Investigations of the physical and chemical structure of archaeological fibres

A thesis submitted to The University of Manchester for the degree of Doctor of Philosophy (Ph. D.) in the Faculty of Engineering and Physical Sciences

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Abstract

Alaa Jabur

Investigations of the physical and chemical structure of archaeological fibres

Archaeological fibres can be defined as natural fibres that belong to different time periods, which found in cemeteries or excavation sites. The preservation conditions cause degradation, mineralisation and sometimes a complete deterioration of these fibres, because the chemical and physical structure of the fibres changed over time in response to the specific burial environments.

The ancient fibres from different archaeological sites were analysed by several non-destructive analytical techniques such as optical Microscopy, Environmental Scanning Electron Microscopy, Attenuated Total Reflectance FTIR and Wide Angle X-Ray Scattering Analysis as well as destructive analytical techniques such as Scanning Electron Microscopy, Transmission Fourier Transform Infrared Spectroscopy, Energy Dispersive X-Ray Spectroscopy and Differential Scanning Calorimetry.

These analytical techniques showed that keratin fibres from a central European climate have a larger damage at the fibre surface compared with frozen conditions. While bog conditions were the best in preserving the surface. FTIR analysis provides information about cystine oxidation changes in keratin fibres. For all ancient keratin fibres showed a silica peak at 1030 cm\(^{-1}\) which affected the symmetric cysteic acid peak at 1040 cm\(^{-1}\). For this reason the asymmetrical cysteic acid peak 1175 cm\(^{-1}\) was used for identification of cystine oxidation changes. Transmission FTIR gives a better view of the overall chemical changes in both cortex and cuticle compared to ATR analysis. All ancient wools and highly medullated Iceman deer hairs showed the highest concentration of cysteic acid compared with human hair and goat hair. Also it was shown that warm conditions have bigger effect on both the degree of oxidation of cystine and the ions uptake from the environment.

The modulated DSC analysis gives a better view on the degree of degradation of hair proteins compared to WAXS analysis. To get a reliable result it is important to correct the DSC data according to the protein content of the fibre.
Declaration

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# Abbreviations

<table>
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<tr>
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<th>Description</th>
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<tbody>
<tr>
<td>ATR</td>
<td>Attenuated Reflectance</td>
</tr>
<tr>
<td>CMC</td>
<td>Cell Membrane Complex</td>
</tr>
<tr>
<td>Cer</td>
<td>Ceramides</td>
</tr>
<tr>
<td>Ch</td>
<td>Cholesterol</td>
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<tr>
<td>ChE</td>
<td>Cholesterol esters</td>
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<tr>
<td>ChS</td>
<td>Cholesterol sulfate</td>
</tr>
<tr>
<td>DSC</td>
<td>Differential Scanning Calorimetry</td>
</tr>
<tr>
<td>EDX</td>
<td>Energy Dispersive x-ray Analysis</td>
</tr>
<tr>
<td>FTIR</td>
<td>Fourier Transform Infrared Spectroscopy</td>
</tr>
<tr>
<td>FFA</td>
<td>Free fatty acids</td>
</tr>
<tr>
<td>$\Delta H_D$</td>
<td>Denaturation enthalpy</td>
</tr>
<tr>
<td>IF</td>
<td>Intermediate filament</td>
</tr>
<tr>
<td>IFAP</td>
<td>Intermediate filament-associated Protein</td>
</tr>
<tr>
<td>IR</td>
<td>Infrared Spectroscopy</td>
</tr>
<tr>
<td>MDSC</td>
<td>Modulated DSC</td>
</tr>
<tr>
<td>18-MEA</td>
<td>18-methyleicosanoic acid</td>
</tr>
<tr>
<td>MIR</td>
<td>Mid-infrared</td>
</tr>
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<td>OM</td>
<td>Optical microscope</td>
</tr>
<tr>
<td>SAXS</td>
<td>Small Angle X-ray Scattering Analysis</td>
</tr>
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<td>SEM</td>
<td>Scanning Electron Microscopy</td>
</tr>
<tr>
<td>$T_D$</td>
<td>Denaturation temperature</td>
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1. Introduction

Natural fibres can be distinguished by their origins, coming from plants or animals [1]. For that natural fibres can be divided into animal fibres which are protein fibres such as wool, hair, fur and silk, and plant fibres, which are cellulose fibres such as flax, cotton, jute, ramie and hemp. In this work, the focus will be on human hair, animal hair (deer hair, wool and goat hair) and plant hair (flax and cotton) depending on the type of the archaeological fibres in this study.

1.1 Animal fibres

Animal fibres are protein fibres, including all keratin fibres such as wool, hair, fur and silk.

1.1.1 Keratin fibres

Keratin fibres are composed primarily of proteins (88%) called keratin. Keratins are a family of fibrous structural proteins. They are tough and insoluble, and form the hard but un-mineralized structures found in vertebrates except fish [2]. The strong nature of keratin fibres is characterized mainly by their high cystine content. Cystine is an amino acid containing two sulphur atoms linked by a disulfide bond. These disulfide bonds forms cross-links providing the toughness and resilience in keratin fibres [3, 4]. Keratins can be classified as having α-helical structure and β-sheet structure, which can be distinguished by their x-ray diffraction patterns. The α-keratin can be found in wool, hair and hoof, while β-keratin can be found in avian keratin and in the stretched form of mammalian keratins [2].

1.1.1.1 General structure and growth of keratin fibres

Keratin fibres are formed by the follicle. Hair follicles are large cavities or sacs, which extend from the surface of the skin through the stratum corneum and the epidermis into the dermis [5]. The process of keratinisation cleavage of the thiol group into disulfide bonds causes the death of the cells and the formation of the mature hair shaft [6].
The follicle creates cells containing self-organising and environmentally-resistant protein materials. These proteins are deposited on the inner surface of the cells during terminal differentiation and are cross-linked by iso-dipeptide bonds, forming the cell envelope [7].

The terminal part of the hair follicle located within the skin is called the hair bulb, which is formed by actively growing cells. At the base of the hair bulb is the dermal papilla, which is located near the centre of the bulb. The dermal papilla has an important role in controlling the growth cycle of hairs and the development of the follicle itself as shown in Figure 1.1.

Basal layers produce hair cells and melanocytes produce hair pigment. At the base of the bulb, blood vessels carry nourishment to the growing hair fibre and the sebaceous gland secretes the natural oils lubricating the hairs, namely sebum [7].

Figure 1.1: Hair structure [7].
The new cells are formed by mitosis and migrate from the base of the bulb upward in the follicle. During their upward movement they differentiate and elongate. Then disulfide bonds are formed through a mild oxidative process, and ultimately the permanent hair fibre is formed.

The colour of the keratin fibres (in human hair) is due to pigment contained in ellipsoidal particles that are synthesised in the hair follicle by specialised cells, called melanocytes. The melanin granules contain small amounts of protein [7].

1.1.1.2 The morphological components of keratin fibres

All keratin fibres are comparable in their morphological structure. A keratin fibre can be divided into three main morphological components: cuticle, cortex and medulla as seen in Figure 1.2.

1.1.1.2.1 Cuticle

The cuticle is the outer layer of the fibre, and consists of flat overlapping cells (scales) that surround the fibre core. These cells are arranged in a partly overlapping structure mainly with the scale edges pointed toward the tip end of the hair fibre [8]. Cuticle cells are elongated, acuminated (gradually tapering to a point) and have ovate or flattened shape [7, 2].

The frequency and the shape of the cuticle scales and the number of scale layers can be used to identify the species, e.g. in forensic studies references. In fine wool fibres the cuticle is only one or two scales thick while in human hair and coarse fibres, it is generally 5 to 10 scales thick [8]. Usually the inner cutical cells are thinner than the outer cutical cells [9], the scales have a protective role for the inner parts of keratin fibres [10].

The cuticle has been shown to contain a high percentage of cystine. Analysis of the cuticle of wool fibre shows only negligible birefringence [11] indicating that it is largely amorphous proteins.
Each cuticle cell contains a thin outer membrane, the epicuticle. Beneath this, three further layers can be identified: the A layer, a resistant layer with a high cystine content; the exocuticle, sometimes called B layer, also rich in cystine and finally, the endocuticle, which is low in cystine as seen in Figure 1.3. Inter cellular cement, the cell membrane complex (CMC) is also present, and contains several thin layers; β-layers, which contains lipids.


1.1.1.2 Cortex

The cortex is the main part of a keratin fibre. It determines the physical and mechanical properties such as strength, swelling and structural integrity [10]. The cortex consists of spindle-shaped cells. A cortex cell contains macrofibrils. Between the macrofibrils are cytoplasmic and nuclear remnants. There are two main types of cortical cell, which can be distinguished according to the arrangement of the macrofibrils within the cell: ortho-cortex (microfibrils are closely packed and matrix has low sulphur content) and para-cortex (macrofibrils are separated by small amount of intermacrofibrillar material and matrix has high sulfur content) [5, 14].

1.1.1.2.1 Macrofibrils

Macrofibrils are the main structure in the cortical cells. They consists of intermediate filaments - originally called microfibrils- which are low in cystine [15] and are embedded in a less organised protein structure made up of Intermediate Filament Associated Proteins (IFAPs) – originally called matrix, which is rich in cystine [16, 17].

1.1.1.2.1.1 Intermediate filaments (IFs)

In α-keratin the peptide chains are twisted like a right-handed screw (α-helix). Two helices combine to form a dimer (helical coil) [18]. These dimers are the actual physical structural elements of the intermediate filaments (microfibrils) as shown in Figure 1.4 [5]. These dimers assemble into protofilaments, and two of them form a protofibril. Four protofibrils are packed to form the intermediate filament (microfibril), which associate to form macrofibrils [16]. Stretching keratin fibres under certain conditions, e.g. in water leads to the unfolding of the α-helix to produce β-sheets [14, 19]. Feughelman et al [20] found that parallel β-sheets are derived from parallel α-helices, while anti-parallel β-sheets are derived from anti-parallel α-helices.
1.1.1.2.1.2 Intermediate filaments associated proteins (IFAPs)

Matrix proteins contain high concentrations of disulfide bonds. It is classified as the amorphous region in keratin fibres [7]. Also proteins high in Glycine and Tyrosine are part of the IFAPs.

1.1.1.2.2 Cell membrane complex (CMC)

The cell membranes can be found between all cells and form a continuous phase in keratin fibres [22]. Cell membranes of cuticle and cortical cells are glued together with adhesive protein materials to form the cell membrane complex as shown in Figure 1.3. The adhesive materials can be removed, e.g. by formic acid [23].

The cell membrane complex contains the δ-layer that joins the lower β-layer of a cuticle cell with the β-layer of the underlying cuticle or cortex cell. CMC is rich in polar amino acids and low in cystine [7, 24]. Also, CMC contains an important lipid called 18-methyleicosanoic acid (18-MEA), which also appears on the outer surface of each cuticle (upper layer) and is connected to epicuticle protein structures through thioester bonds [7].

Figure 1.4: Higher order α-keratin structures [21].
1.1.1.2.3 Medulla

The medulla can be found in the centre along the axis of keratin fibres [25]. It is composed of large, loosely connected keratinized cells [7]. Medulla cells have large intra and intercellular air spaces [4]. These cells have a spherical shape, and are surrounded by a cell membrane complex [11]. It has a porous structure [26] that affects both colour and shine of hair by reflection of light. The medulla has a high lipid and low cystine content. It is, however, rich in citrulline containing protein [27]. Some fine animal fibres do not contain a medulla [7], while human and coarse animal fibres have unique medulla pattern, which can be used as a distinguishing characteristic in fibre identification as seen in Figure 1.5 [28].

![Figure 1.5: Human head hair medulla pattern](image)

Figure 1.5: Human head hair medulla pattern [28].
1.1.1.3 Chemical composition of keratin fibres

Keratin fibres such as animal and human hair are high quality natural protein fibres [29]. Generally keratin fibres consist of 95% proteins and other components such as lipids, pigments, nucleic acids and trace elements [7].

Proteins are made of amino acids which are linked by peptide bonds to form poly-peptide chains [21, 30]. Five groups of amino acids can be found in keratin fibres: acidic amino acids, basic amino acids, amino acids with hydroxyl groups, sulphur-containing amino acids, and amino acids with no reactive group. Because keratin fibres, such as wool, contain cationic groups (due to the presence of protonated side chains in arginine, lysine and histidine) and anionic side groups (due to the dissociated side chains of aspartic and glutamic acid and carboxyl end groups) keratin fibre proteins are amphoteric [5].

Fibre proteins are cross-linked by different types of bonds as illustrated in Figure 1.6. The most important crosslink is the covalent disulfide bond, which is formed by two cysteine residues of the polypeptide chains to form cystine, which is mainly located in cortex and exocuticle proteins. It stabilises keratin fibres and gives toughness and abrasion resistance [31].

Other important cross-links in keratin fibres proteins are the iso-dipeptide bonds. These covalent bonds are formed between lysine and glutamyl or asparagyl residues. These bonds are mainly located in the endocuticle and medulla of the fibre.

The non-covalent bonds are hydrogen bonds, which can easily be broken down and re-formed, and salt bonds, which are formed by electron transfer from the side chain of a basic amino group to the side chain of an acidic amino acid [31]. Also there are other weaker interactions such as coulombic interactions between side chain groups of amino acids. Finally, hydrophobic bonds (only in the presence of water) assist single α-helices to form double α-helical ropes, which ultimately form intermediate filaments [22].

Keratin fibres also contain water, which plays an important role in the physical and mechanical properties. Water absorption increases with increasing humidity very rapidly. 75% of the maximum possible amount of water is absorbed within 4 minutes. Water molecules enter keratin fibres via diffusion and bind to hydrophilic sites in the fibre [7].
Figure 1.6: Structural formulae of five important interactions between amino acid side-chains in keratins [5].

Lipids are also found in keratin fibres, including cholesterol esters (ChE), free fatty acids (FFA), cholesterol (Ch), ceramides (Cer) and cholesterol sulfate (ChS) [7]. Internal lipids are located in the cell membrane complex and contribute to the formation of a stable and strong CMC [32]. External lipids are found on the surface of the keratin fibres [2].

Hair pigments, which are responsible for hair colour, are mainly concentrated in the cortex as melanin, which is produced by specialised cells called melanocytes. There are two types of basic melanin pigments: pheomelanin (which is responsible for the lighter colours such as red and fair) and eumelanin (which produces the dark shades such as brown and black hair). The ratio between the two types of pigments determines the hair colour [7].
1.2 Plant fibres

Plant fibres have been used since historic times as raw materials for different purposes such as textiles, papers and feedstock [33]. There are two main types of cellulose fibres: bast fibres e.g. flax, hemp, jute and ramie fibres and seed fibres e.g. cotton fibres. Other groups which are less important for garments are leaf fibres e.g. sisal and fruit fibres, e.g. coconut. Depending on the original plant the morphological structures of the fibres are different. In this work, the focus will be on flax and cotton fibres depending on the type of the archaeological fibres in this study.

1.2.1 Morphology of cellulose fibres

Flax and cotton are the main examples of cellulose fibres which have been identified among archaeological finds.

1.2.1.1 Flax fibres

Flax is a bast fibre which has a high commercial importance today [34]. The primary outer wall of the single flax fibre contains stiffened pectin due to addition of lignin. The secondary walls contain cellulose molecules in primary fibrils, which are assembled into microfibrils, connected to each other by hemicellulose to produce a primary network [35]. The spirally wound cellulose fibrils are embedded in an amorphous matrix of hemicellulose and lignin as shown in Figure 1.7 [36]. Usually 20 to 25 flax fibre bundles form a ring, which is embedded between the wood and the bark.

![Figure 1.7: Flax fibres. Cell wall in flax cell [36].](image-url)
1.2.1.2 Cotton fibres

Cotton is a seed fibre and the most used textile fibre in the world. The cotton fibre is a unicellular outgrowth of an epidermal cell of the cotton seed. It has a four-layered cell wall as shown in Figure 1.8: the cuticle (consisting of wax and pectin materials), the primary wall (composed of cellulosic crystalline fibrils), the secondary wall (including parallel fibrils with spiral winding), which represents the majority of cellulose within the fibre, and the lumen (composed of the remains of the cell contents). The fibre has a long cylindrical shape [37].

![Cotton fibre diagram](image)

Figure 1.8: Cotton fibre. Morphological structure [38].

1.2.2 Chemical composition of cellulose fibres

Plant fibres vary in their chemical composition depending on the type of the fibre, plant species and age of the plant. The main compounds which can be found in plant fibres are: cellulose, hemicellulose, pectin and lignin as explained in Figure 1.9.

Cellulose is hydrophilic, insoluble in water and most organic solvents and is biodegradable. It can be broken down chemically into glucose units by treating it with concentrated acids at high temperature.
Cellulose consisting of unbranched β (1 - 4) D-glucopyranosyl units, which are arranged in a straight chain polymer. These units are linked by numerous intra-molecular and inter-molecular hydrogen bonds. Because of these bonds, cellulose does not melt before it undergoes thermal degradation [39]. Cellulose has a crystalline and an amorphous phase [40, 41]. The amorphous region has a high absorbance ability for dyes and resins [42].

Hemicellulose is made of sugar monomers. Its branched polymer is made up of short chain units of 500 to 3,000 sugar units. Hemicellulose has little strength due to its random and amorphous structure [1].

Pectin is a complex of polysaccharides that contain four linked α-D-galactosyluronic acid residues [33]. The amount of pectin, the structure and the chemical composition differ between plants and within a plant over time [1].

Lignin is a hydrophobic cross-linked, racemic macromolecule. It can be found in the spaces of the cell wall between cellulose, hemicellulose, and pectin components [1].

Figure 1.9: Chemical structure of (a) cellulose (b) hemicellulose (c) pectin [43].
1.3 Fibres from archaeological sites

Archaeological textiles include all types of individual textile fragments, individual fibres or collections resulting from archaeological excavation [44]. In the case of archaeological fibres the plant fibres (cotton, flax, jute, ramie and hemp) as well as mummy hair and animal materials (hair, fur, silk, and wool) can be expected.

Archaeological fibres vary in their state depending on their preservation conditions. Acidic-alkaline micro-environments, extremes of temperature, extreme aridity, nitrogen-rich bogs and other environmental conditions play an important role in the nature of the preservation process [45]. For example, protein fibres are preserved better in slightly acidic conditions, while cellulose fibres are preserve better in alkaline conditions [45, 44].

Deterioration of archaeological fibres can take several forms: for example, biodegradation, decay, burning, milling and discoloration. Deterioration occurs due to thermal, light, chemical, mechanical or biological influences, all of which can cause weakness of the physical structure and change of the chemical components [44, 46]. Most archaeological fibres appear in different shades of brown due to their preservation conditions [44].

Due to poor preservation conditions, damaged archaeological fibres can cause difficulties in fibre identification by using typical techniques such as optical microscopy and scanning electron microscopy [47]. For this reason several other types of analysis have been carried out, such as lipid, amino acid and DNA analysis, to reveal more information about the archaeological fibres [45].

1.4 Types of preservation site

The decomposition process starts slowly soon after death. But sometimes preservation can stop decomposition due to natural circumstances [48] such as frost and thaw, damp, rain, dry, hot weather and the presence of salt e.g. calcium carbonate [49], leaving mummies, which retain their hair, skin and bones.

However, once mummies and artefacts are exposed to new environmental conditions upon excavation, they can rapidly decay if preservation measures are not employed [48]. There are two types of mummification: natural mummification and artificial mummification.
Natural mummification occurs through the effects of the environment without human assistance. Hot, dry and cold conditions allow bodies to dry out naturally forming well preserved mummies. Artificial mummification occurs when natural processes of decay are blocked artificially by some procedures involving human assistance. Different methods were already used thousands of years ago, such as treatments with resins, and sometimes bandaging or wrapping of some kind. Egyptian mummies were prepared by artificial mummification as part of a burial tradition [50]. This study is focusing on natural mummification.

1.4.1 Cold conditions

Different mummified bodies of humans and animals were found in the permafrost regions, which are located in Alaska, Antarctica, Eskimo, Canada and Greenland. After different types of scientific examination, these bodies showed remarkable histological preservation conditions due to the weather of these regions [51], taking into account, that conditions in glacial periods were much colder than those of today [49].

In freezing conditions, water vapour from ice in bodies is removed by sublimation into cold air currents resulting in preservation by desiccation. Although these conditions provide excellent preservation, the frozen ground prevented bodies from burial leaving them for disposal by animals, also cycles of freezing and thawing cause exposure of the remains to the variation of climate which can affect the preservation process.

The human mummified bodies from frozen regions were subjected to different types of analysis. These analyses showed that these bodies suffered from different diseases such as osteoporosis, trichinosis and other heart diseases. The main reasons for death were vital injuries and in some cases lead poisoning [51].

The most famous example for cold conditions is the Tyrolean Iceman or 'Otzi' (dated 5350-5100 BC). This mummy, its clothes and equipment were discovered in the Italian Alps at 3270 m above sea level [52, 53]. Because of its outstanding state of preservation, the Iceman became one of the most investigated mummies in the world.
1.4.2 Bog conditions (natural chemical preservation)

Peat bogs can be found mainly in north-western Europe. Bog conditions are anaerobic and wet (due to the presence of peat which is waterlogged, the oxygen level is reduced so microorganisms that cause decay cannot survive) and the acidic environment prevents the development of rot-causing microorganisms [54]. This preserves important structures of the mummies, but it leads to dissolution of the bones.

Also the production of spagnan by sphagnum moss in the bog environment (in some cases another chemical found in bog conditions called Alum). This tanned the dead bodies and aided preservation by reacting with the digestive enzymes that are secreted by bacteria, immobilising them on the surface of the bog.

There is a great range of variation in the survival of tissues, hair, nails and other equipment due to anaerobic conditions that are provided by a bog environment. Bodies which have been buried in bogs mainly for two reasons: either they were subjected to criminal punishment or accidents, or to individuals which were rejected by their society.

Bog mummies were subjected to different analyses after all the vegetable fibres had seen removed [55]. They showed the presence of interactions between the bodies and their burial environment, by finding noticeable percentage differences for some elements [51].

The most important examples for bog conditions are: the Lindow man, found at Lindow Moss, Cheshire, England. Scientist found pieces of 2-3 bodies (due to industrial peat cutting machinery). Lindow I was represented by a head only and probably belonged to a female, Lindow II man was fairly complete and Lindow III was represented by parts of the thorax, abdominal area and both arms and legs with no head, but possibly Lindow II and Lindow III belong to the same person. The conditions in the peat bog kept the man’s skin, hair and many of his internal organs well preserved [51, 56].

The Tollund man is a natural mummy of a man, who lived during the 4th century BC. He was found on the Jutland Peninsula in Denmark. His mummy showed variation in the degree of preservation. His body was shrunk, and the left side showed signs of decomposition, while the skin of the right side was well-preserved. The sole of his foot contained superficial incised wounds. He had short red hair due to the influence of the bog water [57, 51].
1.4.3 Hot conditions

Hot, arid, and semiarid environments can provide excellent conditions for preservation of different mummified bodies for humans and animals [48]. Mummies derived from hot and dry conditions can be found mainly in South Africa, Egypt and South America.

Human and animal bodies were prepared for preservation by the removal of inside abdominal parts, preserving them separately. Then the body cavity was filled with different kinds of plant seeds and then sewn up [55] using cloth, mainly linen, and other materials to dry the body, even using chemicals consisting of sodium bicarbonate or sodium carbonate or sodium chloride to prevent decomposition through bacteria [58]. Then dry sand with high temperature will cause rapid desiccation reducing the water content below a point where microorganisms would thrive [51]. Many mummies derived from hot conditions were found, Ginger man is the oldest mummy from hot and dry areas.

Ginger man was discovered at Gebelein, one hundred miles south of Luxor in Egypt, dating to 4400-3100 BC, the time before the pharaohs. He was found buried in a shallow grave cut into the desert sand. The graves were often lined with reed mats, making them like a bed. The body was covered with linen or skins and more mats. Hot, dry sand leads to rapid desiccation resulting in remarkable body, nails and red colour hair preservation [51, 59].

1.4.4 Soily conditions

Soil is made up of different types and sizes of ground rock particles, sand and mineral particles. Each component, and its size, plays an important role in determination of the aeration and drainage characteristics [49]. Also the presence of latrine pits and rubbish disposal, element content and soil pH have a huge impact on the preservation process.

A latrine pit site provides anaerobic conditions, which stop microorganisms that cause decay and provide optimal conditions for the preservation of organic materials [60].

Calcium carbonate content tends to make the soil have alkaline pH. This slows the normal rate of decay caused by soil acidity, leaving a relatively high proportion of organic material (food remnants, organic tools, clothing, and human remains) available for archaeologists to find [49]. Also the presence of metals can support fibre preservation in a process called pseudomorphing. It does occur when textiles were in contact with a metal object. As the
metal corrodes quickly and forms a metal salt, this creates a specific microenvironment that is ideal for the preservation of textiles [44, 45].

The ideal preservation pH depends on the type of fibre. Animal fibres preserve better in a slightly acidic environment, while cellulosic fibres preserve better in alkaline conditions [45]. Also organic materials such as textiles can be preserved by clay, mud and plaster [44].

The most important example of soil burial relates to Hadrian’s wall finds in Vindolanda. A large corpus of organic material was well preserved due to the damp oxygen free soil conditions. Huge wool fragments with different weave types have been found with no presence of cellulosic fibres [61].

1.5 Archaeological background of the investigated keratin fibres

The main archaeological fibres that were used in this study belong to the Tyrolean Iceman, Hadrian’s Wall (Vindolanda, United Kingdom) and Schloesser Brewery (Duesseldorf, Germany).

In addition a wool fabric from Bocholt (Germany) dating from 15th century and human hair from a bog body from northern Germany were investigated. For these fibres, no historical background or details about preservation were known.

1.5.1 Tyrolean Iceman

In September 1991 a well preserved human corpse was found in the Italian Alps, at 3270 m above sea level as seen in Figure 1.10. Together with the mummy (dated 5350-5100 BC), clothes and equipment were discovered [52, 53]. Because of its outstanding state of preservation, the Iceman became one of the most investigated mummies in the world.
The Iceman or 'Otzi' died at the age of 46 years. He had brown eyes and hair, blood type O, was lactose intolerant and suffered from Lyme disease [63]. He was severely wounded by an arrow in his left shoulder, causing bleeding to death [52], and had a serious head wound [64]. Scientists believe that the local climate during the Cooper age in this area provided mild winters, including a humid habitat with mineral rich and free draining soil. After the Iceman’s death his body was covered with snow, desiccated within the snow and froze solid [65].

His belongings as shown in Figure 1.11 were found in good condition with little degradation, due to the glacier preservation conditions [52]. The lifestyle of the Neolithic age has been studied by using the Tyrolean Iceman equipment, which includes his clothing, wooden artefacts and macro and micro food fossils from 'Otzi' intestine. His clothing includes a cap, leggings, a jacket or coat, fragments of skin [64, 66], hair, a bark bag and red deer skin shoes with bear skin soles. His shoes were filled with grass as an insulation layer and stuffed with a network of lime Tilia bark strings [66]. Until now the specific animal species of the Iceman's clothing were not well established, because some scientists believed that his clothes were made from the hair of domesticated animals (sheep, cattle, goats) [67], while others showed that it was made from wild game (red deer, chamois and bear).

