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Development of instrumental methods for the analysis of textile fibres and dyes

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Institute of Chemistry, Faculty of Science and Technology, University of Tartu, Estonia

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Author's contribution

Paper I. Performed all the measurements of the textile fibres, helped to write the manuscript.

Paper II. Lead author in preparing the manuscript. Performed all the experiments.

Paper III. Lead author in preparing the manuscript. Performed all the experiments.

Paper IV. Lead author in preparing the manuscript. Performed most of the experiments (excluding classification with random forest)

Paper V. Performed measurements of the textile fibres.

ABBREVIATIONS

ATR	attenuated total reflectance
DA	discriminant analysis
DAD	diode array detector
DART	direct analysis in real-time
DHB	2,5-dihydroxybenzoic acid
DLaTGS	deuterated lanthanum α alanine doped triglycine sulphate
DMSO	dimethyl sulfoxide
DRIFTS	diffuse reflectance infrared Fourier transform spectroscopy
ESI	electrospray ionisation
FLD	fluorescence detector
FORS	fibre optics reflectance spectroscopy
FT-ICR	Fourier transform ion cyclotron resonance
FT-IR	Fourier transform infrared
GC	gas chromatography
HPLC	high-performance liquid chromatography
HRMS	high-resolution mass spectrometry
I	passed/reflected light
I_0	incident light
LC	liquid chromatography
MALDI	matrix-assisted laser desorption/ionisation
mATR	micro attenuated total reflectance
MS	mass spectrometry
m/z	mass-to-charge ratio
*n*ESI	nano-electrospray ionisation
PCA	principal component analysis
ppm	parts per million
r	reflectance
RF	random forest
SERS	surface-enhanced Raman spectroscopy
UV-Vis	ultraviolet-visible light

1. INTRODUCTION

Historical and archaeological objects are the oldest and probably the most important items to learn about our ancestors, heritage and past. Textiles have a special place among them. Besides practical clothing purpose, they can represent patriotism, sovereignty, peace and war when exploited as flags or banners. Knitting patterns in folk clothes can represent the nationality and origin of the wearer and usage of expensive dyes and fibres can show the social status. To see the historical story behind the object, investigators need to understand how, when and where it was made. These questions become easier to answer with the knowledge of what materials the object consists of.[1] The main aim of this thesis was to develop methods to be able to answer questions "What kind of fibres have been used to make this object?" and "What kind of dyes have been used to dye this object?"

In practice, several different fibre types can be used, many of them having similar properties and appearance, which makes the identification more difficult. Furthermore, in practical situations often contaminated or degraded samples have to be analysed. In many fields, especially in archaeology and conservation, it is very important to use non-destructive identification methods. Thus, this thesis aims, where possible, towards less- or non-invasive methods for identifying textile fibres.

Analysis of textile dyes is very different from fibres, firstly because the amount of dyes in textiles is much smaller thus needing much more sensitive analysis approaches. Also, the variety of dye components is much wider, and thus besides sensitivity, the selectivity of the instrumental methods has a very important role.

For solving these difficulties, in this work, several techniques were used for the development of analysis methods for textile fibres and dyes. For textile fibres, Fourier transform infrared spectroscopy (FT-IR) with different measuring approaches (attenuated total reflectance (ATR), reflectance (r)) and classification was used. In general, a specular reflectance-FT-IR approach has been less used for the analysis of samples with an uneven surface. It was applied in this work as a non-invasive method for textile fibre identification. Method development for textile dyes included a number of instruments like high-performance liquid chromatography (HPLC) with ultraviolet-visible (UV-Vis) detector, fluorescence detector (FLD) and mass spectrometric (MS) detector, and also high-resolution mass spectrometry (HRMS) with matrix-assisted laser desorption/ionisation (MALDI) and electrospray ionisation (ESI). During the work, suitable extraction methods for fibres (case-study samples) and dye sources (seven red plant-, insect- and fungi-based dyes), including right solvents, eluents, parameters etc for instruments were found to identify as many dye components as possible and create an as universal as possible methods for dye analysis.

The overarching objective of this work was to develop a set of methods to determine the composition of textile fibres and dyes.

The specific research objectives of this dissertation are the following:

1. Development of analytical methods for the analysis of textile fibres using FT-IR spectroscopy with different reflection techniques (ATR, reflectance) combined with chemometric classification methods *(publications I, II, IV)*.
2. Development of analytical methods for the determination of chemical composition of different red dyes using a set of chromatographic and mass spectrometric techniques *(publication III)*.
3. Demonstration of the applicability of the developed set of methods for the analysis of textile fibres and dyes on case studies from different cultural heritage objects which some of them representing Estonian history and handicraft *(publications III–V)*.

An additional value of this PhD work are collections of ATR-FT-IR and reflectance-FT-IR spectra of 16 textile fibres and altogether 113 chromatograms, UV-Vis, fluorescence and mass spectra of seven different red dyes that can be used by conservators, forensic and material scientists.

2. LITERATURE OVERVIEW

2.1. Importance of analysis of textile fibres and dyes

Identification of textile fibres and dyes is essential to learn more about the analysed object. In historical aspects, the knowledge of the composition can provide us with information about the age, origin and condition of the textile piece.[2,3] Usage of synthetic fibre indicates that the object was made or repaired after 19th century[4], while usage of cochineal in Estonia might refer to imported dye or textile[5]. Besides learning about history, knowing the composition helps to understand better how to preserve, restore (what types of fibres are needed for restoration) and store the object (some fibres or dyes might be very light-resistant, thus needing darker environment)[2,3,6–8]. Identification of fibres and dyes can also be helpful in forensic investigations, where the origin of an unknown fibre can reveal information about the investigation case[9,10]. Selection of fibre types is wide, and along with that are the quality, properties and price of the textiles. Quick, easy and reliable textile fibre identification can prevent that wrong textile material is used for quality product making either by accident or as fraud.[11–13] Identification of dyes has also many other aspects besides cultural heritage, like searching for potentially harmful synthetic (or even natural) dyes[14,15].

