v*) on the right showing one compound (**5**) alizarin (88.46 %).

These methods again are not as good as the methods involving sugar compounds that are dissolved in water. The only difference in these two acidic

methods compared to when a freshly used dyed wool sample was used is the amount of dye extracted. In the case of the results in Figure 4.30 they are significantly less after the wool sample has been subjected to 400 hours of light box exposure. In the case of the 1M HCl solution the observed compounds are lucidin primeveroside, ruberythric acid, lucidin monoglucoside, alizarin monoglucoside and alizarin. The two mono-glucosides are hydrolysed products of the two disaccharide compounds lucidin primeveroside and ruberythric acid respectively. In the case of a stronger acid being used, 37% HCl solution in H_2O/MeOH (2:1:1, *v/v/v*), the only compound analysed was alizarin.

4.3. Conclusions

The aim of this chapter was to develop a novel, improved, non-hydrolytic technique for analysing glycosidic anthraquinone molecules back extracted from dyed wool samples. The method chosen was the use of solvents mixtures to extract the dye compounds off the wool samples and into the corresponding liquor. Several literature methods were undertaken using dyed wool fibres with madder extract and other new in-house techniques. The use of the methods involving acidic solutions such as 37% HCl solution in H_2O/MeOH (2:1:1, *v/v/v*), 1M HCl solution, 2M TFA solution and formic acid in methanol all produced results with very low yields of glycosidic anthraquinone molecules present or in some instances chemically modified the anthraquinone compound structures. As this was the case these techniques were demonstrated to misrepresent the molecules present on dyed fibres. The use of 0.1% EDTA dissolved in H_2O/DMF (1:1. *v/v*) was shown to be successful at analysing all the products in freshly dyed wool samples but this was shown to not be the case when this technique was used to back extract

photodegraded wool samples. Only the compound alizarin could be detected in these circumstances.

The use of two alternative compounds tartaric acid and sodium citrate in water resulted in glycosidic anthraquinone molecules being analysed in a greater quantity than had been previously analysed. These compounds were chosen to be used as ligands much in the same way as EDTA was used in the literature preparation, to chelate to the aluminium metal mordant and displace the dye molecules. This worked successfully and could be worked on in future work to see what ligand best displaces anthraquinone dye molecules. Adjusting the solvent in both these cases to ethanol had a profound effect on the results observed. The amount of the glycosidic anthraquinone molecules drastically changed, lucidin primeveroside and ruberythric acid. A proposed reasoning for this could be due to the temperature that the water can achieve at refluxing compared to ethanol or that the compounds tartaric and citric acid are preferentially more stable in a water medium, meaning they can penetrate the wool fibres more.

Following on from this idea of trying to displace the dye molecules from the metal-dye bond with a ligand, a new method was devised this time with the use of glucose in water. This produced a result similar to that of tartaric and citric acid with a greater amount of glycosidic anthraquinone molecules analysed. The use of different sugar groups demonstrated to be just as successful showing the fact that this technique of removing the dye molecules using a natural sugar dissolved in water was a new and improved method of successfully analysing the compounds dyed on wool fibres. This was demonstrated to be just as successful when the technique was used on photodegraded wool samples. This technique gave a similar chromatogram to the chromatogram when freshly dyed wool was used proving that glycosidic

compounds can be detected on wool samples that have been aged and subjected to xenon arc lamp for an extended period. So to summarise the aim at the beginning of this chapter was to devise an improved method for back extracting glycosidic anthraquinone dyes from wool and this was achieved with the development of a method using glucose and other naturally occurring sugar compounds which successfully extracted all glycosidic anthraquinone compounds that had been dyed onto the wool samples. This result has been shown to be the case if the sample was a freshly dyed sample or one that has been subjected to an extensive bout of the xenon arc lamp.

5. Conclusions

The conclusions gathered from this PhD work have answered the aims and objectives set out at the beginning. Whilst showing some new areas of research that will help better understand the dyeing process of glycosidic anthraquinone dyes, the back extracting of these glycosidic anthraquinone molecules.

In Chapter 2 and in terms of dyeing with *Rubia tinctorium* on wool, the results gathered showed that alizarin was not the only dye compound (or compound) contributing to the overall coloured appearance at the end. Especially with these early water extractions there was not the vibrant orange colour analysed or visually observed when the same dyeing process was completed with alizarin only. It was also demonstrated that the aglycone alizarin and its glycosylated counterpart alizarin glucoside, have different dyeing properties with the latter observing a Freundlich isotherm and the former observing a Langmuir isotherm. This means that the alizarin glucoside binds to the wool or aluminium metal and also has interactions with other alizarin glucoside molecules through stacking. The alizarin molecule only binds to the wool or aluminium metal. The alizarin molecule was also analysed to have greater affinity to the wool either with no mordant added or with an aluminium pre-mordant in place. This could be due to the size of the individual molecules, alizarin glucoside being a bigger molecule than alizarin. So the amount of alizarin glucoside molecules that can adsorb onto the wool will be less than the amount of alizarin because they occupy more sites on the wool or around the aluminium metal centre.

In Chapter 3 the work was focused upon identifying glycosidic anthraquinone molecules in the extract of *Rubia tinctorium* and understand why different colours

were analysed in the dyeing process within Chapter 2. It was found that using water, a mixture of water and ethanol and ethanol at room temperature as the solvent yielded a very hygroscopic extract. When analysed by ^{1}H NMR spectroscopy and LCMS these extracts were mainly consisting of polysacchrides, free sugars and tannins. To be able to acquire better analysis of the glycosidic anthraquinone molecules within *Rubia tinctorium* another solvent was needed. Using ethanol at reflux temperature was undertaken and the results yielded were drastically better than the other solvents used. The reason for this was a bright orange powder was produced that was easy to handle, could be stored for a period of time, could be weighed out equally for subsequent dyeings and gave a more detailed analysis of the glycosidic anthraquinone molecules extracted. There were two dye compounds analysed by ^{1}H NMR spectroscopy and LCMS initially and they were lucidin primeveroside and ruberythric acid. However further analysis led to four compounds being identified by HPLC when ethanol is used as the solvent, namely lucidin primeveroside, ruberythric acid, alizarin and lucidin-ω-ethyl ether. Lucidin-ω-ethyl ether is not a naturally occurring anthraquinone compound and is only formed by the use of ethanol as the solvent at refluxing temperatures. The advantage of using ethanol as the solvent is the dried extract recovered, which is not moisture sensitive and can be re-dissolved into methanol and many other solvents. The ability to do this led to the compound lucidin primeveroside being successfully separated from the three other anthraquinone compounds present and the crystal structure of this compound being discovered. This discovery will help in the understanding of how the molecule will pack and interact with itself, which could be useful for dyeing studies undertaken with the madder root extract.

In Chapter 4 the work undertaken was to try and discover a benign method of extracting the dye compounds lucidin primeveroside, ruberythric acid from the wool fibres. The use of literature methods formic acid and methanol, hydrochloric acid in a water and ethanol solution, EDTA in a water and DMF solution and trifluoroacetic acid was undertaken as a starting point but quickly showcased that these method were not benign enough or alizarin was the main dye to have been back extracted. The EDTA method was seen as a way forward using a ligand to disrupt the dye-metal-fibre complex. This led to several other compounds being used for this process but the amount of glycosidic anthraquinone molecules analysed contradicted the amount of glycosidic anthraquinone molecules known to be adsorbed upon the wool fibre. The use of glucose dissolved in water proved to be very successful at back extracting three of the compounds, lucidin primeveroside, ruberythric acid and alizarin off the wool. Lucidin-ω-ethyl was never analysed but seeing as this compound was only observed when ethanol was used as the extracting solvent and is in low concentrations gave scope to being not as important as the other three compounds. Back extractions were also undertaken of photodegraded dyed wool fibres. The technique of photodegrading was completed in-house and wide ranges of back extracting solvent mixtures were undertaken. Again glucose dissolved in water was successful at fully retaining the glycosidic anthraquinone molecules. In this system the three compounds lucidin primeveroside, ruberythric acid and alizarin were analysed. The same HPLC profile was observed when the glucose system was undertaken of both the freshly dyed wool and photodegraded dyed wool with just a slight reduction in the amount of dyes observed in the latter.

6. Future Work

The work embarked upon within this thesis has led to the discovery of the first crystal structure of a glycosidic anthraquinone molecule that being lucidin primeveroside and a new technique using glucose dissolved in water to back extract glycosidic anthraquinone molecules from dyed wool fibres.

However this work has led for more answers to be discovered in the area of dyeing wool with *Rubia tinctorium*. There are many examples of crystal structures of alizarin complexed to aluminium but it would be very useful if alizarin glucoside and other anthraquinone glycosides could be complexed to aluminium and crystal structures observed. This would help within the understanding how these anthraquinone glycosides arrange themselves around the aluminium metal centre and if the hydroxyl groups on the sugar moieties bind to the aluminium centres also.

The use of ICP-MS to analyse the fibres after back extracting would also be useful to help in the understanding of the mechanism undertaken in this process. If the same level of aluminium was detected on the fibre after the back extracting process as before then you would have a clearer understanding of the process knowing that the metal to dye bond was broken. If the level of aluminium detected was significantly lower it would be metal to fibre bond that would have been disrupted.

The work undertaken in this work was just on *Rubia tinctorium* and the fibre used was only wool. Taking the procedures from this work and applying them to other natural dyes from botanical sources such was weld, indigo, safflower and even other *Rubia* species to see if the same results are observed. The extraction of other natural sources were attempted in this piece of work but all the samples when dried down

were too hygroscopic and could not be successfully analysed as *Rubia tinctorium* was.

The literature precedent has reported several examples of a two-stage extraction, a mild extraction followed by a harsh acid extraction afterwards.[77,157] Future work involving a glucose extraction as the mild extraction would generate very interesting results. Future work on several glucose back extractions one after the other to observe if more dye compounds can be extracted and anlaysis would generate very interesting results.

Another section for future work would involve a photodegradtion study of glycosidic anthraquinone molecules. The work would firstly begin with how do these compounds degrade under UV-light and natural daylight as individual molecules. Bombarding the glycosidic anthraquinone molecules with UV-light in a control and reproducible process. Following these reactions by HPLC-DAD-MS to know what products are observed in the degradation process. Building on from this, once knowledge of their degradation process was known it would be very interesting to observe if their degradation process altered when dyed upon wool fibres.

7. Experimental

7.1. General procedures and instrumentation

Nuclear magnetic resonance (NMR) spectra were recorded for ^{1}H at 300 and 500 MHz and ^{13}C at 75 and 125 MHz on a Bruker *DPX300* or *DRX500* spectrometer. Chemical shifts are expressed in parts per million (ppm) downfield of tetramethylsilane (singlet at 0 ppm) for proton resonances. The proton coupling constants are reported as corrected values and are given in Hz (multiplicites, s singlet, bs broad singlet, d doublet, t triplet, q quartet). The ^{13}C chemical shifts obtained are referenced in accordance to $CDCl_3$ (77.0 ppm) or $(CD_3)_2SO$ (39.0 ppm) respectively. To aid characterisation 2-D COSY, DEPT and HMQC pulse sequences were utilised. UV/Vis analysis was carried out using a *Jasco V630* UV-Vis spectrophotometer. Mass spectra were obtained using electron ion spray (EIS) on an Agilent technology 1200 series Bruker Daltonics HCT ultra. Solvents were removed at reduced pressure using a Buchi rotary evaporator at 20 mbar, followed by further drying under high vacuum at 0.5 mmHg.

7.1.1. HPLC method

HPLC analyses were carried on an Agilent 1290 Infinity UHPLC using a Diode Array Detector. Peaks were detected at 254 nm at room temperature on a Phenomenex Hyperclone C_{18} column, 5-µm particle size, 250 x 4.6 mm I.D. column equipped with a pre-column. Chromatography was carried out using two solvents: (A) water and 0.1% trifluoroacetic acid solution and (B) acetonitrile and 0.1% trifluoroacetic acid solution. A linear gradient programme was applied: 0-6 minutes 27% B; 6-20 minutes linear increase to 60% B; 20-23 minutes hold on 60% B; 23-

25 minutes linear increase to 70% B; 25-35 minutes hold on 70% B; 35-40 minutes linear decrease to 27% B. The flow rate during the experiment was 1.0 ml min^{-1}. Injections were made by a Basic Marathon autosampler equipped with a 20-µl loop.