Numerous analyses have been conducted to study the daily life of the Iceman. Some scientists used his hair to study his diet, which showed that he was vegetarian with a low portion of animal protein by studying the nitrogen and carbon isotopes [68] as well as the abrasion on his teeth [68]. Others proved that the Iceman was omnivorous due to the presence
of plants and animal fibres in his intestine [65, 63]. Also scientists studied the DNA from his hip bone [63] and the complete sequencing of Iceman's mtDNA. They found that the Iceman is not related to present-day people from the Southern Alps where he lived and died [52, 63]. In this study human, deer and goat hair from the Iceman find are investigated.

Figure 1.11: The Iceman’s equipment: (a) the grass cape made of *brachypodium pinnatum* bundles (b) the birch bark vessel (c) the flint dagger with its sheath of lime bast (d) the sharpening tool made of lime wood, (e) left shoe with the strings made of lime bast and grass as insulation material, (f) the backpack made of hazel and larch laths [65].
1.5.2 Finds from Hadrian’s wall (Vindolanda, UK)

The Hadrian's Wall site was a Roman auxiliary fort in Vindolanda, in northern England as depicted in Figure 1.12. The earliest Roman forts were built of wood and turf around 85 AD [69]. There are four phases of occupation in Vindolanda before 122 AD [61]. In 1814 the first real archaeological work was begun, by the Rev. Anthony Hedley. In 1970, the Vindolanda trust, a registered charity, was founded to administer the site and its museum [69].

![Figure 1.12: Roman settlement at Vindolanda](image)

Thousands of artefacts have been discovered at 4 to 6 metre depths in soil such as textiles, leather goods, wooden object (parts of furniture, tent pegs, combs), shoes, bags, jewellery, bronzes, weapons and animal bones [61, 69]. Hundreds of textiles fragments with no original edges were found in anoxic high acidity soil conditions [61, 71]. Due to these preservation conditions no plant fibres were found [61]. The majority of the textile fragments have brown colour and were made from coarse sheep wool, with the presence of other animal fibres such as cattle and human hair [61, 72].

Large numbers of analyses, such as image analysis have been applied to examine the textiles from Vindolana by The Manchester Ancient Textile Unit (MATU). They showed that fine and coarse woollen weaves of many archaeological textiles have been unaffected by deposition. Diamond twills make up half of the weaves, while others are plain (tabby) and half-basket weave. Twills are fuller and potentially warmer than plain weaves [61]. No
analytical attempt was made to study the structural changes in the animal fibres of these textiles. In this study wool and goat hair were used for analysis from this archaeological site.

### 1.5.3 Finds from Schloesser Brewery (Düsseldorf, Germany)

Düsseldorf lies on the east side of the Rhine where the delta of the River Düssel flows into the Rhine. An archaeological site of importance appeared after demolition of the old brewery building in 1991. Archaeological work began in June 1991. Excavation areas were divided into several sections, depending on specific archaeological periods. The findings groups were identified by numbers, and then further specified with letters. There were 16 findings related to latrine pits at area no.9, which dated back to the second half of the 17\textsuperscript{th} century and to the early 18\textsuperscript{th} century as seen in Figure 1.13.

![Figure 1.13: Finds from Schloesser Brewery: (a) the latrine pit no. 9 (b) conglomerate of fabric and thread fragments from the excavation site no. 9 [73].](image)

Due to the humid and anaerobic environment organic materials were well preserved. The contents of the latrine pits were dark brown, peat like and included household waste filled with excrement residues, undigested food residues, municipal waste and sand layers. Many types of fibres, fabric, leather scraps and clothing accessories were found in the latrine pits. Woven and knitted fabrics were found, which occurred mostly in small strips and also in larger fragments. Striking is a thick bale of cloth strips interspersed with animal hair. Most of
these strips belong to wool fabrics and coarse and fine animal hair such as Ross or cattle, while silk and plant fibres could not detected.

The textile fragments show a brown colour with shades of different brightness, which can be attributed to the used raw materials. Between the tissue fragments a few remains of organic materials are visible such as crop residues or wood as well as soil particles [73]. In this study wool textiles were investigated from this archaeological site.

1.6 Objectives

Different analytical techniques give different views of the changes in archaeological fibres due to their preservation conditions. The object of the research is the correlation of non-destructive and destructive analytical techniques with the focus on structural changes in archaeological fibres, derived from different conditions of preservation, to reveal the actual degree of degradation of the fibres.

Analytical techniques include:

- developing a cleaning procedure for different archaeological samples from different excavation sites;
- differentiating between archaeological keratin and cellulose fibres by using microscopical analysis, including the optical microscope, the Environmental Scanning Electron Microscope – ESEM and the Scanning Electron Microscope SEM;
- employing the Attenuated Total Reflectance ATR technique and Transmission Fourier Transform Infrared Spectroscopy (FTIR) for fibre identification and damage characterisation;
- investigating the thermal properties of archaeological fibres to assess structural changes in archaeological keratin fibres when they are heated in water (High Pressure Differential Scanning Calometry – HPDSC);
- employing Wide angle X-Ray scattering (WAXS) in archaeological samples to measure the structural state of the α-helical fraction in intermediate filaments (IFs);
- employing energy dispersive x-ray (EDX) techniques for elemental analysis;
- optimisation of the chosen analytical techniques for small sample sizes.
References


Chapter 2 - Cleaning archaeological fibres

2.1 Introduction

Many archaeological fibres have survived over thousands of years under special environmental conditions such as freezing, acidic conditions and extreme dry or moist conditions [1, 2]. The preservation conditions determine the preparation and cleaning methods before the archaeological fibres can be investigated. Many problems can affect analytical studies of archaeological fibres, such as fibre degradation and carbonisation [2]. By ageing in an extreme archaeological environment, many fibres have nearly been destroyed and their colours, shades of brown, are due to immersion in different types of media [3]. Moreover, many types of dirt such as dust, soil, insects, etc. have accumulated over the years, and must be removed without damaging the fibres. Against this background the development of appropriate cleaning procedures is a big challenge [4, 5].

2.1.1 Soiling of archaeological fibres

Archaeological fibres are contaminated with different types of soil and other materials, such as sand, dust, fibres fragments and unknown foreign trash. There are many categories of soil, which can be found in archaeological fibres. These categories are listed below.

- Water soluble soil, which includes simple inorganic salts and hydroxides of alkaline metals, hydroxides of other metals and oxygen containing organic materials, such as alcohols, organic acids and sugars. This type of soil can be removed by washing with water.
- Soil that responds to alkaline cleaning agents. This includes soil that contains substances such as oils, body contact smears, fatty foods and products of decayed vegetables and animal matter. This type of soil requires an alkaline medium that can be added to water for cleaning.
- Soil that responds to acid. This type of soil contains metal corrosion products, such as oxides of metals. It shows good cleaning response to acid treatment, depending however, on the acid.
- Soils that are affected by cleaning agents. This type of soil has soap or crudes and can be dissolved rapidly by polyphosphate cleaning agents.
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- Soil that requires enzyme cleaning. This type of soil contains complex biological substances, such as decayed proteins, blood, gelatine, starches and can be cleaned by hydrolysing these substances by enzymes. Cleaning with this method requires neutral pH and temperatures below 45°C to allow the enzymes to work effectively.

- Soil that requires organic solvents. This type contains waxes, oils, resins, rubbers and modern polymers and can be cleaned by non-aqueous solvents [6].

2.1.2 Cleaning techniques

Different cleaning techniques were applied to archaeological fibres. These have been performed using aqueous or non-aqueous treatments, depending on the type of fibre, the amount of soil and the level of damage of these fibres [7].

2.1.2.1 Surface cleaning

Surface cleaning is considering as the first step of cleaning natural fibres by removing all the dust, dirt and grit, which have accumulated on the fibre surface. Removing all of this dirt can be achieved by using a brush, a blower, self-adhesive tape, rubber or vacuum cleaner depending on the type of dirt, type of fibre and the damage of the fibres [8].

2.1.2.2 Wet and dry cleaning procedures

Many studies consider wet cleaning as the most effective way to clean fibres [8], while other studies showed that cleaning with an aqueous medium can change the surface of the fibres as well as their crystalline properties, compared to non-aqueous cleaning [9, 7]. Also, water can wash out some dyes, which have not been set properly [8].

Effective wet cleaning can be achieved by soaking the object in de-ionised or distilled water at the start to prevent the fixation of dirt, then using a flow of water on the objects on a flat surface to prevent weakened fibres being damaged.

Warm water can remove dirt quickly but also can cause swelling of the fibres. For that reason cold water is advisable. At the end, rinsing the object with distilled water is an important step to remove active particles that are left, which can cause more deterioration [8].
Dry cleaning or solvent cleaning can be applied to archaeological textiles. Due to the fact that archaeological fibres have a fragile structure and can be destroyed by sudden dehydration or humidification, specific care is needed during the cleaning process [8]. Dry cleaning has no swelling effect on protein and cellulosic fibres, due to the low moisture content [9]. Usually different types of detergent can be used with small amounts of water in some cases, to improve the cleaning [8].

To remove the soil and dirt from textiles, surface-active agents must be added to reduce the surface tension, so the textile and soil have a close contact with the liquid in both wet and dry cleaning. Tetrachloroethylene can be used in dry cleaning to reduce surface tension [7].

2.1.3 Cleaning archaeological keratin fibres

Keratin fibres usually, withstand slight acidic conditions, but strong acids can destroy peptide groups in the chains. Keratin fibres are sensitive to alkaline conditions. Also they can swell and be plasticised in water [8, 9].

In a study of Columbian archaeological textiles by Bear et al, distilled water or CCl₄ was used to clean keratin samples [10]. Other authors cleaned the hair from an Egyptian mummy, which had been subject to different preservation conditions, with a hydrophilic phase (distilled water) followed by a hydrophobic phase (acetone) [11]. Hansen and Derelian [4] used non-ionic surfactants for cleaning silk tapestries. Besides chemical cleaning procedures, laser technology has also been used to clean archaeological fibres [5, 12].

2.1.4 Objectives

The objectives for this part of the work were to determine, develop and optimise effective cleaning procedures for various archaeological fibres with different preservation conditions and degrees of contamination, and mainly for small sample sizes.

2.2 Experimental work

Various types of archaeological samples derived from different preservation conditions were used in this study. Table 2.1 shows the description of these samples.
Table 2.1: Archaeological samples description.

<table>
<thead>
<tr>
<th>Archaeological find</th>
<th>Archaeological sample</th>
<th>Period of Sample</th>
<th>Preservation conditions</th>
<th>Origin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Iceman</td>
<td></td>
<td>5350 -5100 BC</td>
<td>Under the glacier, about $\approx 0 , ^\circ C$</td>
<td>The Alps, Italy/Austria</td>
</tr>
<tr>
<td>Human hair</td>
<td>91/92B, 91/130, 91/142</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Animal hair, deer</td>
<td>GO II-III, GO V-GO</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>hair</td>
<td>IX, GO XI, GO XIII,</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>91/96, 91/137</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Animal hair, goat</td>
<td>91/110</td>
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</tr>
<tr>
<td>hair</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Vindolanda</td>
<td></td>
<td></td>
<td>In soil, various</td>
<td>Hadrian’s Wall,</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>temperatures</td>
<td>Vindolanda, UK</td>
</tr>
<tr>
<td>Goat hair</td>
<td>A86, goat hair</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Wool</td>
<td>wool, TT/85/38</td>
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<td></td>
<td></td>
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<tr>
<td>Textiles</td>
<td></td>
<td>2$^{nd}$ half 17$^{th}$</td>
<td>Latrine pit</td>
<td>(Schloesser</td>
</tr>
<tr>
<td></td>
<td></td>
<td>century, early 18$^{th}$</td>
<td></td>
<td>Brewery) Old town of</td>
</tr>
<tr>
<td></td>
<td></td>
<td>century</td>
<td></td>
<td>Düesseldorf, Germany</td>
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<tr>
<td>Wool</td>
<td>Wool 2a and Wool 2b</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Textiles</td>
<td>Wool fabric</td>
<td>15$^{th}$ century</td>
<td>n/k</td>
<td>Bocholt, Germany</td>
</tr>
<tr>
<td>Bog body</td>
<td>Bog mummy hair</td>
<td>About 730 AD</td>
<td>Bog conditions</td>
<td>Bernathafeld, (Kreis Aurich), Northern Germany</td>
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</tr>
</tbody>
</table>
2.2.2 Cleaning procedures

2.2.2.1 Cleaning procedure 1

Samples (human, deer and goat hair) were washed with distilled water and left to dry at room temperature (22°C) for 24 hours.

2.2.2.2 Preparing cleaning liquid

10 ml of baby bath (containing Aqua, Coco-Glucoside, Sodium Lauroamphoacetate, Sodium Laureth Sulfate, Polysorbate 20, Cocamidopropyl Betaine, PEG-80 Sorbitan Laurate, PEG-150 Distearate, Citric Acid, Tetrasodium EDTA, Quaternium-15, Parfum, CI 42090) was added to 1 L of distilled water (pH=7.3) to prepare the cleaning liquid (pH= 7.6).

2.2.2.3 Cleaning procedure 2

This procedure was effective for specimen with low soil content. The unclean specimens were immersed in the cleaning liquid for 15 min in a beaker. After this they were placed in a metal sieve ball and immersed in a beaker. The beaker was placed on an infrared hotplate stirrer machine (heat level was 1, which is equal to 26°C and the stirring level was 9 rpm) for 10 min. Later the specimens were washed with distilled water and placed in an ultrasonic bath for different intervals: 2 min, 2 min and 1 min respectively (the specimens were washed with distilled water after every interval). Then the specimens were left to dry for 24 hours at room temperature (20°C).

2.2.2.4 Cleaning procedure 3

This plan was effective for specimens with a high soil content. The unclean specimens were immersed in the cleaning liquid for 15 min in a beaker. After that they were placed in a metal sieve ball and immersed in the beaker. Then the beaker was placed on an infrared hotplate stirrer (heat level was 1, which is equal to 26°C and the stirring level was 9 rpm) for 10 min. Later the specimens were washed with distilled water and placed in an ultrasonic bath for different intervals: 2 min, 2 min, 2 min, 2 min and 1 min respectively (the specimens were washed with distilled water after every interval). Then the specimens were left to dry for 24 hours at room temperature (20°C).
washed with distilled water after every interval). Then the specimens were left to dry for 24 hours at room temperature (20°C).

2.2.3 Determination of the efficiency of the cleaning procedures

2.2.3.1 Optical microscopy

Cleaned fibres were investigated using a LABOVAL 4 optical microscope (CARLZEISS JENA) at different magnifications (x 32, x100 and x 400). Images were obtained by using a Micro Ocular camera (1.3 MP BRESSER).

2.2.3.2 Environmental Scanning Electron Microscopy (ESEM)

Micrographs of the surface of cleaned archaeological samples were obtained using an environmental scanning electron microscope –ESEM-(EDVX / Modal HIT S-3000N 132-2.5 Hitachi).

2.2.3.3 Scanning Electron Microscopy (SEM)

Due to the nonconductive nature of hair, cleaned archaeological fibres were coated with a conductive carbon coating. The samples were vacuum sputter coated with a thin layer of carbon (7nm) and kept in high vacuum ($10^{-2}$ Torr) for 5 minutes by using GATAN PECS. (Oxford, UK). SEM images were obtained with the scanning electron microscopy ZEISSL EVO 60 (Cambridge, UK) at 5 kV accelerating voltage and 250 amp probe current.

2.3 Results and discussion

2.3.1 Archaeological fibres from the Iceman site

The Iceman was preserved in freezing conditions for a long time. For that, different types of ions and dust are expected on the surface of these fibres. ESEM and SEM were used to determine the efficiency of cleaning procedure 1.


2.3.1.1 Human hair

All Iceman human hair 91/92 B, 91/130 and 91/142 was cleaned with distilled water - cleaning procedure 1, which was an effective method, leaving only a few particles on the surface of the fibres as seen in Figure 2.1.

Figure 2.1: SEM. Samples 91/92B (a) before cleaning (b) after cleaning (x1002); sample 91/130 (c) before cleaning (d) after cleaning (x700); sample 91/142 (e) before cleaning (f) after cleaning (x1000).

2.3.1.2 Animal hair

Environmental scanning electron microscopy – ESEM was used for observing the surface of ice man animal hair GO II, GO III, GO V, GO VI, GO VII, GO VIII, GO IX, GO XI, GO
Chapter 2  Cleaning archaeological fibres

XIII, 91/96, 91/110 and 91/137. Images show that washing with distilled water - cleaning procedure 1 was an effective method to clean these samples as seen in Figures 2.2 and 2.6. Only a few particles were left on the surface after cleaning.

Figure 2.2: ESEM. GO II (a) before cleaning (b) after cleaning (x300); GO III (c) before cleaning (d) after cleaning (x200); GO V (e) before cleaning x200 (f) after cleaning (x300).
Figure 2.3: ESEM. GO VI (a) before cleaning (b) after cleaning (x100); GO VII (c) before cleaning (x370); (d) after cleaning (x700); GO VIII (e) before cleaning (f) after cleaning (x100).
Figure 2.4: ESEM. GO IX (a) before cleaning (b) after cleaning (x200); GO XI (c) before cleaning (x200); (d) after cleaning (x300); GO XIII (e) before cleaning (f) after cleaning (x300).
Figure 2.5: ESEM. Samples 91/96. (a) before cleaning (b) after cleaning (x300); 91/137 (c) before cleaning (d) after cleaning (x200).

Figure 2.6: SEM. Sample 91/110. (a) before cleaning (x500); (b) after cleaning (x1000).
2.3.2 Finds from Hadrian’s wall (Vindolanda, UK)

Samples from this find are expected to have many particles from soiling such as dust, ions and insects parts on the surface of their fibres.

2.3.2.1 Sample A86, goat hair

Visual examination of sample A86 goat showed a high percentage of soil on the fibre surface. For this reason cleaning procedure 3 was applied to clean it. ESEM images showed that this cleaning procedure was very effective because it removed a high percentage of the soil as shown in Figure 2.7.

![Figure 2.7: SEM. A86 Goat hair. (a) before cleaning (x3000); (b) after cleaning (x2500).](image)

2.3.2.2 Wool, TT/85/38 fibres

After examining this sample with ESEM, images showed that it had low soil content. For this reason cleaning procedure 2 was applied to clean the Wool TT/85/38 fibres. ESEM images showed that the cleaning procedure was very effective because it removed a high percentage of the soil as seen in Figure 2.8.
Figure 2.8: ESEM. Wool, TT/85/38. (a) before cleaning, (b) after cleaning (x700).

2.3.3 Textiles from Schloesser Brewery (Dusseldorf, Germany)

The samples from the Schloesser Brewery site were found in a latrine pit [13]. Therefore various different contamination particles were expected, such as dust, ions, pieces of ceramics, small plant particles, faeces and insects parts on the surface of the fibres.

2.3.3.1 Wool 2a fibres

Optical microscope images of Wool 2a specimens showed that washing with distilled water was an ineffective method of cleaning this sample as may be seen from Figure 2.9. For this reason multiple cycles of cleaning were applied to clean these fibres, but it was found that they were ineffective, as only a small percentage of soil could be removed as may be seen in Figure 2.10.
Figure 2.9: Optical microscopy. Wool 2a fibres. Cleaning with distilled water (x100).

Figure 2.10: Optical microscopy. Wool 2a multiple cycles of cleaning (x100).

Therefore, cleaning procedure 3 was developed and applied for cleaning. Optical microscopy images showed that this procedure was very effective, because it removed a high percentage of the soil as seen from Figure 2.11.
2.3.3.2 Wool 2b fibres

Optical microscope images of Wool 2b specimens showed that repeated washing with distilled water was not enough to clean this sample. The soil content of sample 2b was very high, for this reason many cleaning cycles were applied to clean the fibres. After all it was found that they were sufficient to clean the sample as may be observed in Figure 2.12.
From previous results for the cleaning procedures and due to the fact that the Wool 2b specimen varied in soil content, procedure 2 was applied to clean the low soil content specimens while procedure 3 was used for high soil content specimens. Optical microscope images showed that both cleaning procedures were very effective for the particular subsamples as may be observed in Figures 2.13 and 2.14.

Figure 2.13: Optical microscopy. Wool 2b fibres. Cleaning procedure 2 (x100).

Figure 2.14: Optical microscopy. Wool 2b fibres. Cleaning procedure 3 (x100).
2.3.4 Wool fabric from 15\textsuperscript{th} century (Germany)

Optical microscopy showed that the wool fabric contained a high percentage of soil as may be seen in Figure 2.15. For that cleaning procedure 3 was applied. Optical microscope images failed to show clearly the surface of wool fabric fibres, for that SEM were images used to show the efficiency of the cleaning procedure as seen in Figure 2.16.

![Figure 2.15: Optical microscopy. Wool fabric. Before cleaning (x100).](image)

![Figure 2.16: SEM. Wool fabric (a) before cleaning (x500); (b) after cleaning (x1000).](image)
2.3.5 Bog mummy hair (north Germany)

Different types of particles such as dust, ions, small plant particles and insects parts can be found at the surface of bog mummy hair. Cleaning bog mummy hair with distilled water - cleaning procedure 1 was a very effective method to remove the particles from the surface of these fibres as illustrated in Figure 2.17.

Figure 2.17: SEM. Bog mummy hair; (a) before cleaning (x800); (b) after cleaning (x500).

2.2.4 Conclusion

Archaeological fibres have survived for different periods of time under special environmental conditions. Some are heavily damaged and their colour has shades of brown due to the immersion in different types of media, with many types of dirt having accumulated over the years. This enabled the development and optimisation of suitable cleaning procedures which are a vital part of the research project.

Different aqueous cleaning procedures were developed and applied to archaeological fibres. These techniques depend on the type of fibre, the amount of contamination and the level of damage to the fibres.

Using optical microscopy, ESEM and SEM it was shown that rinsing with distilled water - cleaning procedure 1. For the Iceman finds and bog mummy hair was an effective cleaning method. This is due to the less contaminated preservation sites.
The archaeological wool includes many specimens with different percentages of soil and other contaminations from their highly contaminated preservation site. For that, developing a suitable more rigorous cleaning procedure was a challenge. Cleaning procedure 2 was suitable for specimens with low soil content, while cleaning procedure 3 was an effective cleaning procedure for specimens with high soil content.
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References


3. Microscopical Investigation

Identification and discrimination of natural fibres, depending on their external surface features, is a very important task for many scientists [1]. For this purpose different kinds of microscopes have been devised, such as the optical microscope - OM [2], the Scanning Electron Microscope -SEM [3], the Environmental Scanning Electron Microscope - ESEM [4], the Transmission Electron microscope - TEM [5, 6] and other types of microscope. Microscopes can be used for different purposes such as fibre identification by analysing the surface scales cells pattern and medulla type and for damage analysis such as mechanical damage and microbiological attack.

3.1 Types of microscopes

3.1.1 Optical microscopy

Optical microscopy has been used for a long time for identifying and distinguishing between keratin and cellulose fibres in different types of textiles by detecting the presence of scale cells at the surface of keratin fibres [1], because this provides information about the external and internal structure of natural and artificial fibres [7]. Optical microscopy also provides a wide range of techniques with low costs for the identification process and provides a colour differentiation tool [8].

The visible light or optical microscope is used to produce enlarged images for samples to identify different features of the sample [9]. The optical microscope uses visible light from beneath the sample, which is mounted on a glass slide and placed on a stage. The sample should be sufficiently thin and bright for the microscope light to pass through. Light reflected from or transmitted by the viewed sample will pass through a series of lenses and form an enlarged image. An Optical microscope works by magnifying the object as much as 1000 times [10].

3.1.1.1 Advantages and limitations of Optical microscope

One big advantage of optical microscopes is the ability to observe living cells with a wide range of biological activity, such as the uptake of food, cell division and movement. Also the
optical microscopy provides colored images at different magnifications up to 1000 times. Also, the low cost of the optical microscopes makes them useful in a wide range of different areas, such as education and the medical sector.

Optical microscopy has limitations such as, it can only show images for light or strongly refracting samples. Also the optical microscope can magnify only to the point of showing objects larger than about 800-200 nanometres (that is the visual light range). Another limitation of optical microscope is the low resolution which is approximately 0.2 micrometres. Increasing contrast for living cells involves staining the different structures with selective dyes, but this process includes killing cells and fixing the sample and may produce artefacts in structural details [11, 10].

3.1.1.2 Identification of natural fibres

Natural fibres are used as raw materials in many products of daily life [12]. Common natural fibres can be hair, wool, silk and cellulose fibres. The fibres can be divided into bast (stem) fibres such as flax, nettle, hemp and jute and seed fibres such as cotton. [13]. Keratin fibres can be identified easily with the optical microscope [14]. The presence of overlapping scales on the fibre surface is a main character of keratin fibres. For plant fibres cotton is easily distinguished from other plant fibres due to their twisted shape. Also polarising microscopy can be used to aid identification. Distinguishing between bast fibres can be a difficult task due to similarities in their surface characteristics [1].

3.1.1.3 Identification of archaeological fibres

In early prehistoric times natural fibres were used in three main concepts: food (some cellulose fibres), cloth and shelter [15]. These archaeological fibres are preserved under different conditions such as extreme temperature, extreme soil pH, extreme dryness, or moist environment with metal presence and in rich–nitrogen bogs. These conditions play a major role for the preservation of these fibres [15, 16]. Archaeological fibres under poor preservation conditions are subject to degradation or carbonization causing fragile structures [8, 2, 15], resulting in many difficulties for fibre identification by using the optical microscopy [15].
Identifying archaeological keratin and plant fibres is possible when these fibres are well preserved by using different microscopical techniques [12]. Compared to plants fibres, keratin fibres are generally more resistant to acidic environmental influences and decay because they are protein fibres, while in alkaline conditions plant fibres are preserved and keratin fibres will be damaged [17, 6]. Plant fibres degrade easily because they are composed of cellulose [16, 17]. The investigation by Ryder and Gabra-Sanders showed that SEM can provide more detailed information about archaeological bast fibres than optical microscopy [18], especially for distinguishing between different bast fibres, which can be a difficult task [1].

### 3.1.2 Electron Microscopy

Optical light microscopy is in many cases a good tool for the differentiation between keratin and cellulose fibres. However, it is limited, especially in the case of highly damaged fibres. Electron microscopy provides higher magnification and a closer view of the distinguishing marks of the fibres.

#### 3.1.2.1 Scanning Electron Microscopy (SEM)

The SEM is one of the most useful microscopes in research. It uses electrons in high vacuum to form an image to show the external morphology [19]. It is also used with other techniques to reveal other information about the sample including chemical composition, crystalline structure and orientation [20].

Samples must be solid, dry and with a conductive surface. In a typical SEM, an electron beam (which has an energy ranging from 0.2 keV to 40 keV) is produced by an electron gun, fitted with a tungsten filament [21]. The electron beam passes through the microscope within a vacuum. The electron beam passes through various electromagnetic fields and is focused by one or two condenser lenses into the sample as illustrated in Figure 3.1.
When the beam hits the sample, it release primary, secondary backscattered electrons and X-rays as shown in Figure 3.2. Specific detectors convert them into a signal to produces the final image [21].

Figure 3.1: Schematic diagram of a SEM [21].