Although the analysis of textiles provides a lot of valuable information, there are nevertheless many problems we might face along the way to results. When exploring historically valuable textile pieces, it has to be kept in mind that damaging the historical object can be unacceptable and thus either only very small sample pieces are available or (in most of the cases) no sample can be taken at all and totally non-destructive analysis methods must be used[16,17]. Besides that, many historical textile items are partially degraded and contaminated, which further complicates the analysis[18]. When focusing on textile dyes, it is important that in many cases, mixtures of different dye sources are used, making the analysis and identification even more complex. For that reason, identifying dyes in historical objects, we need instruments that are very selective and sensitive.[19]

2.2. Overview of different textile fibres

Textiles are flexible woven materials, consisting of fibrous materials which by their chemical composition are different kinds of polymers[20]. By their origin textile fibres can be classified into two groups – natural and man-made fibres. Natural fibres can be both plant and animal origin. Man-made fibres can be either regenerated from natural sources like cellulose or synthesised. Regenerated and synthetic materials are quite new: regenerated textile fibres came into commercial use in the 19th century and first synthetic fibre (polyamide) dates back to the 1930s.[20,21] Due to their perishable nature, there are not that

many ancient textile pieces to learn about textile history from thousands of years from now. There are, however, some prominent exceptions, for example, the Egyptians tombs. In these collections from the tombs, we can learn that linen (flax) fibre was probably the most important textile fibre in ancient Egypt.[22] In Table 1 common textile fibre types are grouped according to their origin.

Table 1. Some common textile fibres classified by their origin.

Natural fibres		**Man-made fibres**	
Animal fibres	**Plant fibres**	**Regenerated fibres**	**Synthetic fibres**
Wool	Cotton	Viscose	Polyester
Silk	Flax (linen)	Acetate, triacetate	Polyamide
	Jute	Lyocell (Tencel)	Polyacrylic
	Sisal	Fibreglass	Elastane
	Hemp		Polyethylene
			Polypropylene

Natural fibres

Natural textile fibres can be classified into two groups – plant fibres, which in their chemical composition are cellulose-based, and animal fibres, which are proteinaceous materials.

The most common **animal fibres** are wool and silk. In Estonia, local wool has been very popular in making carpets, carriage blankets and folk skirts and coats. **Wool** can be obtained from many animals, but most commonly used is sheep wool. Other animals whose fur is used are alpaca, rabbit, yak, goat (e.g. cashmere) etc.[1,20,21] The main components in all of those animal hairs is keratin (α-keratinous), which roughly in its chemical composition is same – proteins consisting different amino acids. The exact sequence of the amino acids is what differentiates between species and other keratin forms (nails, skin, feathers).[23] Another widely used proteinaceous fibre is **silk**. Due to its high qualities like strength, elasticity, softness, durability and ability to bind chemical dyes, silk is still highly valuable fibre despite the huge variety of new man-made fibres. Although silk fibre is obtained from several insects, commercially only filament produced from silk moth *Bombyx mori* is used for textile making. Pure silk contains around 70–80% of fibroin and 20–30% of sericin. Sericin is the glue that is dissolved during processing and thus prepared final silk textile consists mostly of fibroin. Fibroin in its chemical composition is less-complex than keratin, consisting mostly of glycine, alanine, serine and small amounts of cystine.[24]

Most common **natural plant-based fibres** are cotton, linen jute, and hemp. In Estonian history, probably linen, grown locally, was the most important fibre for making clothes like everyday shirts and trousers. Hemp and jute have been used for making ropes and bags. On their chemical composition, all of these are

mainly cellulose with the addition of hemicellulose, fatty substances, pectins, mineral particles and water. The main component – cellulose – is a linear polymer of glucose, which in its simplest form is β-1,4-linked units. Hemicellulose is a more complex group of non-linear polysaccharides. The ratio of cellulose and hemicellulose depends strongly on the fibre type. For example, while cotton is almost entirely cellulose-based, bast fibres like linen can contain significant amounts of hemicellulose.[1,4] Physical appearance of the fibres under a microscope is in many cases very different – cotton fibre is narrow flat with an apparent twist along with the fibre, linen is more round shape with the nodes along with the fibre.[4]

Regenerated fibres

Regenerated textiles are produced from natural organic fibres like cellulose or proteins. The first man-made thermoplastic fibre was **acetate**. Difference between acetate and triacetate is in the number of cellulose hydroxyl groups that are acetylated. Acetate finds its usage in linings and furnishing, while triacetate is used in sportswear and garments that need to keep their shape.[23] The most popular regenerated fibre – **viscose** – was first produced in 1898. Viscose fibres are produced from wood and in their chemical compositions, it is hydrocellulose. Viscose is very hygroscopic, light-resistant and durable.[21] The invention of new generation cellulose-fibre called **lyocell** started in the 1980s. This, at that time, innovative technique includes solving cellulose in an organic solvent (*N*-methyl-morpholine *N*-oxide), after what fibres are spun by extrusion to a spinning bath. Lyocell is mostly sold by its trademark Tencel™.[23]

Synthetic fibres

First synthetic fibre – **polyamide** aka **nylon** – was synthesized for the first time in the 1930s. Synthetic fibres are high-molecular compounds, made from low-molecular components which can origin from coal, crude oil or natural gas.[21] Polyamide/nylon is a common name for all aliphatic polyamides, the most common ones being nylon 6 and nylon 6,6. Nowadays the most commonly used synthetic fibre – **polyester** – got its start in the 1940s. Chemically is polyester a polyethylene-terephthalate.[20,21] **Polyacrylic** (polyacrylonitrile) is a co-polymer, consisting of at least 50% of acrylonitrile monomers. Rest of the polymer consisting of different acrylacid esters, vinylacetate-, acrylamide- and meta-crylamide esters. **Elastane** is elastic fibre, consisting a least 85% of segmented polyurethane.[20]

2.3. Overview of textile dyes

Textile dyes are soluble organic compounds that can give intensive colour in very small quantity. Chemically, for a compound to absorb visible light, thus being colourful, it needs the resonance structure. Specific bonds in molecule absorb certain wavelength in light and we see the transmitted light, which due to

