The Assessment of Natural Pigmentation in Archaeological Wool

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The Assessment of Natural Pigmentation in Archaeological Wool

Annemette Bruselius Scharff

Abstract

Naturally coloured wool contains pigment grains that mainly occur as ellipsoidal organelles (eumelanin) or spherical grains (pheomelanin). Eumelanin is the most commonly occurring pigment, but naturally coloured wool fibres contain both eumelanin and pheomelanin. In black and brown wool, the majority of the grains are eumelanin, whereas red and yellow wool contain mainly pheomelanin. Transmitted light microscopy of whole mounts of the fibres is commonly used for the detection of natural pigment grains. However, it can be difficult to detect the pigment grains exclusively by transmitted light microscopy of whole mounts of fibres. Archaeological fibres can be degraded and soiled, thereby complicating the detection of the pigments. Sometimes, it can be difficult to determine whether the fibres are coloured because of the natural pigmentation or if the colour is caused by a natural dyestuff. This can especially be the case if the pigments are degraded. When analysing the textiles from Lønne Hede (a Danish Iron Age inhumation grave), it was difficult to gain exact information about the natural pigmentation in some of the samples. To investigate this further, four samples of red-brown yarns from patterned fragments were selected for analyses. Earlier dyestuff analyses of the red-brown yarns gave no results, and it was therefore necessary to test the yarns for natural pigmentation. Three different methods were used for the analyses. Transmitted light microscopy of whole mounts of the fibres, transmitted light microscopy of cross-sections dyed with Toluidine Blue O, and transmission electron microscopy of cross-sections. The results showed that it was difficult to detect any pigments by transmitted light microscopy of whole mounts of the fibres. Transmitted light microscopy of dyed cross-sections improved the results making it possible to suggest that some fibres contained pigments and, furthermore, describe the distribution within the fibres. However, in transmission electron microscopy, it was possible to gain exact knowledge about the pigment grains in the fibres and especially information on their condition. This showed that one of the yarns contained a fairly large amount of pigment grains (enough to account for the red-brown colour). However, the pigment grains were severely damaged, which explained why it was difficult to see the pigmentation in the whole mounts of the fibres. Instead of pigment grains, the fibre contained empty holes or partly empty holes. The three other yarns contained very little or no pigment grains.

Keywords: Eumelanin, pheomelanin, identification, transmission electron microscopy

1. INTRODUCTION

Naturally coloured wool contains melanosomes (also called pigment granules) that are special organelles containing a mixture of melanins, amino acids, lipids and possible carbohydrates (Liu et al. 2005; Meredith – Sarna 2006). It is the melanins that are responsible for the colour of the wool and hair and they are produced by the melanocytes in the hair bulb. They are here synthesised in the melanosomes, either as brown-black eumelanosomes or as yellow reddish pheomelanosomes. The eumelanosomes are ovoid organelles that have a highly ordered striated protein matrix on which the eumelanin is densely deposited. The shape of the pheomelanosomes are spherical with less ordered proteinaceous matrices on which the pheomelanin is deposited in a spotty, granular and less dense structure (Castanet – Ortonne 1997). New research on the natural pigmentation in wool is limited because the pigmented fibres today are uncommon and unwanted in modern breeds. Michael Ryder (1990) gives the most extensive description of natural
pigmentation in animal textiles, but otherwise much information is found in the literature on
human hair. The size, shape and the content of the melanosomes in red and black human hair are,
for example, well documented by Yan Liu et al. (2005). They found that the pheomelanosomes
have a very high amino acid content (approximately 44 wt/wt%) and that they are loosely aggre-
gated small granules only 0.2 µm to 0.4 µm in diameter. The eumelanosomes, on the contrary, are
compact rice grain-like structures (maximum 0.5 µm to 1.2 µm) with a much lower amino acid
content (approximately 15 wt/wt%; Liu et al. 2005).

The biochemical steps in the formation of melanins from tyrosin is well documented in the
literature (Prota 1992).

Eumelanin is always present in coloured wool and hair and it is by far the most abundant
melanin, but normally both eumelans and pheomelans are present together. This is well doc-
umented by spectrophotometric (Ozeki et al. 1995; 1996b) and high-performance liquid chro-
matography (HPLC) analyses of melanin degradation products (Ito – Fujita 1985; Ozeki et al.
1996a). However, some studies of black wool and black human hair have shown that these fibres
only contain eumelanins (Aliev et al. 1990; Liu et al. 2005).