7.1.2. LC-MS method

LC analyses were carried out at room temperature on a Phenomenex Hyperclone C_{18} column, 5-µm particle size, 250 x 4.6 mm I.D. column equipped with a pre-column. Chromatography was carried out using two solvents: (A) water and 0.1% formic acid solution and (B) acetonitrile and 0.1% formic acid solution. A linear gradient programme was applied: 0-6 minutes 27% B; 6-20 minutes linear increase to 60% B; 20-23 minutes hold on 60% B; 23-25 minutes linear increase to 70% B; 25-35 minutes hold on 70% B; 35-40 minutes linear decrease to 27% B. The flow rate during the experiment was 1.0 ml/minute. Injections were made by a Basic Marathon autosampler equipped with a 20-µl loop. The method was carried out on an Agilent 1200 LC using a Bruker HCT Ultra Ion Trap for the MS detection and a Diode Array Detector. The ESI (electronspray ionisation) parameters in the NI (negative ion) mode were as follows: spray voltage 4000 V (applied to the spray tip needle), dry gas 10 dm^3 min^{-1}, dry temperature 365 °C, capillary 60 nA, nebulizer 65 psi, nebulising gas N_2.

7.1.3. Scanning Electron Microscope

A Carl Zeiss EVO MA15 scanning electron microscope with the EDS detector was applied for the monitoring of the sample morphology and the determination of major sample components. The determination of major sample components was unsuccessful but several pictures of the samples were taken. SEM parameters were: working distance 8 mm, energy 20 keV, magnification 50-2000x, detectors used were the back scatter and the detector of secondary electrons, with the collection

time of 30 seconds. Samples were mounted in aluminium holders with double sided adhesive carbon tape and coated with an Au/Pd layer. The operating parameters of the Sputter Coater SC 7620 applied for coating of samples with a thin layer of Au/Pd were: power of 230 V (12 A), target Au/Pd, target distance 45 mm, power supply output 800 V, resolution 15 seconds, pump rate 25 L/min, sputtering rate 6 nm/min, coating thickness 1-20 nm, coating uniformity > 10%, gas medium Ar.

7.1.4. Dyeing Experiments

7.1.4.1. Preparation of *Rubia tinctorum* dye-bath

Rubia tinctorum was blended to a fine powder using a Wahl ZX595 Mini Grinder and was dissolved in distilled water at a liquor:fibre ratio (LR) of 40:1. The maximum amount of distilled water to be used was 200 ml due to the size of the dye pots. This liquor was then heated to 90 °C (heating profile shown in Figure 7.1), in a laboratory-scale *Roaches Pyrotec 2000* dyeing machine, in stainless steel sealed dye pots.

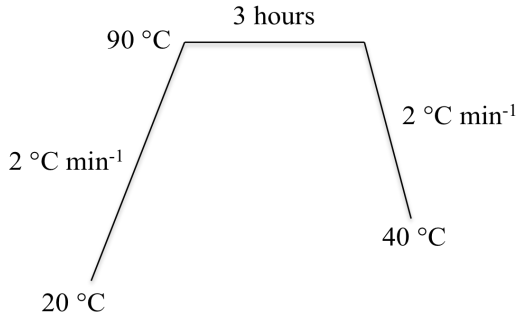


Figure 7.1. Diagram of dye-bath heating profile – method 1.

7.1.4.2. Scouring

Prior to dyeing, all fabrics were scoured using 1 g dm^{-3} sodium carbonate and 1 g dm^{-3} *Sandozin NIN* (non-ionic detergent) using a 40:1 liquor ratio. The wool was placed in a stainless steel, sealed dye pot of 300 cm^3 capacity, and a maximum volume of 200 ml of distilled water was added along with the 1 g dm^{-3} sodium

carbonate and 1 g dm^{-3} *Sandozin NIN*. The dye pot was then heated at 60°C for 15 minutes in a laboratory-scale *Roaches Pyrotec 2000* dyeing machine.

7.1.4.3. Pre-mordanting process

Aluminium potassium sulfate dodecahydrate (0.75 g, 2.91 mmol) and potassium L-tartrate monobasic (0.3 g, 1.59 mmol) were dissolved in distilled water (200 ml), and the scoured wool was immersed in this solution. The mixture was then heated to 90 °C (using the heating profile shown in Figure 7.2) in a laboratory-scale *Roaches Pyrotec 2000* dyeing machine in stainless steel, sealed dye pots of 300 cm^3 capacity. A liquor:fibre ratio (LR) of 40:1 was used. The fabric was removed from the dye pot and stored in sealed plastic bags in darkness until required for dyeing studies.

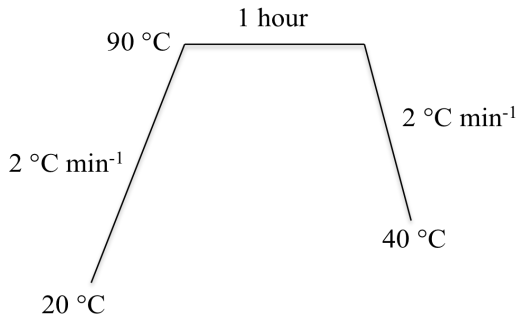


Figure 7.2. Diagram of pre-mordanting heating profile – method 2.

7.1.4.4. Dyeing process

Wool fabric samples were dyed in a laboratory-scale *Roaches Pyrotec 2000* dyeing machine in stainless steel, sealed dye pots of 300 cm^3 capacity. A liquor:fibre ratio (LR) of 40:1 was used. The wool was dyed at 90 °C for 3 hours (using the heating profile shown in Figure 7.1). After the heating profile was complete, the wool was removed from the dye-bath, rinsed thoroughly with cold water, and air-dried (unless post-mordanting was applied).

7.2. Materials

Wool samples were purchased from Whaley's Bradford (http://www.whaleys-bradford.ltd.uk/) and were purchased with the description, Natural Cream Wool, in 10 m rolls with a width of 150 cm. *Sandozin NIN* (non-ionic detergent) was purchased from Clariant UK (www.clariant.com/). Iron(II) sulfate, tin(II) chloride and copper(II) sulfate were all purchased from Sigma Aldrich. Alizarin, purpurin, aluminium potassium sulfate dodecahydrate (alum), potassium L-tartrate monobasic (cream of tartar), ethylenediamine tetraacetic acid (EDTA), tartaric acid, sodium carbonate and sodium citrate were purchased from Sigma Aldrich. Glucose, xylose, fructose, galactose and sodium hydroxide were purchased from Alfa Aesar. Ruberythric acid was purchased from Apin Chemicals. Quinoline, silver (I) oxide, 2,3,4,6-tetra-*O*-pivaloyl-α-D-glucopyranosyl bromide and *Amberlite*® IR-120(plus) resin were purchased from Sigma Aldrich. Sodium methoxide was synthesised in house using sodium metal dissolved in methanol. Water (HPLC grade), methanol (Analytical grade), ethanol (Reagent grade), 37% HCl solution (Reagent grade) and ammonia solution (Analytical grade) were purchased from Fisher Scientific UK. Acetone (HPLC grade), tetrahydrofuran (HPLC grade) trifluoroacetic acid and dimethylformamide (HPLC grade) were purchased from Sigma Aldrich. Distilled water was obtained from on site distillation apparatus. Madder Root was purchased from Aurorasilk (http://www.aurorasilk.com/). All solid reagents were recrystallised where necessary, and all liquid reagents distilled to ensure purity.

7.3. Determination of the ratio of anthraquinones to polysaccharides/free sugars

To determine the ratio of anthraquinone/s (range between 8 ppm and 6.5 ppm) to polysaccharide/free sugars (range between 5 ppm and 3 ppm) in the samples in

Section 7.8, interpretation of the ^{1}H NMR spectra and the following equation was used (Figure 7.3 and Equation 7.1).

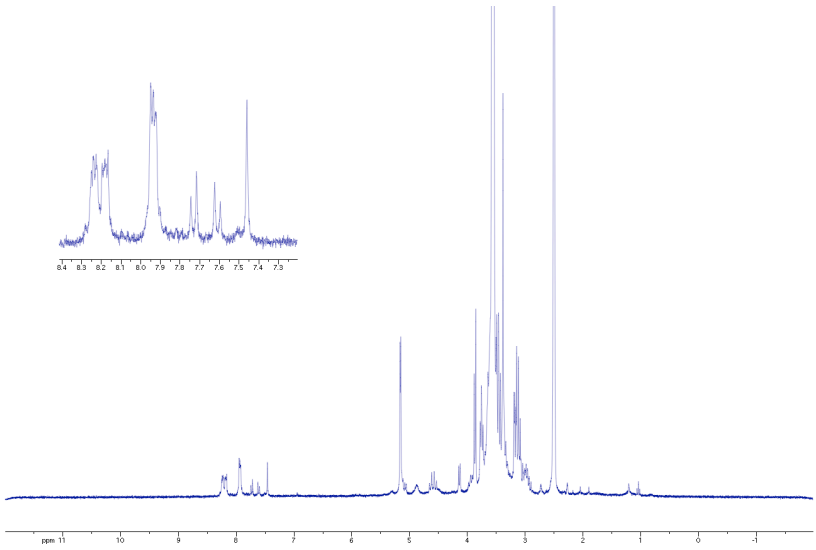


Figure 7.3. ^{1}H NMR spectrum of commercially sourced ruberythric acid in DMSO-d_6.

$$\%_H = \left(\frac{\int\left[\delta_{(8\rightarrow 6.5)}\right]}{\int\left[\delta_{(8\rightarrow 6.5)}\right] + \int\left[\delta_{(5\rightarrow 3)}\right]} \right) \times 100 \qquad (7.1)$$

It should be noted that the % value obtained was the $\%_H$ of the sample (*i.e.* the % of protons in that sample that are assigned to that compound or moiety), and does not directly reflect either %mol or %weight of the components.

7.4. Dyeing studies

7.4.1. Preparation of dye-bath

Madder root was ground-up in a Wahl ZX595 Mini Grinder to fine powder (fresh-ground madder root). Subsequently, 5.0 g of fresh-ground madder, or whole madder root was added to 200 ml of distilled water, and heated to 90 °C (heating profile shown in Figure 7.1) as described in Section 7.1.4.1. The resulting liquor was filtered using a Buchner funnel and water aspirator and reduced down to dryness. A reddish brown solid was collected from the whole madder root extract (0.95 g ± 0.20

g, 19% yield), and a reddish brown solid was collected in the fresh-ground madder extract (1.1 g ± 0.15 g, 22% yield).

7.4.2. Scouring

5 g of wool was placed in 200 ml of distilled water and all wool samples were scoured as described in Section 7.1.4.2.

7.4.3. Pre-mordanting process

5 g of scoured wool was placed in 200 ml of distilled water and all wool samples were pre-mordanted as described in Section 7.1.4.3.

7.4.4. Dyeing process

Wool fabric samples (5.0 g) were dyed as described in Section 7.1.4.4 with different concentrations of alizarin, whole madder root extract and fresh-ground madder extract. For the dyeing studies concentrations of 5%, 10% and 50% on mass of fibre (% omf) were used.

7.4.5. Post-mordanting process

Post-mordanting processes occur after the sample has been dyed using the dyeing process from Section 7.1.4.4. This process was competed in conjuncture with the pre-mordanting process used in Section 7.1.4.3 and the scouring process used in Section 7.1.4.2. With 15 minutes remaining of the dyeing procedure in Section 7.1.4.4 the wool samples were removed from the dye-bath and immersed in clean distilled water at 90 °C. The chosen mordant and potassium tartrate (if applicable) from Table 7.1 were added to the dye-bath. Once the compounds were added the mixture was stirred well, and then the wool was immersed back into the dye-bath and left for a further 30 minutes. Finally, the wool was removed from the dye-bath,

rinsed with clean water, and left to dry. The altered shade of the wool was recorded and tabulated (Table 7.1).

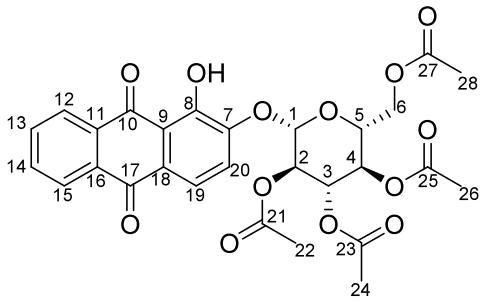
Table 7.1. Mordants used for post-mordanting on dyed wool samples (5 g).

Mordant	Concentration applied (g)	potassium L-tartrate monobasic (g)	Typical Effects of Shading
iron(II) sulfate	0.15	0.30	Dull the original dye
tin(II) chloride	0.15	0.15	Brighten the original dye
copper(II) sulfate	0.4	0	Darken the original dye

In Table 7.1 are the concentrations used of the post-mordants, the amount of potassium L-tartrate and the effects on the colour of the original dyed wool. These effects were only observed using madder as the dye so with other dyes the same effect might not be observed.