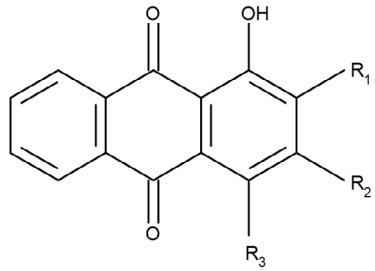
absorbance is now different. Dyes hold that property by consisting of aromatic rings with many different side chains. Some of the common base core aromatics in textile dyes are benzene, naphthalene and anthracene. For colourful compounds, these aromatic structures need right chromophore like for example azo (-N=N) or quinoid fragment.[25]

Dyes can be classified by their origin, chemical composition, dyeing method or usage. By their origin, natural textile dyes can be extracted from plants, insects, mushrooms etc. Besides a wide variety of natural dyes, starting with the synthesises of mauvine in 1856, the list has been and is still expanding with many synthetic dyes. Classification according to chemical composition can divide dyes into groups of azo, anthraquinone, flavonoid, indigoid, gallotannin, carotenoid, anthocyanidin etc. Another important way to classify dyes is according to their dyeing technique, which can differ from each other greatly and depend on chosen fibre and dye type. Direct dyeing is the simplest method involving soaking or boiling dye source in water along with fibres. This method for durable dyeing is suitable only for certain dyes with polar groups that can form strong bonds with fibre. The more used method is mordant dyeing, where mordants such as metal ions, tannins etc are used to create chelation between fibre and dye. Some of the examples of popular metal mordants through history are alum ($KAl(SO_4)_2 \cdot 12H_2O$), tin(II)chloride and potassium dichromate. Vat dyeing is a special technique used with indigo and shellfish, where the soluble form of the dye is soaked to the textile fibre and after the fibre is taken out of the bath and exposed to oxygen, the insoluble colourful dye is formed during oxidation.[5,25–27]

2.3.1. Natural red textile dyes

Variety of dye sources is wide and even wider is the colour option, since the exact colour depends on the dyeing method, used mordants and fibre[5]. In this chapter, only natural red dye sources that are used in this works experimental part, are generally described.

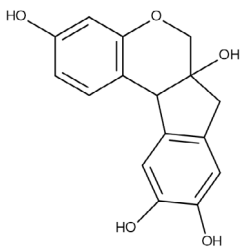
Dyer's madder (*Rubia tinctorum L*) is one of the most popular sources for dyeing, used already in classical antiquity. The main source for dyes in this plant are the roots, which contain numerous different anthraquinone aglycones and glycosides (anthraquinone with primeverose group), the most important one being alizarin and its primeveroside called ruberythric acid. Addition to alizarin, other important components that have dyeing effect are purpurin, pseudo-purpurin, rubiadin, munjistin, lucidin etc. More stable glycosides are also found for pseudopurpurin and rubiadin, addition to lucidin primeveroside and rubianin. The core structure of the anthraquinone along with substitute groups for some of the components is given in Figure 1.[5,28]



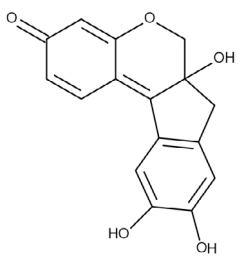
Alizarin – R_1=OH, R_2=H, R_3=H
Ruberythric acid – R_1=$C_{11}H_{19}O_{10}$, R_2=H, R_3=H
Pseudopurpurin – R_1=COOH, R_2=OH, R_3=OH
Rubiadin – R_1=CH_3, R_2=OH, R_3=H
Munjistin – R_1=COOH, R_2=OH, R_3=H
Purpurin – R_1=OH, R_2=H, R_3=OH
Lucidin – R_1=CH_2OH, R_2=OH, R_3=H
Xantopurpurin – R_1=H, R_2=OH, R_3=H
Nordamnacanthal – R_1=COOH, R_2=OH, R_3=H

Figure 1. The core structure of the anthraquinone compounds in dyer's madder with substitute groups for characteristic dye components.

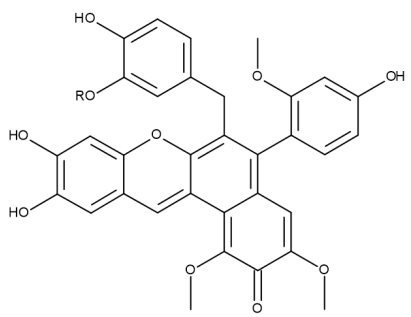
The main dyeing component in **redwood** (brazilwood, *Caesalpinia echinata*) is brazilein, which is produced during the autooxidation of brazilin (both structures given in Figure 2)[5,29,30]. **Logwood** (*Haematoxylum campechianum*) contains also small amounts of brazilein and brazilin, but the main component in this wood is hematoxylin, which forms hematein after autooxidation. Hematoxylin and hematein are structurally very similar to brazilein and brazilin.[5]



Brazilin



Brazilein



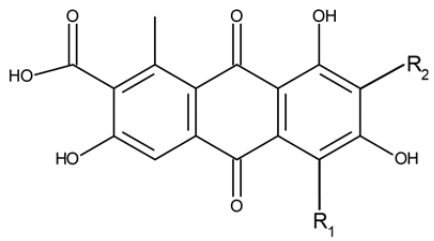
Santalin A – R=H
Santalin B – R=CH_3

Figure 2. Chemical structures of dyes from logwood, redwood and sandalwood.