Figure 3.2: Electrons and x-rays from a sample in the SEM [21].
3.1.2.1 Advantages and Limitation of Scanning Electron Microscopy

The SEM is capable of performing several types of analysis by using the sample signals. These signals include secondary electrons (which produce sample SEM images), diffracted backscattered electrons (used to determine crystal structures and orientations of minerals) and photons (used for elemental analysis and continuum X-rays) [20].

To obtain SEM images, samples must fit into the microscope chamber, and must be solid and dry to prevent water to vaporise in the vacuum [20]. Non-metal samples must be coated with a conductive layer such as gold or carbon to prevent build up of electrons on the surface, which would lead to cloudy and obscured images [22]. The sample afterwards cannot be used for other analyses.

3.1.2.2 Environmental Scanning Electron Microscopy (ESEM)

Environmental Scanning Electron Microscopy - ESEM can be used to reveal information about the microstructure of a fibre’s surface [4]. ESEM was developed in the last fifteen years to study materials which do not need to be desiccated and coated with gold-palladium [23] or any other preparation. ESEM analysis is considered to be “non-destructive” because it does not lead to damage or material loss.

An ESEM utilises a scanning electron beam and electromagnetic lenses to focus the electron beam on the specimen surface. The beam electrons interacts with the specimen surface layer and produce various signals that are collected with detectors. The signals are modulated to produce a digital image [24].

In ESEM the lower part of the specimen chamber is closed off by shutting the main valve, and a large-bore pipe allows the evacuation of the upper portion of the column. The top of the specimen chamber will not be closed completely, to allow the electron beam to enter it.

The principle of ESEM is to have a small enough pinhole between two different vacuum levels. Vacuum will not 'diffuse' from one level to another through the pinhole. That will create a very good vacuum at the electron gun, at the top and mid-portion of the column, but not in the specimen chamber where there can be a relatively poor vacuum, without endangering the electron gun as illustrated in Figure 3.3 [23, 25].
3.1.2.2.1 Advantages and limitation of Environmental Scanning Electron Microscopy

ESEM machines can be operated relatively easily, while the specimens can be examined faster avoiding any type of complex preparation methods [23]. Also, using ESEM does not produce any modification of the natural surface or create artefacts [19]. Biological specimens can be maintained fresh and alive. Therefore, ESEM constitutes a radical breakthrough from conventional electron microscopy. Also ESEM can operate at high and low pressure, rough and high vacuum and with high or low beam accelerating voltage [25].

ESEM was chosen for this study because it produces images that reveal detailed information about surface structure with a wide range of magnifications without damaging the fibres. The main limitation of ESEM is that the distance in the specimen chamber over which the electron beam remains usable is limited in the gaseous environment. This distance varies from around 10 mm to a fraction of a millimetre as the gas pressure may vary from low vacuum to atmospheric pressure. At low pressure level, the ESEM will revert to a typical SEM operation [23].

Figure 3.3 Schematic diagram of an ESEM [26].
3.1.2.3 Studying archaeological fibres by SEM and ESEM

Under different burial conditions, natural fibres undergo different types of damage, depending on the environmental conditions of preservation [16]. ESEM is the most important tool to collect micro structural data of archaeological fibres, because it provides a direct view of samples without any preparation [4] or modification the fibre surface.

3.1.2.3.1 Fibre identification

SEM has been used widely to identify archaeological natural fibres because it provides more details that can be obtained in identification compared with optical microscopy [18]. Keratin fibres can be distinguished from plant fibres by the presence of overlapping scales on the fibre surface [27, 28], also the scale type and the direction of spinning twist in different textiles can be identified [27].

3.1.2.3.2 Identification of fibre damage

Archaeological textiles have been subject to damage and degradation [7, 15]. Thermal, light, chemical, mechanical and biological sources may have affected the proteinaceous and cellulosic archaeological materials and may have them subject to damage [29]. Many of them are nearly destroyed because their fibres decayed and their colour has shades of brown due to the immersion of the objects [17].

Archaeological fibres can show different types of damage which can be caused by a variety of light, micro-organisms and insects [17], or by mechanical damage.

- Mechanical damage

Mechanical damage can happen to archaeological cellulose and keratin fibres due to poor preservation conditions. This damage includes: flattening of the yarn (fractures in fibres and fibrillation) and abrasive action (fibre fatigue, brush ends, fibre loss, thin places, peeling and broken fibres) [7].
• **Environmental damage**

This type of damage can occur due to weathering, e.g. photo degradation [17, 7]. Keratin fibres are subject to oxidation, which causes breakdown of the cell membrane complex and oxidises cystine residues of the matrix of the cortex and of other fibre components rich in cystine, such as the A-layer and the exocuticle [30, 31]. Photo-degradation causes changes in fibre colour and a reduction in tensile strength. Archaeological cellulose fibres may also be oxidised but without disruption of the fibre structure [7].

Microbial attack is the most common damage in archaeological fibres. Lack of oxygen, humidity, warm conditions and the presence of other decaying materials encourages microbial attack [17]. In burial soil, fungi and bacteria can cause mechanical damage by penetrating and growing within fibres [2], producing special enzymes, such as celluloselyase and proteolytic enzyme to break down the crystalline and amorphous regions in plant and animal fibres [7]. Cellulose fibres are more susceptible to microbial attack than keratin fibres [17].

Microbial damage in keratin fibres can be characterised by the loss of scale structure [7, 12], longitudinal damage [27], broken fibre ends, fibrillation, brittleness and weight loss. In cellulose fibres diagonal or longitudinal splitting and segmentation are observed [2], due to micro-organism activity, that cause cleavage of glycosidic bonds and digestion of the primary wall of the fibre cells [16].

### 3.1.4 Objectives

The purpose of this section of the study is to identify fibres in archaeological samples as well as to determine the damage to the surface in more detail and in correlation with the preservation situation, using optical microscopy, SEM and ESEM.
Chapter 3

Microscopical analysis

3.2 Experimental work

3.2.1 Optical Microscope

Cleaned archaeological fibres were investigated by using LABOVAL 4 optical microscope (CARLZIESS JENA) at different magnifications (x 32, x100 and x 400). Images were obtained by using a Micro Ocular camera (1.3 MP BRESSER company).

3.2.2 Scanning Electron Microscopy (SEM)

Due to the nonconductive nature of hair, cleaned archaeological fibres were coated with a conductive carbon coating. The samples were vacuum sputter coated with a thin layer of carbon (7nm) and kept in high vacuum (\(-10^{-2}\) Torr) for 5 minutes by using GATAN PECS (Oxford, UK). SEM images were obtained with the scanning electron microscope ZEISS EVO 60 (Cambridge, UK) at 5 kV accelerating voltage and 250 A probe current.

3.2.3 Environmental Scanning Electron Microscopy (ESEM)

Micrographs of the surface of cleaned archaeological samples were obtained using an environmental scanning electron microscope –ESEM-(EDVX / Modal HIT S-3000N 132-2.5 Hitachi Company). Some samples have been identified by SEM only.

3.3 Results and discussion

Optical microscopy, SEM and ESEM images of virgin keratin fibres (human hair, deer hair, goat hair and wool) were used for identification of archaeological keratin fibres and for detecting the surface damage in these fibres.

3.3.1 Archaeological fibres from Iceman site

3.3.1.1 Human hair

Images obtained by optical microscopy, SEM and ESEM of virgin human hair showed the presence of flattened and narrow scales with smooth edges on the surface of the fibres and the medulla appears as one unbroken line, which is a main character of keratin fibres [32] as shown in Figure 3.4.
A comparison between virgin human and Iceman human hair was made. The optical microscope images of samples 91/92B, 91/130 and 91/142 failed to show the medulla (except sample 91/130) or the scales as seen in Figures 3.5, 3.6 and 3.7. This is could be due to the thickness of the cortex layer that can block the microscope light from passing through. ESEM and SEM images showed that these fibres are human hair fibres, due to presence of flattened and narrow scales with smooth scales edges on the surface. Images also showed an absence of any damage on the external surface of these fibres. SEM images were very effective for showing the surface scales compared with ESEM.
Figure 3.4: Virgin human hair. Optical photomicrograph (a) x100  (b) x400; ESEM (c) x700  (d) x1500; SEM (e) x1000  (f) x3500.
Figure 3.5: Sample 91/92 B. Optical photomicrograph (a) x100; SEM (b) x1002; ESEM (c) x400.

Figure 3.6: Sample 91/130. Optical photomicrograph (a) x400; SEM (b) x800; ESEM (c) x700.

Figure 3.7: Sample 91/142. Optical photomicrograph (a) x400; SEM (b) x300; ESEM (c) x1000.
3.3.1.2 Animal hair (deer hair)

A comparison between virgin deer hair and samples GO II, GO III, GO V, GO VI, GO VII, GO VIII, GO IX, GO XI, GO XIII, 91/96 and 91/137 fibres was made by using optical microscopy, SEM and ESEM.

The optical microscope images showed that these fibres are coarse deer hair due to the presence of a high percentage of medulla (fragmental) in comparison with the large distances between vacuolated cells. A specific feature of this species is a thin cortex layer, which is the main characteristic of highly medullated keratin fibres [33, 34]. Undercoat deer fibres showed the presence of scales on the surface of the fibres and an absence of medulla as shown in Figures 3.8 and 3.19.

While the ESEM and SEM images showed that the Iceman animal fibres contain a high percentage of flattened scales arranged transversely to the longitudinal hair axis in a deeply imbricate type. Their free margins were curved and quite regular, which is the main characteristic of coarse deer hair. SEM images were very effective in showing the surface scales compared to ESEM. Optical microscopy, SEM and ESEM images show the presence of mechanical damage (fibre fracture) in many of these fibres, which leads to fibre fragility.
Figure 3.8: Optical photomicrograph. Virgin coarse deer hair (a) x100, (b) x400; undercoat deer hair (c) x32, (d) x100; ESEM, virgin coarse deer hair (e) x200, (f) x1000; SEM (g) x750, (h) x1500.
Figure 3.9: GO II. Optical photomicrograph (a) x100; SEM (b) x371; ESEM (c) x300.

Figure 3.10: GO III. Optical photomicrograph (a) x400; SEM (b) x500; ESEM (c) x200.

Figure 3.11: GO V. Optical photomicrograph (a) x100; SEM (b) x194; ESEM (c) x200.
Figure 3.12: GO VI. Optical photomicrograph (a) x100; SEM (b) x1000; ESEM (c) x100.

Figure 3.13: GO VII. Optical photomicrograph (a) x100; SEM (b) x750; ESEM (c) x1300.

Figure 3.14: GO VIII. Optical photomicrograph (a) x100; SEM (b) x250; ESEM (c) x700.
Figure 3.15: GO IX. Optical photomicrograph (a) x100; SEM (b) x1000; ESEM (c) x270.

Figure 3.16: GO XI. Optical photomicrograph (a) x400; SEM (b) x1000; ESEM (c) x300.

Figure 3.17: GO XIII. Optical photomicrograph (a) x100; SEM (b) x500; ESEM (c) x300.
3.3.1.3 Animal hair (goat hair)

Optical microscope images were ineffective for identifying the surface of virgin goat hair. This is due to the thickness of the cortex layer that contains melamine pigment with low percentage of medulla as illustrated in Figure 3.20. For this reason virgin white goat hair was tested under the optical microscope. The optical microscope, SEM and ESEM images showed that the surface of goat hair fibres was rough. The thick scales were imbricated, very elongated transversely and arranged in a herringbone pattern at oblique angles with respect to the longitudinal axis of the hair as a main characteristic of goat hair [34].
Optical microscopic images for Sample 91/110 showed the presence of medulla as a large unbroken line with unclear large areas of cuticle and cortex layers. ESEM and SEM images were partially ineffective to show the type of these fibres, due to the removal the cuticle layer on the surface of these fibres by mechanical damage as seen in Figure 3.21. For this reason another types of identification analysis was needed such as Attenuated Total Reflectance - ATR.

Figure 3.20: Optical photomicrograph (a) virgin dark goat hair x400; (b) virgin white goat hair x400; SEM (c) x2500; ESEM (d) x3000.
3.3.2 Keratin fibres from Hadrian's Wall (Vindolanda, UK)

3.3.2.1 A86, goat hair

The comparison with virgin goat hair as shown in Figure 3.22, using optical microscopy images for sample A86; goat hair failed in fibre identification, due to the thickness of the cortex layer with a high percentage of melamine pigment. SEM images for sample A86 showed that these fibres are keratin fibres. Due to presence of scales which are imbricated, very elongated and arranged in a herringbone pattern at an oblique angle with respect to the longitudinal axis of the hair, these fibres are assumed to be goat hair. SEM images showed an absence of any damage at the external surface of the fibres as seen in Figure 3.15. SEM images were very effective in showing the surface scales compared with ESEM. For that, another method is needed for fibre identification such as Attenuated Total Reflectance - ATR.
3.3.2.2 Wool TT/85/38 fibres

Using optical microscopy, SEM and ESEM images of virgin wool fibres showed the absence of a medulla and therefore no vacuolated cell patterns were visible in the normal wool. The surface scales were large rhomboidal asymmetric and protruding with free margins. They are tooth-shaped conferring an imbricate crown-like appearance with defined scales edge on the surface of the fibres, which is main characteristic of wool fibres [34] as seen in Figure 3.23.

Optical photomicrograph and SEM images of Wool, TT/85/38 fibres (which are part of a fabric) are compared with virgin wool and show the presence of flat scales with well defined scale edges and no medulla. Because these fibres were part of fabric, it is suggested that these fibres are wool fibres as seen in Figure 3.24 a, b, c. SEM images show the presence of fibrillation, fibre fracture and flatted fibres as seen in Figure 3.16 b, c, d), which is due to mechanical damage and microbial attack. These results are in a good agreement with Cooke and Lomas' study about fibre damage in Vindolanda finds [35]. ESEM images show the presence of flat imbricate crown-like scales on the surface of some fibres, which is a characteristic of wool fibres. Also loss of cuticle scales in some fibres is shown in Figure 3.24 d.
Figure 3.23: Virgin wool fibres. Optical photomicrograph (a) x400; SEM (b) x2500; ESEM (c) x2000.

Figure 3.24: Wool, TT/85/38. Optical photomicrograph (a) x400; SEM (b) x2500; ESEM (c) x1000; (d) x700.
3.3.3 Textiles from Schloesser Brewery (Düsseldorf, Germany)

3.3.3.1 Wool 2a fibres

Optical microscope images of wool 2a (which are part of a fabric) were compared with virgin wool as in Figure 3.23. They showed the presence of flat scales with well defined scale edges on the surface of the fibres and the absence of medulla, which are the main characteristics of wool fibres as shown in Figure 3.25. Because these fibres were part of a fabric, it is confirmed that the fibres of wool 2a are sheep’s wool.

Using an SEM failed to identify the type of these fibres. ESEM, however, showed the presence of flat imbricate crown-like scales with defined scales edge on the surface of the fibres, with an appearance characteristic of wool fibres. These results show good agreement with the results obtained by the optical microscopy. In ESEM images a plain weave was detected in wool 2a and yarn with general fibrillation as shown in Figure 3.26. ESEM and SEM images also revealed information about microbial attack in some of these fibres. The microbial attack leads to longitudinal fracture in some of these fibres and a loss of the cuticle. A good agreement is obtained between these results and Lewis' (1981) work, as he reported the loss of cuticle scales and fibrillation in degraded keratin fibres in soil burial [36].

Figure 3.25: Wool 2a fibres. Optical photomicrograph (a) x32; (b) x400.
3.3.3.2 Wool 2b fibres

Wool 2b images compared with virgin wool images as illustrated in Figure 3.23 and 3.27. In the comparison, the optical microscope images of wool 2b fibres (which are part of a fabric) as shown to be keratin fibres, mainly wool, due to the presence of flat scales with well defined scales edge and an absence of medulla. Also other fibres were detected which could be cellulose fibres as shown in Figure 3.27. Close inspection of the fibres showed the presence of different keratin fibres such as rabbit hair fibres in different specimens in this sample as seen in Figure 3. 28. In the textiles from the old town in Düsseldorf, plant fibres were absent [37].
Both SEM and ESEM images show that wool 2b fibres are wool fibres due to the presence of large imbricate crown-like appearance flat scales with well defined scales edge on the surface of the fibres and an absence of medulla as seen in Figure 3.29 and 3.30a. These results are in good agreement with the description of the textiles from the old town in Düsseldorf.

SEM and ESEM images also give a variety of information about different types of damage which can be detected in wool 2b, such as flattening of the yarn crown which leads to breakdown of fibres and fibrillation as shown in Figure 3.30b. The weave of the textile could not be determined in wool 2b. Other types of fibre damage also were detected in sample 2b such as: abrasive action, which has led to fibre fracture and scale damage as invisible in
Figure 3.30c and microbial attack that has led to changes in colour, brittleness and surface damage as invisible in Figure 3.30d.

Figure 3.29: Wool 2b. SEM (a) x250, (b) x1000.
3.3.4 Wool fabric from 15th century (Germany)

A comparison was made between images of virgin wool fibres as shown in Figure 3.23 and wool fabric fibres. Optical microscopy images did not show a clear scale type at the surface of these fibres, but they showed the presence of flattening of the yarn crown which leads to fibrillation as seen in Figure 3.31.

The SEM images of wool fabric fibres showed the presence of a large imbricate crown-like appearance flat scales with well defined scales edge on the surface of the fibres and an absence of medulla in Figure 3.32, which are the main characteristic of wool fibres. Because these fibres were part of a fabric, it is suggested that the fibres are wool fibres.
3.3.5 Bog mummy hair (north Germany)

Bog mummy hair was compared with virgin human hair in Figure 3.33. The comparison showed that these fibres are human hair, due to the presence the flattened and narrow scales with smooth scales edge in both optical and SEM images. Also the optical microscope images showed the presence of the medulla as one unbroken line which is a main characteristic of human hair as shown in Figure 3.34. There was no mechanical damage detected for these fibres and the fibres show excellent preservation conditions.
Figure 3.33: Virgin human hair. Optical photomicrograph (a) x100; SEM (b) x1500; ESEM (c) x700.

Figure 3.34: Bog mummy. Optical photomicrograph (a) x400; SEM (b) x500; (c) x1600.
3.4 Conclusions

Optical microscopy, SEM and ESEM have been used to identify and distinguish between keratin and cellulose fibres and for damage analysis. The different techniques provide information about the external and internal structure of natural fibres.

3.4.1 Archaeological fibres from the Iceman site

Microscopical analysis showed that Iceman fibres are human, deer and goat hair fibres. Images of the Iceman’s human hair showed the absence of any substantial damage on the external surface of these fibres.

Ancient deer hair showed mechanical damage through longitudinal fracture in some fibres with no microbial attack. Sample 91/110 was identified as goat hair with a removed cuticle layer at the surface of some of these fibres through mechanical damage.

3.4.2 Finds from Hadrian’s wall (Vindolanda, UK)

Ancient fibres from Vindolanda proved to be goat and wool fibres. In A86, goat hair there was no damage of the external surface of the fibres, while wool TT/85/38 fibres showed fibrillation, fibre fracture and flattened fibres due to mechanical damage and microbial attack.

3.4.3 Textiles from Schloesser Brewery (Düsseldorf, Germany)

Microscopical analysis showed that the ancient textiles from the old town in Düesseldorf site are wool samples. It also showed damage for these fibres, which include abrasive action which has led to fibre fracture and scale damage as well as microbial attack that has to a change in colour and brittleness.
3.4.4 Wool fabric from 15th century (Germany)

Microscopical analysis showed that wool fabric from 15th century contains wool fibres and has mechanical damage, including the flattening of the yarn crown, which has to fibrillation.

3.4.5 Bog mummy hair (north Germany)

Bog mummy hairs have been confirmed as human hair with no or little damage at the external surface.

In general the comparison between archaeological fibres that come from to different preservation conditions, wool fibres from warm conditions show substantial damage of the external surface compared with the Iceman animal hair, which derived from frozen conditions. Ancient human hair from frozen and bog conditions showed no or little damage to the external surface.
References


Chapter 4 - FTIR Investigations

4. Fourier Transform Infrared Spectrometer

4.1 Introduction

The Fourier Transform Infrared Spectroscopy - FTIR technique measures the extent of infrared light which is absorbed or transmitted or reflected from a sample at each wavelength. The FTIR technique is employed as a chemical analytical tool to determine the presence of a particular substance in a sample [1, 2]. It can also be used for the identification of the chemical nature of natural fibres and for damage analysis of these fibres.

The main advantage of Fourier transform spectrometers is their rapid data collection and high light intensity at the detector and in consequence a high signal to noise ratio. Different types of detectors, light sources and other optical components are chosen to optimise different requirements of the infrared spectrum. IR spectroscopy can by divided into three main techniques:

A. Reflection spectroscopy. This technique studies the light that is reflected or scattered from the sample.

B. Transmission spectroscopy. This is highly interrelated to absorption spectroscopy. The technique is based on determining the fraction of light that passed through the sample.

C. Absorption spectroscopy. This is widely used in chemistry and life sciences to identify and determine the concentration of chemical substances. This analytical technique is based on measuring the amount of light absorbed by the sample at a given wavelength [1].

4.1.1 Attenuated Total Reflectance (ATR)

To provide rapid information at the molecular level Attenuated Total Reflectance - ATR can be used. The ATR technique is a non-destructive technique as the solid, liquid or gas samples can be examined directly without any preparation. A beam of infrared light is passed through an ATR crystal and will be reflected at the surface crystal. Usually a diamond crystal is the ideal material for ATR due to its mechanical properties particularly with hard solid samples. Then the reflected beam is collected by a detector. The penetration depth of the beam into the sample is typically between 0.5 and 2 µm. Solid samples have to be in direct contact with the
ATR crystal, to prevent trapped air from distorting the results [1, 2]. In the case of the studied fibres, the ATR technique can be used for fibre identification [3] and to detect damage in the S-O vibrational region.

### 4.1.2 Transmission FTIR

The transmission technique involves IR radiation passing through the sample and detecting that portion of the beam that is transmitted, i.e. not absorbed, creating a molecular fingerprint of the sample. It is then measured versus the respective wavelength to generate a spectrum. Like a fingerprint no two molecular structures produce the same infrared spectrum. This makes infrared spectroscopy useful for several types of analysis [4, 5].

There are various sampling techniques used in the transmission FTIR mode, the main two are.

- **Compressed Pellets.** The method includes grinding the dry sample together with pure, dry potassium bromide (KBr) to a fine powder. The mixture is then transferred to a compression die and placed under high pressure to form a pellet that is transparent to infrared light.

- **IR Sampling Cards.** With this method a wide range of samples can be used, including inorganic and organic liquids of low volatility, solids in volatile solvents, insoluble solids, semisolids, pastes, lubricants, paints, etc. The microporous sample substrate permits rapid solvent evaporation when the sample is applied as a solution. The two most popular types of cards are polytetrafluoroethylene cards which provide an absorption free background from 4000 to 1400 cm\(^{-1}\) and polyethylene substrates which provide an absorption free background for the entire spectrum [5].

### 4.1.3 FTIR and Protein Structure

The IR spectrum can be categorised into three smaller ranges: Near IR (13,000 to 4,000 cm\(^{-1}\)), Mid IR (4,000 to 200 cm\(^{-1}\)) and Far IR (200 to 10 cm\(^{-1}\)). This FTIR investigation of keratin fibres focuses on the mid IR region, between 4000 and 400 cm\(^{-1}\). In the mid-infrared (MIR) spectrum, different functional chemical groups lead to characteristic fundamental vibrations [6].
To provide information about the chemical composition of various polymers, including amino acids from proteins, FTIR has widely been used in the research of natural protein polymers such as keratin fibres.

Proteins are made up of different amino acids. In general, keratins may have about twenty different amino acids, with side chains of different structure. These amino acids are held together by strong and fairly rigid bonds called peptide bonds to form the primary structure of the peptide chain. Peptide bonds are covalent bonds, which form between the amino-end on one amino acid molecule, and the carboxylate-end of another amino acid molecule as shown in Figure 4.1. Peptide chains also have the ability to form hydrogen bonds. These hydrogen bonds are crucial for the formation of a secondary structure [7].

In the case of keratin fibres, the atoms that take part in peptide bonds lead to a number of vibrational bands that can be observed in the IR spectrum of proteins. Characteristic bands found in the infrared spectra of proteins and polypeptides include the Amide A and B, Amide I, Amide II, and Amide III bands and the S-O vibrational region which absorb in the near to 3430 to 3295 [9], 1700 to 1600, 1600 to 1435, 1200 to 1350 and 1000 to 1250 cm\(^{-1}\) regions, respectively [9, 10, 11, 12, 13]. Figure 4.2 shows the most important areas in the protein spectrum.
Table 4.1 shows the absorptions associated with the Amide A and B bands (not shown), which primarily originate from the bending vibrations of the N—H bond and OH (H₂O) stretching vibrations. The Amide I band is predominantly (80%) attributed to the C=O stretching mode of the amide functional group, while the remaining contribution (20%) arises from C-N stretching. The absorption associated with the Amide II band primarily originates from the bending vibrations of the N—H bond and C-N stretching vibrations. The amide III absorption is caused by coupling C-N stretching vibrations with N-H in plane bending vibrations, and the weak contributions from C-C and C=O stretching [9]. Other vibration modes also appear in the Mid-IR range, including the amino acid side chains, such as C-H deformations (1471-1460 cm⁻¹), CH₂ and CH₃ deformations (1453 to 1443 cm⁻¹ and 1411 to 1399 cm⁻¹), and the cystine oxide stretches, such as the asymmetric and symmetric cysteic acid (1175 cm⁻¹ and 1040 cm⁻¹), cystine monoxide (1071 cm⁻¹) stretches and symmetric cystine dioxide (1121 cm⁻¹) [9, 14].
Table 4.1: The Peak Assignments for Cystine Derivatives and Amino Acids in the mid-IR Spectrum [9].

<table>
<thead>
<tr>
<th>Wavenumbers (cm⁻¹)</th>
<th>Assignment</th>
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<tbody>
<tr>
<td>3550 to 3315</td>
<td>Amide A and B NH (Primary amine) and OH (H₂O)</td>
</tr>
<tr>
<td>2960 and 2885</td>
<td>CH</td>
</tr>
<tr>
<td>1655</td>
<td>Amide I. 80% (C=O stretch) [α-helix / β-sheets], and 20% (C-N stretch)</td>
</tr>
<tr>
<td>1550</td>
<td>Amide II 60% (C-N stretch) and 40% (N-H stretch)</td>
</tr>
<tr>
<td>1241</td>
<td>Amide III 30% (N-H stretch), 30% (C-N stretch) and weak contributions (C-C and C=O stretch)</td>
</tr>
<tr>
<td>1175</td>
<td>Sulfonate, S-O asym, stretch</td>
</tr>
<tr>
<td>1121</td>
<td>Cystine dioxide (R-SO₂-S-R)</td>
</tr>
<tr>
<td>1071</td>
<td>Cystine monoxide (R-SO-S-R)</td>
</tr>
<tr>
<td>1040</td>
<td>Sulfonate, S-O sym, stretch</td>
</tr>
</tbody>
</table>

4.1.4 Uses of ATR and Transmission FTIR techniques

4.1.4.1 Fibre identification

The ability to accurately identify natural fibres by using FTIR/ATR and Transmission FTIR has been used in a variety of previous investigations. Zemaityte et al [3] used FTIR techniques as a tool to distinguish between different types of animal fibres. They carried out a comparison of the spectra. They found that the spectrum for natural black wool can be distinguished from that for white wool.