The pigment granules are normally found in the cortex cells of the wool fibres, and predomi-
nantly in the nuclear remnant (NR) of the paracortex cells, or in between the macrofibrills of
the orthocortex (Ryder 1990; Scharff – Bolt Jørgensen, 2017). They are opaque and can therefore
be documented by transmitted light microscopy of whole mounts or cross-sections of the fibres.
Light microscopy is the most common method for documentation of natural pigmentation
in wool (Wildmann 1954; Ryder 1990; Wilson et al. 2007a; 2010). However, when dealing with
archaeological textiles, it is not always easy to distinguish between natural pigmentation, dye-
stuffs, soiling and degradation products. Furthermore, the state of preservation of the pigment
granules can blur their visibility. M. Ryder (1990) records an apparent breakdown of the pigment
that, with time, can give a diffuse effect. He mentions that this process is not thoroughly under-
stood, and makes no mention of the circumstances under which this breakdown takes place.
Andrew Wilson et al. (2007b) mention the burial environment as another reason why the
pigment granules may have become invisible by transmitted light microscopy. The question is
therefore how to be sure whether a fibre is coloured by natural pigmentation or not?

A reproducible method for quantification of the pigment granules that also gives information
on the colour of the yarn is a topic that will not be discussed further here. This is described
and discussed by M. Ryder (1981; 1990; Ryder – Walton Rogers 2000) and his proposed method
was used by Penelope Walton (1988; Walton Rogers 1997, 38) and Lise Bender Jørgensen (Bender
Jørgensen – Walton 1986). This paper only investigates methods for identifying the natural pig-
ment granules in the fibres, and evaluates their ability to detect pigment granules and their state
of degradation. The two methods that are tested and compared here are transmitted light micro-
scopy and transmission electron microscopy. The material analysed is new brown and white
wool, and waterlogged Iron Age wool fragments from Lønne Hede in Denmark. The selection of
the fragments was based on two criteria: 1) The fragments should be patterned in dark and white
or blue colours so that it was certain it was not the time in the soil which had coloured the wool;
2) That no dye components were detected when analysing the dark-coloured wool yarns (Vanden
Berghe et al., in preparation).

2. MATERIALS AND METHODS

New brown and white wool from North Ronaldsay sheep and four archaeological fragments were
sampled for analysis. Two were taken from the grave excavated in 1969 – red yarns from the
original blue and red checked, tabby-woven shawl, and the blue and red striped waistband from
the skirt. Two more were taken from graves 1 and 2, excavated in 1995 – one red-brown yarn from the cream and brown striped twill (sample Tx 1.6) and one brown yarn from the diamond twill (sample Tx 2.3). Whole mounts of the samples were embedded in Euparal (refractive index \(RI = 1.48\)), and cross-sections were cut with a glass knife from both blocks of glycol methacrylate (Technovit H7100) into approximately 3 µm sections and an epoxy embedding medium (Spurr) into approximately 1µm sections. The sections were collected from the knife edge using tweezers and stretched in water by carefully applying them to the top of water droplets placed on microscope slides. When the water had evaporated, the sections were stuck to the microscope slides and half were stained immediately. Half of the sections remained unstained. The stained glycol methacrylate (GMA) sections were treated with Aniline Blue Black (ABB; 1 g in 100 ml 7% acetic acid) and half of the Spurr sections were stained with Toluidine Blue O (TBO; 1% TBO and 2% borax in distilled water). The sections were mounted in polystyrene dissolved in xylene (DePeX; \(RI = 1.529\)) with glass coverslips on top. The transmission electron microscopy protocol for making grids for the analysis is described in detail by Annemette Bruselius Scharff and Lise Bolt Jørgensen (2017).

A Nikon eclipse 80i microscope with 4 Nikon Plan Acromat Objectives (10x/0.25, 20x/0.40, 40x/0.65 and 100x/1.25 oil were used for the transmission light microscopy analyses of whole mounts and cross-sections. Köhler Illumination was applied, and for the oil objective a drop of Nikon immersion oil Type A, \(N_D = 1.515\), were placed on the cover slip before the 100x objective was moved into position. Transmission electron microscopy grids were analysed with a Jeol 1010 CX transmission electron microscope at 80 kV.