Sandalwood (*Pterocarpus santalinus L.*) composition is more unique because it contains a row of different compounds that are poorly soluble in water. While those components are more difficult to extract from the heartwood, they are more durable to light and washing in the textile. Main dye components in sandalwood are santalin A, santalin B (see Figure 2) and many other flavonoids, like santal, pterocarpin, maackiain etc. Besides dyes, wood also contains tannins which act as a mordant during the dyeing process.[5,31]

Discovery of America in the 15th century changed the market and trade of dyes and reason for that is a parasite insect called **cochineal** (*Dactylopius coccus*). Cochineal insect consists mainly of carminic acid and small amounts of

kermesic acid, flavokermesic acid (see Figure 3) and isomers for carminic acid. Cochineal gives very intensive, bright red and durable colour, with different shades depending on the use of mordants.[5,32,33] Another insect used for dyeing is kermes (*Kermes vermilio*), which in the 15th century was one of the most expensive and luxurious dye. Dyeing components in kermes are kermesic acid and flavokermesic acid, which have especially good colour fastness in silk and wool.[5]



Flavokermesic acid – R_1=H, R_2=H
Kermesic acid – R_1=OH, R_2=H
Carminic acid – R_1=OH
R_2= $C_6H_{11}O_5$ (glycose unit)

Figure 3. Core structure of the anthraquinone compound in insects with substitute groups for characteristic dye components.

Bloodred webcap (*Cortinarius sanguineus*) is a poisonous mushroom, commonly spread in Europe and North-America. Dyeing compounds in bloodred webcap are different anthraquinones and their glycosides like emodin, dermorubin, dermocybin etc. But what makes this dye source very special and distinct from others is its content of chloro-compounds like 5-chlorodermorubin, 7-chloroemodin and 5,7-dichloroemodin.[5,34,35]

2.3.2. Red dyes and dyeing in Estonian history

The oldest plant-dyed textiles in Estonia date back to 14th century, which were presumably dyed red with northern bedstraw (*Galium Boreale*) or alder bark. Until the 19th century, plant-based dyes were the only ones used, which were replaced with the rise of aniline dyes. But before synthetic dyes, many foreign natural red dye sources were also used like dyer's madder, cochineal, kermes and brazilwood. From local plants, shades of red can also be obtained from blueberries and blackberries. For getting different shades and more intensive colours, mordant usage was well known also in Estonia. Besides traditional metal mordants, natural mordants included e.g cranberry juice, urine, sour kvass and sauerkraut.[36]

2.4. Instrumental techniques used to analyse textile fibres and dyes

The wide variety of textile fibres and even richer choice of textile dyes makes the identification and characterization of these materials challenging. Especially difficult is the analysis of dyes, since their concentration in samples is very low, compared to fibres, which are the main components. Since these two materials are very different by their abundance in textile samples, very different instruments are used for their analysis. The most widely used techniques in the analysis of textile fibres and dyes are described below.

2.4.1. Analysis of textile fibres

Textile fibres are solid polymeric materials, many of them insoluble in any solvent[20]. Due to that, mostly analytical techniques suitable for solid samples are used. In Table 2 the advantages and disadvantages of different techniques used for textile fibre analysis are presented. Below are described more thoroughly instrumental techniques that are used in the experimental part of this thesis.

Table 2. Different instrumental techniques used for the analysis of textile fibres.

Technique	Advantages	Disadvantages
Visual microscopic analysis [4,20,37,38,II,IV]	Quick, easy, inexpensive, none or very limited sample preparation, can be non-destructive.	Difficult to distinguish between man-made fibres, might need cleaning when fibres are contaminated or have additives (glue, paint, etc), in many cases impregnation with water or glycerine is needed.
Raman spectroscopy [9,10,39,40]	Can be non-invasive, easy, quick, can be portable.	Can be damaging to the sample, textile dyes (fluorescence) are a great disturbance, distinguishing between cellulose-based fibres is complicated or impossible.
FT-IR spectroscopy [3,7,13,41–48,I,II,IV,V]	Quick, easy, inexpensive, can be non-invasive (reflectance mode) or slightly destructive (ATR mode), distinguishing between man-made fibres is possible, textile dyes do not interfere, can be portable.	Distinguishing between cellulose-based plant fibres is complicated or impossible, additives on the fibres can interfere with the interpretation of the fibre.
Pyrolysis-gas chromatography (GC) [20,49,50]	An information-rich technique that can distinguish small differences in the polymer structure. Technique is very sensitive and with good reproducibility.	Destructive technique, sample preparation is time- and sample-consuming, fragmenting technique, complex chromatograms and for the interpretation special software is needed, rather expensive equipment.

Microscopic analysis is probably the quickest, easiest and most used technique for the identification of different natural textile fibres (e.g. cellulose-based fibres). However, most of the modern man-made fibres are almost identical under microscope, thus impossible to identify using only visual observation.[20,37]

For polymeric organic compounds like fibres, different spectroscopic approaches like infrared and Raman spectroscopies are very suitable analysis techniques.[3,10,12,13,39,41–45] Advantages of these techniques do not rely only on the capability of analysing solid samples, though this is a very important aspect, but also on their easy and quick usage, little or no sample preparation at all and in many cases non-destructiveness[20,41,II,IV].

FT-IR (Fourier transform infrared) spectroscopy has shown remarkably good results in analysing textile fibres easily, quickly, in many cases non-destructively, both in qualitative and quantitative way[3,13,42–45,II]. For these reasons, it was chosen as the main method of fibre identification in this thesis, with the aim to expand the capabilities of this technique.

FT-IR is a vibrational spectroscopic technique for gaining information about chemical bonds present in molecules of the sample. When irradiating the sample with infrared radiation, molecules absorb that radiation at specific wavenumbers (each chemical bond absorb at certain wavenumber) and start to vibrate. When measuring the incident light (I_0) and passed/reflected light (I) at a range of 4000-225 cm^{-1}, it is possible to calculate absorbance intensity, and with Fourier transform calculations, obtain the infrared spectrum. Every compound has its unique FT-IR spectrum and identification of unknown samples is possible with a comparison of them with standard sample spectrum.[51,52]

The most common FT-IR sampling techniques are transmittance[51,52], attenuated total reflectance (ATR)[13,51–53], specular reflectance[51,52,54] and diffuse reflectance (DRIFTS)[51,52,55,56]. For the analysis of solid samples like fibres, transmittance is not well suitable since very low concentrations are needed, or in case of solid samples, very thin layers must be used.