Garside and Wyeth [15] used FTIR to distinguish between cellulosic fibres by comparing the spectrum of six different fibres. They found minor differences in the composition of the various cellulosic plant fibres, while the archaeological cellulose fibres show large discrepancies. But in spite of difficulties in identifying material with 100 % accuracy it is a valuable tool [3].
4.1.4.2 Damage analysis in keratin fibres

ATR and Transmission FTIR spectroscopy has been used to assess the extent of damage in keratin fibres [16]. In the ATR technique, the IR penetration depth is approximately 2.0 µm. Therefore, the structural damage can be studied for the exocuticle layer only (which is rich in cystine content) of keratin fibres. The area in the spectrum which is of special interest for monitoring the degree of fibre damage is the S-O vibrational region (950 to 1200 cm$^{-1}$), which shows the modification of cystine [17, 18]. In Transmission FTIR, the IR radiation passes through the whole sample. Therefore the structural damage can be studied in the S-O vibration range for cuticle and cortex (which has an overall bigger content of cystine compared to the cuticle layer), and in the Amide A, Amide I, Amide II and Amide III range for the damage in α-helix proteins.

4.1.4.2.1 Oxidation products of cystine in keratin fibres

Keratin fibre damage occurs due to the influence of weathering, daily hair care (physical factors) and chemical treatments during hair dressing such as bleaching, permanent waving [19] and permanent hair straightening [20]. The damage due to an initial oxidation reaction causes removal of 18-methyleicosanoic acid (18-MEA) and free lipids from the surface and between cuticle cells and breakdown of the cell membrane complex. Oxidation reactions also cause cleavage of disulfide bonds between cystine residues in the matrix of the cortex and other hair components rich in cystine, such as the A-layer and the exocuticle [21, 22, 23, 24]. Also hair pigments and other amino acid (methionine, tyrosine, lysine, and histidine) functional groups are attacked and oxidatively degraded [21].

During weathering photo-degradation can occur from the effect of sunlight. In virgin human hair cystine oxidation products have been detected at the tip due to the influence of weathering [25], causing a decrease in strength of the cuticle layer [26]. Also the H$_2$S gas (formed in the process of the natural decomposition of organic matter) is produced in wool through degradation of cysteine. This is enhanced by the presence of UV, high temperature (50 °C) [27] and photochemical activity. A portion of the sulfur is converted to hydrogen sulfide, some of which is subsequently oxidized to sulfuric acid as a sign of degradation [28].
Both bleaching and hair permanent waving are examples of chemical damage of human hair. Bleaching treatment by hydrogen peroxide is about in the pH range of 9 to 11. The oxidation process attacks the thioester groups that bind 18-methyleicosanoic acid at the surface proteins. This reaction partially removes the hydrophobic surface barrier and it creates sulfur acids (primarily sulfonate groups) on and in the fibre surface [21]. The oxidation process also causes cleavage of disulfide bridges in cuticle proteins and the cortical matrix forming cysteic acid. This leads to a decrease in intermolecular cross links, resulting in fragmentation of the cuticle proteins into smaller peptides [19].

Two schemes have been proposed for the oxidative degradation of disulfide bonds as shown in Figure 4.3, sulphur - sulfur (S–S) fission process (during bleaching) and a carbon - sulfur (C–S) fission process [21].

![Figure 4.3: Schemes for disulfide bond fission](image)

Permanent waving consists of a reduction and oxidation process. The reduction process causes production of thiol groups, which are re-oxidised to disulfide bonds by oxidation [29]. Kizawa et al [19] suggested that both processes cause the release of the S100A3 protein which has an adhesive function between the cuticle layers, from the inner part of the endocuticle, causing the cuticle to delaminate into large fragments.

Different functional groups are involved in both oxidative fission schemes as shown in Figures 4.3 and 4.4 such as cysteinyl residues, or mercaptan groups in keratin fibres. The intermediate oxidation products of cystine are: disulfide monoxide, dioxide [17], trioxide, and tetroxide [30], which can be detected in the S-O vibrational region. The IR bands that are typically monitored in the S-O vibrational region are: cysteine- S-thiosulfate (1022 cm⁻¹), cysteic acid (1042 cm⁻¹), cystine monoxide (1075 cm⁻¹) and cystine dioxide (1120 cm⁻¹) [18].
Sulfonic acid is unstable, while trioxide, and tetroxide are more sensitive to alkali pH than dioxide and monoxide [21] as listed in Table 4.2.

<table>
<thead>
<tr>
<th>Disulfide oxides</th>
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<tbody>
<tr>
<td>-S-S-</td>
<td>Disulfide</td>
</tr>
<tr>
<td>-SO-S-</td>
<td>Monoxide</td>
</tr>
<tr>
<td>-SO₂-S-</td>
<td>Dioxide</td>
</tr>
<tr>
<td>-SO₂-SO-</td>
<td>Trioxide</td>
</tr>
<tr>
<td>-SO₂-SO₂-</td>
<td>Tetroxide</td>
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<tr>
<th>Sulfur acids</th>
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<tbody>
<tr>
<td>-SH</td>
<td>Mercaptan</td>
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<tr>
<td>-S-OH</td>
<td>Sulfenic acid</td>
</tr>
<tr>
<td>-SO₂H</td>
<td>Sulfinic acid</td>
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<tr>
<td>-SO₃H</td>
<td>Sulfonic acid</td>
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Figure 4.4: The oxidation of the mercaptan groups and cysteiny1 residues in keratin fibres [30].

### 4.1.4.2.2 Damage of α- helical IFs proteins in keratin fibres

Primary, secondary and quaternary structures can be found in fibrous proteins, such as human hair and animal fibres, with no tertiary structure [31]. Secondary structures are based on the interaction of the hydrogen bonds. In secondary structures two important structures can be found in keratins, the α- helix and the β sheet as illustrated in Figure 4.5. The α- helix arises
through the formation of H-bonds between the amide hydrogen and the carbonyl oxygen of a peptide bond as H-bond donors and acceptors, respectively.

The β sheet arises from the formation of H-bonds in backbone residues between neighbouring chains, with the donor (amide) and acceptor (carbonyl) atoms pointing sideways rather than along the chain, as in the alpha helix. The β sheets can be either anti-parallel, in which neighbouring chains run in opposite directions or parallel, and which extend in the same direction, respectively.

Depending on the amino acid sequence in the primary structure, secondary structures form. Helix forming amino acids includes alanine, cysteine, leucine, methionine, glutamic acid, glutamine, histidine, and lysine. Beta sheet formers include valine, isoleucine, phenylalanine, tyrosine, tryptophan, and threonine, while serine, glycine, aspartic acid, asparagine, and proline are found most often in turns [7].

Figure 4.5: Systematically structures in the secondary structure of fibrous proteins [7].

Keratin fibres consist of three major regions: the cuticle, cortex and medulla. The cortex layer consists of spindle shape cells, which contain intermediate filaments IFs, with a predominantly α-helical structure, embedded in an amorphous, high sulphur protein matrix [30].

In stressed keratin fibres such as hair, both α-helix and β-sheet structures can be found. The mechanism of the transition of α-helix structures to β-sheet is not fully understood. Kreplak et
al suggested that the transition is due to an unfolding – refolding process of the IFs keratin molecules [32] at high humidity [10, 33, 34].

The IR spectrum of keratin fibres in general shows typical absorption IR bands for peptide bonds (CONH), which have typical vibration for α-helical, β-sheet and β-turn and disordered structures. The vibration of these bonds can be related to different regions such as Amid A, Amid I, Amid II and Amid III [35, 9, 12] as listed in Table 4.1.

To study changes in the α-helical structure, the Amid I is generally chosen rather than other regions [36, 11], because Amid A has high frequency vibrations of the N—H bond, which belong to non-helical peptide and the α-helical peptide. It also exhibits a water band near 3400 cm\(^{-1}\) causes shifting to lower wave numbers [36].

The Amide II region is less significant for study of the secondary structures, because it contains vibrations of the N—H bond, which shows spectral shift under hydration conditions [11, 9, 36]. The Amide III region shows very weak intensity of α-helical conformation at 1271 cm\(^{-1}\) [35], due to irregular conformation [37].

The Amide I absorption is sensitive to the secondary structure of the proteins [13]. Amid I consists mainly of the C=O stretching bond with minor contributions from C-N-H bending with the peptide backbone. This makes this region ideal to study the proteins' secondary structure [11].

In virgin wool the Amide I region shows an absorption peak at 1651 to 1657 cm\(^{-1}\) [38, 10, 39], which is characteristic for the α-helical structure. Bands at 1631, 1624 and 1615 cm\(^{-1}\) are characterised as being typical for β-sheet structures [38, 10, 13], while bands at 1690, 1680, 1674 and 1668 cm\(^{-1}\) are assigned to various types of β-turns and disordered structures [38, 39, 40].

A water band can be found in Amide I centred at 1640 cm\(^{-1}\), which causes a spectral shift of α-helix and β-sheet bonds [41, 42]. To avoid this problem, spectrometers are routinely purged with either N\(_2\) or dry air [41, 10] or using dried KBr [12, 38, 43].

Many studies have been conducted on the Amide I region. Dumas and Miller [39] used Transmission FTIR to show that the Amide I region in virgin human tissue (hair and skin) was significantly different for the three hair layers, in both line shape and Amide I peak position. Also, the Amide I region in keratin fibres, which were subjected to different
physical and chemical treatments were studied. Cardamone et al. [38] studied the Amide I region of wool by Transmission FTIR. They found a decrease in α-helical structure and an increase in β-sheet structure content and a less rigid orientation in reduced hydrolysis wool. An increase in β-sheet absorption was observed compared to the α-helical structure in stretched wool, in the Amide I region by using the ATR technique [10]. Odylha et al. [44] showed the evolution of a structure at about 1670 cm$^{-1}$ and 1720 cm$^{-1}$ due to an increase in disordered structures and possible formation of fatty acids from the oxidation of lipids in light aged wool.

4.1.5 Mineralisation in archaeological materials

Keratin fibres are usually ion exchanger and an exposure to earth and water can facilitate their interaction with some elements [45]. Consequently archaeological materials may undergo a range of different alterations after burial due to biological and physico-chemical processes [46]. FTIR/ATR has been used to understand the exceptional preservation of the organic remains by providing information on both chemical composition and the crystallinity of these remains [46, 47].

During burial keratin fibres are prone to biodegradation. The disulfide bond and peptide bond are the main reactive sites in keratin fibres such as wool. In alkaline conditions, disulfide bonds will break down and form cysteic acid, leading to the less reactive lanthionine cross-link. Also the structural integrity of keratin fibres is destroyed. Finally, peptide bonds break and the fabric is dissolved. In acidic conditions disulfide bonds are more stable [48].

The presence of some metals leads to the preservation of the morphology of keratin fibres by a process called mineralisation. This process occurs in positive casts (minerals form within the fibre) and negative casts (minerals deposited on the fibre surface) [48]. Corrosion of these metals is toxic to micro-organisms so microbiological degradation is inhibited, which leads to preserved keratin fibres [49, 50].

Mineralisation of organic materials during preservation has been repeatedly shown. Cabanes and Albert [47] showed the accumulation of sodium nitrate and calcium carbonate on organic remains from the surroundings in the archaeological Cova des Pas site. Mansilla et al. [45] observed the absorption of sodium and calcium from adhered minerals in mummy hair,
causing preservation of the mummy hair with less ordered scales and rough surfaces. By using the FTIR technique other investigations showed the presence of carbonate and phosphate in preserved human bones at 1640 and 1030 cm\(^{-1}\) [46], and the presence of glucose in skin at 1030, 1080, 1118 and 1151 cm\(^{-1}\) by using FTIR /ATR analysis [51]. Hallegot et al [52] found calcium in ancient hair obtained from mummified remains.

Silica dioxide SiO\(_2\), which formally can be considered to be orthosilicic acid H\(_4\)SiO\(_4\), accumulate on the surface of virgin wool during growth [53]. In nature organic materials that are embedded in soil may undergo silicification by vitreous and crystalline silica minerals [43, 54] such as Si-CH\(_2\)CH\(_3\), which shows a vibration band at 1020-1000 cm\(^{-1}\), Si-F at 1030 cm\(^{-1}\) and Si-OCH(CH\(_3\))\(_2\) at 1055-1030 cm\(^{-1}\). [55].

4.1.6 Objectives

The objective of this chapter is to focus on oxidation of cystine on the surface of archaeological fibres by using the ATR technique. Also the state of oxidation of cystine in the main part of the fibres (cortex) by using Transmission FTIR as a measure for the state of preservation.

4.2 Experimental work

4.2.1 Sample Preparation

Cleaned archaeological samples and virgin fibres were used in both attenuated reflectance and transmission FTIR spectroscopy.

4.2.1.1 ATR Sample Preparation

FTIR-ATR requires no special sample preparation. ATR spectra of cleaned archaeological samples and virgin fibres were obtained by using a NICOLET 5700 FT-IR for an angle of incidence of 45° with a Diamond-ATR Smart Orbit Accessory (IR penetration 2 \(\mu\)m) to provide information about the surface of the hair fibres. Before collecting the sample spectrum, the background spectrum was obtained. 64 sample scans were taken for each sample at a resolution of 4 cm\(^{-1}\) from 500 to 4000 cm\(^{-1}\). Three repeats were made. All spectrum manipulations were carried out using the OMNIC software (Nicolet).
4.2.1.2 KBr Pellet preparation

From each archaeological sample snippets were dried at 110 °C for one hour to remove moisture. A bottle of sealed KBr powder was left in a 150 °C oven overnight before pellet preparation. After removal from the oven, 200 mg dried KBr was weighed immediately and ground to a fine powder. About 4 to 5 mg of samples snippets were added and mixed well with KBr using a mortar and pestle by hand. This mixture was then transferred to a Graseby Specac die with a barrel diameter of 15 mm. Using a Carver press at a pressure of 10 kbar under vacuum for 2 to 3 min, a clear glassy disk was formed of about 4.5 mm thickness. This pellet was used to record the spectrum.

FT-IR spectra of samples, which are in a pellet form, were acquired with a 5700 FT-IR spectrometer (Thermo Nicolet) equipped with KBr optics and a deuterated triglycine sulfate (DTGS) detector. The FT-IR data analysis was conducted using the infrared spectra analysis software package OMNIC. All recorded spectra were averaged over 64 scans of 4000 to 500 cm\(^{-1}\) at a resolution 4 cm\(^{-1}\). A background spectrum was obtained as a reference through a KBr pellet without fibres before obtaining the spectrum of a sample. All the spectra were recorded five times at different points to obtain reproducible results. The transmittance spectra were then converted to absorbance spectra for further investigation.

4.2.2 Spectral Processing

All of the OMNIC spectral (.SPA) files from ATR and Transmission measurements (KBr) were imported and saved as .CSV files before transferring the data to a Microsoft Excel 2007 spreadsheet. To bring peak amplitude to a target level, peaks were normalised by setting the 1232 cm\(^{-1}\) band (Amide III region), according to Odlyha et al [44], or on the peak at 1451 cm\(^{-1}\), the CH\(_2\)-stretching area [56]. The parts of the spectrum containing bands of interest (after normalisation) were imported into Peakfit peak separation and analysis software (sigma plot, version 1.2), and were converted into second derivative spectra by using the Gaussian deconvolution derivative method. The PeakFit software identified the various peak positions based on the second derivative. The second derivative spectra were reduced to minimum signal-to-noise ratio spectra using an automatic smoothing function, and the achieved spectra were then imported to a Microsoft Excel 2007 spreadsheet for further investigation.
4.3 Results and discussion

ATR technique was used for fibre identification and to determine external changes in the S-O vibrational region by detecting the oxidation products of cystine at the surface of archaeological fibres. Transmission FTIR shows the internal changes in the S-O vibrational region as a measure for the state of preservation.

4.3.1 Fibre identification

The ATR technique has been used widely to identify different types of keratin and cellulose fibres [3, 15]. Keratin fibres are protein fibres. All keratin fibres show similarities in their FTIR spectrum, which is totally different from the FTIR spectrum of cellulose fibres, for that a differentiation between keratin and cellulose fibres can be performed. The differences between the spectra of the two types of fibre can be seen in Figure 4.6.

![Normalised ATR spectra of virgin keratin and cellulose fibres.](image)

**Figure 4.6:** Normalised ATR spectra of virgin keratin and cellulose fibres.

4.3.1.1 Archaeological fibres

Keratin fibres can be identified by characteristic absorption bands assigned mainly to peptide bonds (CONH) [13]. For that ATR spectra of archaeological fibres are compared with virgin keratin fibres.

Previous and present microscopical examinations showed that all Iceman fibres [57], wool 2a, wool fabric and bog mummy hair were keratin fibres. ATR spectra of these
archaeological samples were compared with virgin keratin fibres. They showed a high degrees of similarity between them for the S-O vibrational (the differences in this region are due to different percentages of cystine oxidation products) region and Amide regions (1500 to 1250 cm\(^{-1}\)), which gives evidence that these samples are keratin fibres as seen in Figure 4.7.

![Normalised ATR spectra of selected archaeological keratin fibres.](image)

Figure 4.7: Normalised ATR spectra of selected archaeological keratin fibres.

### 4.3.1.2 Wool 2b fibres from Schloesser Brewery (Düsseldorf, Germany)

A clear identification for wool 2b fibres by microscopical analysis was not possible due to the high mechanical damage of the fibres. For that the ATR technique was used for fibre identification. The ATR spectrum revealed that the specimens were keratin fibres, through comparison with virgin wool and flax fibres (because these fibres were commonly used in fabric at that time), with no presence of any other cellulosic fibres as can be seen in Figures 4.8 and 4.9. These results show that microscopical examination was not significant to identify the archaeological fibres.
4.3.2 Chemical changes in archaeological fibres

The ATR technique was used to detect the oxidation products of cystine in the S-O vibrational region on the surface of archaeological fibres. While transmission FTIR shows the internal changes in S-O vibrational region and mainly in the cortex and cuticle layers.
The area in the spectra which is of special interest for monitoring the degree of fibre damage is the S-O vibrational region (950 to 1200 cm\(^{-1}\)), which shows the modification of cystine [17].

The FTIR spectra of keratin fibres are usually normalised with a specific peak height to correct for different protein contents, due to mineralisation and fibre degradation, and in order to bring peak amplitudes to a common level. Peak normalisation of keratin fibres can be based on the Amide III region 1232 cm\(^{-1}\), according to Odlyha et al [44], or on the peak at 1451 cm\(^{-1}\) for CH\(_2\) stretching [56]. Normalisation results of archaeological samples and virgin fibres showed that normalisation with the peak at 1232 cm\(^{-1}\) reduces the differences between different spectra for each sample. Furthermore, the normalisation with this wave number will not affect the S-O vibrational area and the Amide I region.

The S-O vibration area of keratin fibres shows overlapping peaks for the oxidation products of cystine as shown in Figure 4.10. To reveal the hidden peaks and to determine their changes, a peak analyser program is applied. There are two semi-quantitative methods which can be used, second derivative and peak deconvolution.

![Figure 4.10: ATR spectra of virgin and bleached human hair (mean of three repeats).](image)

The second derivative method is helpful to identify the peaks of oxidation products, as it shows local minima at the original peak positions as invisible in Figure 4.11, while peak
deconvolution removes the smearing or boarding of peaks arising through the measurement. Both methods give relative measures for the amount of oxidation products.

The main focus of the analysis of the S-O vibrational region is semi-quantification of cystine oxidation products. The comparison of both methods revealed that the deconvolution technique gave less reproducible results than the second derivative method. For this reason the second-derivative method was used for further analysis. This method allows the semi-quantification of cysteic acid and other oxidation products independently of the number of peaks. The second derivative analysis has been used widely [12, 44, 56].

A valley in the second derivative corresponds to the wavenumber of maximum absorbance of a band in the original spectrum. Since the peak depth of the second derivative spectrum is a negative value, the absolute peak depth was employed. The differences between the peak depths are associated with the fission of protein chains by different changes under preservation conditions, causing different amounts of cystine to be oxidised to cysteic acid.

Figure 4.12 shows the progressively increasing absolute peak depths for cysteic acid in virgin and bleached human hair. A slight shift in the peak position (± 2 to 4 wavenumbers units) for cysteic acid and other cystine oxidation products in the second derivative is observed in some archaeological fibres. This is attributed to a change in disulfide conformation as a result of partial disulfide bond rupture [58].
4.3.2.1 ATR technique

4.3.2.1.1 Archaeological fibres from the Iceman site

The identification analysis showed that the Iceman fibres contain human hair and animal fibres (deer and goat hair). It is compared with virgin hair to detect the changes in the S-O vibrational region.

4.3.2.1.1.1 Human hair

Keratin fibres are ion exchangers with many elements which are available in the preservation environment. They undergo silicification by silica minerals [54] containing Si-CH₂CH₃, which shows a vibration band at 1020 to 1000 cm⁻¹, Si-F at 1030 cm⁻¹ and Si-OCH(CH₃)₂ at 1055 to 1030 cm⁻¹ [55]. Also other elements and compounds were found such as glucose in skin at 1030, 1080, 1118 and 1151 cm⁻¹ [51]. In the case of archaeological fibres, which were found embedded in different types of soil, silica oxides were commonly expected to be found at the surface and inside these fibres.
Archaeological Iceman human hair (91/92 B, 91/130 and 91/142) was compared with virgin human hair to study the changes in the S-O vibrational region for detecting the oxidation products of cystine. Figure 4.13 shows the presence of a silica peak at 1030 cm\(^{-1}\), which interferes with the symmetric cysteic acid peak at 1042 cm\(^{-1}\). For that asymmetric cysteic acid peak at 1175 cm\(^{-1}\) is used to give a reliable result to show the chemical changes in the S-O vibrational region.

Figure 4.13: ATR spectra of sample 91/92B, S-O vibrational region, second derivative.

Figure 4.14 shows the decrease in peak height for symmetric cysteic acid at 1042\(^{-1}\) due to the interference of the silica peak as seen in Figure 4.15, which originates from contamination with elements and particles during preservation in the glacier (see chapter 5). Also a slight increase was detected in the peak for asymmetric cysteic acid at 1175 cm\(^{-1}\) in both samples 91/130 and 91/142, respectively, compared with virgin human hair. This result shows that these fibres had fewer changes in the S-O vibrational region compared to sample 91/92B, which showed a large decrease in both symmetric and asymmetric cysteic acid, respectively compared with virgin human hair.

Although cystine oxides (monoxide and dioxide) have been indicated [30], the exact quantities of these intermediate oxidation products vs. cysteic acid were not reported, due to their instability. For this reason cystine oxidation products such as cystine monoxide and cystine dioxide were not used as evidence of change in the S-O vibrational region at the surface of archaeological fibres.
Figure 4.14: ATR. The 2\textsuperscript{nd} derivative peak height for cysteic acid at 1042 and 1175 cm\textsuperscript{-1} for Iceman human hair (mean ± S.E).

Figure 4.15: ATR. The 2\textsuperscript{nd} derivative silica peak height for Iceman human hair fibres (mean ± S.E).
4.3.2.1.2 Animal fibres (deer hair)

The same steps of comparison have been performed for archaeological deer hairs (samples Go II, Go III, Go V, Go VI, Go VII Go VIII, Go IX, Go XI, Go XIII, 91/96 and 91/137). The changes in the S-O vibrational region can be studied by measuring the peak heights for cysteic acid (1042 and 1175 cm\(^{-1}\)) in the 2\(^{nd}\) derivative spectrum in archaeological deer hair and compared with virgin coarse deer hair.

Figure 4.16 shows the presence of cysteic acid at wave numbers 1042 and 1175 cm\(^{-1}\). The results shows that the peak height of cysteic acid at 1042 cm\(^{-1}\) is higher in virgin coarse deer hair compared with archaeological animal fibres. This is attenuated due to the presence of a silica peak as seen in Figure 4.17, because the surface of these fibres is contaminated with different elements and particles (see chapter 5). The peak height of cysteic acid at 1175 cm\(^{-1}\) is higher in most archaeological animal fibres compared to virgin coarse deer hair (except Go XI and Go XIII samples). This is attenuated due to the oxidation process of cystine to cysteic acid under glacier preservation conditions.

Figure 4.16: ATR. The 2\(^{nd}\) derivative peak heights of cysteic acid at 1042 and 1175 cm\(^{-1}\) for Iceman deer hair (mean ± S.E).
4.3.2.1.1.3 Animal fibres (goat hair)

Bielanski [53] showed that silica dioxide $\text{SiO}_2$ accumulates naturally on the surface of virgin wool during growth, as well as through contamination with dust particles. For that silica is expected to be found at the surface of virgin keratin fibres.

Figure 4.18 shows the comparison between sample 91/110 and virgin goat hair. The results show no change for asymmetric cysteic acid at 1175 cm$^{-1}$ and a huge decrease in symmetric cysteic acid at 1042 cm$^{-1}$ due to the effect of the presence of a silica peak at 1030 cm$^{-1}$, because the fibre surfaces are contaminated with silica and different elements (see chapter 5). This result strongly supports the assumption that the surface of these fibres shows actually no changes in the S-O vibrational region.
4.3.2.1.2 Finds from Hadrian’s wall (Vindolanda, UK)

4.3.2.1.2.1 A86, goat hair

To study the changes in the S-O vibrational region, a comparison has been made between A86, goat hair and virgin goat hair as shown in Figure 4.19. The comparison shows a decrease in peak height of cysteic acid at 1042 cm\(^{-1}\) due to the presence of silica oxides, as these fibres were embedded in soil during the preservation period. Also, there was an increase of cysteic acid at 1175 cm\(^{-1}\), which gives evidence that there were oxidation changes at the surface of these fibres.
4.3.2.1.2. Wool, TT/85/38

Archaeological wool, TT/85/38 sample was compared with virgin wool fibres to study the changes in the S-O vibrational region to detect the oxidation products of cystine. The comparison shows the presence of bunte salt and cysteic acid at the surface of virgin wool fibres due to the weathering process, while archaeological wool fibres show the presence of bunte salt and a decrease in peak height or absence of cysteic acid at 1042 and 1175 cm\(^{-1}\) respectively in this sample as seen in Figure 4.20 due to fibre mechanical damage and microbial attack. These results show good agreement with the Cooke and Lomas studies about fibre damage in Vindolanda finds [59].

![Figure 4.20: ATR. The peak heights of cystine oxidation products for virgin wool and TT/85/38 fibres (mean ± S.E).](image)

4.3.2.1.3 Finds from Schloesser Brewery (Düsseldorf, Germany)

4.3.2.1.3.1 Wool 2a fibres

The comparison between sample 2a wool and virgin wool shows the increase of the peak height of cysteic acid at 1042 cm\(^{-1}\) due to the absence of a silica peak effect and a decrease in peak height of cysteic acid at 1175 cm\(^{-1}\), respectively. Also the comparison showed an absence of silica and cystine S-thiosulfate (bunte salt) due to complete conversion to cysteic
acid, in the S-O vibrational region as seen in Figure 4.21. This result gives evidence of changes in the S-O vibrational region at the surface of wool 2a fibres.

![Figure 4.21: ATR. The peak heights of cystine oxidation products for finds from Dusseldorf, Germany (mean ± S.E).](image)

4.3.2.1.3.2 Wool 2b fibres

Archaeological wool 2b fibres were compared with virgin wool fibres to detect the oxidation products of cystine. The comparison showed a slight increase in peak height at 1042 cm\(^{-1}\) and a decrease in peak height at 1175 cm\(^{-1}\), respectively, at the surface of sample fibres as shown in Figure 4.21 due to the presence of changes in the S-O vibrational region.