### 3. RESULTS

<table>
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<tr>
<th>TLM / TEM</th>
<th>Paraffin/ epoxy</th>
<th>Sections Embedding medium</th>
<th>Staining ABB TBO</th>
<th>New brown wool</th>
<th>Lønne Hede 1969 Checked shawl type 4 red</th>
<th>Lønne Hede 1969 Waist band type 7 red</th>
<th>Lønne Hede 1995 Tx 1.6p red-brown</th>
<th>Lønne Hede 1995 Tx 2.3 red-brown</th>
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</thead>
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<tr>
<td>TLM</td>
<td></td>
<td>Whole mounts Paraffin oil</td>
<td>No</td>
<td>Observed as pearls on a string</td>
<td>Fibres observed with granules that looked like pearls on a string</td>
<td>No granules observed</td>
<td>Pigments? Darker lines observed in light brown fibres</td>
<td>Observed as pearls on a string</td>
</tr>
<tr>
<td>TLM</td>
<td>GMA</td>
<td>Cross section DePeX (RI = 1.52)</td>
<td>No</td>
<td>Visible</td>
<td>Sometimes visible</td>
<td>No granules</td>
<td>No granules</td>
<td>Sometimes visible</td>
</tr>
<tr>
<td>TLM</td>
<td>GMA</td>
<td>Cross section DePeX (RI = 1.52)</td>
<td>ABB</td>
<td>Visibility improved</td>
<td>Visibility improved</td>
<td>No granules</td>
<td>No granules</td>
<td>Visibility improved</td>
</tr>
<tr>
<td>TLM</td>
<td>Spurr</td>
<td>Cross section DePeX (RI = 1.52)</td>
<td>No</td>
<td>Visible</td>
<td>Sometimes visible</td>
<td>No granules</td>
<td>No granules</td>
<td>Sometimes visible</td>
</tr>
<tr>
<td>TLM</td>
<td>Spurr</td>
<td>Cross section DePeX (RI = 1.52)</td>
<td>TBO</td>
<td>Visible, but can be overruled by intense colouring of the NR</td>
<td>Sometimes visible</td>
<td>No granules</td>
<td>No granules</td>
<td>Sometimes visible</td>
</tr>
<tr>
<td>TEM</td>
<td>Spurr</td>
<td>Cross section Grids -</td>
<td>-</td>
<td>Visible as round black spots mainly in NR</td>
<td>Vary from round black spots to empty holes</td>
<td>Seldom detection of round black spots</td>
<td>No granules</td>
<td>Vary from round black spots to empty holes</td>
</tr>
<tr>
<td>TEM</td>
<td>Spurr</td>
<td>Longitudinal cuts Grids -</td>
<td>-</td>
<td>Mainly ovoid seldom spherical</td>
<td>Mainly ovoid seldom spherical</td>
<td>-</td>
<td>-</td>
<td>Mainly ovoid seldom spherical</td>
</tr>
</tbody>
</table>

*Tab. 1: The results of the observations of pigment granules in wool fibres, when samples were observed by transmitted light microscopy (TLM) and transmission electron microscopy (TEM).*
4. DISCUSSION

In whole mounts of new brown fibres, the pigment granules were clearly visible in fibres that were not heavily pigmented, and at magnifications such as 200x or 400x they could be observed as dark lines in a transparent non-coloured keratin matrix. It was at 1,000x magnification, that the pigment granules were first observed as pearls on a string (Fig. 1). The brown lines that were observed at 200x and 400x magnification in three of the four archaeological samples could suggest some natural pigmentation, but when observed at 1,000x magnification only two samples (Type 4 and Tx 2.3) had fibres that showed the characteristic pearl-like structure and this was not observed in every fibre. The third sample (Tx 1.6p) had only dark diffuse lines at 1,000x magnification. This could be because of the state of preservation of the granules. M. Ryder (1990) records an apparent breakdown of the granules that over time produces a diffuse effect. When this happens, he suggests looking at coarser fibres, because they are usually more densely pigmented than the finer ones. This was not possible here, because the samples tested were all very fine fibres from the Danish Iron Age. Whole mount analyses of archaeological fibres, therefore, do not always give a clear picture of the pigment content, their distribution, and their state of preservation.

In GMA cross-sections of new brown wool, the granules were visible and it was possible to see the distribution within the fibres, even when heavily pigmented. Staining with ABB improved the visibility of the pigment granules in archaeological fibres, but when dealing with non-degraded fibres, it was not necessary to stain them. ABB colours protein blue, while the melanin keeps its brown dark colour.