Currently, the most used sampling technique is ATR-FT-IR. This approach enables to analyse all different kind of samples – solids, liquids, thick samples and very small sample pieces. With ATR-mode, a sample is placed onto the ATR crystal (made of e.g. diamond or germanium), pressed against it and IR beam is passed through the crystal under a fixed and well-defined angle with respect to the crystal surface. For total reflectance to occur, the sample's refractive index must be lower than the refractive index of the crystal and the angle between the beam and the surface normal must be higher than the critical angle (e.g. 40 deg for a diamond, 22 deg for germanium when the refractive index for the sample is 1.5[52]). Even though total reflectance occurs and the beam is reflected back into the crystal, it does interact with the sample that is pressed against the crystal surface. Part of the beam is absorbed – attenuated by the sample. Radiation with different wavenumbers is absorbed to a different extent and as a result, ATR-FT-IR spectrum is recorded.[46,51,52] ATR-FT-IR has shown excellent results in the analysis of textile fibres[3,13,43]. But besides many advantages of ATR, it still is a contact method, and the high pressure needed for

close contact between samples and the crystal is potentially damaging to the samples. This limits the use of ATR in case of valuable fragile samples that cannot be impacted in any way.

For these cases, non-contact reflectance approaches can be more preferred techniques. There are two non-contact reflectance techniques used in FT-IR – specular reflectance and diffuse reflectance (DRIFTS), collectively termed as reflectance-FT-IR (r-FT-IR). With specular reflectance-FT-IR approach, the infrared radiation is directed onto the sample under a certain angle – while most of the radiation is reflected back, some of it is absorbed in the surface layer of the sample, so that the spectrum of the reflected radiation differs from the spectrum of the incident radiation.[46,51,52] In general, specular reflectance-FT-IR application is analysing samples with very even mirror-like surface[46]. However, in many cases it is impossible to gain such surface, especially without altering the samples, thus some scientists in the field of cultural heritage have tried applying specular reflectance-FT-IR to samples with rough surface[48,57,IV]. DRIFTS method is used to analyse samples with a very rough and uneven surface. In this case, an incident beam is reflected diffusely and special mirrors are used to gathered diffusely reflected light and direct it into the detector.[46] In many cases of using specular reflectance-FT-IR instrument, the recorded spectra are formed via a combination of specular reflectance and diffuse reflectance components[57,58]. Although this might make the interpretation of spectra more complicated, it is easier to work that way and the recorded spectra still have shape characteristic to the materials used and are thus suitable for characterisation and identification of fibres.[48,57,IV]

2.4.2. Analysis of textile dyes

For textile dye analysis, a range of different instruments has been used and, in this chapter, some of them are discussed. In Table 3 the techniques are compared, according to their advantages and limitations in the analysis of textile dyes.

Table 3. Instrumental techniques used for the analysis of textile dyes.

Technique	Description	Advantages	Disadvantages
LC-UV-Vis/ DAD [2,18,28,30,59–67,III]	LC for components separation and detection by absorbance in the UV (200–400 nm) and Vis ranges (400–780 nm) for component identification. DAD enables recording the whole spectrum.	LC's ability of component separation is very useful since dyes are often complex mixtures; detection in the Vis range is selective to dyes. The technique is mature and abundantly used for analysing dyes.	Components must be dissolved for LC. Sample preparation destructive and time-consuming. UV-Vis is often not sensitive enough for all the components and sometimes not selective enough (absorbance spectra are wide and uncharacteristic). Without standards, difficult to distinguish between similar components.
LC-FLD [67,68,III]	LC for components separation, FLD for detecting components that emit fluorescence light.	LC's ability of component separation is very useful. If a compound is fluorescent, then detection is typically more selective and sensitive than UV-Vis absorbance.	Components must be dissolved for LC – sample preparation destructive and time-consuming. Not all compounds fluoresce, might need derivatisation. Without standards, difficult to distinguish between similar components.
LC-MS [2,19,26,67,69–73,III]	LC for components separation, MS for components identification (possible to identify all components that ionize under selected conditions).	LC's ability of component separation is very useful. MS is highly sensitive, very selective for identification, enhanced by the use of tandem-MS. The technique is mature and abundantly used for analysing dyes.	Components must be dissolved for LC – sample preparation destructive and time-consuming. For MS the right detection mode (negative or positive ion) and conditions have to be used for all the components to ionise.
GC-MS [74–78]	GC for components separation, MS for components identification (possible to identify all components that ionize under selected conditions).	GC's ability of separation is very useful. MS is highly sensitive and very selective for identification. The technique is mature and abundantly used.	Components must be volatile and thermally stable. Needs destructive and time-consuming extraction from fibres. Extensive fragmentation. Complex mass spectrum, interpretation based on reference spectra (library). As dye components are not volatile, they need derivatisation which might be time- and resource-consuming and not always efficient.

Technique	Description	Advantages	Disadvantages
Direct HRMS [79–81,III]	With direct MS dyes are analysed from fibre or solution without previous chromatographic separation. High resolution and high *m/z* accuracy enable identifying components more reliably (not just nominal mass-to-charge ratio (*m/z*)). Direct analysis from the fibre can be done with MALDI or DART.	Soft ionisation methods (ESI, MALDI) can be used, no extensive fragmentation. Very information-rich mass spectra. Accurate *m/z* values enable reliably identifying compounds. Possible to analyse directly from fibre without sample preparation when using MALDI.	Mass spectra are complex and might be complicated to interpret since there's no previous separation of compounds. When using ESI/APCI or other ionisation where solution form is used, the sample needs destructive and time-consuming preparation. For MALDI, suitable matrix substance must be found.
Raman / SERS [82–87]	With Raman spectroscopy it is possible to gain information about chemical bonds in compounds, thus characterise the structure. With SERS Raman spectra are recorded from dyes on the fibre surface by enhancing the Raman signal and quenching the interfering fluorescence using metal surface.	Sensitive method – very small samples amounts can be used (as small as single fibre with 1mm length). Recording spectra is a fast procedure.	Sample surface must be coated with metal nanoparticles. Mordant dye components might need extraction from fibre (due to dye-metal cation complex), which introduces challenging sample preparation. Interpretation of mixtures can be difficult.
FORS [22,88,89]	FORS enables recording dye spectra in the UV-Vis range directly from the dyed fibre surface.	A non-invasive method, analysis directly from fibre – does not need extraction. Possible to analyse *in-situ*. Recording a spectrum is fast and simple.	Spectra are usually not characteristic enough for positive identification. In the case of mixtures, peaks overlap. Useful rather as a preliminary method. When analysing directly from fibre, dye concentration cannot be changed (both too low and too high might be a problem).
Fluorescence spectroscopy / microfluorometry [8,22,90–94]	Fluorescence emission spectra are measured with the fibre optic system directly from fibre or from solution. 3D spectrum can be obtained when recording emission spectra at different excitation wavelengths.	Due to fibre optics, does not need an extraction and is a non-invasive method. Often quite selective, since not all dyes fluoresce. Compounds give special emission spectra at different excitation wavelengths.	Not all compounds fluoresce. In mixtures, spectra from dyes might overlap and/or interfere.