Also the comparison shows the absence of silica and a decrease in the bunte salt peak height due to the incomplete conversion to cysteic acid.

4.3.2.1.4 Wool fabric from 15\(^{th}\) century (Germany)

Figure 4.22 shows the comparison between virgin and archaeological wool fabric. The comparison shows a decrease in peak height of cysteic acid at 1042 cm\(^{-1}\) due to the presence of silica oxides, because these fibres were embedded in soil during their preservation period. Also there was a decrease of cysteic acid at 1175 cm\(^{-1}\) due to the incomplete conversion of
cystine S-thiosulfate (bunte salt) to cysteic acid, which gives evidence that there were oxidative changes at the surface of these fibres.

Figure 4.22: ATR. The peak heights of cystine oxidation products for archaeological wool fabric (mean ± S.E).

4.3.2.1.5 Bog mummy hair (north Germany)

The comparison between bog mummy hair and virgin human hair shows a decrease in peak height for cysteic acid at 1042 and 1175 cm\(^{-1}\), respectively as shown in Figure 4.23 and also the absence of bunte salt, due to absence of oxidative changes in the S-O vibrational region at the surface of these fibres.
4.3.2.2 Transmission FTIR

The transmission FTIR technique was used to detect the changes in keratin fibres in the S-O vibrational region, which include mainly the cortex (which has a large absolute content of cystine compared with the cuticle layer) and cuticle layers.

The damage in the S-O vibrational region can be studied by measuring the height in the 2nd derivative spectrum of the cystine oxidation products; peak heights such as cysteine-S-thiosulfate (1022 cm$^{-1}$), cysteic acid (1042 and 1175 cm$^{-1}$) for archaeological fibres.

4.3.2.2.1 Archaeological fibres from the Iceman site

4.3.2.2.1.1 Human hair

Archaeological Iceman human hair (samples 91/92B, 91/130 and 91/142) are compared with virgin human hair to detect the damage in the S-O vibrational region. Figure 4.24 shows an increase in peak height of cysteic acid at 1042 (it was lower in virgin human hair and sample 91/142 due to the presence of the silica peak at 1030 cm$^{-1}$) and 1175 cm$^{-1}$, respectively, in archaeological human hair fibres due to the oxidation of cystine to cysteic acid in both cuticle and cortex.
The comparison also shows the absence of S-thiosulfate (bunte salt) except sample 91/142, compared with virgin human hair, due to the complete conversion of bunte salt to cysteic acid.

Figure 4.24: Transmission FTIR. The peak heights of cystine oxidation products and silica for the Iceman human hair (mean ± S.E).

4.3.2.2.1.2 Animal fibres (deer hair)

Figure 4.25 shows a comparison between archaeological Iceman deer hair (samples Go II, Go III, Go V, Go VI, Go VII Go VIII, Go IX, Go XI, Go XIII, 91/96 and 91/137) with virgin deer hair to detect the changes in the S-O vibrational region. The comparison shows an increase in peak heights for cysteic acid at 1042 and 1175 cm\(^{-1}\), respectively, in archaeological deer hairs due to the oxidation of cystine to cysteic acid in both cuticle and cortex.

The comparison also shows the decrease of S-thiosulfate (bunte salt) compared with virgin deer hair due to the incomplete conversion to cysteic acid.
Figure 4.25: Transmission FTIR. The peak heights of cystine oxidation products and silica for the Iceman deer hair (mean ± S.E).

4.3.2.2.1.3 Animal fibres (goat hair)

A comparison between archaeological and virgin goat hair was made to again analyse the changes in the S-O vibrational region. The results shows a decrease in both peaks for cysteic acid at 1042 and 1175 cm\(^{-1}\) as well for bunte salt at 1022 cm\(^{-1}\) in archaeological goat hairs due to high degree of cystine degradation and conversion to hydrogen sulfate in both cuticle and cortex, there is an absence of silica within the fibres as may be seen in Figure 4.26.
Figure 4.26: Transmission FTIR. The peak heights for cystine oxidation products and silica for the Iceman in comparison to virgin goat hair (mean ± S.E).

### 4.3.2.2.2 Finds from Hadrian’s wall (Vindolanda, UK)

#### 4.3.2.2.2.1 A86, goat hair

Figure 4.27 shows an increase in peak height of cysteic acid at 1042 cm⁻¹ (due to the presence of silica at 1030 cm⁻¹) and 1175 cm⁻¹, respectively in archaeological goat hair. This is due to oxidation of cystine to cysteic acid in both cuticle and cortex. The comparison also shows a decrease of S-thiosulfate (bunte salt) compared with virgin goat hair due to the incomplete conversion of bunte salt to cysteic acid.
Figure 4.27: Transmission FTIR. The peak heights for cystine oxidation products and silica for A86, goat hair (mean ± S.E).

4.3.2.2.2 Wool TT/85/38

A comparison between archaeological and virgin wool was made to again detect changes in the S-O vibrational region. Figure 4.28 shows an increase in peak heights of cysteic acid at 1042 and 1175 cm\(^{-1}\), respectively in wool TT/85/38 due to the oxidation of cystine to cysteic acid in both cuticle and cortex. The comparison also shows the decrease of S-thiosulfate (bunte salt) compared to virgin wool due to the incomplete conversion to cysteic acid, with the absence of silica.
Figure 4.28: Transmission FTIR. The peak heights for cystine oxidation products for Wool TT/85/38 (mean ± S.E).

4.3.2.2.3 Finds from Schloesser Brewery (Düsseldorf, Germany)

4.3.2.2.3.1 Wool 2a fibres
The S-O vibrational region of wool 2a fibres was compared with virgin wool fibres. The comparison showed that peak heights of cysteic acid at both wave numbers were lower in the archaeological wool fibres as shown in Figure 4.29 due to high degree of cystine degradation and conversion to hydrogen sulfate in both cuticle and cortex, with no presence of cystine S-thiosulfate and silica.

4.3.2.2.3.2 Wool 2b fibres
The comparison with virgin wool fibres shows that peak heights for cysteic acid at both wave numbers were higher in wool 2b fibres due to the oxidation of cystine in both cuticle and cortex as shown in Figure 4.29. Also the bunte salt peak height was higher compared to virgin wool due to the incomplete cystine oxidation process. Silica was absent.
4.3.2.4 Wool fabric from 15\textsuperscript{th} century (Germany)

Figure 4.30 shows a comparison between wool fabric and virgin wool fibres for the S-O vibrational region. The comparison shows an increase in peak heights of cysteic acid at 1042 and 1175 cm\textsuperscript{-1}, respectively, in the wool fabric fibres, which is attenuated due to the oxidation of cystine to cysteic acid in both cuticle and cortex. The comparison also shows a slight increase of S-thiosulfate (bunte salt) compared with virgin wool, due to the incomplete conversion to cysteic acid and the high levels of silica.
Figure 4.30: Transmission FTIR. The peak heights for cystine oxidation products for wool fabric compared to virgin wool (mean ± S.E).

4.3.2.2.5 Bog mummy hair (north Germany)

A comparison between bog mummy and virgin human hair was made to detect the changes in the S-O vibrational region as seen in Figure 4.31. The comparison shows a large increase in peak heights for symmetric cysteic acid at 1042 cm\(^{-1}\) (due to the absence of the influence of a silica peak) and an increase in asymmetric cysteic acid at 1175 cm\(^{-1}\), respectively, in bog mummy hair. These are due to the oxidation of cystine to cysteic acid at both cuticle and cortex. The comparison also shows the decrease of S-thiosulfate (bunte salt) compared with virgin wool due to the conversion to cysteic acid.
4.3.2.2.3 Comparison between ATR and Transmission FTIR

In ATR spectroscopy the IR radiation does not penetrate the whole keratin fibre, so it can only investigate the cuticle layer and possibly the outer layers of the cortex, depending on the type of fibre. This is because the depth of IR penetration in ATR depends on the refractive index of the crystal and of the sample [56]. In this case IR penetration is about 2 µm when using a diamond crystal. The transmission FTIR technique provides information mainly for the cortex (due to the high percentage of cortex in keratin fibre) and the cuticle layer. For this reason ATR data were adjusted to one hundred percent to enable a comparison between attenuated reflectance and transmission modes of FTIR spectroscopy for detecting oxidation effects in archaeological keratin fibres.

The results of the comparison show that the peak heights of cysteic acid at 1042 cm$^{-1}$ by using ATR and transmission FTIR were less then that at 1175 cm$^{-1}$, due to the influence of the silica peak at 1030 cm$^{-1}$ as shown in Figures 4.32 and 4.33.

Wool TT/85/38 shows the absence of asymmetric cysteic acid at 1175 cm$^{-1}$ by using the ATR technique for unknown reason as seen in Figure 4.33, while all ancient fibres showed increased peak heights of symmetric and asymmetric cysteic acid by using transmission FTIR as seen in Figures 4.32 and 4.33. These results were expected, because transmission FTIR
shows the oxidation changes in both cortex and cuticle of keratin fibres, while ATR shows the oxidation changes of the cuticle layer only.

Figure 4.32: Comparison between Transmission FTIR and ATR. The peak heights for cysteic acid at 1042 cm\(^{-1}\) for selected ancient keratin fibres (mean ± S.E).

Figure 4.33: Comparison between Transmission FTIR and ATR. The peak heights for cysteic acid at 1175 cm\(^{-1}\) for selected ancient keratin fibres (mean ± S.E).
The comparison also showed an increase of bunte salt peak height in transmission FTIR due to the high percentage of cortex compared to cuticle (except for sample A 86, goat hair, where the bunte salt was concentrated only at the surface of the fibre) as shown in Figure 4.34.

![Figure 4.34: Comparison between Transmission FTIR and ATR. The peak height for bunte salt for selected ancient keratin fibres (mean ± S.E).](image)

### 4.4 Conclusions

Both ATR and transmission FTIR techniques may be used for fibre identification and to detect the oxidation products of cystine in the S-O vibrational region of archaeological fibres as a measure for the state of preservation.

#### 4.4.1 Archaeological fibres from the Iceman site

The surface of the Iceman keratin fibres (by using the ATR technique) shows low variation in asymmetric cysteic acid at 1175 cm$^{-1}$ with high variation in symmetric cysteic acid at 1042 cm$^{-1}$. This is due to the absence of the influence of the silica peak at 1030 cm$^{-1}$. The results show that Iceman deer hairs have the highest amount of cysteic acid compared with human hair and goat hair, as a result of cystine oxidation to cysteic acid as invisible in Figures 4.35 and 4.36. This is attenuated to morphological differences between human and goat hair (which has a thick cuticle layer) and deer hair (highly medullated keratin fibres).
Using transmission FTIR showed that Iceman deer hairs have the highest concentration of cysteic acid compared with human hair and goat hair, with less variation at both 1042 and 1175 cm\(^{-1}\), respectively as may be seen in Figures 4.35 and 4.36. This is due to the absence of the influence of the silica peak at 1030 cm\(^{-1}\). Because using transmission FTIR provides information mainly for the cortex, which has no silica and cuticle due to the fact that the accumulation of trace elements can be observed mainly in the cuticle and medulla (see chapter 5) [60].

Figure 4.35: ATR. The peak heights of cysteic acid for different archaeological fibres (a) at 1042 cm\(^{-1}\) (b) at 1175 cm\(^{-1}\) (mean ± S.E).
4.4.2 Finds from Hadrian’s wall (Vindolanda, UK)

Wool, TT/85/38 shows a high degree of cystine oxidation to cysteic acid in both cortex and surface compared with A86, goat hair as may be seen clearly in Figures 4.35 and 4.36. This is due to morphological difference between goat hairs (which have a thick cuticle layer) and wool fibres (which have a thin cuticle layer).
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Vindolanda goat hair had more cysteic acid in both cortex and surface layers at 1175 cm\(^{-1}\) (cysteic acid was lower at 1042 cm\(^{-1}\) due to the influence of the silica peak at 1030 cm\(^{-1}\)) compared with Iceman goat hair (sample 91/110) as may be seen in Figures 4.35 and 4.36. These results give evidence that frozen conditions have less impact on oxidation of cystine compared with the Vindolanda preservation conditions (central Europe climate).

4.4.3 Textiles from Schloesser Brewery (Düsseldorf, Germany)

ATR results for both wool 2a and wool 2b surfaces were similar. Transmission FTIR results for wool 2b show the presence of more cysteic acid at 1042 and 1175 cm\(^{-1}\), respectively, in the cortex layer compared with wool 2a as seen in Figures 4.35 and 4.36. This leads to the conclusion that wool 2b fibres has more chemical changes of cortex.

4.4.4 Wool fabric from 15\(^{th}\) century (Germany)

Wool fabric fibres showed more oxidation changes in the cortex compared to the surface of these fibres as shown in Figures 4.35 and 4.36. The ATR technique can only investigate mainly the cuticle layer, while the transmission FTIR technique provides information for the cortex (due to the high percentage of cortex in keratin fibre) as well as for the cuticle layer.

In addition, the wool fabric showed fewer changes in the S-O vibrational region for both the surface and cortex layers compared with other ancient wool fibres which derived from other preservation conditions. Wool 2b showed the most oxidative changes in the S-O vibrational region, while wool, TT/85/38 and wool 2a were vary in their cysteic acid content compared to other ancient wool fibres as shown in Figure 4.37.
Figure 4.37: The 2nd derivative peak heights of cysteic acid at 1042 and 1175 cm\(^{-1}\) for different ancient wools (a) ATR (b) Transmission FTIR (mean ± S.E).

### 4.4.5 Bog mummy hair (north Germany)

ATR results for bog human hair showed a large decrease in cysteic acid peak height at 1142 cm\(^{-1}\) (due to the influence of the silica peak at 1030 cm\(^{-1}\)) with no change of cysteic acid at 1175 cm\(^{-1}\), compared to virgin human hair. The results of transmission FTIR show an increase of both cysteic acid peak heights at 1142 and 1175 cm\(^{-1}\), respectively, which provide evidence that oxidation changes can be found only in cortex layers in these fibres as seen in Figure 4.37.

ATR results for bog mummy hair and for the Iceman human hair surface show a variation in the peak height of symmetric cysteic acid at 1042 cm\(^{-1}\) (due to the influence of the silica peak at 1030 cm\(^{-1}\)), and nearly the same for the peak height of asymmetric cysteic acid at 1175 cm\(^{-1}\) as seen in Figure 4.38. Bog mummy hair cortex, shows less severe oxidation changes compared with the Iceman human hair.
The surface of all archaeological keratin fibres from different preservation conditions show high variations of cysteic acid at 1042 cm\(^{-1}\) due to the influence of the silica peak at 1030 cm\(^{-1}\). Iceman deer hairs show the highest oxidative changes at 1175 cm\(^{-1}\). Ancient wool fibres (which have a thin cuticle layer) and Iceman deer hair (highly medullated keratin fibres) show the highest oxidative changes at the cortex layer at both 1142 and 1175 cm\(^{-1}\), respectively, compared with other ancient keratin fibres.

The conclusion from the above results is that, the degree of oxidation seems to be highly influenced by the morphology of the fibres and not only by the preservation conditions.
References


Chapter 5 - Energy Dispersive X-ray analysis

5.1 Introduction

Energy dispersive X-ray spectroscopy - EDX is an x-ray technique used for elemental analysis or chemical characterization [1]. This technique is non-destructive and has a sensitivity of >0.1% for elements heavier than carbon. The technique can provide qualitative, semi-quantitative and quantitative analysis, and can also provide spatial distribution of elements through mapping. EDX analysis can be used with several applications including Scanning Electron Microscopy - SEM, Transmission Electron Microscopy - TEM and Scanning Transmission Electron Microscopy - STEM [2].

5.1.1 The principles of Energy dispersive X-ray spectroscopy (EDX)

Many materials are electrically non-conducting. To provide a path for the incident electrons to flow to ground, a surface coating must be applied, e.g. with vacuum-evaporated carbon (~10nm thick). Carbon coating has a minimal influence on X-ray intensities due to its low atomic number and does not add unwanted peaks to the X-ray spectrum [3].

EDX is usually attached to SEM or TEM. The high-energy electron beam is focused onto the carbon coated sample. The incident beam will excite an electron in an inner shell. Ejecting it creates an electron vacancy, which is filled by an electron from the outer shell as seen in Figure 5.1, resulting in the emission of an x-ray to balance the energy difference between the two electrons' energy shells [4]. The atomic structure of the element from which the x-rays are emitted allows the elemental composition of the specimen to be measured. X-ray energy is sensed by the detector and converted into voltage signals, which can be counted and measured by a pulse processor. Then the data are analysed and displayed as a spectrum showing peaks corresponding to the elemental composition of the sample [1, 2].
The EDX technique can provide qualitative analysis (by determining the elements that are present in an 'unknown' specimen by identifying the lines in the X-ray spectrum using tables of energies or wavelengths), and quantitative analysis by counting the number of pulses generated in the detector by X-ray photons, which are emitted randomly from the sample [5].

6.1.2 EDX for the study of keratin fibres

Different types of biopolymers such as proteins, nucleic acids, and carbohydrates can be studied by EDX. Keratin fibres can be subject to EDX analysis, to provide useful information for forensic purposes, to diagnose human diseases, contamination with toxic elements [6, 7], and environmental pollution [7, 8].

Keratin fibres are composed mainly of proteins (88%), which are made of amino acids bonded together by peptide bonds between the carboxyl (-COOH) and amino (-NH₂) groups of adjacent amino acid residues. Each amino acid has functional groups, along with a side-chain (R group). The key elements of keratin fibres are carbon, nitrogen, oxygen, and sulfur (due to the presence of disulfide bonds in cystine residue) [9]. Also keratin fibres contain many trace elements such as silicon, calcium, iron, zinc, magnesium, sodium, potassium, aluminium and other elements [7].

Figure 5.1: EDX - Scheme, simulated trajectories of electrons [5].
The surface of virgin wool had a relative elemental composition of about 80.2% carbon, 17.6% oxygen and 2.2% sulfur (nitrogen was not measured due to its low energy level, which is difficult to detect by EDX) and other trace elements [10, 11]. A key element such as sulphur is not always distributed homogenously across the virgin hair diameter [12] and also shows large variations for different types of keratin fibres. Sulfur can be reduced to hydrogen sulfide, if metal ions are available in metal-sulfide form such as iron sulfide, which produce hydrogen sulfide and iron [13].

There are two types of trace elements in keratin fibres, endogenous elements (those elements taken directly or indirectly from the bloodstream in the body during hair growth by binding to sulfhydryl groups that are present in follicular proteins) and exogenous elements (those originating from external sources such as dust, tap water and cosmetic treatments) [7, 8].

Different endogenous elements in keratin fibres have been detected. Cu, Ca, Fe, Si, Zn, Na, I, Mn, and Mg are examples of these elements, which can be detected after surface contamination removal by washing with distilled water and acetone [14]. The majority of elements are considered to have accumulated longitudinally on the outer surface of the hair above the scalp level. Internally, most elemental signals (especially Al) decrease longitudinally once exposed to the environment, with the exception of Si, which showed an increase [15]. The accumulation of these elements can be observed in the cuticle and medulla (due to the protein structure and its chemistry rather than aggregation in medulla voids or associations with other features such as melanin) regions rather than the cortex [15].

The concentration of endogenous elements shows variations in distribution from the scalp to the tip of the hair depending on age, sex, nutrition, the position of the hair itself and the influence of the environment [8, 12, 16]. This is due to the movement of elements through the inside of the hair from the scalp to the tip as well as to weathering of the hair surface by the environment (washing or combing) over a long period of time [8]. Grace [17] showed that there is no effect of high Zn intakes on Ca, Cu, Fe, K, Mg, Mn, Na, P, S content for a 12 months old wool fleece.

Exogenous elements in keratin fibres can be absorbed from air, solutes present in washing or swimming water and hair treatments used for cosmetic, medical or hygienic purposes. These Elements are deposited directly onto the hair surface and can easily be removed by washing.
Bate and Dyer [19] reported that large amounts of Au, Hg, Zn, Cu, Fe, Cr, Co, Mn, Ba and Sr are absorbed by hair in simulated perspiration at pH 5.5. Samarasinghe [20] showed that Cu\(^{2+}\) ions were found to show the greatest absorption by wool fibres, whereas Zn\(^{2+}\) and Mn\(^{2+}\) were found to be absorbed the least from the environment. Brady et al [21] suggested that keratin fibre beds could be used for removing low concentrations of mercuric ions from waste waters with short contact times. Bed capacity was about 0.3-0.6 mg/g depending on the pH conditions. He isolated mercuric ions from brine solutions at high pH by filtration through beds of unmodified wool. Also he used the wool absorption capacity to separate low concentrations of copper contaminants from nickel solutions. In acidic conditions, chromium (III) salts can be detected in wool fibres [22]. All these publications show that keratin fibres are able to absorb ions from the environment easily.

The outer surface of each cuticle cell of keratin fibres contains a fatty layer of 18-methyleicosanoic acid (18-MEA) and is connected to the epicuticle protein structures by covalent thioester linkages [23, 24]. The presence of this fatty layer makes the surface of wool hydrophobic. Therefore the water absorption properties are hindered. Damage of the fatty acid layer causes changes in the hydrophilic property of keratin fibres [24] by increasing the interaction with other molecules such as metal ions in the surrounding environment [25]. Especially the polar groups of amino acid in proteins of wool fibres are able to bind other charged ions, salts, acids and bases [11]. Also the carboxylic acid groups can interact with many other cationic compounds such as silver ions [25].

Damage due to oxidation and reduction reactions can cause breakdown of the cell membrane complex and oxidation reactions can be induced by cleavage of cystine disulfide bonds [26]. This leads to lower molecular weights, reduces the sulfur content of the proteins [27] and decreases the strength of the cuticle layer [28]. In alkaline conditions, the relative sulphur content in the fibre surface can be reduced, due to the removal of cuticle-associated high cystine proteins, as significant surface damage [10].
5.1.3 Studying archaeological keratin fibres

Archaeological keratin fibres have been found under different preservation conditions. These fibres were found embedded in different types of media and exposed to humidity, extreme temperatures and microbial attacks [29] and mechanical pressing by the weight of soil [30]. These conditions cause degradation, mineralisation and severe damage to these fibres. This affects their physical and chemical structure and makes fibre identification, determination of chemical composition and other laboratory analyses a difficult task [29, 31, 32].

Due to different preservation conditions, archaeological fibres have lost their natural pigmentation such as the Vindolanda archaeological textiles [33, 34], and exhibit shades of brown [29, 33] due to immersion in soil. The main factors that affect the adhesion between soil and keratin fibres are (a) the cohesive properties of soil and (b) the shear resistance of the soil, due to the surface form and roughness of the fibres [35]. Many particles from the soil of various sizes, including dust and minerals, can be detected at the fibre’s surface. These minerals include metal salts of iron, copper, bronze and other elements [36].

Aggregation of metal salts on the fibre surface and inside the fibre structure cause mineralisation, which leads to preserved keratin fibres as the corrosion proteins of these metals were toxic to micro-organisms so that microbiological degradation is inhibited [37, 35]. Mineralisation processes can occur in positive casts (minerals form within the fibre) and negative casts (minerals deposited on the fibre surface) [38]. The processes depend on metal concentration, soil pH, temperature and moisture [37, 35].

Changes in elemental composition in ancient keratin fibres are expected as a result of leaching, ion exchange, biological activity and/or precipitation of insoluble inorganic elements [13]. Mansilla et al [39] observed absorption of sodium and calcium from adhered minerals in mummy hair causing preservation of the mummy hair with less ordered scales and a rough surface. Hallegot et al [40] found calcium ions in ancient hair obtained from mummified remains. Grime et al [41] showed line profiles of S, Cu and As in Iceman hair. These results show clearly the difference between the surface concentration of Cu and the internal concentration of As, which are associated with the production of copper artefacts (As is a common constituent of copper ores).
Bielanski [42] stated that silicon dioxide $\text{SiO}_2$ accumulates on the surface of virgin wool during growth. Silica minerals [43, 44] such as $\text{Si-CH}_2\text{CH}_3$ can be found on the surface of organic materials that have been embedded in soil [45]. Kempson et al. studied the changes of element composition in ice mummy human hair -KDT- (it was subject to a highly variable environment with possible periods of drying and certainly a series of freeze–thaw events). He showed the presence of excess elemental (Al, Si, Fe and Ni) concentrations, which may result from mineralisation at the hair surface [13].

5.1.4 Objectives

The objective of this part of the project is to apply energy dispersive x-ray analysis to determine the absorption of trace elements (especially the presence of silica, due to the presence of a silica peak in the FTIR spectrum at 1030-1033 cm$^{-1}$, that influences the cysteic acid peak at 1042 cm$^{-1}$) at the surface and inside the archaeological fibres from different preservation sites.

5.2 Experimental work

5.2.1 Sample preparation

Due to the nonconductive nature of keratin fibres, cleaned archaeological and virgin fibres were coated with conductive carbon. The samples were vacuum sputter coated with a thin layer of carbon (~3nm) and kept in high vacuum (~$10^{-2}$ Torr) for 5 min by using GATAN PECS. (Oxford, UK).

5.2.1 Preparation of cross sections

To investigate the trace elements content of the cortex and medulla, EDX of fibre cross sections was performed. In most cases only few ancient fibres were available. These fibres also showed reduced strength which made a simple bundle technique with a hand microtome a difficult task. For that, cross sectional EDX analysis was obtained for two archaeological samples (sample 91/92B human hair and wool 2b) by a modified technique.

Cross sections of single ancient keratin fibres were obtained by embedding them in a bundle of synthetic fibres (which have a much smaller diameter compared with ancient fibres).
Because of the difference in diameter, cross sections of keratin fibres can easily be distinguished from synthetic fibres as illustrated in Figure 2.5.

![Figure 5.2: Cross section of ancient and synthetic fibres x600.](image)

The fibre bundle is pulled through a heat shrinkable tube very tightly. After shrinking the tube on a hot plate, one end of the tube was cut straight. Some collodion was placed on the cut end to stick the fibre together. After drying, thin sections were cut with a razor blade on a sample holder. It is important that the first section has to be discarded because the fibre ends were covered with glue.

### 5.2.3 EDX analysis

The elemental composition was obtained by scanning electron microscopy XL 30 (Philips). Three repeats (spots) were obtained for each sample at a 10 kV accelerating voltage, 3x spot size and 250 Amp probe current. The penetration of the beam is ~7.5µm and the diameter of covered area is ~1.5µm. Also single particle measurements were obtained for each sample.
5.2.4 Scanning Electron Microscopy (SEM)

SEM images were obtained by scanning electron microscopy on a ZEISS EVO 60 (Cambridge, UK) at 5 kV accelerating voltage and 250 Amp probe current.

5.3 Results and discussion

Using EDX provides information about the trace elements’ composition on the archaeological keratin fibre surfaces (the trace elements contained were normalised to one hundred percent after subtraction of the key protein elements) by comparing them with virgin keratin fibres to determine any changes occurring at the surface. The penetration of the beam is \( \sim 7.5\mu m \) and the diameter of the covered area is \( \sim 1.5\mu m \) for each measurement.