When focusing up and down in the microscope, the state of preservation of the melanin granules became unclear. Granules that at one position were brown turned out to look like empty holes at a slightly changed focus position (see Figs 2, 3). Sometimes, the apparent empty holes were only slightly brown when the focus was changed, especially in sample Tx 2.3. This made it more uncertain whether the fibres actually contained melanin granules or if the slightly brown areas were caused by granules that had partly or totally degraded. Two of the archaeological samples (Type 7 and Tx 1.6p) showed no signs of natural pigmentation in the fibres. It was thus not possible to detect any pigment granules in the GMA cross-section of the the sample Tx 1.6p that only showed unclear brown lines when observed at 1,000x magnification in the whole mount.

Epoxy is a harder material compared to GMA and it was possible to cut 50 nm sections for these analyses. It was used for embedding fibres to be analysed with transmission electron microscopy. As part of the transmission electron microscopy protocol, cross-sections (1 μm thick) were mounted on micro slide sections and observed under the microscope. Two sections were applied to each microscope slide, one unstained and one stained with TBO, before they were mounted with DePeX. This proved to be very helpful because it was difficult to find the non-
stained section in the microscope. DePeX has a refractive index that is very close to that of wool keratin (RI = 1.55), which provides very little contrast when observing it. On the other hand, it makes the melanin granules more visible. In principle, the melanin granules should be very visible under these conditions. It was also possible to locate brown opaque spots in an otherwise transparent wool fibre. However, it gave the best results when the TBO stained cross-sections were observed first. This gave information on the ortho and para cortex, and often it was also possible to see the natural pigment granules (Fig. 4). These granules are mainly found in the NR of the paracortex cells, but because TBO stains the NR more intensely than the rest of the paracortex cells, the granules can sometimes be more difficult to see here. Observation of the non-stained sections can help clarify whether there are granules in the NR or not. A disadvantage of the 1 µm thin sections is that they can be difficult to transfer from the knife to the microscope slide without folding. This is observable in two of the three fibres in Fig. 4. These thin sections did not greatly improve the information on the pigment content compared to the GMA cross-sections. When the granules were intact, it was possible to see them in both types of cross-sections. When the granules were partly degraded, it was difficult to get a clear picture of the pigment content (Fig. 5). When cross-sections of the fibres were analysed in transmission electron microscopy, it was first possible to gain exact information on the amount and preservation status of the individual granules (Fig. 6). With
transmission electron microscopy, it was also possible to suggest which kind of melanin granules were found in the fibres. In longitudinal sections, it could often be detected whether the granules were the eumelanin containing rice-grain-shaped melanosomes or if they were the spherical ones containing pheomelanin, because the granules always lie parallel to the length of the fibre.

It was interesting to observe that the granules were one of the first structures to degrade in the analysed fibres from Lønne Hede. The textiles come from graves that were buried in a waterlogged, slightly acid environment, which apparently had a very damaging effect on the melanin granules. Other environments, where the microbial activity is dominant, have the opposite effect on wool and hair. Wilson et al. (2007a) have, for example, shown that, when hair was buried in soil with microbial activity (in loam at a pasture site), the natural pigment granules were the last components to degrade.

Fig. 5: TBO stained epoxy cross-section of red-brown fibres from textile sample Tx 2.3. Pigment granules are observed, but it is difficult to get a clear picture of the total pigmentation of the fibres. © A. B. Scharff.

Fig. 6: Transmission electron microscopy observation of the same two fibres' cross-sections as seen in Fig. 5. The fibres are full of holes partly filled with pigment granules. Only a few intact granules can be seen. © L. Bolt Jørgensen, A. B. Scharff.
5. CONCLUSION

It can be possible to detect natural pigmentation in whole mounts of fibres, when the pigmentation is not too intense and when the pigment granules are still found intact in the fibres. To be certain, the fibres must be observed at 1,000x, at which magnification the pigment granules can be seen as pearls on a string. The best way to observe the distribution of the granules within the fibres is in cross-sections. GMA cross-sections stained with ABB give very good results, but again it can be a problem to observe the granules when they are degraded. Transmission electron microscopy is the only method that gives exact information on the amount, distribution and condition of the granules, and it could furthermore be possible to suggest which kind of melanin is present in the fibres. However, this method is costly and time consuming, and therefore something to consider when whole mounts and GMA cross-sections give inconclusive results, or when exact knowledge on the kind and the condition of the melanin granules is required.

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