7.5. Alizarin vs. alizarin glucoside isotherm study

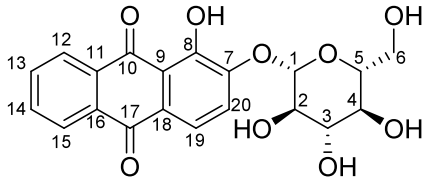
7.5.1. Synthesis of alizarin glucoside (2-(β-D-glucopyranosyloxyl)-1-hydroxyl-9, 10-anthraquinone



7.1

2,3,4,6-Tetra-*O*-acetyl-α-D-glucopyranosyl bromide (10.0 g, 0.024 mol) and 1,2-dihydroxy-9,10-anthraquinone (3.0 g, 0.0125 mol) were added to a 1 litre round bottom flask and quinoline (25 ml) along with Ag_2O (6.25 g) were added, then the

round bottom flask was covered in aluminium foil and a subaseal placed in the neck of the flask and the mixture was left stirring for 2 hours at room temperature. After 2 hours at room temperature, chloroform (250 ml) was added and the mixture filtered through celite. The filtrate was vigorously shaken with 5% H_2SO_4 (100 ml). The chloroform layer was washed with water (30 ml x 3) and dried over anhydrous calcium chloride. After filtration, the solvent was evaporated under reduced pressure and ethanol (15 ml) was added. The residue partially dissolved. The residual solid was isolated by filtration and the solution was then left for crystallisation in the refrigerator for 24 hours. The resultant solid was then filtered and dried at room temperature. The resultant solid (5.1 g) was dissolved in ethyl acetate (30 ml) and ethanol (45 ml) at 60 °C and once dissolved left to recrystallise in the refrigerator. A fine yellow powder was obtained and was filtered and dried at room temperature. 2(2,3,4,6-Tetra-*O*-acetyl-β-D-glucopyranosyloxyl)-1-hydroxyl-9,10-anthraquinone (**7.1**; 4.4 g ± 0.7 g, 7.7 mmol, 62% yield), m.p. 200-203 °C, literature m.p. 201-205 °C, ^{1}H NMR, δH (500 MHz, $(CD_3)_2SO + D_2O$); 8.26 – 8.17 (m, 2H, H-13 and H-14), 8.00 – 7.90 (m, 2H, H-12 and H-15), 7.75 (d, 1H, H-19, J = 7.5 Hz), 7.57 (d, 1H, H-20, J = 7.5 Hz), 5.71 (d, 1H, H-1, J = 7.5 Hz), 5.42 (t, 1H, H-3, J = 7.5 Hz), 5.16 (dd, 1H, H-2, J = 20 Hz, 7.5 Hz), 5.05 (t, 1H, H-4, J = 7.5 Hz), 4.28 (m, 1H, H-5), 4.22 (dd, 1H, H-6, J = 12 Hz, 5.5 Hz), 4.11 (dd, 1H, H-6', J = 12 Hz, 2 Hz), 2.05 – 2.95 (m, 12H, CH_3 groups);^{13}C nmr, δH (125 MHz, $(CD_3)_2SO + D_2O$); 188.4 (s) (Ar-C=O), 180.9 (s) (Ar-C=O), 169.9 (s) (CH_3-C=O), 169.5 (s) (CH_3-C=O), 169.2 (s) (CH_3-C=O), 168.9 (s) (CH_3-C=O), 152.1 (s), 149.8 (s), 134.9 (s), 134.1 (s), 133.2 (s), 132.8 (s), 127.2 (s), 126.58 (s), 126.3 (s), 121.6 (s), 119.3 (s), 116.7 (s), 97.1 (s), 71.5 (s), 70.8 (s), 70.2 (s), 67.5 (s), 61.2 (s), 20.1 (s), 20.1 (s), 20.0 (s), 19.9 (s).



7.2

2(2,3,4,6-Tetra-*O*-acetyl-β-D-glucopyranosyloxyl)-1-hydroxyl-9,10-anthraquinone (4.4 g, 7.7 mmol) was dissolved in tetrahydrofuran (50 ml) and methanol (50 ml). Once the compound was completely dissolved sodium methoxide (100 mg, 1.8 mmol) was added and the mixture stirred at room temperature for 16 hours. The solution was adjusted to pH 6 with the addition of Amberlite® IR-120 (plus) resin slowly with continuous stirring until the solution turned yellow. The solution was then filtered and evaporated to dryness. It was then dissolved in tetrahydrofuran (40 ml) at 66 °C and allowed to recrystallise in the refrigerator. A fine yellow powder was obtained and was filtered and dried at room temperature. 2-(β-D-glucopyranosyloxyl)-1-hydroxy-9,10-anthraquinone (**7.2**; 2.53 g ± 0.09 g, 6.3 mmol, 81% yield), m.p. 236-240 °C, literature m.p. 236-240 °C, ^{1}H NMR, δH (500 MHz, $(CD_3)_2SO + D_2O$); 8.22 – 8.12 (m, 2H, H-13 and H-14), 7.93 – 7.87 (m, 2H, H-12 and H-15), 7.68 (d, 1H, H-19, J = 8.5 Hz), 7.52 (d, 1H, H-20, J = 8.5 Hz), 5.15 (d, 1H, H-1, J = 7.5 Hz), 3.73 – 3.69 (m, 1H, sugar proton), 3.52 – 3.43 (m, 2H, sugar protons), 3.40 – 3.32 (m, 2H, sugar protons), 3.22 (t, 1H, H-3, J = 7.5 Hz);^{13}C nmr, δH (125 MHz, $(CD_3)_2SO + D_2O$); 188.78 (s) (Ar-C=O), 188.4 (s) (Ar-C=O), 151.8 (s), 151.3 (s), 135.7 (s), 134.8 (s), 133.6 (s), 133.2 (s), 127.2 (s), 127.1 (s), 126.2 (s), 120.8 (s), 120.7 (s), 116.6 (s), 99.9 (s), 77.3 (s), 76.6 (s), 73.2 (s), 69.7 (s), 60.8 (s).

7.5.2. Dyeing studies of alizarin vs. alizarin glucoside (no mordant used)

Wool fabric samples (1.0 g) were scoured as described in Section 7.1.4.2 and dyed as described in Section 7.1.4.4, with different concentrations of alizarin and alizarin glucoside. These concentrations are given as percentage on mass of fibre (% omf). For example, if 5% omf of alizarin was used on 1.0 g of wool then 0.05 g of alizarin would be required. For the dyeing isotherm studies concentrations of the two compounds were diluted from a stock solution of 25 g in a litre of distilled water. The concentrations were as follows 20%, 10%, 5%, 2%, 1%, 0.5%, 0.2%, 0.1% and 0.05% omf of alizarin and alizarin glucoside respectively. The dye-bath liquor was collected and analysed by UV-vis.

7.5.3. Dyeing studies of alizarin vs. alizarin glucoside (pre-mordant used)

Wool fabric samples (1.0 g) were scoured as described in Section 7.1.4.2, pre-mordanted as described in Section 7.1.4.3 and dyed as described in Section 7.1.4.4, with different concentrations of alizarin and alizarin glucoside. These concentrations are given as percentage on mass of fibre (% omf). For example, if 5% omf of alizarin was used on 1.0 g of wool then 0.05 g of alizarin would be required. For the dyeing isotherm studies concentrations of the two compounds were diluted from a stock solution of 25 g in a litre of distilled water. The concentrations were as follows 20%, 10%, 5%, 2%, 1%, 0.5%, 0.2%, 0.1% and 0.05% omf of alizarin and alizarin glucoside respectively. The dye-bath liquor was collected and analysed by UV-vis.

7.5.4. Analysis of alizarin and alizarin glucoside dye-baths

The dye-baths were collected from Section 7.5.2 and 7.5.3 and a sample of the solution (7 ml) was decanted into a separate vessel. Tetrahydrofuran (7 ml) was

added to the sample of the dye-bath solution (7 ml) and a UV-vis spectrum of the solution was recorded. This was completed for all the solutions in the dyeing studies. Further information on this technique is explained in Section 7.6.

7.6. UV-Visible spectrophotometry

The exhausted dye-bath liquors were measured using a *Jasco V630* UV-Visible/NIR spectrophotometer in the visible region of the spectrum (360-700 nm), at 10 nm intervals. Concentrations were calculated from calibration graphs at the wavelength of maximum absorption (λ_{max}). Absorbance of the diluted dye solution was measured at λ_{max} and percentage dye-bath exhaustion (E%) calculated using equation 7.2, where A_0 and A_1 represent absorbance of dye solution before and after dyeing, respectively.

$$E\% = 100 \times \left(1 - \frac{A_1}{A_0}\right) \tag{7.2}$$

7.7. Colour measurement

After drying, samples were analysed using a *Datacolor SF600 Spectraflash* colour spectrophotometer connected to a personal computer using *DCI Colour Tools* software. From reflectance values (R) at a specified wavelength (λ) of the dyeings, the colour strength (K/S) of the sample was calculated using the Kubelka-Munk equation (7.3).

$$K/S = \frac{\left(1 - R_\lambda\right)^2}{2R_\lambda} \quad (7.3)$$

7.8. Extraction studies

7.8.1. Solvent studies

7.8.1.1. Water extraction

Madder root (1 g) was pulverised in a Wahl ZX595 Mini Grinder and dissolved in HPLC water (120 ml). The solution was heated to 60 °C for 24 hours. Sample aliquots (3 ml) were taken at time intervals of 1, 2, 3, 4, 6 and 24 hours. Each aliquot was analysed by UV-Vis spectroscopy (typical λ_{max} of 531 nm). The resulting liquid was reduced down to a solid by rotary evaporation and a ^{1}H NMR spectrum was recorded in DMSO-d_6. ^{1}H NMR, δH (500 MHz, $(CD_3)_2SO$ + D_2O); 8.30 – 8.14 (m, 2H), 8.00 – 7.92 (m, 2H), 7.74 (d, 1H, J = 8.5 Hz), 7.63 (d, 1H, J = 8.5 Hz), 7.48 (s, 1H), 3.0 –5.5 (m, vb). The ^{1}H NMR spectrum analysed a 1:1 ratio of lucidin and alizarin molecules by analysis of the aromatic protons.

7.8.1.2. Ethanol extraction

Madder root (1 g) was pulverised in a blender and dissolved in ethanol (120 ml). The solution was heated to 60 °C for 24 hours. Sample aliquots (3 ml) were taken at time intervals of 1, 2, 3, 4, 6 and 24 hours. Each aliquot was analysed by UV-Vis spectroscopy (typical λ_{max} of 424 nm). The resulting liquid was reduced down to a solid by rotary evaporation and a ^{1}H NMR spectrum was recorded in DMSO-d_6. ^{1}H NMR, δH (500 MHz, $(CD_3)_2SO$ + D_2O); 8.30 – 8.14 (m, 2H), 8.00 – 7.92 (m, 2H), 7.74 (d, 1H, J = 8.5 Hz), 7.63 (d, 1H, J = 8.5 Hz), 7.48 (s, 1H), 3.0 –5.5 (m, vb). The ^{1}H NMR spectrum analysed a 1:1 ratio of lucidin and alizarin molecules by analysis of the aromatic protons.

7.8.1.3. 1M NaOH solution extraction

Madder root (1 g) was pulverised in a blender and dissolved in 1M NaOH solution (120 ml). The solution was heated to 60 °C for 24 hours. Sample aliquots (3 ml) were taken at time intervals of 1, 2, 3, 4, 6 and 24 hours. Each aliquot was analysed by UV-Vis spectroscopy (typical λ_{max} of 506 nm). The resulting liquid was reduced down to a solid by rotary evaporation and a ^{1}H NMR spectrum was recorded in DMSO-d_6. ^{1}H NMR, δH (500 MHz, $(CD_3)_2SO$ + D_2O); δ 3.0 ppm – 5.5 ppm. The ^{1}H NMR spectrum analysed no aromatic protons.

7.8.2. pH studies

7.8.2.1. Alizarin

Alizarin (0.01 g) was dissolved in a solution consisting of HPLC water (250 ml) and HPLC acetone (250 ml). 1M HCl and 1M NaOH were added to adjust the pH to 2 and 13 respectively, to have 12 samples of alizarin dissolved in pH solutions ranging from pH 2 to pH 13. The amount of acid or base added was less than 20 ml for initial pH increments, with exception to achieve pH 13. Sample aliquots (3 ml) were taken for each pH and were analysed by UV-Vis spectroscopy, results shown in Chapter 3.

7.8.2.2. Purpurin

Purpurin (0.025 g) was dissolved in solution consisting of HPLC water (250 ml) and HPLC acetone (250 ml). 1M HCl and 1M NaOH were added to shift the pH to 2 and 13 respectively, to have 12 samples of purpurin dissolved in pH solutions ranging from pH 2 to pH 13. The amount of acid or base added was less than 20 ml for initial pH increments, with exception to achieve pH 13. Sample aliquots (3 ml) were taken for each pH and were analysed by UV-Vis spectroscopy, results shown in Chapter 3.