One of the most used approaches for analysing textile dyes is chromatographic separation with different detectors. Since dyes are a rather diverse group of molecules with different sizes, mostly polar features and often not volatile, liquid chromatography (LC) is the most used separation technique in this field. Liquid chromatography is an analytical separation technique using liquid mobile phase to carry analytes through a solid stationary phase to separate them by their chemical and physical properties[59]. For textile dye analysis, liquid chromatography with UV-Vis detection has been the most widespread technique since the original developments by Wouters and Verhecken[60,95]. For several decades, numerous research groups have relied on this approach, where liquid chromatography is used to separate compounds and UV-Vis or diode array detection (DAD) is used to detect colourful compounds[2,18,28,30,61–66]. When using diode array detection, dye compounds can also be characterized by UV-Vis spectra addition to detection. However, the low specificity and characteristic of UV–Vis spectral shapes and low sensitivity has led to an understanding that HPLC with mass spectrometric detection (MS) is a much more suitable technique for identifying such a wide variety of different dye components[2,19,26,69–73]. MS coupled with LC enables to characterizing each chromatographic peak with its mass spectrum containing the signal corresponding to the mass-to-charge ratio (*m/z*) of the ion formed from the corresponding compound. With tandem mass spectrometry, it is possible to study fragmentation and with this information learn more about the structures of the compounds.[26,71]

MS is highly universal. It can detect all components in the solution that can be ionised. The high universality can in some point be a disadvantage as well – when using low-resolution detection then in complex mixtures several components might have the same nominal *m/z*, thus identification of the relevant components might be more difficult. Usage of high-resolution mass spectrometry (HRMS) enables obtaining *m/z* with sufficient accuracy so that in many cases molecular composition can be deduced from the *m/z* ratio without the use of a standard substance[19,96]. The HRMS used in this thesis was Fourier transform ion cyclotron resonance (FT-ICR) mass spectrometer, which, besides high *m/z* accuracy, is also able to separately detect signals corresponding to similar *m/z* values with high resolution and thus differentiate between compounds with close *m/z*[97]. With HRMS different ionization methods can be used[98]. In this work matrix-assisted laser desorption/ionization (MALDI)[79,97–99] and electrospray ionization (ESI)[98,100] have been used, suitable for a variety of complex materials, including those that are (partly) polymeric, with low volatility and cannot be easily separated by chromatographic methods. With direct analysis, without chromatographic separation, mass spectra can have a very large number of peaks and can be complicated to interpret even with the use of HRMS. To reduce the complexity, soft ionisation methods without extensive fragmentation can be used. (MALDI) is a soft ionisation method with the great advantages of (1) not needing fully dissolved components (the solutions can be slightly hazy) and (2) mass spectra can be obtained from solid samples without extraction/ dissolution. In the case of MALDI, the sample is mixed with matrix substance

and with the help of laser and matrix substance, molecules are ionized from the dried sample mixture. MALDI produces ions directly from the solid state. In ESI high voltage is applied to liquid flow, creating an aerosol of charged droplets. Solvent from these charged droplets is then evaporated with heated inert gas to form ions. ESI is a good ionisation source for analysing compounds also in the negative mode[100]. As many dye components have acidic groups (-OH groups attached to aromatic systems in many anthraquinones like alizarin, purpurin, as well as carboxylic acid groups in kermesic and flavokermesic acid)[5], ESI in negative mode was chosen for this work. For very small sample amounts (often the case in cultural heritage), nano-ESI (nESI) approach is preferred with flow-rates as low as 25–50 nl/min.[98]

3. EXPERIMENTAL SECTION

3.1. Analysed standard samples

Standard samples in this work are not high-purity chemicals, but rather a collection of daily used textile pieces and textile dye sources. The identity of the fibres was confirmed by comparing the IR spectra of the same materials of different origin against each other. Textile dyes were analysed with several instruments and results were confirmed with available literature to ensure the suitability as a standard.

Textile fibres
Different textile materials were used for creating the collection of FT-IR spectra of fibres. For this research, 61 single-component textile fibres of 16 different types were used: wool, silk, cotton, linen, jute, sisal, viscose, cellulose acetate (acetate), Tencel™ (lyocell), fibreglass, polyester, polyamide, polyacrylic, elastane, polyethylene and polypropylene (see articles I, II and IV). Additionally, 52 two-component fibre mixtures were analysed for article II (Table 1 in article II). Samples were obtained from different companies from Estonia (Kreenholm Manufaktuur OÜ, Estonian National Opera), fabric stores (AS Abakhan Fabrics Estonia) and private collections.

Textile dyes
The textile dyes and dye sources (Dyer's madder, redwood, logwood, sandalwood, kermes, cochineal, bloodred webcap) were obtained from the Estonian National Museum, Kremer Pigmente GmbH & Co. KG, Aichstetten, Germany and a private collection.