The results of measurements for all ancient keratin fibres showed a large diversity of trace element content in the subsamples due to variation between the measuring spots along the fibre surface (the graphs just shows the trace element content, because all keratin fibres contain C, N, O and S).

5.3.1 Archaeological fibres from the Iceman site

5.3.1.1 Human hair

EDX analysis of virgin human hair and archaeological human hair (samples 91/92, 91/130 and 91/142) was used to determine the trace element composition changes at the surface of these fibres. Figure 5.3 shows the diversity of the trace element content in the subsamples due to variation between the measuring spots along the fibre surface.

The comparison seen in Figure 5.4 shows that Si, P, Fe and Ca are the main elements that are found in the cuticle layer of these archaeological fibres. These result from mineralisation at the hair surface, which were adsorbed from the surrounding environment. The presence of Si is in good agreement with the FTIR result as shown in chapter 4, where the presence of a silica peak was detected at 1030 cm\(^{-1}\), which influenced the cysteic acid peak at 1042 cm\(^{-1}\).

Also there was a decrease of Mg content (except for sample 91/142) due to leaching from the original hair into the melt water. Na and K content were higher in the virgin human hair,
which may be due to the influence of modern factors such as sweating artefacts and washing with shampoo [13].

Figure 5.3: Sample 91/92B. The diversity of relative trace element contents in subsamples.

Figure 5.4: Relative content of trace elements (normalised to 100%) at the surface of archaeological Iceman human hair (mean ± standard error).

Single particle measurements were obtained to give an idea about the expected element contents for the surrounding environment at particular archaeological sites as listed in Table 5.1.
Table 5.1: Relative content (in%) of different elements for a particle at the surface of the Iceman human hair (single measurement).

<table>
<thead>
<tr>
<th></th>
<th>C</th>
<th>N</th>
<th>O</th>
<th>S</th>
<th>Si</th>
<th>Ca</th>
<th>Na</th>
<th>Mg</th>
<th>P</th>
<th>Al</th>
<th>Fe</th>
<th>K</th>
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<td>Virgin human hair</td>
<td>49.54</td>
<td>24.70</td>
<td>15.98</td>
<td>8.74</td>
<td>0.20</td>
<td>0.69</td>
<td>0.09</td>
<td>0.06</td>
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</tr>
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<td>16.91</td>
<td>13.57</td>
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<td>0.00</td>
<td>0.02</td>
<td>0.08</td>
<td>0.05</td>
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<tr>
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<td>53.72</td>
<td>21.86</td>
<td>15.15</td>
<td>6.05</td>
<td>0.00</td>
<td>0.74</td>
<td>0.00</td>
<td>0.04</td>
<td>0.02</td>
<td>2.17</td>
<td>0.17</td>
<td>0.11</td>
<td></td>
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<td>Sample 91/142</td>
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<td>17.59</td>
<td>36.42</td>
<td>6.09</td>
<td>3.56</td>
<td>0.12</td>
<td>1.13</td>
<td>3.43</td>
<td>0.34</td>
<td>0.53</td>
<td>0.70</td>
<td>0.16</td>
<td>0.37</td>
</tr>
</tbody>
</table>

To find out whether there is a difference between the surface adsorption and absorption of trace elements inside the fibre, a cross sectional comparison between virgin human hair and sample 91/92B was made. Figure 5.5 shows sample 91/92B fibres (with a large diameter), which are embedded in a bundle of synthetic fibres (with small diameter). From the difference in diameter, cross sections of ancient fibres were easily distinguished from synthetic fibres.

![Figure 5.5: Cross sections: (a) sample 91/92B (x500); (b) virgin human hair (x200).](image)

The comparison between virgin human hair and sample 91/92B shows an increase of Ca and K ions in sample 91/92B due to element absorption from the environment as shown in Figure
5.6. This conclusion is supported by the low content of Ca and the absence of K at the surface of these fibres due to fibre cleaning.

Figure 5.6: Relative content of trace elements (normalised to 100%) in the cross section of sample 91/92B (mean ± standard error).

A comparison between cross section and surface element content for virgin and Iceman human hair was made as seen in Figure 5.7. For virgin human hair, large amounts of different endogenous elements such as Si, P, Na, Al and Mg were detected in the cross section compared to the surface. This is due to the fact that these elements accumulate in the cuticle and the medulla of keratin fibres [15], and are removed from the surface by daily hair washing.

Figure 5.7 shows the comparison between the cross section and the surface elements content for sample 91/92B. The comparison shows the presence of Si, Na, Al and an excess of P, Mg, Ca and K due to absorbance of these elements from the surrounding environment.
Chapter 5  
Energy Dispersive X-ray analysis

Figure 5.7: Relative content from the Iceman find of trace elements (normalised to 100%) (a) virgin human hair (b) sample 91/92B (mean ± standard error).

5.3.1.2 Animal hair (deer hair)

EDX measurements of Iceman deer hair (GO II, GO III, GO V, GO VI, GO VII, GO VIII, GO IX, GO XI, GO XIII, 91/96 and 91/137) also show strong diversity of trace element content in subsamples due to variation between the measuring spots along the fibre surface as seen in Figure 5.8.

Table 5.2 shows a comparison of trace element content between Iceman deer hair and virgin deer hair. Si, P, Al, Mg and Fe are the main elements that were found at the surface of the ancient deer hair, due to absorbance from the surroundings. Na is found only in GO VIII and
sample 91/92B, which could be due to an artefact from sweat (after touching the fibres with unprotected hands). Ca and K were found only in sample 91/96. These results show good agreement with FTIR results (see chapter 4) for the influence of presence of a silica peak which affects the cysteic acid peak at 1042 cm\(^{-1}\). For that single particle elements content was obtained to give an idea about the surrounding environment as listed in detail in Table 5.3.

Table 5.2: Relative content of trace element at the surface of Iceman deer hair (mean of three measurements and standard errors S.E).

<table>
<thead>
<tr>
<th></th>
<th>Si</th>
<th>S.E</th>
<th>P</th>
<th>S.E</th>
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<th>S.E</th>
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<th>S.E</th>
<th>Fe</th>
<th>S.E</th>
<th>Ca</th>
<th>S.E</th>
<th>K</th>
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<td>Virgin deer hair</td>
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<td>0.00</td>
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<tr>
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<td>3.64</td>
<td>32.56</td>
<td>10.04</td>
<td>21.46</td>
<td>3.91</td>
<td>25.68</td>
<td>12.56</td>
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<td>GO V</td>
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<td>5.62</td>
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<td>85.43</td>
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</table>

Table 5.3: Relative content of different element for a particle at the surface of Iceman deer hair (single measurement).

<table>
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<tr>
<th></th>
<th>C</th>
<th>N</th>
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<th>S</th>
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<td>13.28</td>
<td>10.01</td>
<td>49.21</td>
<td>0.89</td>
<td>12.72</td>
<td>1.80</td>
<td>0.30</td>
<td>7.63</td>
<td>2.03</td>
<td>1.28</td>
<td>0.84</td>
<td></td>
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<tr>
<td>GO V</td>
<td>55.77</td>
<td>27.44</td>
<td>10.86</td>
<td>5.49</td>
<td>0.18</td>
<td></td>
<td>0.11</td>
<td>0.16</td>
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<td></td>
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</tr>
<tr>
<td>GO VI</td>
<td>5.24</td>
<td>3.68</td>
<td>53.38</td>
<td>0.15</td>
<td>17.45</td>
<td>0.59</td>
<td>1.19</td>
<td>0.16</td>
<td>14.24</td>
<td>3.93</td>
<td></td>
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<td></td>
</tr>
<tr>
<td>GO VII</td>
<td>52.52</td>
<td>11.52</td>
<td>31.14</td>
<td>0.53</td>
<td>0.28</td>
<td>0.72</td>
<td>0.57</td>
<td>0.27</td>
<td>1.25</td>
<td>0.75</td>
<td>0.45</td>
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<td></td>
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<tr>
<td>GO VIII</td>
<td>11.18</td>
<td>6.55</td>
<td>51.71</td>
<td>1.16</td>
<td>11.20</td>
<td>1.88</td>
<td>0.79</td>
<td>0.27</td>
<td>10.32</td>
<td>2.95</td>
<td>1.98</td>
<td></td>
<td></td>
<td></td>
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<td>GO IX</td>
<td>32.41</td>
<td>23.11</td>
<td>33.19</td>
<td>9.85</td>
<td>0.17</td>
<td>0.10</td>
<td>0.31</td>
<td>0.27</td>
<td>0.16</td>
<td>0.32</td>
<td>0.11</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>GO XI</td>
<td>49.08</td>
<td>14.76</td>
<td>30.99</td>
<td>4.18</td>
<td>0.05</td>
<td>0.10</td>
<td>0.02</td>
<td>0.06</td>
<td>0.02</td>
<td>0.48</td>
<td>0.26</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>GO XIII</td>
<td>32.33</td>
<td>10.24</td>
<td>35.70</td>
<td>3.53</td>
<td>7.54</td>
<td>0.07</td>
<td>0.14</td>
<td>0.33</td>
<td>0.05</td>
<td>5.63</td>
<td>2.59</td>
<td>1.86</td>
<td>0.10</td>
<td></td>
</tr>
<tr>
<td>Sample 91/96</td>
<td>9.66</td>
<td>0.00</td>
<td>50.06</td>
<td>0.59</td>
<td>16.78</td>
<td>0.09</td>
<td>1.65</td>
<td>0.80</td>
<td>0.24</td>
<td>15.36</td>
<td>1.21</td>
<td>3.45</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sample 91/137</td>
<td>27.45</td>
<td>46.73</td>
<td>2.22</td>
<td>11.40</td>
<td></td>
<td></td>
<td>0.18</td>
<td>9.83</td>
<td>2.18</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
5.3.1.3 Animal hair (goat hair)

Figure 5.9 shows that Al, Na and Fe are increased at the surface of sample 91/110 (Iceman goat hair) compared with virgin goat hair, due to the mineralisation of these fibres. Cl and K were absent from the surface of ancient fibres, which could be due to leaching from the original hair into the melt water.

![Figure 5.9: Relative content of trace elements (normalised to 100%) at the surface of sample 91/110 (mean ± standard error).](image)

5.3.2 Finds from Hadrian’s wall (Vindolanda, UK)

5.3.2.1 A86, Goat hair

The comparison between virgin and sample A86 goat hair shows the presence of Si (which supports the results of ART analysis) and an increase of Al and Fe at the surface of the ancient fibres due to absorbance from the surroundings as shown in Figure 5.10. Cl and K are absent from the surface of ancient fibres, which can be due to leaching, ion exchange, biological activity and/or precipitation of insoluble inorganic elements [13], as well as fibre cleaning.
Figure 5.10: Relative content of trace elements (normalised to 100%) at the surface of A86 goat hair and virgin goat hair (mean ± standard error).

5.3.2.2 Wool, TT/85/38

The comparison was mainly between archaeological and virgin wool fibres. Mainly silicon was detected at the surface of wool TT/85/38 fibres, due to absorbance from the surrounding environment as shown in Figure 5.11. This result was expected, because these fibres had undergone mechanical damage and microbial attacks, including flattening of the fibre (due to losing the cortex layer), loss of cuticle scales in some fibres, fibrillation and fibre fracture (see chapter 3). Also the fibres were selected from the yarns in a piece of ancient textile, which makes the element absorbance less. No other elements were detected at the surface of these fibres, especially since the accumulation of these elements can be observed in the cuticle and medulla regions rather than the cortex [15]. Also these results show a good agreement with FTIR result (see chapter 4), when the presence of a silica peak at 1030 to 133 cm\(^{-1}\) was detected.
5.3.3 Textiles from Schloesser Brewery (Düsseldorf, Germany)

Microscopical analysis showed extensive mechanical damage in Dusseldorf wool fibres including longitudinal fractures and loss of more than half the fibre body in many fibres due to poor preservation conditions in the latrine pit (embedded in soil) as shown in chapter 3.

5.3.3.1 Wool 2a fibres

Figure 5.12 shows the trace elements content of wool 2a fibres. Silicon content is substantially increased at the surface of these fibres, due to absorbance from the surrounding environment that has a variety of elements, an example is shown in Table 5.4. P and Na are decreased with the absence of Ca and K due to leaching, ion exchange, biological activity and/or precipitation of insoluble inorganic elements [13] as well as through fibre cleaning.
Figure 5.12: Relative content of trace elements (normalised to 100%) at the surface of textiles from the old town in Düsseldorf fibres compared to virgin wool (mean ± standard error).

Table 5.4: Relative content of different elements for a particle at the surface of textiles from the old town in Düsseldorf fibres compared to virgin wool (single measurement).

<table>
<thead>
<tr>
<th></th>
<th>C</th>
<th>N</th>
<th>O</th>
<th>S</th>
<th>Si</th>
<th>Ca</th>
<th>Na</th>
<th>Mg</th>
<th>P</th>
<th>Al</th>
<th>Fe</th>
<th>K</th>
<th>Cl</th>
</tr>
</thead>
<tbody>
<tr>
<td>Virgin wool fibres</td>
<td>46.04</td>
<td>20.37</td>
<td>28.82</td>
<td>3.36</td>
<td>0.11</td>
<td>0.11</td>
<td>0.36</td>
<td>0.01</td>
<td>0.08</td>
<td>0.03</td>
<td>0.33</td>
<td>0.38</td>
<td></td>
</tr>
<tr>
<td>Wool 2a</td>
<td>16.33</td>
<td>0.00</td>
<td>38.73</td>
<td>0.15</td>
<td>34.14</td>
<td>0.90</td>
<td>4.03</td>
<td>0.02</td>
<td>0.13</td>
<td>5.35</td>
<td>0.22</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Wool 2b</td>
<td>46.15</td>
<td>20.35</td>
<td>20.07</td>
<td>7.11</td>
<td>0.21</td>
<td>2.08</td>
<td>0.72</td>
<td>0.68</td>
<td>0.15</td>
<td>2.46</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

5.3.3.2 Wool 2b fibres

Figure 5.12 and Table 5.4 show large differences between the element contents adsorbed from the environment for samples wool 2a and wool 2b. This result is also supported by SEM images for the surface of these samples, which show that the wool 2a fibres are totally incrusted due to cuticle loss as seen in Figure 5.13. These results were expected due to the fact that excavation areas at old town of Dusseldorf were divided into several sections depending on specific archaeological periods. The findings groups are identified by numbers, and then specified separately with letters depending on the digging depth. There were 16 findings related to latrine pits at area no.9, which dated between the second half of the 17th century and the early 18th century [46].
Figure 5.13: SEM images: (a) wool 2a (b) wool 2b (x10000)

Wool 2b fibres show the presence of Ca. This was expected due to the preservation in a latrine pit (embedded in soil) as listed in Table 5.4. A decrease in P and Na and the absence of Si, Al, Mg and K are observed because these fibres had undergone mechanical damage and microbial attack that destroyed the cuticle layer as shown in chapter 3, where these elements are located [15] as invisible in Figure 5.12. These results show good agreement with FTIR result (see chapter 4), where the absence of the influence of the silica peak at 1030 to 1033 cm$^{-1}$ on the cysteic acid peak at 1042 cm$^{-1}$ was noticed.

To find out if there is a difference between the surface adsorption and absorption of trace elements inside the fibre, the cross sectional comparison between virgin wool and wool 2b was made. SEM images for the cross sections show the irregular edges of wool 2b compared with virgin wool, due to mechanical damage and microbial attack as shown in Figure 5.14.
Figure 5.14: SEM images of the cross section: (a) wool 2b (x550); (b) virgin wool (x200).

Also the comparison shows that Si, P, Al and Ca are observed for the cross section of wool 2b fibres, due to element absorbance from the environment as shown in Figure 5.15 and in Table 5.4.

Figure 5.15: Relative content of trace elements (normalised to 100%) in the cross section of wool 2b compared to virgin wool (mean ± standard error).
A comparison between the cross sections and the surface element content for virgin wool and wool 2b was made as in Tables 5.5 and 5.6. In virgin wool, large amounts of different endogenous elements such as Si, P, Na, Al, Mg and K were detected at the surface compared with the cross section. This is due to the fact that these elements accumulate in the cuticle and medulla of keratin fibres [15]. Wool 2b showed an excess of Si, Al, Mg and K in the cross section due to the absorbance of these elements from the environment and damage to the surface of these fibres.

Table 5.5: Trace element contents in the cross section and at the surface of virgin wool (mean of three measurements and standard error).

<table>
<thead>
<tr>
<th>Virgin wool</th>
<th>Si</th>
<th>S.E</th>
<th>P</th>
<th>S.E</th>
<th>Na</th>
<th>S.E</th>
<th>Al</th>
<th>S.E</th>
<th>Mg</th>
<th>S.E</th>
<th>K</th>
<th>S.E</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cross section</td>
<td>0.18</td>
<td>0.11</td>
<td>0.09</td>
<td>0.01</td>
<td>0.23</td>
<td>0.12</td>
<td>0.18</td>
<td>0.10</td>
<td>0.50</td>
<td>0.10</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Surface</td>
<td>22.16</td>
<td>4.55</td>
<td>7.32</td>
<td>2.89</td>
<td>24.95</td>
<td>5.69</td>
<td>2.38</td>
<td>2.38</td>
<td>7.89</td>
<td>7.38</td>
<td>35.35</td>
<td>13.36</td>
</tr>
</tbody>
</table>

Table 5.6: Trace element contents in the cross section and at the surface of wool 2b (mean of three measurements and standard error).

<table>
<thead>
<tr>
<th>Wool 2b</th>
<th>Si</th>
<th>S.E</th>
<th>P</th>
<th>S.E</th>
<th>Na</th>
<th>S.E</th>
<th>Al</th>
<th>S.E</th>
<th>Mg</th>
<th>S.E</th>
<th>K</th>
<th>S.E</th>
<th>Ca</th>
<th>S.E</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cross section</td>
<td>0.51</td>
<td>0.42</td>
<td>0.82</td>
<td>0.74</td>
<td>0.10</td>
<td>0.07</td>
<td>0.20</td>
<td>0.13</td>
<td>0.11</td>
<td>0.05</td>
<td>0.23</td>
<td>0.06</td>
<td>2.64</td>
<td>1.75</td>
</tr>
<tr>
<td>Surface</td>
<td>0.38</td>
<td>0.20</td>
<td>2.16</td>
<td>1.55</td>
<td>0.84</td>
<td>0.66</td>
<td>0.16</td>
<td>0.16</td>
<td>96.55</td>
<td>1.86</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

5.3.4 Wool fabric from 15th century (Germany)

Figure 5.16 shows the trace element contents of ancient wool fabric compared with virgin wool. The comparison shows the presence of Si, which supports the FTIR result as shown in chapter 4. Also the comparison shows an increase of P, Ca, and Fe content due to mineralisation at the ancient wool surface, also a decrease in Si, Na and K as a result of leaching, ion exchange, precipitation of insoluble inorganic elements [13], as well as fibre cleaning.
5.3.5  Bog mummy hair (north Germany)

Bog mummy hair shows a diversity of trace element content in subsamples between measuring spots along the fibre surface (repeat 3 shows a presence of proteinous elements and an absence of trace elements, but it was used during calculations to maintain the consistency of the results) as seen in Figure 5.17. Si was detected at the surface of these fibres which supports the FTIR results (see chapter 4). Also, there was an excess of P, Al and Mg compared to virgin human hair, due to absorption and mineralisation of these fibres as an effect of the environment as shown in Figure 5.18. The presence of Ca and K just at the surface of virgin human hair is attenuated to the influence of modern factors such as washing with shampoo. For this reason only single element content was determined to give an idea about the surrounding environment as shown in Table 5.7.
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Figure 5.17: Bog mummy hair. The diversity of trace element contents in subsamples. Repeat 3 shows an absence of trace elements.

Figure 5.18: Relative content of trace elements in the surface of bog mummy hair compared to virgin human hair (mean ± standard error).

Table 5.7: Relative content of different elements for a particle at the surface of bog mummy hair (single measurement).

<table>
<thead>
<tr>
<th></th>
<th>C</th>
<th>N</th>
<th>O</th>
<th>S</th>
<th>Si</th>
<th>Ca</th>
<th>Na</th>
<th>Mg</th>
<th>Al</th>
</tr>
</thead>
<tbody>
<tr>
<td>Virgin human hair</td>
<td>49.54</td>
<td>24.70</td>
<td>15.98</td>
<td>8.74</td>
<td>0.20</td>
<td>0.69</td>
<td>0.09</td>
<td>0.06</td>
<td></td>
</tr>
<tr>
<td>Bog mummy</td>
<td>27.33</td>
<td>21.83</td>
<td>44.89</td>
<td>3.86</td>
<td>1.04</td>
<td></td>
<td></td>
<td></td>
<td>1.05</td>
</tr>
</tbody>
</table>
5.4 Conclusions

5.4.1 Archaeological fibres from the Iceman site

Iceman goat hair shows in comparison to the highly medullated fibres (deer hair) a lower absorption of most ions more comparable to Iceman human hair. Ca and K show the lowest concentrations that were absorbed by Iceman deer hair as shown in Figure 5.19. These elements were detected in the cuticle layer of these archaeological fibres as a result of mineralisation.

![Figure 5.19: Relative content of trace elements (which is set to 100%) at the surface of Iceman keratin fibres, human hair (blue), deer hair (brown) and goat hair (yellow) (mean).](image)

5.4.2 Finds from Hadrian’s wall (Vindolanda, UK)

Goat hair from Vindolanda shows strong absorption of many ions compared with wool TT/85/38, which showed only large amounts of Si at the surface of these fibres as shown in Figure 5.20. This result is due to mechanical damage and microbial attack, which reduce the element absorbance.
Figure 5.20: Relative content of trace elements (normalised to 100%) at the surface of Vindolanda keratin fibres (mean ± standard error).

Figure 5.21 shows a comparison between virgin and different ancient goat hairs. Si is found in the virgin goat hair as well as in the archaeological fibres. Fe is the only element that can be found in both Iceman goat hair (sample 91/110) and Vindolanda goat hair (A86, goat hair).

Figure 5.21: Relative content of trace elements (normalised to 100%) at the surface of Iceman goat hair (red), Vindolanda goat hair (green) compared to virgin goat hair (mean ± standard error).
5.4.3 Textiles from Schloesser Brewery (Düsseldorf, Germany)

Wool 2a shows an excess in Si content, while wool 2b shows an excess of Ca at the surface of these fibres. This is due to differences of mechanical damage between them as a result of the difference of the depth location in every section of the historical latrine pit.

5.4.4 Wool fabric from 15th century (Germany)

Wool fabric shows the presence of Si and an excess of P, Mg, and Fe at the surface due to mineralization. Comparing the trace element content for different ancient wools, all fibres showed Si in various concentrations. Wool TT/85/38 from the Vindolanda and wool 2a from the latrine pit in Düesseldorf show very high concentrations of Si as seen in Figure 5.22.

![Figure 5.22: Relative content of trace elements (which is set to 100%) at the surface of different ancient wool (wool TT/85/38 from Vindolanda, wool 2a and wool 2b from the latrine pit) (mean).](image)

5.4.5 Bog mummy hair (north Germany)

Bog mummy hair shows the presence of Si, Al and Na, with an excess of P and Mg at the surface of these fibres compared with virgin human hair. This is due to the absorption and mineralisation of these fibres as an effect of the environment.
Figure 5.23 shows a comparison of the trace element content between virgin, Iceman and bog human hair. Al, Mg, Na were found in all hair samples. Si and P were found only in the archaeological hair samples (Iceman and bog hair), while Fe was only found in the Iceman human hair. Also the comparison shows the absence of Ca in the bog mummy hair. The presence of Ca and K in virgin hair is attributed to cosmetic treatments.

![Figure 5.23: Relative content of trace elements (which is set to 100%) at the surface of virgin and different ancient human hair (Iceman find and bog find) (mean ± standard error).](image)

In general, Iceman keratin fibres (deer hair not shown in the graph due to its higher scale) were the fibres, which were most affected by the preservation environment, due to the enrichment of trace elements that were detected at the surface of these fibres, and which had been absorbed from environment. All keratin fibre samples from different preservation sites show the presence of Si in different concentrations. Also, all element concentrations were similar with the exception of wool sample 2b, while no Fe and Ca were detected at the surface of bog mummy hair as seen in Figure 5.24.
Figure 5.24: Relative content of trace elements (which is set to 100%) at the surface of different ancient keratin fibres. Iceman human hair (green), A86, goat hair from Vindolanda (yellow) and wool 2a and wool 2b from the latrine pit (red) (mean).

*(Trace element content for deer hair is not shown in the graph due to its higher scale).*
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Chapter 6 - Thermal analysis

6.1 Introduction

Bio-macromolecules such as proteins, lipids and nucleic acids in aqueous solution are in equilibrium between the native (folded) conformation and the denatured (unfolded) conformation. Differential Scanning Calorimetry (DSC) is a technique that is used to study thermodynamic properties of biological molecules by controlled temperature changes [1]. In addition, DSC can be used to study the degree of crystallinity in different polymers [2], which can be employed to answer many question in scientific research and in the field of civil or criminal law [3].

6.1.1 Differential Scanning Calorimetry (DSC)

The DSC instrument provides a measurement tool for the heat absorbed or evolved from the sample when heated. DSC can be classified into two types: heat-flux DSCs and power-compensated DSC. In a heat flux DSC, both sample material pan and an empty reference pan are placed on a thermoelectric disk surrounded by a furnace. They are heated by the thermoelectric disk. There would be a temperature difference between the sample and reference pans, which is measured by area thermocouples. In a power-compensated DSC, the sample and reference pans are placed in separate furnaces heated by separate heaters at the same temperature. The difference in thermal power required to maintain them at the same temperature is measured and plotted as a function of temperature or time [4, 5] as shown in Figure 6.1.
Using the DSC technique one can determine: the denaturation temperature – $T_D$ (the temperature where 50% of the polymer is in its folded conformation and the other 50% is unfolded), the denaturation enthalpy - $\Delta H_D$ and the moisture content depending on the melting temperature [4].

### 6.1.2 Thermal Stability of Polymers

There are different types of natural polymers such as proteins, carbohydrates and lipids. These polymers can undergo several physical and structural changes when they are heated, accompanied by endothermic or exothermic changes during thermal measurement.

#### 6.1.2.1 Thermal Stability of Proteins

Proteins have complex structures. Through the application of heat the protein structure can be partial or totally denaturated, by unfolding it through breaking the hydrogen bonds without any change to covalent bonds in the polypeptide backbone of the molecule as illustrated in Figure 6.2 [7]. The denaturation process can be reversible or irreversible. In the case of proteins temperatures above 45°C are generally considered to lead to irreversible denaturation.
In the case of hair proteins, the helical domains of the IFs in the protein unfold to random coils. This leads to a change in the molecular conformation, which results in a more flexible, disorganised, opened polypeptide chain structure.

Hydrophobic interactions have a dominant role in determining the stability of the folded state of the protein rather than other interactions, such as hydrogen bonds, Van der Waals forces and electrostatic interactions [8]. The stability of the folded state decreases at both higher and lower temperatures, and the denatured proteins often show irreversible change at high temperatures.

Figure 6.2: Hydrogen bonds in a simple α-helix and the denaturated state of a protein [9].

### 6.1.2.1.1 Thermal Stability of Keratin Fibres

Factors that are responsible for the stability of native bio-macromolecules are hydrophobic interactions, hydrogen bonding, conformational entropy, and the physical environment [10]. In the case of keratin fibres, the peak temperature is the denaturation temperature, while the area of the peak with respect to the base line is considered to be denaturation enthalpy, which is the energy needed for helix denaturation [11].