7.8.2.3. Ruberythric acid

Commercially sourced ruberythric acid (0.22 g) was dissolved in a solution consisting of HPLC water (250 ml) and HPLC acetone (250 ml). 1M HCl and 1M NaOH were added to shift the pH to 2 and 13 respectively, to have 12 samples of ruberythric acid dissolved in pH solutions ranging from pH 2 to pH 13. The amount of acid or base added was less than 20 ml for initial pH increments, with exception to achieve pH 13. Sample aliquots (3 ml) were taken for each pH and were analysed by UV-Vis spectroscopy, results shown in Chapter 3.

7.8.2.4. Alizarin glucoside

Synthesised alizarin glucoside (0.01g) from Section 7.5.1 was dissolved in a solution consisting of HPLC water (250 ml) and HPLC acetone (250 ml). 1M HCl and 1M NaOH were added to shift the pH to 2 and 13 respectively, to have 12 samples of alizarin glucoside dissolved in pH solutions ranging from pH 2 to pH 13. The amount of acid or base added was less than 20 ml for initial pH increments, with exception to achieve pH 13. Sample aliquots (3 ml) were taken for each pH and were analysed by UV-Vis spectroscopy, results shown in Chapter 3.

7.8.2.5. Madder root

Madder root (0.2 g) was blended to a fine powder using a Wahl ZX595 Mini Grinder and dissolved in a solution consisting of HPLC water (250 ml) and HPLC acetone (250 ml). 1M HCl and 1M NaOH were added to shift the pH to 2 and 13 respectively, to have 12 samples of madder root dissolved in pH solutions ranging from pH 2 to pH 13. The amount of acid or base added was less than 20 ml for initial pH increments, with exception to achieve pH 13. Sample aliquots (3 ml) were taken for each pH and were analysed by UV-Vis spectroscopy, results shown in Chapter 3.

7.8.3. Commercially sourced ruberythric acid

Ruberythric acid (0.2 g) was ground up in a pestle and mortar and placed under a high vacuum for 2 hours to remove any residual moisture. A ^{1}H NMR spectrum of this was then recorded in DMSO-d_6. ^{1}H NMR, δH (500 MHz, $(CD_3)_2SO$ + D_2O); 8.30 – 8.14 (m, 2H), 8.00 – 7.92 (m, 2H), 7.74 (d, 1H, J = 8.5 Hz), 7.63 (d, 1H, J = 8.5 Hz), 7.48 (s, 1H), 3.0 –5.5 (m, vb). The ^{1}H NMR spectrum analysed a 1:1 ratio of lucidin and alizarin molecules by analysis of the aromatic protons.

7.8.4. Scaled-up extraction studies

7.8.4.1. Water/ethanol extraction

Madder root (17.0 g) was ground up using a Wahl ZX595 Mini Grinder until it was fine and of consistent particle size. This was dispersed in 50/50 v/v water/ethanol (500 ml) solution. This was stirred at reflux for 3 hours. It was then filtered using a Buchner funnel and water aspirator and reduced down to dryness. This solid was dried under high vacuum. The result was a red/black solid weighing 3.37 g ± 0.72 g (19 % average yield). A ^{1}H NMR spectrum was recorded in DMSO-d_6. ^{1}H NMR, δH (500 MHz, $(CD_3)_2SO$ + D_2O); 8.30 – 8.14 (m, 2H), 8.00 – 7.92 (m, 2H), 7.74 (d, 1H, J = 8.5 Hz), 7.63 (d, 1H, J = 8.5 Hz), 7.48 (s, 1H), 3.0 –5.5 (m, vb). The ratio of anthraquinones to polysaccharides was >96% in favour of the polysaccharides.

7.8.4.2. Water then ethanol extraction

Madder root (8.0 g) was ground up using a Wahl ZX595 Mini Grinder until it was fine and of a consistent particle size. This was dissolved in water (250 ml) and heated to reflux with stirring for 3 hours. It was then filtered using a Buchner funnel and water aspirator and reduced down to dryness. The resulting solid was weighed and a ^{1}H NMR spectrum was recorded in DMSO-d_6. The ratio of anthraquinones to polysaccharides was >98% in favour of polysaccharides. ^{1}H NMR, δH (500 MHz,

$(CD_3)_2SO + D_2O$); 8.30 – 8.14 (m, 2H), 8.00 – 7.92 (m, 2H), 7.74 (d, 1H, J = 8.5 Hz), 7.63 (d, 1H, J = 8.5 Hz), 7.48 (s, 1H), 3.0 –5.5 (m, vb). The ^{1}H NMR spectrum analysed a 1:1 ratio of lucidin and alizarin molecules by analysis of the aromatic protons.

The residual madder root was left to dry in the air for 24 hours and subsequently extracted further with ethanol (250 ml) at room temperature for 24 hours. The tincture was then filtered using a Buchner funnel and water aspirator and reduced down to dryness. This solid was placed under high vacuum until dry. The resulting solid was weighed, and a ^{1}H NMR spectrum was recorded in DMSO-d_6. ^{1}H NMR, δH (500 MHz, $(CD_3)_2SO + D_2O$); δ 3.0 ppm – 5.5 ppm. No anthraquinones were present, only polysaccharides/ sugars were observed in this sample.

7.8.4.3. Room temperature ethanol extraction

Madder root (8.0 g) was ground up in a blender until it was fine and of a consistent particle size. This was dissolved in ethanol (250 ml) and stirred at room temperature for 3 hours. It was then filtered using a Buchner funnel and water aspirator and reduced down to dryness. This solid was placed under high vacuum until dry. The resulting solid was weighed and a ^{1}H NMR spectrum was recorded in DMSO-d_6. The ratio of anthraquinones to polysaccharides was >99% in favour of the polysaccharides. ^{1}H NMR, δH (500 MHz, $(CD_3)_2SO + D_2O$); 8.30 – 8.14 (m, 2H), 8.00 – 7.92 (m, 2H), 7.74 (d, 1H, J = 8.5 Hz), 7.63 (d, 1H, J = 8.5 Hz), 7.48 (s, 1H), 3.0 –5.5 (m, vb). The ^{1}H NMR spectrum analysed a 1:1 ratio of lucidin and alizarin molecules by analysis of the aromatic protons.

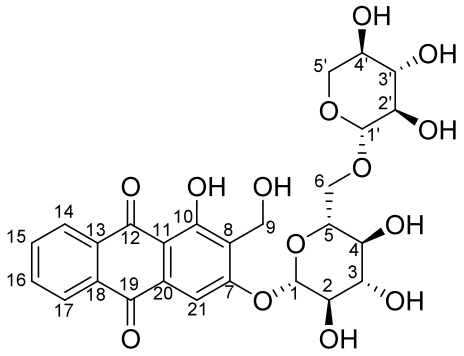
7.8.4.4. Hot ethanol extraction

Madder root (17.0 g) was ground up using a Wahl ZX595 Mini Grinder until it was fine and of consistent particle size. This was dispersed in ethanol (500 ml) and

heated to reflux with consistent stirring for 3 hours. It was then filtered using a Buchner funnel and water aspirator and reduced down to dryness This was placed under high vacuum until dry. The result was an orange powder (2.52 g ± 0.46 g, 14 % average yield). A ^{1}H NMR spectrum was recorded in DMSO-d_6. ^{1}H NMR, δH (500 MHz, $(CD_3)_2SO$ + D_2O); 8.30 – 8.14 (m, 2H), 8.00 – 7.92 (m, 2H), 7.74 (d, 1H, J = 8.5 Hz), 7.63 (d, 1H, J = 8.5 Hz), 7.48 (s, 1H), 3.0 –5.5 (m, vb). The ratio of anthraquinones to polysaccharides was 10%:90%. The ^{1}H NMR spectrum analysed a 1:1 ratio of lucidin and alizarin molecules by analysis of the aromatic protons. Madder extract (0.003 g) was dissolved in water (3 ml) and subjected to the HPLC method in Section 7.1.1. Peaks were detected at a wavelength of 254 nm; peak (**1**) retention time = 5.84 min (λ_{max} = 406, $[M-H]^-$; m/z = 563), lucidin primeveroside; peak (**2**) retention time = 6.43 min (λ_{max} = 415, $[M-H]^-$; m/z = 533), ruberythric acid; peak (**3**) retention time = 17.94 min (λ_{max} = 430, ($[M-H]^-$, m/z = 239), alizarin; peak (**4**) retention time = 23.59 min (λ_{max} = 412, $[M-H]^-$; m/z = 298), lucidin-ω-ethyl ether.

7.8.5. Recrystallisation of madder root extract

Madder root extract (2.2 g), from the hot ethanol extraction (Section 7.8.4.4), was dissolved in 150 ml of methanol. This was sonicated until all the extract dissolved. The solution was then left in the freezer for several days. The resulting liquor was decanted and several yellow fine-needled crystals resided at the bottom of the flask. An X-ray crystal structure was recorded. The resulting pure compound was analysed by ^{1}H NMR spectroscopy in DMSO-d_6.The yellow needles were collected by vacuum filtration and shown to be lucidin primeveroside (**7.3**; 156 mg ± 20 mg, yield: 7%) by NMR and X-Ray crystallography.



7.3

The resulting pure compound was analysed by ^{1}H and ^{13}C NMR spectroscopy in DMSO-d_6 + D_2O. m.p. 300-301 °C; ^{1}H NMR, δH (500 MHz, $(CD_3)_2SO$ + D_2O); 8.19 – 8.17 (m, 1H, H-15 or H-16), 8.14 – 8.12 (m, 1H, H-15 or H-16), 7.92 – 7.86 (m, 2H, H-14 and H-17), 7.40 (s, 1H, H-21), 5.10 (d, 1H, H-1 glucose, J = 7.2 Hz), 4.61 (d, 1H, one of the $\underline{CH_2}OH$, J = 11.42 Hz), 4.53 (d, 1H, one of the $\underline{CH_2}OH$, J = 11.4 Hz), 4.12 (d, 1H, H-1' xylose, J = 7.5 Hz), 3.92 (d, 1H, sugar proton, J = 9.8 Hz), 3.69 – 3.59 (m, 3H, sugar protons), 3.42 – 3.26 (m, 4H, sugar protons), 3.07 (t, 1H, sugar proton, J = 8.8 Hz), 3.01 – 2.96 (m, 2H, sugar protons); ^{13}C nmr, δH (125 MHz, $(CD_3)_2SO$ + D_2O); 187.6 (s) (C=O), 182.3 (s) (C=O), 162.3 (s), 162.2 (s), 135.6 (d), 135.5 (d), 134.4 (s), 133.3 (s), 133.2 (s), 127.6 (s), 127.2 (s), 123.7 (s), 111.9 (s), 106.6 (s), 104.4 (s), 100.8 (s), 76.6 (s), 76.1 (s), 76.1 (s), 73.7 (s), 73.5 (s), 69.9 (s), 69.5 (s), 68.5 (s), 65.9 (s), 51.4 (s) (CH_2OH).

X-ray crystallography: A suitable single crystal was selected and immersed in an inert oil. The crystal was then mounted on a glass capillary and attached to a goniometer head on a Bruker X8 Apex diffractometer using a graphite monochromated Mo-K_α radiation (λ = 0.71703 Å) using 1.0 ϕ-rotation frames. The crystal was cooled to 150 K by an Oxford cryostream low temperature device. The full data set was recorded and the images processed using DENZO and

SCALEPACK programs. The crystal was analysed using the X-ray crystallography machine by Mr. Colin Kilner.

Structure solution by direct methods was achieved through the use of SHELXS86, SIR92 or SIR97 programs, and the structural model defined by full matrix least squares on F^2 using SHELX97. Molecular graphics were plotted using POV-RAY via the XSEED program. Editing of CIFs and construction of tables of bond lengths and angles was achieved using WC and Platon. Unless otherwise stated, hydrogen atoms were placed using idealised geometric positions (with free rotation for methyl groups), allowed to move in a "riding model" along with the atoms to which they were attached, and refined isotropically.

7.9. Back extractions of dyed wool samples

7.9.1. Preparation of dyed wool samples

Madder root (17.0 g) was ground up using a Wahl ZX595 Mini Grinder until it was fine and of consistent particle size. This was dispersed in ethanol (500 ml) and heated to reflux with constant stirring for 3 hours. It was then filtered and the liquor reduced to a solid. This was placed under high vacuum until dry. The result was an orange powder (2.52 g ± 0.46 g, 14 % average yield). Wool samples (5 g) were prepared and dyed using the methods and profiles stated in Sections 7.1.4.2 – 7.1.4.4.