3.2. Analysis of textile fibres

3.2.1. Analysis with FT-IR spectroscopic techniques

FT-IR spectroscopy was used for articles I, II, IV and V to analyse the composition of textile fibres. For fibre analysis, two different FT-IR instruments were used – ATR-FT-IR spectrometer and FT-IR microspectrometer with ATR and reflectance mode.

ATR-FT-IR spectrometer
For recording ATR-FT-IR spectra from fibres, Thermo Scientific Nicolet 6700 FT-IR spectrometer with Smart Orbit micro-ATR accessory was used. The instrument has DLaTGS detector, Vectra Aluminium interferometer and sealed and desiccated optical bench with CsI optics. Smart Orbit is a single-bounce diamond crystal ATR accessory with a refractive index of 2.4, active sample area diameter 1.5 mm. Parameters used in measurements were: resolution 4 cm^{-1}, spectral range 225-4000 cm^{-1}, zero filling factor 0, apodization window was

Happ-Genzel. Thermo Electron's OMNIC 9 software was used to collect and process the IR spectra.

For better spectrum quality, the number of scans recorded for each spectrum was 128, except for semi-quantitative analysis in article II, where 32 scans were used. The number of scans was optimised to save time needed for spectra registration. For each sample piece, several spectra were recorded from different parts of the fibre piece. This action was needed to test the homogeneity of the textile piece and to be sure that the sample only consisted of one type of fibre. All in all, the collection of one-component fibres now consists of 10 individual spectra from each analysed textile piece.

For the analysis with ATR-FT-IR spectrometer textile samples do not need any sample preparation. A textile sample was placed on the ATR crystal and pressure was applied. During the measurements of standards textile fibres, different approaches were tested to obtain spectra with the highest quality possible. Better quality spectra were obtained when the textile piece was rather tightly knitted and thicker. For thin cloths, textile pieces were folded, forming multilayer pieces and then spectra were recorded. As most of the new cloth pieces are very durable and strong, quite strong pressure was applied to get more intense spectra. Measurements with real-life samples were carried out in a somewhat different way. As textile fibres degrade with time, they tend to get more brittle, thus experiments with pressing sample onto crystal must be done with extreme care. For real-life samples, if sample ought not to be damaged, only light pressure was applied when recording spectrum.

FT-IR microscpectrometer

Thermo Scientific Nicolet iN10 MX integrated FT-IR microscope (FT-IR microspectrometer) was used in reflectance (r) and ATR modes. Measurements were done using mercury-cadmium-terruride (MCT) detector cooled with liquid nitrogen, spectral range 550/600-4000 cm^{-1}, resolution 4 cm^{-1} and 64 number of scans for standard collection (article IV) and 8 scans for homogeneity study (article II). For the analysis in reflectance mode, the sample was placed on the gold plate, which was also used as a background. For the analysis in ATR mode, the sample was placed on the metal plate and the slide-on ATR objective with a conical germanium crystal (Slide-On MicroTip Ge ATR crystal) was used. The micro-ATR tip allows analysing samples as small as 3 microns. As the pressure area in ATR-FT-IR microspectrometer is much smaller than at regular ATR-FT-IR spectrometer, less damage is done with this method on real-life samples. In the reflectance mode measurement area is adjustable: in most cases aperture 150x150 μm was used since it was small enough to analyse small parts of the sample, but large enough for good quality spectra. For smaller sample pieces aperture down to 25x25 μm was used. The data were collected and processed using Thermo Electron's OMNIC PICTA software.

Similarly, to measurements with ATR-FT-IR spectrometer, from each sample piece, several spectra were recorded in reflectance and micro-ATR (mATR) mode as well. As this FT-IR microspectrometer enables mapping and

also spectrum recording is faster due to MCT detector, more spectra were recorded from each sample. Standard spectra collection now consists of 2068 mATR- and 1662 reflectance-FT-IR spectra.

3.2.2. Classification of textile fibres with different chemometric methods

Classification methods for identification of textile fibres were developed with Thermo Scientific TQ Analyst™ Professional Edition 9.0 and with an in-house written Python script. With TQ Analyst Pro software discriminant analysis was used and random forest classification with the *sklearn* library with Python.

TQ Analyst™ Professional is Thermo Scientific software that enables doing qualitative and quantitative analysis for infrared spectra. In this work, only classification methods were used. Experimental sections in articles II and IV describe all the important aspects of developing the discriminant analysis method for identifying textile fibres.

An in-house python script was written for analysing data for article IV. Preliminary method development included random forest, support vector machine and kNN (k-nearest neighbours algorithm) methods. Random forest method was chosen for subsequent classification.

3.3. Analysis of textile dyes

Analysis of textile dyes was done with five instrument sets (HPLC with DAD, FLD and MS detection and FT-ICR-MS with MALDI and ESI ion sources). Analysis of dye standards was done using natural dye sources, needing extraction phase. From real-life fibre samples, extraction of dyes was also needed. In the next chapters, all the steps of the analysis are described.

3.3.1. Extraction of dyes from the dye sources

Prior to analysis, dyes were all extracted from dye sources: insects, plants and mushroom. For extraction, an in-house method was developed combining recipes and instructions from literatute[5,36,101,102]. But to avoid contamination only Milli-Q grade water (Milli-Q® Advantage A 10, Millipore) and pure ethanol (Keemiakaubad AS, 96,7%) were used. The extraction method was developed to be easy and quick enough so that now and then new solutions can be made for additional analyses. General extraction process included weighing right amount (1 g) of dye source into a beaker, soaking the dye source in Milli-Q water (or 40% ethanol in Milli-Q water) for 16 hours and then heating the solution on the stove to make the extraction more effective (see the example in Figure 4). The exact extraction method is more thoroughly described in the experimental section of article III.