The DSC technique has been used to study the denaturation behaviour of α - helical crystallites in keratin fibres, the role of cross-linked IFAPs and the effect of physical and
chemical treatments on keratin fibres [11, 12]. The morphological structure of keratin fibres has two phases: the crystalline phase, which includes the α-helical intermediate filaments (IF) with their helical fractions and the amorphous (non-crystalline) phase, which includes the intermediate filament associated proteins - IFAPs as illustrated in Figure 6.3 [13, 14].

![Figure 6.3: Model of the α-helical structure in intermediate filaments (IFs) of a keratin fibre (human hair) [14].](image)

During thermal analysis, a keratin fibre show changes due to the break of the cross links, such as disulfide bonds, hydrogen bonds and salt links and finally the rupture of peptide bonds, leading to denaturation [15]. Istrate et al [14] suggest various thermal denaturation stages: decomposition which includes cleavage of disulfide and some C-S bonds, unfolding of the helical material (reversible action) and an irreversible denaturation of the IF structure.

### 6.1.3 DSC analysis of Keratins

There are two DSC methods, dry DSC and DSC with water, which are used to study the thermal behaviour of keratin fibres. The denaturation peak for keratin fibres is very dependent on the DSC technique. It can be observed at 138°C in an aqueous medium and at 230 to 250°C when dry [4, 16].
In dry DSC increasing the temperature lets the moisture content of the keratin fibre evaporate, which causes a shift of the endothermic effects sometimes to above 200ºC [4,17]. However, this effect is always secondary in size compared to a large background peak due to general keratin pyrolysis [11]. Tonin [17] showed that the DSC curve can be differentiated by a second order derivative in order to resolve the peaks to identify the structural changes in treated wool by using the dry DSC technique. Zoccola et al [18] used dry DSC to show the difference in endothermic peaks between wool and horn hoof, due to the denaturation of crystalline fractions with different molecular weights.

Using DSC with water makes keratin fibres softer with water uptake [19]. Raising the temperature during the DSC experiment causes some chemical changes in the keratin fibre by inducing a drop of viscosity of the matrix around the IFs. Once the viscosity has decreased enough the denaturation process occurs between 110 and 160 ºC [20, 21].

Due to the disappearance of the protective role of the matrix, the α-helical domains in the IFs unfold as a result of the high temperature. This process involves a transition from a compact structure to a more flexible, disorganised structure. The hydrophobic regions of individual protein molecules (exposed to water) tend to cluster together, which causes a collapse of the IF structure (irreversible denaturation reaction) [14].

Wortmann and Deutz [22] suggested that a DSC curve reflects the progress of the helix-coil transition in the IFs of keratin fibres. Using the DSC technique with keratin fibres shows two important events, denaturation of the α-form crystallites and thermal degradation of other components e.g. damage of cystine disulfide linkages [16]. This is more pronounced in DSC with water than with dry DSC [23]. The cleavage of disulfide bonds can be reduced by increasing the steam pressure in modified wool [24].

6.1.4 Objectives

The objectives of this part of the study relate to using DSC to assess structural changes in archaeological keratin fibres when they are heated in water (High Pressure Differential Scanning Calometry – HPDSC) after optimising the sample size to be suited for archaeological samples.
6.2 Experimental work

6.2.1 Sample preparation

Virgin fibres and archaeological samples were cut into snippets (1mm in length). Then samples were weighted and 3 to 5 mg used in the HPDSC experiments. DSC with water was employed for keratin samples.

6.2.2 Thermal analysis

The HPDSC experiments were performed using a TA Modulated DSC thermal analyzer (DSC-100), with a modulation of $\pm 1^\circ$C/min, using pressure resistant (25 bar), stainless steel pans for a temperature range 80°C to 180°C (heating rate 3°C/min) [12]. The DSC device was calibrated using indium and palmitic acid, with high purity. Virgin fibres and archaeological samples were weighed into a sample container, 40 µl of water was added, and the containers were sealed with a lid using the sample encapsulating press. Samples were stored over night to achieve equilibrium of water content and distribution. A sealed empty pan was used as a reference. To reach reproducibility of results, at least two samples for each type of fibre were measured.

Sample 91/110 goat hair was only available in a small portion. For that it was difficult to be investigated by DSC.

6.2.3 Optimisation of sample size

Virgin wool fibres were used to optimise the DSC technique for small sample sizes. Different wool weights were analysed (5, 2.0, 1.5, 1.0, 0.5 and 0.3) mg. The data were collected and analysed using the TA Instruments Universal Analysis 2000 Program, (Version 4.5A, Build 4.5.0.5.).

6.3 Results and discussion

DSC in water was used to study the thermal behaviour of keratin fibres when they were heated. The denaturation enthalpy ($\Delta H_D$) measures the stability of the IFs (the backbone of keratin fibres), while the denaturation temperature ($T_D$) gives information about the changes
in IFAPs, which is kinetically controlled by their crosslink density [11]. In virgin keratin fibres most $T_D$ fall into the temperature range of 140 – 150°C [12].

### 6.3.1 Optimisation of Sample Size

Archaeological sample are precious samples, therefore optimising sample size for the DSC technique is very important. To determine the minimum sample size at which the machine still gives accurate information (sensitivity of the machine), different weights of virgin brown wool (there is no difference with white wool) were tested. The results show the relationship between wool fibre weight and denaturation enthalpy as shown in Figure 6.4. Minimum weights with a small value of standard error were 1 to 1.5 mg.

![Figure 6.4: The relationship between fibre weight and denaturation enthalpy in wool fibres (mean ± standard error).](image)

Results showed a comparable denaturation enthalpy down to 1mg of virgin wool. For smaller sample weights the enthalpy drops. Hence 1 to 1.5 mg samples are ideal to use to study the thermal properties of virgin keratin fibres.

In amino acid analysis, the protein content of virgin wool under standard conditions is about 80% (private communication with Dr. G. Wormann). Archaeological fibres undergo degradation and mineralisation processes during their preservation, which reduces the actual
protein content of the fibres. Table 6.1 shows the protein content for different archaeological and virgin keratin fibres, measured by amino acid analysis (unpublished data, Dr. G. Wormann).

Table 6.1: Protein content of virgin and archaeological fibres under standard conditions.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Protein Content %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Virgin deer hair</td>
<td>69</td>
</tr>
<tr>
<td>Sample Go II</td>
<td>30</td>
</tr>
<tr>
<td>Sample Go III</td>
<td>59</td>
</tr>
<tr>
<td>Sample Go V</td>
<td>75</td>
</tr>
<tr>
<td>Sample Go VI</td>
<td>57</td>
</tr>
<tr>
<td>Sample Go V II</td>
<td>58</td>
</tr>
<tr>
<td>Sample Go V III</td>
<td>48</td>
</tr>
<tr>
<td>Sample Go IX</td>
<td>57</td>
</tr>
<tr>
<td>Sample Go XI</td>
<td>52</td>
</tr>
<tr>
<td>Sample Go X III</td>
<td>54</td>
</tr>
<tr>
<td>Virgin human hair</td>
<td>76</td>
</tr>
<tr>
<td>Sample 91-92 B</td>
<td>56</td>
</tr>
<tr>
<td>Sample 91/130</td>
<td>60</td>
</tr>
<tr>
<td>Virgin wool</td>
<td>74</td>
</tr>
<tr>
<td>Virgin goat hair</td>
<td>74</td>
</tr>
<tr>
<td>Sample wool 2b</td>
<td>25</td>
</tr>
<tr>
<td>sample wool fabric</td>
<td>60</td>
</tr>
<tr>
<td>Sample wool TT 85/38</td>
<td>68</td>
</tr>
</tbody>
</table>

The reduction of protein content has an effect on the measured denaturation enthalpy, which is calculated on the basis of the sample weight. Figure 6.5 shows the consequences, for the example of deer hair, when a comparison is made between virgin and ancient samples with respect to protein content.
Figure 6.5: DSC in water under standard conditions (dry hair sample + water). Endotherm peak before and after sample weight correction.

For this reason the corrected sample weight is calculated on the basis of 100% protein (with 3 to 5 mg sample size) to make sure that the degradation of the intermediate filaments are measured in the ancient fibres and not the difference in the protein content.
6.3.2 **Archaeological fibres**

To understand the thermal behaviour of keratin fibres, DSC results of archaeological keratin fibres that derived from different preservation conditions are used to make comparison with references fibres.

6.3.2.1 **Archaeological fibres from the Iceman site**

6.3.2.1.1 **Human hair**

The DSC peaks for archaeological Iceman human hairs (samples 91/92, 91/130 and 91/142) are compared with virgin human hair as shown in Figure 6.6.

The denaturation enthalpy of Iceman human hair samples decreases by up to two thirds (samples 91/92B and 91/142) and up to half in sample 91/130 compared to virgin human hair due to damage in the α-helical material as seen in Figures 6.7. The comparison in Figure 6.8 shows no change in denaturation temperature in both samples 91/130 and 91/142, which indicates that there is no damage in crosslinks in the matrix IFAPs, while the DSC curve for sample 91/92B shows an increase in denaturation temperature due to new crosslinks in the matrix.
Figure 6.6: DSC peak (the protein content is not corrected)  (a) virgin human hair  (b) sample 91/92B (archaeological Iceman human keratin fibres).
Figure 6.7: Archaeological Iceman human keratin fibres. Denaturation enthalpy $\Delta H_D$ (the protein content is not corrected) (mean ± standard error).

Figure 6.8: Archaeological Iceman human keratin fibres. Denaturation temperature $T_D$ (the protein content is not corrected) (mean ± standard error).
6.3.2.1.2 Animal hair (deer hair)

In comparison with virgin coarse deer hair, samples (GO II, GO III, GO V, GO VI, GO VIII, GO IX and 91/137) show a large decrease in denaturation enthalpy of up to two thirds due to damage in the α-helical material. The DSC curve for archaeological deer hair (GO VII, GO XI, GO XIII and 91/96) showed a smaller decrease in denaturation enthalpy as shown in Figure 6.9.

It is interesting to note that a general increase in denaturation temperature as seen in Figure 6.10 due to the stabilisation of matrix proteins is observed. The nature of the stabilisation was not investigated. Ionic forces, as well as new cross links may be responsible for this effect.

Figure 6.9: Archaeological Iceman animal keratin fibres. Denaturation enthalpy $\Delta H_D$ (the protein content is not corrected) (mean ± standard error).
Figure 6.10: Archaeological Iceman animal keratin fibres. Changes in denaturation temperature $T_D$ (the protein content is not corrected) (mean ± standard error).

### 6.3.2.2 Finds from Hadrian’s wall (Vindolanda, UK)

#### 6.3.2.2.1 Sample A86, goat hair

A comparison between samples A86, goat hair and virgin goat hair showed a decrease in both $\Delta H_D$ and $T_D$, due to degradation processes in both IFs and IFAPs, respectively as shown in Figures 6.11 and 6.12.

Figure 6.11: Archaeological goat hair in comparison to virgin goat hair. Denaturation enthalpy $\Delta H_D$ (the protein content is not corrected) (mean ± standard error).
6.3.2.2.2  Wool, TT/85/38

In comparison to virgin white wool, wool TT/85/38 shows a large decrease in denaturation enthalpy down to 3.40 J/g due to damage to the α-helical material as shown in Figure 6.13 and 6.14, however there was no change in denaturation temperature. This result shows that the degradation is pronounced in the α-helical material of the IFs only with no change in the crosslinked matrix compared to virgin wool.

![Graph showing denaturation temperature and enthalpy comparison]

Figure 6.12: Archaeological goat hair in comparison to virgin goat hair. Denaturation temperature $T_D$ (the protein content is not corrected) (mean ± standard error).

Figure 6.13: Wool TT/85/38 hair in comparison to virgin wool. Denaturation enthalpy $\Delta H_D$ (the protein content is not corrected) (mean ± standard error)
205

6.3.2.3 Textiles from Schloesser Brewery (Düsseldorf, Germany)

The comparison between virgin wool and wool 2a and wool 2b show a large decrease in

denaturation enthalpy due to a decrease of α-helical material in the archaeological fibres as

seen in Figures 6.15. The comparison shows slight change in denaturation temperature in

wool 2a, which means there is only a slight damage in the cross-linked matrix IFAPs. \( T_D \)

increased in wool 2b due to the formation of new cross links in the matrix as shown in Figure

6.16.

Figure 6.15: Archaeological wool 2a and wool 2b in comparison to virgin wool.
Denaturation enthalpy \( \Delta H_D \) (the protein content is not corrected) (mean ±standard error).
6.3.2.4 Wool fabric from the 15th century (Germany)

$\Delta H_D$ of sample wool fabric fibres is much decreased compared with virgin wool down to 1.26 J/g due to a pronounced degradation in the $\alpha$-helical material. However, $T_D$ increased in these archaeological keratin fibres due to the stabilization of matrix proteins through formation of new cross links in the matrix as seen in Figures 6.17 and 6.18.

Figure 6.17: Archaeological wool fabric in comparison to virgin wool. Denaturation enthalpy $\Delta H_D$ (the protein content is not corrected) (mean ± standard error).
Figure 6.18: Archaeological wool fabric in comparison to virgin wool. Denaturation temperature $T_D$ (the protein content is not corrected) (mean ± standard error).

### 6.3.2.5 Bog mummy hair (north Germany)

The comparison between the virgin human hair and sample bog mummy hair showed a huge decrease in denaturation enthalpy up to two thirds and denaturation temperature due to degradation in both IFs and IFAPs respectively and this may be seen in Figures 6.19 and 6.20).

Figure 6.19: Bog mummy hair in comparison to virgin human hair. Denaturation enthalpy $\Delta H_D$ (the protein content is not corrected) (mean ± standard error).
6.4 Conclusions

DSC analysis has been used to assess structural changes in archaeological keratin fibres that derived from different preservation conditions.

6.4.1 Archaeological fibres from the Iceman site

The DSC analysis of Iceman human and deer hair samples shows substantial damage in the \( \alpha \)-helical material. There is no damage for the cross links in the matrix IFAPs for human hair and a slight decrease for ancient deer hair, respectively as seen in Figure 6.21. This means that highly medullated deer fibres had more changes to the cross links of the IFAPs compared with the Iceman human hair.
Also, Iceman human hair, which derives from frozen conditions show less damage in both IFs and IFAPs compared with bog mummy hair as seen in Figure 6.22.
6.4.2 Finds from Hadrian’s wall (Vindolanda, UK)

Both samples A86, goat hair and wool, TT/85/38 show the presence of the degradation processes in both the IFs and the IFAPs respectively. Also the results show that the fine fibres of wool, TT/85/38 were more affected by preservation conditions than the A86, goat hair as shown in Figure 6.23.

Figure 6.23: Finds from Vindolanda. (a) Denaturation enthalpy (b) Ddenaturation temperature (mean ± standard error).

Wool TT/85/38 shows the presence of degradation processes just for the IFs compared with virgin wool and other ancient wool fibres, which derived from other preservation conditions as shown in Figures 6.23 and 6.24.

Figure 6.24: Different archaeological wool fibres. (a) changes in denaturation enthalpy (b) changes in denaturation temperature (mean ± standard error).
6.4.3 Textiles from Schloesser Brewery (Düsseldorf, Germany)

Both samples wool 2a and wool 2b showed significant damage to the α-helical material, while the denaturation temperature was increased in wool 2b, as a result of the stabilization of the matrix proteins through new cross links. Also both samples wool 2a and wool 2b which derived from a latrine pit show a large degradation in their IFs compared with other ancient wool fibres as seen in Figure 6.24.

6.4.4 Wool fabric from 15th century (Germany)

Wool fabric showed a pronounced degradation in the α-helical material and stabilisation of matrix proteins due to new cross link in the matrix compared with virgin wool and other ancient wool fibres as seen in Figure 6.24.

6.4.5 Bog mummy hair (north Germany)

Bog mummy hair showed a significant decrease in denaturation enthalpy and denaturation temperature due to degradation in both IFs and IFAPs, respectively, compared with virgin and Iceman human hair as seen in Figure 6.22.

In general, all Iceman archaeological samples (human and deer hair) show various degrees of α-helical material damage with no damage to cross links at the matrix IFAPs, compared with other archaeological samples from different archaeological sites. All ancient wool from different preservation sites shows the most damage in the α-helical material compared with other ancient fibres (human and deer hair). They also show variations in denaturation temperatures depending on the preservation environments for each archaeological group as shown in Figures 6.25 and 6.26.

Bog conditions had more impact on both the IFs and the IFAPs on bog mummy hair compared with Iceman human hair which derived from frozen conditions.
Figure 6.25: Different archaeological fibres. Denaturation enthalpy (mean ± standard error).

Figure 6.26: Different archaeological wool fibres. Denaturation temperature (mean ± standard error).
References


6. Materials solutions. (2013). Materials solutions. Available at: https://www.google.co.uk/search?hl=en&site=imghp&tbm=isch&source=hp&biw=1034&bih=651&q=dsc+analysis&oq=DSC+analy&gs_l=img.1.0.0j0i24l9.2190.9350.0.12677.9.8.0.1.1.0.74.467.8.8.0...0.0.0..1ac.1.17.img.HY0j94_cqDM#facrc=_&imgdii=_&imgrc=OvBhpG xKDfk75M%253A%3BUxd7O15mRlMxGM%3Bhttp%253A%252F%252Fwww.materialssolutions.info%252Fthermal%252Fthermal.html%3B933%3B301 . Accessed at: [21/4/2014].


Chapter 7 - Wide Angle X-Ray Scattering

7.1 Introduction

Wide angle X-ray Scattering (WXRS) is a non-destructive technique, which can be employed to investigate the structure and fraction of crystals in solid polymers.

X-ray techniques include wide angle x-ray scattering (WXRD) and small-angle X-ray scattering (SAXS). In WXRD the distance between the sample and detector is shorter and thus diffraction maxima occur at larger angles. SAXS probes structures in the nanometer to micrometre range by measuring scattering intensity at very small scattering angles; while WAXS concentrates on scattering angles 2θ larger than 3°. Also crystalline solid samples can be examined by WXRD, while samples in aqueous solution can be only examined by SAXS [1].

7.1.1 The working principle of Wide Angle X-ray Scattering

X-rays have a similar nature to visible light through with shorter wavelength. This is of advantage to show small distances between atoms [2]. A solid sample is mounted on a goniometric head, which orients in the direction of the x-ray beam and allows it to pass through the sample. X-rays are scattered by the electrons of the samples atoms as illustrated in Figure 7.1 [3, 4]. The reflection of the x-rays is collected by a detector to generate a diffraction pattern or a diffraction peak depending on the x-ray machine.

![Figure 7.1: Wide angle x-ray diffraction machine](image)

Figure 7.1: Wide angle x-ray diffraction machine [5]
X-rays interact with a target material by scattering X-rays from atoms within the solid materials. In materials with a regular structure (i.e. crystalline), the scattered X-rays undergo constructive and destructive interference. This is the process of diffraction. The diffraction of X-rays by crystals is described by Bragg's Law. In 1912 Bragg, found a relationship among several factors: the distance between similar atomic planes called d-spacing, which is measured in Å, and the diffraction angle, called the θ angle, which is measured in degrees. For practical reasons the diffractometer measures the angle twice, hence it is called ‘2θ.’ The wavelength of incident x-radiation (λ) is equal to 1.54 Angstroms for CuKα. These factors are combined in Bragg's Law:

\[ n \lambda = 2d \sin \theta \]

Where:

- \( n \) = integer,
- \( \lambda \) = wavelength in angstroms,
- \( d \) = interatomic spacing in angstroms,
- \( \theta \) = the diffraction angle in degrees [6].

### 7.1.2 Keratins and Wide angle X-ray Scattering

WXRD is used to study the crystalline part of different types of natural polymers such as keratin. Keratins can be classified when they are examined by x-ray diffraction into four types of diffraction pattern: α-pattern, β-pattern, amorphous pattern and feather pattern as shown in Figure 7.2. The differences in the spacing of equatorial and meridional reflections in these patterns give a distinct pattern.

The α-pattern has meridional reflections at 5.15 Angstroms (Å) and an equatorial reflection at 9.8 Å, while β- and feather patterns have a prominent equatorial reflection at ~4.7 Å. The amorphous pattern consists of two narrow halos at a spacing of ~4.5 Å and 9.5 Å [7].
Figure 7.2: Classifications of keratins on the bases on their wide angle x-ray diffraction. (a) α-pattern (b) β-pattern (c) amorphous keratin. [7].

*Feather pattern not available

7.1.2.1 Structural changes in keratin fibres

Keratin fibres consist of cuticle, cortex and medulla. The cortex is responsible for most of the physical and mechanical properties of the fibre. Each cortical cell contains many macrofibrils, which contain intermediate filaments (IFs), the crystalline part of the fibre that is embedded in the amorphous matrix of the intermediate filament associated proteins (IFAPs), is rich in cystine [8, 9, 10]. The peptide chains of the IFs form α-helices. Two helices combine to form a dimer (helical coil) [11]. These dimers are the actual physical structural elements of the IFs [12]. Wide angle x-ray diffraction provides information about the structural state of the α-helical fraction in the intermediate filaments (IFs). It has been reported that stretching keratin fibres under certain conditions, such as in a steam expansion [13] or at increased temperature in an aqueous environment [14], causes structural changes by unfolding of the α-helix in the intermediate filaments to produce β-sheets [15, 16, 17]. Also mechanical damage leads to a decrease in the crystallinity of keratin fibres [18]. Treating keratin fibres chemically by reduction, as oxidation produces a decrease in α-helical content and an increase of random coil structure due to cleavage of disulfide bonds [10, 14], while coating wool with silver causes strong adhesion of the silver with the fibre surface due to the adsorption and interaction of silver with sulfur moieties related to the cysteine group without causing damage to the α-helical content [19].
Fonollosa et al [20] reported that x-ray scattering analysis was able to detect minor damage in keratin fibres by the transition of crystal lipids to the liquid crystalline state. These changes are reversible in some cases [21].

Some problems were found during x-ray scattering analysis. For example, sample flatness, roughness, and positioning constraints usually preclude in-line measurements. These constraints are removed if the incident X-ray beam is parallel [22]. Also Astbury [23] found that water penetrates between the protein chains in wool causing a lateral swelling, which leads to an increase of the longer spacing. Furthermore, other swelling agents also give rise to similar spacing changes, while some, at sufficient concentration, interpenetrate the crystalline structure so strongly as to destroy the X-ray pattern altogether.

### 7.1.3 Objectives

WAXS is used to determine the structural state of the $\alpha$-helical fraction of the intermediate filaments (IFs) in archaeological keratin samples.

### 7.2 Experimental work

#### 7.2.1 Measurement technique

Bundles of cleaned archaeological and virgin keratin fibres (approximately 20 hairs) were laid parallel on a flat nickel disc and examined by wide angle x-ray diffraction. A Philips x-ray diffractometer is used with 1.54Å, CuK$\alpha$ radiation. The generator intensity was 45 kV, and the generator current was 40 Amps. The fibres were scanned from 2$\theta$ 5 to 70°, with a step size of 0.070°. To maintain reproducibility of results, at least two samples for each type of fibre were measured.

#### 7.2.2 Processing of results

The results were saved as ASC files (as graphs, not images), before transferring the data to Microsoft Excel as seen in Figure 7.3. By using a peak deconvolution application within Excel, the graph was analysed by four peaks as seen in Figure 7.4. The first peak relates to the $\alpha$-helical proteins, the second peak relates to the amorphous region in the fibre, while the third and fourth peaks relates to non-protein parts of the fibre.
The relative amount of α-helical proteins was calculated from the area of fitted distributions from scanned equatorial reflexes by using a peak deconvolution program. The relative α-helix content was calculated by multiplying the fraction of α-helical content divided by the summation of α-helical structure and β-structure by 100. In addition, the results were imported into Peakfit, a peak separation and analysis software (version 1.2), and were
converted into second derivative spectra by using the Gaussian deconvolution derivative method. The software decides the peak position based on the second derivative method.

*WXRD for archaeological fibres (91/110, 91/130, 91/142 and wool 2a) could not be obtained due to the small sample size.

7.3 Results and discussion
Keratin fibre diffraction patterns derived from \( \alpha \)-helices and \( \beta \)-sheet structures can be distinguished due to the difference in the parameter values associated with structural repeat units and peptide orientation relative to the fibre axis. In respect of this fact, Zimenkov et al [1] showed the presence of an equatorial reflection at 9.3-9.8 Å, which corresponds to the \( \alpha \)-helical coiled and \( \sim 4.5 \) Å associated with the amorphous region and the changes due to different types of stress[12]. Also due to the fact that archaeological keratin fibres were subject to different types of preservation conditions, an equatorial reflection may be shifted for these fibres.

7.3.1 Archaeological fibres from the Iceman site
7.3.1.1 Human hair
X-ray diffraction patterns of the Iceman human hair were compared with virgin human hair. The results of the archaeological samples show typical diffraction patterns of \( \alpha \)-keratins with a high degree of noise due to low protein content as shown in Figure 7.5.
Sample 91/92B human hair showed shifts in the diffraction pattern of the $\alpha$-keratins with prominent 20 peaks. Hence the second derivative method was applied to the original data to calculate the correct peak position of the $\alpha$-helical coiled and amorphous region as listed in Tables 7.1 and 7.2. The reason for this approach is that the peak deconvolution program (based on Excel), gives an estimation of the peak maxima in the graph due to the underlying formula as well as giving the percentage of $\alpha$-helix content. The second derivative method gives only accurate results with respect to the peak maxima.
The results for the peak maxima of keratin fibres when using the second derivative method fit very well with the data of α-helix proteins found by both Zimenkov et al and Zahn et al [1, 12]. These results give certainty about the presence of the α-helix peak in the WXRS results.

Table 7.1: Original and corrected equatorial reflection associated with α-helix for sample 91/92B.

<table>
<thead>
<tr>
<th>Fibre name</th>
<th>Repeat 1</th>
<th>Repeat 2</th>
<th>Average</th>
<th>S.E</th>
<th>Repeat 1</th>
<th>Repeat 2</th>
<th>Average</th>
<th>S.E</th>
</tr>
</thead>
<tbody>
<tr>
<td>Virgin human hair</td>
<td>9.07</td>
<td>8.94</td>
<td>9.01</td>
<td>0.06</td>
<td>9.51</td>
<td>9.58</td>
<td>9.55</td>
<td>0.04</td>
</tr>
<tr>
<td>Sample 91/92B</td>
<td>9.00</td>
<td>8.64</td>
<td>8.82</td>
<td>6.92</td>
<td>9.79</td>
<td>9.80</td>
<td>9.80</td>
<td>0.00</td>
</tr>
</tbody>
</table>

Table 7.2: Original and corrected equatorial reflection associated with the amorphous region for sample 91/92B.