7.9.2. Initial back extractions of dyed wool using known methods

7.9.2.1. Formic acid method

Formic acid (10 ml) was added to methanol (190 ml) and a dyed wool sample (5.0 g) from Section 7.9.1 was immersed in the solution. This was heated to reflux and stirred for 30 minutes. The wool sample was then removed from the solution and the

solution decanted and stored for further analysis. The wool sample was air dried overnight and then subjected to K/S analysis. The decanted solution (3 ml) was subjected to the HPLC method in Section 7.1.1. Peaks were detected at a wavelength of 254 nm; peak (**1**) retention time = 6.52 min (λ_{max} = 406, $[M-H]^-$; m/z = 563), lucidin primeveroside; peak (**2**) retention time = 6.92 min (λ_{max} = 415, $[M-H]^-$; m/z = 533), ruberythric acid; peak (**3**) retention time = 18.5 min (λ_{max} = 430, ($[M-H]^-$, m/z = 239), alizarin.

7.9.2.2. HCl method

37% HCl solution (100 ml) was carefully added to a solution of methanol (50 ml) and water (50 ml) and a dyed wool sample (5.0 g) from Section 7.9.1 was immersed in the solution. This was heated to reflux and stirred for 10 minutes. The wool sample was then removed from the solution and the solution decanted and stored for further analysis. The wool sample was air dried overnight and then subjected to K/S analysis. The decanted solution (1 ml) was added to water (9 ml) and this diluted solution (3 ml) was subjected to the HPLC method in Section 7.1.1. Peaks were detected at a wavelength of 254 nm; peak (**1**) retention time = 18.04 min (λ_{max} = 430, ($[M-H]^-$, m/z = 239), alizarin.

7.9.2.3. EDTA method

EDTA (2.0 g) was dissolved in a solution of water (100 ml) and dimethylformamide (100 ml) and a dyed wool sample (5.0 g) from Section 7.9.1 was immersed in the solution. This was heated to reflux and stirred for 30 minutes. The wool sample was then removed from the solution and the solution decanted and stored for further analysis. The wool sample was air dried overnight and then subjected to K/S analysis. The decanted solution (3 ml) was subjected to the HPLC method in Section 7.1.1. Peaks were detected at a wavelength of 254 nm; peak (**1**) retention time = 6.21

min (λ_{max} = 406, $[M-H]^-$; m/z = 563), lucidin primeveroside; peak (**2**) retention time = 6.86 min (λ_{max} = 415, $[M-H]^-$; m/z = 533), ruberythric acid; peak (**3**) retention time = 18.39 min (λ_{max} = 430, ($[M-H]^-$, m/z = 239), alizarin.

7.9.3. Analysis of madder extract after dyeing

After the madder extract was used in the dyeing process in Section 7.9.1 the dye-bath solution was decanted and collected. The dye-bath solution (3 ml) was subjected to the HPLC method in Section 7.1.1. Peaks were detected at a wavelength of 254 nm; peak (**1**) retention time = 5.87 min (λ_{max} = 406, $[M-H]^-$; m/z = 563), lucidin primeveroside; peak (**2**) retention time = 6.64 min (λ_{max} = 415, $[M-H]^-$; m/z = 533), ruberythric acid; peak (**3**) retention time = 17.92 min (λ_{max} = 430, ($[M-H]^-$, m/z = 239), alizarin.

7.9.4. A further study into the back extraction of madder dyed wool samples from Section 7.9.1

7.9.4.1. Tartaric acid method A

Tartaric acid (5.0 g) was dissolved in ethanol (200 ml) and a dyed wool sample (5.0 g) from Section 7.9.1 was immersed in the solution, heated to reflux and stirred for 30 minutes. The wool sample was then removed from the solution and the solution decanted and stored for further analysis. The wool sample was air dried overnight and then subjected to K/S analysis. The decanted solution (3 ml) was subjected to the HPLC method in Section 7.1.1. Peaks were detected at a wavelength of 254 nm; peak (**1**) retention time = 17.74 min (λ_{max} = 430), alizarin; peak (**2**) retention time = 23.30 min (λ_{max} = 412), lucidin-ω-ethyl ether.

7.9.4.2. Tartaric acid method B

Tartaric acid (5.0 g) was dissolved in water (200 ml) and a dyed wool sample (5.0 g) from Section 7.9.1 was immersed in the solution, heated to reflux and stirred for 30 minutes. The wool sample was then removed from the solution and the solution decanted and stored for further analysis. The wool sample was air dried overnight and then subjected to K/S analysis. The decanted solution (3 ml) was subjected to the HPLC method in Section 7.1.1. Peaks were detected at a wavelength of 254 nm; peak (**1**) retention time = 5.91 min (λ_{max} = 406), lucidin primeveroside; peak (**2**) retention time = 6.52 min (λ_{max} = 415), ruberythric acid; peak (**3**) retention time = 18.07 min (λ_{max} = 430), alizarin.

7.9.4.3. Sodium citrate method A

Sodium citrate (5.0 g) was dissolved in ethanol (200 ml) and a dyed wool sample (5.0 g) from Section 7.9.1 was immersed in the solution, heated to reflux and stirred for 30 minutes. The wool sample was then removed from the solution and the solution decanted and stored for further analysis. The wool sample was air dried overnight and then subjected to K/S analysis. The decanted solution (3 ml) was subjected to the HPLC method in Section 7.1.1. Peaks were detected at a wavelength of 254 nm; peak (**1**) retention time = 5.85 min (λ_{max} = 406), lucidin primeveroside; peak (**2**) retention time = 6.46 min (λ_{max} = 415), ruberythric acid; peak (**3**) retention time = 18.03 min (λ_{max} = 430alizarin; peak (**4**) retention time = 23.48 min (λ_{max} = 412), lucidin-ω-ethyl ether.

7.9.4.4. Sodium citrate method B

Sodium citrate (5.0 g) was dissolved in water (200 ml) and a dyed wool sample (5.0 g) from Section 7.9.1 was immersed in the solution, heated to reflux and stirred for 30 minutes. The wool sample was then removed from the solution and the solution

decanted and stored for further analysis. The wool sample was air dried overnight and then subjected to K/S analysis. The decanted solution (3 ml) was subjected to the HPLC method in Section 7.1.1. Peaks were detected at a wavelength of 254 nm; peak (**1**) retention time = 5.89 min (λ_{max} = 406), lucidin primeveroside; peak (**2**) retention time = 6.50 min (λ_{max} = 415), ruberythric acid; peak (**3**) retention time = 18.04 min (λ_{max} = 430), alizarin.

7.9.4.5. 2M TFA method

Trifluoroacetic acid (30 ml) was added to water (172 ml) and a dyed wool sample (5.0 g) from Section 7.9.1 was immersed in the solution. This was heated to reflux and stirred for 10 minutes. The wool sample was then removed from the solution and the solution decanted and stored for further analysis. The wool sample was air dried overnight and then subjected to K/S analysis. The decanted solution (3 ml) was subjected to the HPLC method in Section 7.1.1. Peaks were detected at a wavelength of 254 nm; peak (**1**) retention time = 5.61 min (λ_{max} = 406, $[M-H]^-$; m/z = 563), lucidin primeveroside; peak (**2**) retention time = 6.20 min (λ_{max} = 415, $[M-H]^-$; m/z = 533), ruberythric acid; peak (**3**) retention time = 7.17 min (λ_{max} = 406, ($[M-H]^-$, m/z = 431), lucidin monoglucoside; peak (**4**) retention time = 8.42 min (λ_{max} = 415, $[M-H]^-$; m/z = 401), alizarin monoglucoside; peak (**5**) retention time = 17.81 min (λ_{max} = 430, ($[M-H]^-$, m/z = 239), alizarin.

7.9.4.6. Glucose method

Glucose (5.0 g) was dissolved in water (200 ml) and a dyed wool sample (5.0 g) from Section 7.9.1 was immersed in the solution. This was heated to reflux and stirred for 180 minutes. The wool sample was then removed from the solution and the solution decanted and stored for further analysis. The wool sample was air dried overnight and then subjected to K/S analysis. The decanted solution (3 ml) was

subjected to the HPLC method in Section 7.1.1. Peaks were detected at a wavelength of 254 nm; peak (**1**) retention time = 5.87 min (λ_{max} = 406, $[M-H]^-$; m/z = 563), lucidin primeveroside; peak (**2**) retention time = 6.47 min (λ_{max} = 415, $[M-H]^-$; m/z = 533), ruberythric acid; peak (**3**) retention time = 17.97 min (λ_{max} = 430, ($[M-H]^-$, m/z = 239), alizarin.

7.9.5. Back extractions using different sugar components

7.9.5.1. Xylose method

Xylose (5.0 g) was dissolved in water (200 ml) and a dyed wool sample (5.0 g) from Section 7.9.1 was immersed in the solution. This was heated to reflux and stirred for 180 minutes. The wool sample was then removed from the solution and the solution decanted and stored for further analysis. The wool sample was air dried overnight and then subjected to K/S analysis. The decanted solution (3 ml) was subjected to the HPLC method in Section 7.1.1. Peaks were detected at a wavelength of 254 nm; peak (**1**) retention time = 5.59 min (λ_{max} = 406), lucidin primeveroside; peak (**2**) retention time = 6.17 min (λ_{max} = 415), ruberythric acid; peak (**3**) retention time = 17.73 min (λ_{max} = 430), alizarin.

7.9.5.2. Sucrose method

Sucrose (5.0 g) was dissolved in water (200 ml) and a dyed wool sample (5.0 g) from Section 7.9.1 was immersed in the solution. This was heated to reflux and stirred for 180 minutes. The wool sample was then removed from the solution and the solution decanted and stored for further analysis. The wool sample was air dried overnight and then subjected to K/S analysis. The decanted solution (3 ml) was subjected to the HPLC method in Section 7.1.1. Peaks were detected at a wavelength of 254 nm; peak (**1**) retention time = 5.59 min (λ_{max} = 406), lucidin

primeveroside; peak (**2**) retention time = 6.16 min (λ_{max} = 415), ruberythric acid; peak (**3**) retention time = 17.69 min (λ_{max} = 430), alizarin.

7.9.5.3. Fructose method

Fructose (5.0 g) was dissolved in water (200 ml) and a dyed wool sample (5.0 g) from Section 7.9.1 was immersed in the solution. This was heated to reflux and stirred for 180 minutes. The wool sample was then removed from the solution and the solution decanted and stored for further analysis. The wool sample was air dried overnight and then subjected to K/S analysis. The decanted solution (3 ml) was subjected to the HPLC method in Section 7.1.1. Peaks were detected at a wavelength of 254 nm; peak (**1**) retention time = 5.61 min (λ_{max} = 406), lucidin primeveroside; peak (**2**) retention time = 6.19 min (λ_{max} = 415), ruberythric acid; peak (**3**) retention time = 17.73 min (λ_{max} = 430), alizarin.

7.9.5.4. Galactose method

Galactose (5.0 g) was dissolved in water (200 ml) and a dyed wool sample (5.0 g) from Section 7.9.1 was immersed in the solution. This was heated to reflux and stirred for 180 minutes. The wool sample was then removed from the solution and the solution decanted and stored for further analysis. The wool sample was air dried overnight and then subjected to K/S analysis. The decanted solution (3 ml) was subjected to the HPLC method in Section 7.1.1. Peaks were detected at a wavelength of 254 nm; peak (**1**) retention time = 5.615 min (λ_{max} = 406), lucidin primeveroside; peak (**2**) retention time = 6.202 min (λ_{max} = 415), ruberythric acid; peak (**3**) retention time = 17.742 min (λ_{max} = 430), alizarin.

7.9.6. Back extractions using different molarities of HCl solution in H_2O/MeOH

7.9.6.1. 1M HCl method

37% HCl solution (16.7 ml) was carefully added to a solution of methanol (91.6 ml) and water (91.6 ml) and a dyed wool sample (5.0 g) from Section 7.9.1 was immersed in the solution. This was heated to reflux and stirred for 10 minutes. The wool sample was then removed from the solution and the solution decanted and stored for further analysis. The wool sample was air dried overnight and then subjected to K/S analysis. The decanted solution (1 ml) was added to water (9 ml) and this diluted solution (3 ml) was subjected to the HPLC method in Section 7.1.1. Peaks were detected at a wavelength of 254 nm; peak (**1**) retention time = 5.88 min (λ_{max} = 406, $[M-H]^-$; m/z = 563), lucidin primeveroside; peak (**2**) retention time = 6.47 min (λ_{max} = 415, $[M-H]^-$; m/z = 533), ruberythric acid; peak (**3**) retention time = 7.44 min (λ_{max} = 406, ($[M-H]^-$, m/z = 431), lucidin monoglucoside; peak (**4**) retention time = 8.71 min (λ_{max} = 415, $[M-H]^-$; m/z = 401), alizarin monoglucoside; peak (**5**) retention time = 18.00 min (λ_{max} = 430, ($[M-H]^-$, m/z = 239), alizarin.

7.9.6.2. 3M HCl method

37% HCl solution (51.5 ml) was carefully added to a solution of methanol (74.2 ml) and water (74.2 ml) and a dyed wool sample (5.0 g) from Section 7.9.1 was immersed in the solution. This was heated to reflux and stirred for 10 minutes. The wool sample was then removed from the solution and the solution decanted and stored for further analysis. The wool sample was air dried overnight and then subjected to K/S analysis. The decanted solution (1 ml) was added to water (9 ml) and this diluted solution (3 ml) was subjected to the HPLC method in Section 7.1.1. Peaks were detected at a wavelength of 254 nm; peak (**1**) retention time = 7.45 min

(λ_{max} = 406, $[M-H]^-$; m/z = 431), lucidin monoglucoside; peak (**2**) retention time = 18.02 min (λ_{max} = 430, $[M-H]^-$; m/z = 239), alizarin.

7.9.6.3. 5M HCl method

37% HCl solution (83.5 ml) was carefully added to a solution of methanol (58.2 ml) and water (58.2 ml) and a dyed wool sample (5.0 g) from Section 7.9.1 was immersed in the solution. This was heated to reflux and stirred for 10 minutes. The wool sample was then removed from the solution and the solution decanted and stored for further analysis. The wool sample was air dried overnight and then subjected to K/S analysis. The decanted solution (1 ml) was added to water (9 ml) and this diluted solution (3 ml) was subjected to the HPLC method in Section 7.1.1. Peaks were detected at a wavelength of 254 nm; peak (**1**) retention time = 7.46 min (λ_{max} = 406), lucidin monoglucoside; peak (**2**) retention time = 18.05 min (λ_{max} = 430), alizarin.

7.9.6.4. 6M HCl method

37% HCl solution (100 ml) was carefully added to a solution of methanol (50 ml) and water (50 ml) and a dyed wool sample (5.0 g) from Section 7.9.1 was immersed in the solution. This was heated to reflux and stirred for 10 minutes. The wool sample was then removed from the solution and the solution decanted and stored for further analysis. The wool sample was air dried overnight and then subjected to K/S analysis. The decanted solution (1 ml) was added to water (9 ml) and this diluted solution (3 ml) was subjected to the HPLC method in Section 7.1.1. Peaks were detected at a wavelength of 254 nm; peak (**1**) retention time = 18.04 min (λ_{max} = 430, $[M-H]^-$; m/z = 239), alizarin.

7.9.6.5. 10M HCl method

37% HCl solution (167 ml) was carefully added to a solution of methanol (16.5 ml) and water (16.5 ml) and a dyed wool sample (5.0 g) from Section 7.9.1 was immersed in the solution. This was heated to reflux and stirred for 10 minutes. The wool sample was then removed from the solution and the solution decanted and stored for further analysis. The wool sample was air dried overnight and then subjected to K/S analysis. The decanted solution (1 ml) was added to water (9 ml) and this diluted solution (3 ml) was subjected to the HPLC method in Section 7.1.1. Peaks were detected at a wavelength of 254 nm; peak (**1**) retention time = 18.05 min (λ_{max} = 430), alizarin.

7.9.7. Photodegradation of wool samples dyed with madder extract from Section 7.9.1

To implement the photodegradation of dyed wool samples by light irradiation, bespoke equipment was constructed. The apparatus consisted of a wooden box of dimensions 1m x 1m x 0.44m. Within the box both the irradiation element and the sample area was contained within a circular steel frame of 0.84 m diameter and 0.385 m height. Dyed wool samples were mounted on to cardboard cut-outs and stapled in place then they were mounted at varying distances from the centre on a spherical section of mesh to enable uniform irradiation and energy delivery. Illumination was provided by a xenon arc bulb, wherein the bulb was an XBO 450 w/4 xenon short arc lamp, producing 450 watts with a luminous flux of 13000 lm.[189] The light source element was located 0.24 m from the base. The setup is shown diagrammatically in Figure 7.4.

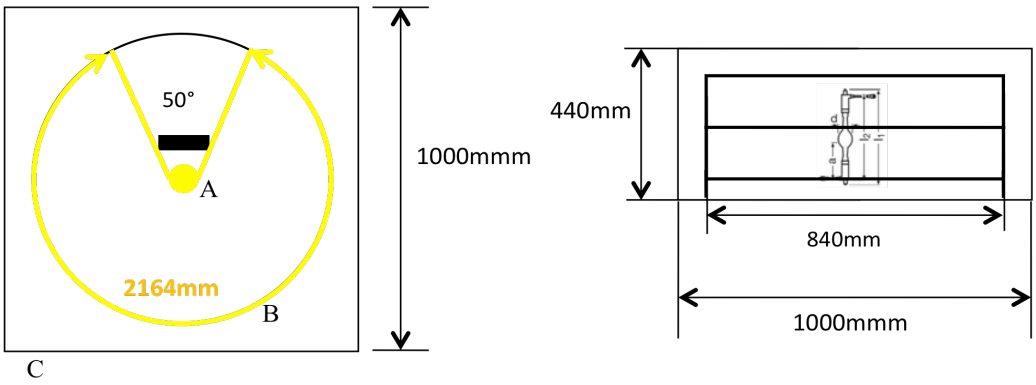


Figure 7.4. Schematic diagram of the light box. (**A**) the Orsam XBO 450 W/4 xenon arc lamp, (**B**) wire frame to hold the samples in place and (**C**) wooden box to support the whole structure.[175]

Owing to a 310° range of irradiation and the height of the box the maximum sample size was about 2.27 m x 0.46 m, the number of samples irradiated was dependent on the specific test procedure or requirement of processing. The delivery of energy to the dyed wool sample was controlled by the duration of exposure, in every instance the samples were exposed to 400 hours of light and the distance from the bulb. The box was fitted with a fan to expel ozone and facilitate air exchange. Testing was conducted in ambient conditions and the temperature and humidity were recorded using two sensors that were placed in the box.

7.9.8. Light fastness testing

Blue wool reference samples were purchased from the Society of Dyers and Colourists. Eight reference samples were purchased in total and they were cut into strips measuring 3 cm high and 10 cm long and mounted on to a cardboard cut-out. The blue wool samples were stapled, in descending order from blue wool 1 to blue wool 8, to the cardboard with 5 mm spacing between each sample. A separate piece of cardboard measuring 20 cm high and 5 cm long was placed over the right hand side of all the blue wool samples, as seen in Figure 7.5.

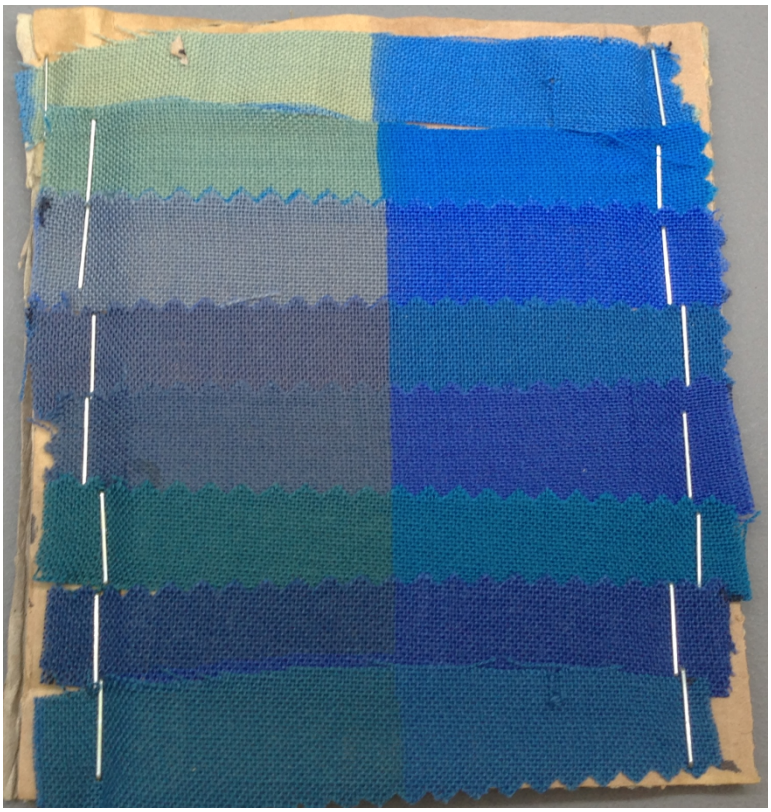


Figure 7.5. Blue wool samples after 400 hours of photodegradation showing the difference between the uncovered side and the covered side. Blue wool 1 is on the top and descends to Blue wool 8 at the bottom.

This was to determine how much degradation the blue wool sample went through. This sample was mounted to the light box on the spherical mesh surrounding the light source and was subjected to the 400 hours of light that the dyed wool samples were also subjected to. Once the 400 hours were completed the sample was removed from the machine and the cardboard covering halve the samples was removed to determine the amount of light exposure. Each consecutive number represents a doubling of the period of time needed to produce fading.[1] All the blue wool references including blue wool 8 showed signs of degradation, as can be seen in Figure 7.5. This system was implemented so that the work could be repeated to the same level of degradation.

7.9.9. Back extraction of photodegraded wool samples dyed with madder extract

7.9.9.1. Glucose method

Glucose (5.0 g) was dissolved in water (200 ml) and a dyed wool sample (5.0 g) from Section 7.9.7 was immersed in the solution. This was heated to reflux and stirred for 180 minutes. The wool sample was then removed from the solution and the solution decanted and stored for further analysis. The wool sample was air dried overnight and then subjected to K/S analysis. The decanted solution (3 ml) was subjected to the HPLC method in Section 7.1.1. Peaks were detected at a wavelength of 254 nm; peak (**1**) retention time = 5.55 min (λ_{max} = 406), lucidin primeveroside; peak (**2**) retention time = 6.43 min (λ_{max} = 415), ruberythric acid; peak (**3**) retention time = 17.66 min (λ_{max} = 430), alizarin.

7.9.9.2. Fructose method

Fructose (5.0 g) was dissolved in water (200 ml) and a dyed wool sample (5.0 g) from Section 7.9.7 was immersed in the solution. This was heated to reflux and stirred for 180 minutes. The wool sample was then removed from the solution and the solution decanted and stored for further analysis. The wool sample was air dried overnight and then subjected to K/S analysis. The decanted solution (3 ml) was subjected to the HPLC method in Section 7.1.1. Peaks were detected at a wavelength of 254 nm; peak (**1**) retention time = 5.58 min (λ_{max} = 406), lucidin primeveroside; peak (**2**) retention time = 6.16 min (λ_{max} = 415), ruberythric acid; peak (**3**) retention time = 17.70 min (λ_{max} = 430), alizarin.

7.9.9.3. EDTA method

EDTA (2.0 g) was dissolved in a solution of water (100 ml) and dimethylformamide (100 ml) and a dyed wool sample (5.0 g) from Section 7.9.7 was immersed in the

solution. This was heated to reflux and stirred for 30 minutes. The wool sample was then removed from the solution and the solution decanted and stored for further analysis. The wool sample was air dried overnight and then subjected to K/S analysis. The decanted solution (3 ml) was subjected to the HPLC method in Section 7.1.1. Peaks were detected at a wavelength of 254 nm; peak (**1**) retention time = 17.67 min (λ_{max} = 430), alizarin.

7.9.9.4. 1M HCl method

37% HCl solution (16.7 ml) was carefully added to a solution of methanol (91.6 ml) and water (91.6 ml) and a dyed wool sample (5.0 g) from Section 7.9.7 was immersed in the solution. This was heated to reflux and stirred for 10 minutes. The wool sample was then removed from the solution and the solution decanted and stored for further analysis. The wool sample was air dried overnight and then subjected to K/S analysis. The decanted solution (1 ml) was added to water (9 ml) and this diluted solution (3 ml) was subjected to the HPLC method in Section 7.1.1. Peaks were detected at a wavelength of 254 nm; peak (**1**) retention time = 5.60 min (λ_{max} = 406), lucidin primeveroside; peak (**2**) retention time = 6.199 min (λ_{max} = 415), ruberythric acid; peak (**3**) retention time = 7.16 min (λ_{max} = 406), lucidin monoglucoside; peak (**4**) retention time = 8.42 min (λ_{max} = 415), alizarin monoglucoside; peak (**5**) retention time = 17.87 min (λ_{max} = 430), alizarin.