<table>
<thead>
<tr>
<th>Fibre name</th>
<th>Repeat 1</th>
<th>Repeat 2</th>
<th>Average</th>
<th>S.E</th>
<th>Repeat 1</th>
<th>Repeat 2</th>
<th>Average</th>
<th>S.E</th>
</tr>
</thead>
<tbody>
<tr>
<td>Virgin human hair</td>
<td>4.38</td>
<td>4.04</td>
<td>4.21</td>
<td>0.17</td>
<td>4.18</td>
<td>4.42</td>
<td>4.30</td>
<td>0.12</td>
</tr>
<tr>
<td>Sample 91/92B</td>
<td>4.25</td>
<td>4.30</td>
<td>4.28</td>
<td>2.96</td>
<td>4.19</td>
<td>4.11</td>
<td>4.15</td>
<td>0.00</td>
</tr>
</tbody>
</table>

Also the results showed no change in crystallinity of sample 91/92B fibres compared to virgin human hair. This may be due to the forming of new bonds to preserve the main peptide chain structure as shown in Figure 7.6.

![Figure 7.6: Relative α-helix content of virgin and archaeological 91/92B fibres (mean ± standard error).](image-url)
7.3.1.2 Animal hair (deer hair)

Iceman animal hair samples (GO II, GO III, GO V, GO VI, GO VII, GO VIII, GO IX, GO XI, GO XIII, 91/96 and 91/137) show shifts in the diffraction pattern of α-keratins with a prominent minor peak at 9 to 10° and a peak around 20 to 22°, corresponding to crystalline spacing of ~9.8Å and 4.5 Å, by using the peak deconvolution program. The second derivative method was applied to the original data to calculate the correct peak position for the α-helix and the amorphous region. The correct peak maxima were calculated by the second derivative method as listed in Tables 7.3 and 7.4, while the change in α-helical content was calculated by using the peak deconvolution program.

Table 7.3: Original and corrected equatorial reflections associated with the α-helix for Iceman deer hair.

<table>
<thead>
<tr>
<th>Fibre name</th>
<th>Using peak deconvolution Å</th>
<th>Using second derivative Å</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Repeat 1</td>
<td>Repeat 2</td>
</tr>
<tr>
<td>Virgin deer hair</td>
<td>9.04</td>
<td>8.95</td>
</tr>
<tr>
<td>GO II</td>
<td>9.14</td>
<td>9.24</td>
</tr>
<tr>
<td>GO III</td>
<td>9.13</td>
<td>9.02</td>
</tr>
<tr>
<td>GO V</td>
<td>9.34</td>
<td>9.01</td>
</tr>
<tr>
<td>GO VI</td>
<td>8.98</td>
<td>9.12</td>
</tr>
<tr>
<td>GO VII</td>
<td>8.98</td>
<td>9.02</td>
</tr>
<tr>
<td>GO VIII</td>
<td>9.07</td>
<td>9.24</td>
</tr>
<tr>
<td>GO IX</td>
<td>9.34</td>
<td>8.88</td>
</tr>
<tr>
<td>GO XI</td>
<td>8.33</td>
<td>9.03</td>
</tr>
<tr>
<td>Sample 91/96</td>
<td>8.89</td>
<td>9.07</td>
</tr>
<tr>
<td>Sample 91/137</td>
<td>9.14</td>
<td>9.02</td>
</tr>
</tbody>
</table>
Table 7.4: Original and corrected equatorial reflection associated with the amorphous region for Iceman deer hair.

<table>
<thead>
<tr>
<th>Fibre name</th>
<th>Using peak deconvolution Å</th>
<th>Using second derivative Å</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Repeat 1</td>
<td>Repeat 2</td>
</tr>
<tr>
<td>Virgin deer hair</td>
<td>4.09</td>
<td>3.96</td>
</tr>
<tr>
<td>GO II</td>
<td>4.15</td>
<td>4.14</td>
</tr>
<tr>
<td>GO III</td>
<td>4.19</td>
<td>4.04</td>
</tr>
<tr>
<td>GO V</td>
<td>4.15</td>
<td>4.16</td>
</tr>
<tr>
<td>GO VI</td>
<td>3.96</td>
<td>4.03</td>
</tr>
<tr>
<td>GO VII</td>
<td>4.07</td>
<td>4.09</td>
</tr>
<tr>
<td>GO VIII</td>
<td>4.05</td>
<td>4.06</td>
</tr>
<tr>
<td>GO IX</td>
<td>4.01</td>
<td>4.04</td>
</tr>
<tr>
<td>GO XI</td>
<td>4.06</td>
<td>3.96</td>
</tr>
<tr>
<td>GO XIII</td>
<td>4.13</td>
<td>3.95</td>
</tr>
<tr>
<td>Sample 91/96</td>
<td>4.04</td>
<td>4.11</td>
</tr>
<tr>
<td>Sample 91/137</td>
<td>3.87</td>
<td>4.05</td>
</tr>
</tbody>
</table>

Figure 7.7 shows a slight change in crystallinity compared with virgin deer hair in most of the Iceman deer hair samples (except GO V and sample 91/137, where the crystallinity increased). This may be due to the formation of new bonds to preserve the main peptide chain folding [14].

Figure 7.7: Relative α-helix content of Iceman animal fibres compared with virgin deer hair (mean ± standard error).
7.3.2 Keratin fibres from Hadrian's Wall (Vindolanda, UK)

7.3.2.1 A 86, goat hair

Shifts in the diffraction pattern for A86, goat hair were found, which were corrected by using the second derivative method as listed in Table 7.5 and 7.6. The comparison shows a decrease in crystallinity due to change in α-helical content as seen in Figure 7.8.

Table 7.5: Original and corrected equatorial reflection associated with α-helix for A86, goat hair.

<table>
<thead>
<tr>
<th>Fibre name</th>
<th>Repeat 1</th>
<th>Repeat 2</th>
<th>Average</th>
<th>S.E</th>
<th>Repeat 1</th>
<th>Repeat 2</th>
<th>Average</th>
<th>S.E</th>
</tr>
</thead>
<tbody>
<tr>
<td>Virgin goat hair</td>
<td>9.32</td>
<td>8.85</td>
<td>9.08</td>
<td>0.24</td>
<td>9.65</td>
<td>9.51</td>
<td>9.58</td>
<td>0.07</td>
</tr>
<tr>
<td>A 86, goat hair</td>
<td>9.03</td>
<td>9.02</td>
<td>9.02</td>
<td>0.01</td>
<td>9.64</td>
<td>9.80</td>
<td>9.72</td>
<td>0.08</td>
</tr>
</tbody>
</table>

Table 7.6: Original and corrected equatorial reflection associated with the amorphous region for A86, goat hair.

<table>
<thead>
<tr>
<th>Fibre name</th>
<th>Repeat 1</th>
<th>Repeat 2</th>
<th>Average</th>
<th>S.E</th>
<th>Repeat 1</th>
<th>Repeat 2</th>
<th>Average</th>
<th>S.E</th>
</tr>
</thead>
<tbody>
<tr>
<td>Virgin goat hair</td>
<td>3.65</td>
<td>3.95</td>
<td>3.80</td>
<td>0.15</td>
<td>4.22</td>
<td>4.00</td>
<td>4.11</td>
<td>0.11</td>
</tr>
<tr>
<td>A 86, goat hair</td>
<td>3.94</td>
<td>3.96</td>
<td>3.95</td>
<td>0.01</td>
<td>4.36</td>
<td>4.32</td>
<td>4.34</td>
<td>0.02</td>
</tr>
</tbody>
</table>

Figure 7.8: Relative α-helix content of archaeological A86, goat hair compared to virgin goat hair (mean ± standard error).
7.3.2.2 Wool, TT/85/38

Archaeological wool TT/85/38 fibres showed an unexpected diffraction pattern for a wool sample with only a minor peak at 9° and a peak around 20°, corresponding to crystalline spacing of ~ 9.8 Å and ~ 4.5 Å. To calculate the correct peak position of the α-helix and the amorphous region, both the deconvolution program and the second derivative methods were applied. The results showed the presence of a small peak corresponding to the α-helix, which is too small to be used for the calculation of the α-helix amount as seen in Figure 7.9.

![Figure 7.9: Wool TT 85/38 x-ray diffraction peak.](image)

7.3.3 Textiles from Schloesser Brewery (Düsseldorf, Germany)

A comparison was performed between archaeological wool 2b and virgin wool. It showed a shift in the diffraction pattern of wool 2b α-keratins when using peak deconvolution. The second derivative method was applied to the original data to calculate the correct peak position, as listed in Tables 7.7 and 7.8. In comparison, a slight decrease in crystallinity in wool 2b fibres was detected as shown in Figure 7.10.
Table 7.7: Original and corrected equatorial reflection associated with α-helix for wool 2b.

<table>
<thead>
<tr>
<th>Fibre name</th>
<th>Using peak deconvolution Å</th>
<th>Using second derivative Å</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Repeat 1</td>
<td>Repeat 2</td>
</tr>
<tr>
<td>Virgin white wool</td>
<td>8.92</td>
<td>9.15</td>
</tr>
<tr>
<td>Wool 2b</td>
<td>8.56</td>
<td>9.20</td>
</tr>
</tbody>
</table>

Table 7.8: Original and corrected equatorial reflection associated with the amorphous region for wool 2b.

<table>
<thead>
<tr>
<th>Fibre name</th>
<th>Using peak deconvolution Å</th>
<th>Using second derivative Å</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Repeat 1</td>
<td>Repeat 2</td>
</tr>
<tr>
<td>Virgin white wool</td>
<td>4.15</td>
<td>4.03</td>
</tr>
<tr>
<td>Wool 2b</td>
<td>4.08</td>
<td>4.05</td>
</tr>
</tbody>
</table>

Figure 7.10: Relative α-helix content of archaeological wool 2b fibres compared to virgin wool (mean ± standard error).

7.3.4 Wool fabric from the 15th century (Germany)

The comparison between virgin white wool and archaeological wool fabric showed shifts in the diffraction pattern when using peak deconvolution, which was corrected by applying the second derivative method as listed in Tables 7.9 and 7.10. The comparison shows a large decrease in crystallinity as may be seen in Figures 7.11 and 7.12.
Table 7.9: Original and corrected equatorial reflection associated with α-helix for wool fabric.

<table>
<thead>
<tr>
<th>Fibre name</th>
<th>Using peak deconvolution Å</th>
<th>Average</th>
<th>S.E</th>
<th>Using second derivative Å</th>
<th>Average</th>
<th>S.E</th>
</tr>
</thead>
<tbody>
<tr>
<td>Virgin white wool</td>
<td>8.92</td>
<td>9.03</td>
<td>0.11</td>
<td>9.50</td>
<td>9.73</td>
<td>0.23</td>
</tr>
<tr>
<td>Wool fabric</td>
<td>10.24</td>
<td>9.08</td>
<td>1.16</td>
<td>10.63</td>
<td>10.19</td>
<td>0.43</td>
</tr>
</tbody>
</table>

Table 7.10: Original and corrected equatorial reflection associated with the amorphous region for wool fabric.

<table>
<thead>
<tr>
<th>Fibre name</th>
<th>Using peak deconvolution Å</th>
<th>Average</th>
<th>S.E</th>
<th>Using second derivative Å</th>
<th>Average</th>
<th>S.E</th>
</tr>
</thead>
<tbody>
<tr>
<td>Virgin white wool</td>
<td>4.15</td>
<td>4.09</td>
<td>0.06</td>
<td>4.23</td>
<td>4.14</td>
<td>0.09</td>
</tr>
<tr>
<td>Wool fabric</td>
<td>3.69</td>
<td>4.02</td>
<td>0.33</td>
<td>4.18</td>
<td>4.26</td>
<td>0.07</td>
</tr>
</tbody>
</table>

Figure 7.11: Equatorial scan of the x-ray diffraction pattern (peak deconvolution program) (a) virgin wool (b) wool fabric.
7.3.5 Bog mummy hair (north Germany)

Bog mummy hair showed shifts in the diffraction pattern after using peak deconvolution. This was corrected by applying the second derivative method as listed in Tables 7.11 and 7.12. The data for the peak maxima of keratin fibres after using the second derivative method fit very well with the data for α-helix proteins. The comparison between virgin human hair and bog mummy hair show a decrease in α-helical content as may be seen in Figures 7.13 and 7.14.

Table 7.11: Original and corrected equatorial reflection associated with the α-helix for bog mummy hair

<table>
<thead>
<tr>
<th>Fibre name</th>
<th>Using peak deconvolution Å</th>
<th>Using second derivative Å</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Repeat 1</td>
<td>Repeat 2</td>
</tr>
<tr>
<td>Virgin huam hair</td>
<td>9.07</td>
<td>8.94</td>
</tr>
<tr>
<td>Bog mummy hair</td>
<td>8.91</td>
<td>8.95</td>
</tr>
</tbody>
</table>
Table 7.12: Original and corrected equatorial reflection associated with the amorphous region for bog mummy hair.

<table>
<thead>
<tr>
<th>Fibre name</th>
<th>Using peak deconvolution Å</th>
<th>Using second derivative Å</th>
</tr>
</thead>
<tbody>
<tr>
<td>Virgin human hair</td>
<td>4.38 4.04 4.21 0.17</td>
<td>4.18 4.42 4.30 0.12</td>
</tr>
<tr>
<td>Bog mummy hair</td>
<td>3.96 4.05 4.00 0.04</td>
<td>4.38 4.08 4.23 0.15</td>
</tr>
</tbody>
</table>

Figure 7.13: Equatorial scan and deconvolution analysis of x-ray diffraction patterns. (a) virgin human hair (b) bog mummy hair.
Chapter 7

Wide Angle X-Ray Scattering

7.4 Conclusions

WAXS can be used to measure the structural state of the \( \alpha \)-helical fraction of the intermediate filaments (IFs) in archaeological samples.

7.4.1 Archaeological fibres from the Iceman site

Iceman human hair, which derived from glacier conditions shows no change in crystallinity compared with virgin human hair, while most Iceman deer hairs show a slight change in crystallinity due to the formation of new bonds to preserve the main peptide chain fold compared with virgin deer hair and Iceman human hair. These results give evidence that frozen conditions had an impact only on the highly medullated keratin fibre crystallinity compared with Iceman human hair as seen in Figure 7.15, due to their thin cuticle and cortex layers.

Figure 7.14: Relative \( \alpha \)-helix content of bog mummy hair compared with virgin human hair (mean ± standard error).
7.4.2 Finds from Hadrian’s wall (Vindolanda, UK)

Central European climate of Vindolanda decreased the α-helix crystallinity of ancient goat hair. However the α-helical crystallinity for wool TT/85/38 was difficult to measure due to the destroyed cortex layer in some of these fibres (see chapter 3).

7.4.3 Textiles from Schloesser Brewery (Düsseldorf, Germany)

Wool 2b fibres, which derive from a latrine pit, show a slight decrease of α-helical crystallinity compared with virgin wool and wool fabric as shown in Figure 7.16.
Figure 7.16: Relative α-helix content of different ancient wools compared with virgin wool (mean ± standard error).

7.4.4 Wool fabric from 15th century (Germany)

Wool fabric shows a large decrease in crystallinity compared with virgin wool and wool 2b as seen in Figure 7.16. These results give evidence that the wool fabric preservation conditions had a huge impact on the wool fibre crystallinity compared with the Düsseldorf preservation conditions.

7.4.5 Bog mummy hair (north Germany)

Bog preservation conditions show a pronounced impact on bog mummy hair crystallinity compared with virgin and Iceman human hair as shown in Figure 7.17. This means that bog preservation conditions had more impact on ancient human hair crystallinity compared with frozen preservation conditions.
In general, the preservation conditions of wool fabric induced the largest reduction of keratin fibres’ crystallinity. Vindolanda and bog preservation conditions were mild in their effect, while the Iceman and the old town of Düsseldorf preservation conditions had less influence on fibre crystallinity as may be clearly seen in Figure 7.18.

Figure 7.17: Relative $\alpha$-helix content of different ancient human hair (mean ± standard error).

Figure 7.18: Relative $\alpha$-helix content of different ancient keratin fibres (mean ± standard error).
References


Chapter 8 – Conclusions

The objective of this study was the determination of the state of preservation of archaeological keratin fibres. One major task was the comparison of different analytical tools and their validation. The following analytical tools were chosen.

1. Surface analysis, which includes: Microscopical analysis to determine overall morphological changes. ATR, transmission FTIR and EDX analysis to determine the chemical changes, mainly of the cuticle layer.

2. Analysis of inner parts of the keratin fibre, which includes: Transmission FTIR analysis to determine the chemical changes mainly of the matrix (IFAPs) and the cortex layer. DSC (denaturation method) and WAXS analysis (non denaturation method) used to determine the changes of the IFs.

The microscopical studies have shown that Iceman deer hair (highly medullated keratin fibres) suffered strong mechanical damage. This damage include longitudinal fracture in some fibres with no microbial attack (due to frozen conditions). This is attenuated to a thin cuticle and cortex layer compared with Iceman human hair as seen in Figures 8.1a and b.

It seems that some fibres of the Iceman goat hair suffured removal of the cuticle layer due to mechanical damage. No mechanical damage at the external surface was detected in ancient A86, goat hair from Vindolanda as seen in Figures 8.1c and d. This means that frozen conditions are harshest on the external surface of ancient goat hair, which could be due to the repeated freezing and thawing processes [1].

The results show the absence of any mechanical damage on the external surface of the Iceman and bog mummy human hair, which could be due to their thick cuticle layer. These results give evidence that frozen and bog conditions preserved very well the external surface of ancient human hair.
Different ancient wool fibres, which have thin cuticle layers show variations of surface damage. The preservation conditions at Vindolanda and the old town in Düesseldorf site have led to fibrillation, fibre fracture, flattened fibres (in wool, TT/85/38) and scale damage (wool 2a and wool 2b), as main mechanical damage and microbial attack. The unknown preservation conditions of wool fabric from 15th century have led to mechanical damage including the presence of flattening the yarn crown, with no microbial attack, which is shown in Figure 8.2.
Figure 8.2: Different ancient wool fibres. SEM. (a) Wool, TT/85/38 (x1000); (b) Wool 2a (c) Wool 2b (x2500).

In the comparison between archaeological keratin fibres from different preservation conditions, wool fibres from the central European climate show large damage at the external surface compared with frozen conditions. Highly medullated fibres (deer hair) are easy to attack physically, due to the highly porous structure of the medulla, which provides a large surface for damage. Bog preservation conditions were the best in preserving the external surface.

ATR, transmission FTIR and EDX analysis were used to determine the chemical changes (oxidation of the cystine) of the cuticle of keratin fibres. In all archaeological samples from different preservation conditions cystine oxidation processes have taken place. Usually the formation of symmetrical cysteic acid is determined at 1042 cm\(^{-1}\). All ancient keratin fibres show the influence of a silica peak at 1030 cm\(^{-1}\) on the cysteic acid peak at 1042 cm\(^{-1}\). This is due to the uptake of silica compounds from the environment by keratin fibres. For that asymmetrical cysteic acid at 1175 cm\(^{-1}\) was used for the comparison. All graphs showed variations during measurements at the peak height due to different selected areas.

The highly medullated Iceman deer hairs showed the highest degree of cystine oxidation and also high absorption of most ions (except Ca) from the environment, compared with thick cuticle layer human hair and goat hair. The results also show that the formation of cysteic acid increased with silicon uptake for some fibres. Iceman goat hair, which has a thicker cuticle layer than the human hair, showed the lowest amount of cysteic acid content and the lowest absorbed ions for fibres from frozen conditions as seen in Figures 8.3, 8.4 and 8.5.
This means that morphological structures are affected both by oxidation of cystine and ion uptake in frozen conditions.

Figure 8.3: ATR. Selected ancient keratin fibres. Cysteic acid at 1175 cm\(^{-1}\).

Figure 8.4: EDX. Selected Ancient keratin fibres. Relative content of Silica.
Figure 8.5: Different Iceman keratin fibres. Relative content of different trace elements. Human hair (green), deer hair (red) and goat hair (blue).

Both the Iceman and bog mummy hair showed a comparable degree of cystine oxidation and silicon uptake, while bog mummy hair also show less absorption of other trace elements as seen in Figures 8.3, 8.4 and 8.6. These results provide evidence that frozen conditions, which have a wet environment (due to the continuous freezing and thawing process) have more effect on ion uptake rather than only the oxidation of cystine.

The oxidation processes of cystine as well as the silicon uptake were higher for A86, goat hair which came from a central European climate compared with the Iceman goat hair. Both of them vary in their ion uptake depending on ion enrichment in the environment as shown in Figures 8.3 and 8.5. These results show evidence that warm conditions have more effect on both the degree of oxidation of cystine and on ion uptake from the environment.
Figure 8.6: Different ancient keratin fibres. Relative content of different trace elements - Iceman human hair (green), Iceman goat hair (brown).

Archaeological wool fibres which have a thin cuticle layer showed high variation for cystine oxidation processes and silicon uptake. The wool fabric shows less oxidation change with a lower concentration of silicon compared with other ancient wool fibres. Wool 2b shows the strongest oxidation with a low content of silicon, which was the opposite case to wool 2a. The differences between the wool samples from the latrine pit might be explained by the environmental differences within the layers of the archaeological site. Wool, TT/85/38, had no cysteic acid at 1175 cm\(^{-1}\) with a strong presence of silicon compared with other ancient wool fibres as shown in Figures 8.3, 8.4 and 8.6.

In conclusion, the results obtained from ATR and EDX show that there is no general relationship between the degree of oxidation of the surface proteins and the conditions at the preservation site. But the high degree of medullation in deer hair seems to have an influence. In addition, the degree of surface ion uptake seems to play a role for cystine oxidation. Even the high iron uptake of some hair samples did not lead to extraordinary cystine oxidation.

To investigate the changes of the inner parts of keratin fibres transmission FTIR analysis was used to determine the chemical changes mainly of the matrix proteins (IFAPs) of the cortex.

For all ancient keratin fibres the silica peak at 1030 cm\(^{-1}\) showed a small influence on the cysteic acid peak at 1042 cm\(^{-1}\). This is because most of the cystine is located in the matrix of
the cortical cells, while silica is concentrated at cuticle and medulla of keratin fibres. For this reason asymmetrical cysteic acid at 1175 cm$^{-1}$ was used during comparison. Also all ancient fibres show increases of peak heights of both symmetric and asymmetric cysteic acid in the matrix compared to the cuticle. Transmission FTIR shows the oxidation changes in both cortex and cuticle of keratin fibres, while ATR shows the oxidation changes mainly of the cuticle layer.

The highly medullated Iceman deer hairs show the highest cysteic acid peak of the matrix, compared with human hair and goat hair. Iceman goat hair, which has a thicker cuticle layer than human hair, shows the lowest content of cysteic acid as shown in Figure 8.7. This means that this morphological structure is affected by the oxidation of cystine under frozen conditions.

This agrees with ATR results, Iceman human hair shows a higher oxidation level in the matrix compared to bog mummy hair. It might be that, frozen conditions, which have a wet environment (due to the continuous freezing and thawing process), have more effect on the oxidation of cystine as seen in Figure 8.7. Iceman goat hair and A86, goat hair from Vindolanda show variations in the chemical changes of the matrix.

All ancient wools which have a thin cuticle layer and highly medullated Iceman deer hairs show the highest concentration of cysteic acid compared with human hair and goat hair (thick cuticle layer fibres). Ancient wool from a central European climate shows higher chemical changes in the matrix compared with Iceman deer hairs. This result provides evidence that cystine oxidation inside the fibre is promoted by warm conditions.

In general, Transmission FTIR gives a better view of the chemical changes in both cortex and cuticle layers compared to ATR analysis.
Both WAXS (non-denaturation method) and DSC (denaturation method) have been used to determine the changes of the IFs. A non-denaturation method can be used to determine the amount of structural molecules which are neither disturbed by the sample preparation or by the analysis as such. The denaturation method in addition shows the split of the peptide bonds (which can be caused by e.g. microbial attack) and the S-S bonds, as well as the effect of other cross links and strong interactions e.g. ionic bonds.

In WAXS the \(\alpha\)-helical protein content in virgin keratin fibres is determined as 20.7\% for human hair, 14.2\% for deer hair, 17.5\% for goat hair and 21.2\% for wool. In comparison to the virgin fibres, there is only a moderate decrease in \(\alpha\)-helical material found. The only exception is wool fabric from Germany which showed less than 5\% of \(\alpha\)-helical content. This is because the history of this sample is unknown, so no explanation can be given so far as seen in Figure 8.8.

Figure 8.7: Transmission FTIR (second derivative peak height). Cysteic acid in the inner part of selected ancient keratin fibres.
Figure 8.8: WAXS. Relative α-helix content for archaeological keratin fibres. (mean ± standard error).

The DSC results for denaturation enthalpy of ancient keratin fibres show large variation compared to the results of WAXS. The changes are much larger compared to virgin keratin fibres (11.58 J/g for human hair, 11.07 J/g for deer hair, 6.02 J/g for goat hair and 8.67 J/g for wool). No changes in denaturation temperature are observed compared to virgin keratin fibres, due to the high degree of stabilisation in the matrix proteins as shown in Figures 8.9 and 8.10. Since a large degree of oxidation has been detected in the cortex by transmission FTIR, it is assumed that specially pronounced anionic forces are responsible for the stability.

Figures 8.8, 8.9 and 8.10 show the decrease in crystallinity for Iceman deer hair (highly medullated fibres). Also they show variations in the damage of the α-helical material of IFs with no damage in the cross links of the matrix IFAPs, compared to Iceman human hair (which has a thick cuticle layer). Frozen conditions in the glacier had this greater impact on the state of the IFs, depending on the morphology of keratin fibres.

Bog preservation conditions caused large damage to the α-helical material of the IFs and the cross links of the matrix IFAPs compared with Iceman human hair as seen in Figures 8.8, 8.9 and 8.10. These results provide evidence that bog preservation conditions had more impact on the state of IFs and the cross links of the matrix IFAPs of keratin fibres compared with those from frozen conditions.
Figure 8.9: Changes in denaturation enthalpy $\Delta H_D$ for archaeological keratin fibres (mean ± standard error).

Figure 8.10: Changes in denaturation temperature $T_D$ for archaeological keratin fibres (mean ± standard error).
The central European climate of Vindolanda caused a decrease in crystallinity and degradation in both IFs and IFAPs in sample A86, goat (thick cuticle layer) compared with wool TT/85/38 (thin cuticle layer). Therefore the central European climate had more impact on the state of IFs and the cross links of the matrix IFAPs depending on the morphology of keratin fibres as shown in Figures 8.8, 8.9 and 8.10.

All ancient wools (thin cuticle layer) from different preservation conditions show more degradation in α-helical intermediate filaments IFs, due to the damage of peptide bonds compared with other ancient keratin fibres (human, deer and goat hair) as seen in Figure 8.9. Also the results show variation in the damage to crystallinity and stabilization of matrix proteins, due to the formation of new cross links compared with other archaeological keratin fibres (human, deer and goat hair) as shown in Figures 8.8 and 8.10.

In general all preservation conditions lead to good preservation of keratin fibres. Except the latrine pit preservation conditions, which were the worst due to the microbial active environment, that caused fibre degradation.

In general DSC analysis (denaturation method) gives a better view of the state of the IFs as well as of the state of the cross links in the matrix, compared to WAXS analysis (non denaturation method).

From all the results above, the main damage can be detected in the cortex rather than the surface of archaeological human, deer and goat hair. However, for archaeological wool from different preservation conditions, the damage can be detected at both the surface and the cortex layer due to the thin layer of the cuticle.

The chosen analytical tools highlight different facets of the changes caused by the preservation environments. Microscopy and surface analysis show obvious changes in the morphology of the fibre and chemical changes of the cuticle. Because the mechanical stability of the keratin fibres this is important for e.g. storage of the samples in a museum.
References