7.9.9.5. 6M HCl method

37% HCl solution (100 ml) was carefully added to a solution of methanol (50 ml) and water (50 ml) and a dyed wool sample (5.0 g) from Section 7.9.7 was immersed in the solution. This was heated to reflux and stirred for 10 minutes. The wool sample was then removed from the solution and the solution decanted and stored for further analysis. The wool sample was air dried overnight and then subjected to K/S

analysis. The decanted solution (1 ml) was added to water (9 ml) and this diluted solution (3 ml) was subjected to the HPLC method in Section 7.1.1. Peaks were detected at a wavelength of 254 nm; peak (**1**) retention time = 17.85 min (λ_{max} = 430), alizarin.

8. References

1. Hofenk de Graaff, J. H., The Colourful Past: Origins, Chemistry and Identification of Natural Dyestuffs, Abegg-Stiftung and Archetype Publications Ltd., Riggisberg and London, **2004**.
2. Bruenello, F., The Art of Dyeing: in the history of mankind, Neri Poza Editore, Vicenza, **1973**.
3. Bechtold, T., Mussak, R., Handbook of Natural Colorants, John Wiley & Sons Ltd., **2009**.
4. Martin, H., *Chimia*, **1995,** 49, 45.
5. Robertson, S. M., Dyes from Plants, Van Nostrand Reinhold Company, New York, **1973**.
6. Cardon, D., Natural Dyes: Sources, Tradition, Technology and Science, Archetype Publications Ltd., London, **2007**.
7. Koren, Z., *J. Soc. Dyers Colour*, **1994,** 110, 273.
8. Eisner, T., Nowicki, S., Goetz, M., Meinwald, J., *Science*, **1980**, 208, 1039-1042.
9. Schweppe, H., Handbuch der Naturfarbstoffe: Vorkommen, Verwendung, Nachweis, ecomed, Landsberg/Lech, **1992**.
10. Glover, B., *J. Soc. Dyers Colour*, **1998**, 114, 4–7.
11. Taylor, G. W., *Rev. Prog. Colouration*, **1986**, 16, 53–61.
12. Zarkogianni, M., Mikropoulou, E., Varella, E., *Color. Tech.,* **2011**, 127, 18-27.
13. Kiel, E. G., Heertjes, P. M., *J. Soc. Dyers Colour,* **1965,** 81, 98.
14. Micheal, M., Tera, F., Aboelanwar, S., *Colourage*, **2003,** 1, 31.

15. Sousa, M., Miguel, C., Rodrigues, I., Parola, A., Pina, F., de Melo, J., Melo, M., *Photochem. Photobiol. Sci.* **2008,** 7, 1353.

16. Ferreira, E., Hulme, A., McNab, H., Quye, A., *Chem. Soc. Rev.* **2004,** 33, 329.

17. Blackburn, R. S., Lecture slides: *Text5108*, University of Leeds, **2010**.

18. Orna, M. V., *J. Chem. Ed.*, **1980**, 57, 256.

19. Orna, M. V., *J. Chem. Ed*., **1980**, 57, 264.

20. Dyke, S. F., Floyd, A. J., Sainsbury, M., Theobald, R. S., Organic Spectroscopy An Introduction, Longman Group Limited, New York, **1978**.

21. Christie, R. M., Colour Chemistry, The Royal Society of Chemistry, **2001.**

22. Nassau, K., The Physics and Chemistry of Colour: 2nd Edition, Wiley & Sons Ltd., **2001**.

23. Sternhell, S., Kalman, J. R., Organic Structures From Spectra, John Wiley & Sons Ltd., **1986**.

24. Zhang, X., Laursen, R. A., *Anal. Chem*., **2005**, 77, 2022-2025.

25. McAllister, L. Modification of natural dyes for superior dyeing capability, high fastness and wide colour gamut, PhD Thesis, University of Leeds, **2012**.

26. Cresswell, C. J., Runquist, O. A., Campbell, M. M., Spectral Analysis of Organic Compounds, Longman Group Ltd., **1972**.

27. Marasinghe, P. A. B., Gillispie, G. D., *Chem. Phys*. **1989**, 136, 249.

28. Kunkely, H., Vogler, A., *Inorg. Chem. Comm*., **2007**, 10, 355.

29. Smith, P. T., Zaklika, K. A., Thakur, K., Walker, G. C., Tominaga, K., Barbara, P. F., *J. Phys. Chem*., **1991**, 95, 10465.

30. Hall, D., Nobbs, C. L., *Acta. Cryst*., **1966**, 21, 927.

31. Mantzouris, D., Karapanagiotis, I., Valianou, L., Panayiotou, C., *Anal. Bioanal. Chem.*, **2011,** 399, 3065.

32. Chenciner, R., Madder Red: A history of luxury and trade, Curzon Press, **2000**.

33. Schaefer, G., The Cultivation of Madder, CIBA Review 39, 1398-1406.

34. Halleux, R., Les Alchimistes Grecs, Tome 1, Papyrus de Leyde – Papyrus de Stockholm – Fragments et Recettes, Paris, **1981**.

35. Forbes, R. J., Studies in Ancient Technology IV, Leiden, **1964**.

36. Cooksey, C., *Biotech. Histochem.*, **2009**, 84, 123.

37. http://www.mobot.org/MOBOT/research/APweb/ date accessed: 08/06/2010.

38. Ozgen, U., Kazaz, C., Secen, H., Calis, I., Coskun, M., Houghton, P., *Turk. J. Chem*., **2009**, 33, 361.

39. Derksen, G., Lelyvel, G., Van Beek, T., Capelle, A., De Groot, E., *Phytochem. Anal.*, **2004**, 15, 397.

40. Farizadeh, K., Montazer, M., Yazdanshenas, M., Rashidi, A., Malek, R., *J. App. Poly. Sci.*, **2009**, 113, 3799.

41. Singh, R., Geetanjali,, *J. Serb. Chem. Soc.*, **2005**, 113, 937.

42. De Santis, D., Moresi, M., *Indust. Crop. Prod*., **2007**, 26, 151-162.

43. Brown, T. M., *Ed. Chem*., **1999**, 36 (1), 20-22.

44. Derksen, G. C. H., Niederlander, H. A. G., van Beek, T. A., *J. Chrom. A*, **2002**, 978, 119-127.

45. Jager, I., Hafner, C., Welsch, C., Schneider, K., Iznaguen, H., Westendorf, J., *Mut.Res*., **2006**, 605, 22-29.

46. Attar-ur-Raham, Bioactive Natural Products (Part G), Elsevier, **2002**.

47. Dogan, Y., Baslar, S., Huseyin Mert, H., Ay, G., *Eco. Bot.,* **2003**, 57 (4), 442.

48. http://www.gutenberg-e.org/lowengard/C_Chap36.html date accessed 21/01/14.

49. https://www.nms.ac.uk/turkey_red/colouring_the_nation/research/dyeing_and_printing_techniques/the_turkey_red_process.aspx date accessed 21/01/14.

50. Schweppe, H., Winter, J., Madder and alizarin artist' pigments: a handbook of their history and characteristics, E. West FitzhHugh, Washington and Oxford, **1997**.

51. Schunck, E., Q. *J. Chem. Soc*., **1860**, 12, 198.

52. Perkin, W. H., *J. Chem. Soc*., **1880**, 37, 554.

53. Thomson, R., Naturally Occurring Quinones IV: Recent Advances, Blackie Academic & Professional, Chapman and Hall, **1997**.

54. Cuoco, G., Mathe, C., Archier, P., Vieillescazes, C., *J. Cul. Herit.*, **2011**, 11, 98-104.

55. Blackburn, R. S., Biodegradable and sustainable fibres, Woodhead Publishing in Series Textiles, Woodhead Publishing Ltd., Cambridge, **2005**.

56. Simpson, W. S., Crawshaw, G. H., Wool: Science and Technology, Woodhead Publishing Ltd., Cambridge, **2002**.

57. http://www.csiro.au/en/Organisation-Structure/Divisions/Materials-Science--Engineering/Innovation/The-chemical-and-physical-structure-of-merino-wool.aspx date accessed: 01/01/2013.

58. Christie, J. R., *Wool Sci. Rev*., **1987**, 64, 25-43.

59. Baker, J. R., Principles of biological microtechnique, Wiley Ltd., Metheun, London, **1958**.

60. Zarkogianni, M., Mikropoulou, E., Varella, E., Tsatsaroni, E., *Col. Tech.*, **2010,** 127, 18.

61. Wouters, J. and Verhecken, A., *Stud. Con*., **1989**, 34, 189-200.

62. Weigle, Palmy., Ancient Dyes For Modern Weavers, Watson-Guptill Publications. 1974.

63. de Sousa, A., Bessler, K., Lemos, S., Ellena, J., Gatto, C., *J. Inorg. Gen. Chem.,* **2009,** 635, 106.

64. Kiel, E. G. and Heertjes, P. M., *J. Soc. Dyers and Colourists*, **1963**, 79, 21-27.

65. Kiel, E. G. and Heertjes, P. M., *J. Soc. Dyers and Colourists*, **1963**, 79, 61.

66. Soubrayal, P., Dana, G., *Mag. Res. Chem.,* **1996,** 34, 638.

67. Wunderlich, C. H. and Bergerhoff, G., *Chemische Berichte*, **1994**, 127, 1185-1190.

68. Fain, V. Y., Zaitsev, B. E., Ryabov, M. A., *Russian J. Org. Chem.,* **2004,** 30, 390.

69. Shabanova, A. G., Sladkov, A. M., Uvarov, A. V., *Russian J. Phys. Chem.,* **1965,** 39, 763.

70. Corfield, M. C. and Robson, A., *Biochem. J*., **1995**, 59 (1), 62-68.

71. Sanyova, J., Dyes in History and Archaeology 21, Archetype Publications Ltd., **2002**, 208-212.

72. Manhita, A., Ferreira, T., Candeias, A., *Anal. Bioanal. Chem*., **2011**, 400, 1501-1514.

73. http://en.wikipedia.org/wiki/Stacking_(chemistry) date accessed: 01/01/2013.

74. Clayden, J., Greeves, N., Wouthers, P. and Warren, S., Organic Chemistry, Oxford University Press, **2005**.

75. Emsley, J., *Chem. Soc. Rev*., **1980** , 9, 91-124.

76. Atkins, P., de Paula, J., Atkins' Physical Chemistry: 8th Edition, W. H. Freeman and Company, Oxford University Press, **2006**.

77. Wouters, J., Grzywacz, C. M., Claro, A., *Stud. Conserv*., **2010**, 55, 186-203.

78. Germer, R., "Die angebliche mumie der Teje, probleme interdisziplinärer arbeiten." Studien zur Altägyptischen Kultur 11, **1984**.

79. Doursther, H., Dictionnaire universel des poids et mesures anciens et modernes, contenant des tables de monnaies de tous les pays, **1965**.

80. Sandberg, G., The Red Dyes: Cochineal, Madder ad Murex Purple, Lark Books, **1994**.

81. Karpov, H. V. M., **1859**, 120-121.

82. Miller, P., The Method of Cultivating Madder, London, **1758**.

83. Girardin., Matieres textiles et matures tinctoriales, **1861**.

84. Nie, W. L. J. de, De ontwikkeling der Nederlandsche textielververij van de XIVe tot XVIIIe eeuw, Dissertation, Leiden, **1937**.

85. Nicolai, G., Ludimagistrum in pago Wonswerdt. nemo artifex nascitur, Friesland, The Netherlands, **1648**, syns b. van de verwe f:13, 14.

86. Teli, M. D., Paul, R., Pardeshi, P. D., *Colourage*, **2001**, 51.

87. Sequin-Frey, M., *J. Chem. Ed*., **1981**, 58(4), 301.

88. Sousa, M., Miguel, C., Rodrigues, I., Parola, A., Pina, F., de Melo, J., Melo, M., *Photbiol. Sci.*, **2008**, 7, 1353.

89. Ozgen, U., Kazaz, C., Secen, H., Calis, I., Coskun, M., Houghton, P., *Turk. J. Chem.,* **2009,** 33, 561.

90. Singh, R., Geetanjali. A., *J. Serb. Chem. Soc.,* **2005,** 70, 937.

91. Farizadeh, K., Montazer, M., Yazdanshenas, M., Rashidi, A., Malek, R., *J. App. Pol. Sci.,* **2009,** 113, 3799.

92. Mouri, C., Laursen, R., *Microchem. Acta*, **2012**, 179, 105.

93. Degano, I., Ribechini, E., Modungo, F., Colombini, M. P., *App. Spec. Rev.*, **2009**, 44, 363.

94. Wouters, J., Grzywacz, C. M., Claro, A., *Stud. Conserv.*, **2011**, 56 (3), 231-249.

95. Rosenberg, E., *Anal. Bioanal. Chem.,* **2008**, 391, 33-57.

96. Bacci, M., *Sensor. Actuat. B-Chem*., **1995**, 29, 190-196.

97. Gulmini, M., Idone, A., Diana, E., Gastaldi, D., Vaudan, D., Aceto, M., *Dyes and Pigments*, **2013**, doi:10.1016/j.dyepig.2013.02.010.

98. Gardner, D. J., *Practical Raman Spec.*, Spinger-Verlag ISBN 978-0-387-50254-0, **1998**.

99. Thomas, J., Buzzini. P., Massonnet, G., Reedy, B., Roux, C., *Food Sci. Int*., **2005**, 152, 189-197.

100. Jeanmarie, D. L., Van Duyne, R. P., *J. Electroanal. Chem.*, **1977**, 81, 1.

101. Albrecht, M. A., Creighton, J. A., *J. Am. Chem. Soc.*, **1977**, 99, 5215.

102. Gale, R. J., Spectroelectrochemistry: Theory and Practice, Plenum: New York, **1988**.

103. Moscovits, M., *Rev. Mod. Phys.*, **1985**, 57, 783.

104. Brosseau, C. L., Gambardella, A., Casadio, F., Grzywacz, C. M., Wouters J., Van Duyne, R. P., *Anal. Chem.*, **2009**, 81, 3056.

105. Le Ru, E. C., Blackie, E., Meyer, M., Etchegoin, P. G., *J. Phys. Chem.*, **2007**, 111, 13794.

106. Xu, X., Li, H., Hasan, D., Ruoff, R. S., Wang, A. X., Fan, D. L., *Adv. Funct. Mater*., **2013**, doi: 10.1002/adfm.201203822.

107. Leona, M., Stenger, J., Ferloni, E., *J. Raman Spec.*, **2006**, 37, 981.

108. Leona, M., Lombardi, J. R., *J. Raman Spec.*, **2007**, 38, 853.

109. Chen, K., Leona, M., Vo-Dinh, T., *Sens. Rev.*, **2007**, 27, 109.

110. Whitney, A. V., Casadio, F., Van Duyne, R. P., *App. Spec.*, **2007**, 61, 994.

111. Pfister, R., 'Teinture et alchimie dans l'Orient hellénistique', *Seminarium Kondakowianum,* Prague, **1934**, 7, 1–59.

112. Rafaelly, L., Heron, S., Nowik, W., Tchapla, A., *Dyes and Pigments*, **2008**, 71, 191-203.

113. Wouters, J., *Stud. Conserv*., **1985**, 30, 119-128.

114. Wouters, J., Verhecken, A., *J. Soc. Dyers Colourists*, **1991**, 107, 266-269.

115. Zhang, X., Laursen, R. A., *Anal. Chem*., **2005**, 77, 2022-2025.

116. Shibayama, N., Yamaoka, R., Sato, M., Dyes in History and Archaeology 20, Archetype Publications Ltd., **2005**, 51-69.

117. Sanyova, J., *Mircochim. Acta,* **2008**, 162, 361-370.

118. Sanyova, J., Reisse, J., *J. Cult. Herit.,* **2006**, 7, 229-235.

119. Valianou, L., Karapanagiotis, I., Chryssoulakis, Y., *Anal. Bioanal. Chem*., **2009**, 395, 2175-2189.

120. Tiedemann, E. J., Yang, Y. Q., *J. Am. Inst. Conserv*., **1995**, 34, 195-206.

121. Grzywacz, C. M., Bomin, S., Yuquan, F., Wouters, J., in *ICOM Committee for Conservation, 15th Triennial Conference, New Delhi, 22–26 September 2008*: *Preprints*, ed. J. Bridgland, Allied Publishers, New Delhi, **2008,** Volume I, 534–541.

122. Serrano, A., van Bommel, M., Hallett, J., *J. Chrom. A,* **2013**, 1318, 102-111.

123. Taujenis, L., Olsauskaite, V., *Chemija*, **2012**, 23, 210.

124. Ohno, H., Fukaya, Y., *Chem. Lett.*, **2009**, 38, 2-7.

125. Sun, N., Rodriquez, H., Rahman, M., Rogers, R. D., *Chem. Comm.*, **2011**, 47, 1405-1421.

126. Usuki, T., Yasuda, N., Yoshizawa-Fujita, M., Rikukawa, M., *Chem. Comm.*, **2011**, 47, 10560-10562.

127. Lovejoy, K. S., Lou, A. J., Davis, L. E., Sanchez, T. C., Iyer, S., Corley, C. A., Wilkes, J. S., Feller, R. K., Fox, D. T., Koppisch, A. T. and Del Sesto R. E., *Anal. Chem*., **2012**, 84, 9169-9175.

128. Drivas, I., Chemical modification of natural dyes and their application in textiles, PhD Thesis, University of Leeds, **2010**.

129. McAllister, L. Modification of natural dyes for superior dyeing capability, high fastness and wide colour gamut, PhD Thesis, University of Leeds, **2012**.

130. Derksen, G. C. H., van Beek, T. A., *Nat. Prod. Chem*., **2002**, 26, 629-684.

131. El-Emary, N.A., Backheet, E. Y., *J. Phytochem*., **1998**, 48(1), 277-279.

132. Fragoso, S., Guash, J., Acena, L., Mestres, M. and Busto, O. J., *Food. Sci. Tech.*, **2011**, 46, 2569-2575.

133. Sundrarajan, M., Raji, S., Sclvam, S., *Colourage*, 2009, 56(8), 60.

134. Frackowiak, A., Skibinski, P., Gawel, W., Zaczynska, E., Czarny, A. and Gancarz, R., *Euro. J. Med. Chem*., **2010**, 45, 1001-1007.

135. Cegarra, J., Puente, P., Valldeperas, J., The Dyeing of Textile Materials, Texilia: **1992**.

136. Zhang, Z. Z., Sparks, D. L., Scrivner, N. C., *Clays and Clay Minerals,* **1992,** 40, 355.

137. Langmuir, I., *J. Am. Chem. Soc.,* **1916,** 38, 2221.

138. Langmuir, I., *J. Am. Chem. Soc.,* **1918,** 40, 1361.

139. Proctor, A., Toro-Vasquez, J. F., *J. Am. Org. Chem. Soc.,* **1996,** 73, 1627.

140. Guilhem, J., *Acta. Crystallogr.,* **1961**, 14, 88.

141. Guilhem, J., Determination par la diffraction des rayons X, de la structure cristalline des deux dihydroxyanthraquinones, l'anthrarufine et l'alizarine, PhD thesis, Facult_e des sciences de Paris, l'Universit_e de Paris, **1967**.

142. Guilhem, J., *Bull. Soc. Chim. Fr*., **1967**, 1666.

143. Orban, N. Boldizsar, I. Szücs, Z. Danos, B., *Dyes and Pigments*, **2008**, *77*, 249–257.

144. Westendorf, J., Poginsky, B., Marquardt, H., Groth, G., Marquardt, H., *Cell Bio. And Toxic.*, **1988**, 4(2), 225-239.

145. Boldizsár, I., Szucs, Z., Füzfai, Zs., Molnár-Perl, I., *J. Chromatogr. A,* **2006**, 1133, 259–274.

146. Itokawa, H., Mihara, K., Takeya, K., *Chem. Pharm. Bull*., **1983**, 31, 2353-2358.

147. Calis, I., Tasdemir, D., Ireland, C., Sticher, O., *Chem. Pharm. Bull*., **2002**, 50, 701-702.

148. El-Emary. N. A., Backheet. E. Y., *Phytochemistry*, **1998**, 49, 277—279.

149. Ozgen, U., Kazaz, C., Secen, H., Coskun, M., *Turk. J. Chem*., **2006**, 30, 15-20.

150. Surowiec, I., Nowick, W., Trojanwicz, M., *J. Sep. Sci.*, **2004**, 27, 209-216.

151. Xian, Z., Good, I., Laursen, R., *J. Arch. Sci.,* **2008**, 35, 1095-1103.

152. Laursen, R. A., Xian, Z., *Dyes His. Arch*., **2005**.

153. Drivas, I., Blackburn, R. S., Rayner. C. M., *Dyes and Pigments*, **2010**, 88 (1), 7-17.

154. http://www.chemaxon.com/marvin/sketch/index.php date accessed : 24/3/2010.

155. Serrano, A., Sousa, M. M., Hallett, J., Lopes, J. A., Oliveira, M. C., *Anal. Bional. Chem.*, **2011**, 401, 735-743.

156. Mantzouris, D., Karapanagiotis, I., Valianou, L., Panayiotou, C., *Anal. Bioanal. Chem.,* **2011**, 399, 3065–3079.

157. Lech, K., Jarosz, M., *Anal. Bioanal. Chem*., **2011**, 399, 3241–3251.

158. Novotna, P., Pacakova, V., Bosakova, Z., Stulik, K., *J. Chrom. A,* **1999**, 863, 235-241.

159. Petroviciu, I., Albu, F., Medvedovici, A., *Mircochem. J.*, **2010**, 95, 247-254.

160. Xian. Z., Laursen, R. A., *Int. J. Mass Spec.,* **2009**, 284, 108-114.

161. Ackacha, M. A., Polec-Pawlak, K., Jarosz, M., *J. Sep. Sci.*, **2003**, 26, 1028-1034.

162. Koren, Z. C., *Dyes Hist. Archael.*, **1993**, 11, 25-33.

163. Halpine, S. M., *Stud. Conser.*, **1996**, 41, 76-94.

164. Hofmann-de Keijzer, R., Van Bommel, M. R., *Dyes Hist. Archaeol*., **2005**, 20, 70–79.

165. Joosten, I., Van Bommel, M. R., Hofmann-de Keijzer, R., Reschreiter, H., *Microchim. Acta.,* **2006**, 155, 169–174.

166. Wouters, J., Rosario-Chirinos, N., *J. Am. Inst. Conserv.*, **1992**, 31, 237-255.

167. Surowiec, I., Quye, A., Trojanowicz, M., *J. Chrom. A,* **2006**, 1112, 209-217.

168. Szostek, B., Orska-Gawrys, J., Surowiec, I., Trojanowicz, M., *J. Chrom. A*, **2003**, 1012, 179-192.

169. Balakina, G. G., Vasiliev, V. G., Karpovam, E. K., Mamatyuk, V. I., *Dyes Pigm.*, **2006**, 71, 54-60.

170. Kirby, J., White, R., *Natl. Gallery. Tech. Bull.*, **1996**, 17, 56-80.

171. Schweppe, H., *J. Am. Inst. Conserv.,* **1979**, 19, 14-23.

172. Surowiec, I., Orska-Gawrys, J., Biesaga, M., Trojanowicz, M., Hutta, M., Halko, R., Urbaniak-Walczak, K., *Anal. Lett.*, **2003**, 36, 1211-1229.

173. Campbell, L., Dunkerton, J., Kirby, J., Monnas, L., *Natl. Gallery. Tech. Bull*., **2001**, 22, 29-41.

174. Colombini, M. P., Andreotti, A., Baraldi, C., Degano, I., Lucejko, J. J., *Mircochem. J.,* **2007**, 85, 174-182.

175. Wright, M. T., Properties of Nonwoven Assemblies Containing Mechanically Processed and Photo-aged PPTA Fibres, PhD Thesis, University of Leeds, **2012**.

176. Feller, R. L., *Accelerated Aging: Photochemical and Thermal Aspects*, The J. Paul Getty Trust, USA, **1994**.

177. Manhita, A., Ferreira, V., Vargas, H., Ribeiro, I., Candeias, A., Teixeira, D., Ferreira, T., Dias, C. B., *Mircochem. J.*, **2011**, 98, 82-90.

178. Odlyha, M., Theodorakopoulos, C., Campana, R., *AUTEX Res. J.,* **2007**, 7, 9-18.

179. Miller, I. J. and Smith, G. J., *J. Soc. Dyes and Color.,* **1995**, 111, 103-106.

180. Pospisil, J. and Nespurek, S., *Progress in Poly. Sci.,* **2000,** 25, 1261-1335.

181. Ahn, C., Obendorf, S. K., *Tex. Res. J.,* **2004**, 74, 949-954.

182. Orska-Gawrys, J., Surowiec, I., Kehl, J., Rejniak, H., Urbaniak-Walczak, K., Trojanwicz, M., *J. Chrom. A,* **2003**, 989, 239-248.

183. http://www.osram.com/osram_com/products/lamps/specialty-lamps/xbo/xbo28795/index.jsp?search_result=%2fosram_com%2fsearch%2fadvanced_search.jsp%3faction%3ddosearch%26inp_searchterm_1%3dXBO%2b450%2bw%252F4%26inp_searcharea%3dall%26start%3d3%26entries%3d10%26website_name%3dosram_com&productId=ZMP_85060 date accessed: 01/01/2013.