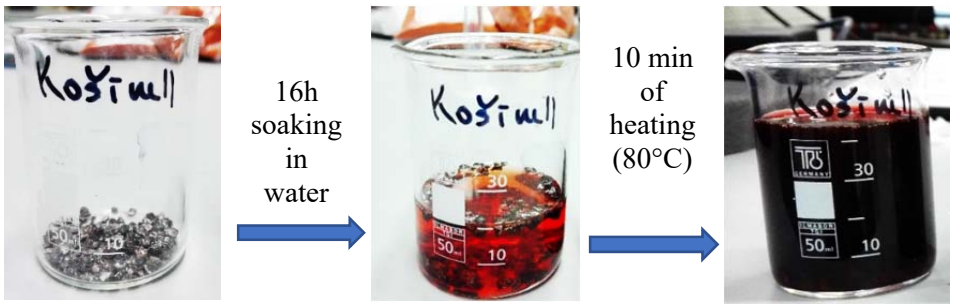


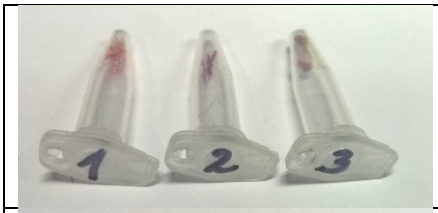
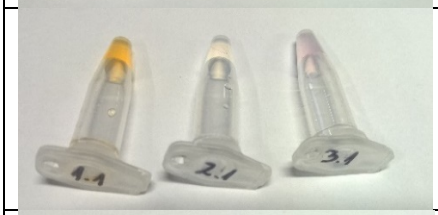
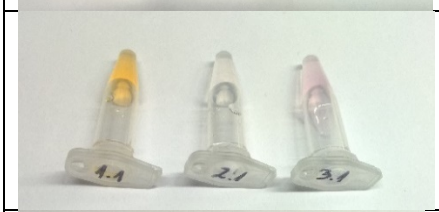
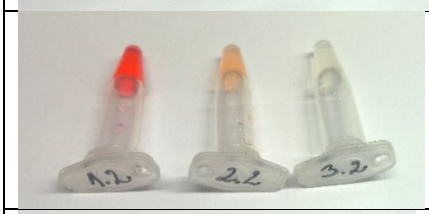
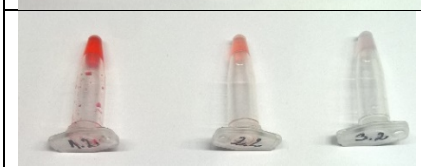
Figure 4. Illustration of the extraction process from cochineal dye source.

3.3.2. Extraction of dyes from textile fibres

From used case-study samples (see section 4.3), dyes were extracted with a multi-step method, which was developed by combining different methods available in the literature[22,69,103,104]. Multi-step extraction was chosen because it is known from the literature that different dye components extract better with different solvents. Many dyes can easily be extracted from fibres without hard HCl method[105,106], while some mordant dyes might need stronger acid to break the fibre-metal-dye complex. Softer extraction methods are needed to use in the beginning, since many dye components might not be durable for stronger acid methods. For example, many flavonoids can decompose due to strong acids and thus lot of information can be lost[106].

Multi-step exctraction consisted of three steps and in Table 4 workflow for three different red textile fibres is presented (real-life samples from carriage blanket and carpet from Estonian National Museum, results from sample nr 1 in Table 4 are more discussed in article III section 3.3.2 and section 4.3.1 in this thesis). It can be seen that the first mild methanol extraction step is able to dissolve yellow compounds from the first sample. Second sample solution got a very slight pink colour, while the solution from the third sample is with more intense colour. The second, dimethyl sulfoxide (DMSO, Sigma Aldrich, 99,9%), step gives quite similar results, adding a stronger colour to the third sample. Harsh HCl (Sigma Aldrich, p.a ≥37%) extraction step seems to be suitable to the first and second sample, which solutions gain a rather strong orange colour. The third sample solution, however, does not change much colour, probably meaning that essential dyes were already extracted with the first two steps.

Table 4. Extraction of red dyes from three different fibres. Fibre nr 1 is from a carriage blanket and fibres 2 and 3 from a carpet (provided by Estonian National Museum).

	Weighing textile fibre samples about 0.5–1 mg.
	100 µL of 2:1 (v/v) methanol/Milli-Q water and heating for 20 minutes at 60–70 °C.
	Solution from previous step + 100 µL of DMSO and heating for 20 minutes at 60–70 °C.
	100 µL HCl solution (3 M HCl in water/methanol 1:1, v/v) and heating for 20 minutes at 60–70 °C.
	Solutions from previous steps were evaporated to dryness under a nitrogen stream and reconstituted in methanol/ water (2:1, v/v) to 60 µL.

3.3.3. HPLC-DAD-FLD-MS

For dye analysis, *Agilent Infinity 1290* liquid chromatography with *Agilent 1290 Infinity* diode array detector, *Agilent 1200 Series* fluorescence detector and *Agilent Technologies 6495 Triple Quad* mass spectrometer was used. For chromatographic separation, gradient elution with methanol (B) and 0.1% formic acid in Milli-Q water (A) was used with a *Zorbax RRHD SB-C18* column (2.1 × 50 mm and a particle size of 1.8 µm). The eluent flow was 0.3 mL min^{-1}. The utilized gradient was: 0–5 min, 5% B; 5–60 min, 5%–100% B; 60–80 min, 100% B; 80–81 min, 100%–5% B; 81–101 min, 5% B. The MS was used in the negative ion mode with the following ionization source (Agilent Jet Stream)

parameters: nebulizer pressure of 20 psi, drying gas at 14 L min^{-1} and 250°C, sheath gas flow at 11 L min^{-1} and 350°C, and a capillary voltage of 3500 V.

For chromatographic separation, such a long gradient was chosen to make the method more universal to be used later for all the other dye sources as well. A gradient from 0–60 min is for component separation, 60–101 min to rinse the system, including column, for next injection. As with mass spectrometric detection, it is possible to separate peaks that do not have full chromatographic separation, a gradient was not adjusted in different part for full chromatographic separation. Before injection, extracted dye solutions from natural sources were filtered using 13 mm polytetrafluoroethylene (PTFE) filters with a pore size of 0.45 μm (Whatman). The exact amount of the sample injection depended on the dye source and intensity of the component peaks in the DAD absorption, FLD fluorescence emission and mass spectra, but was between 1–10 μL.

To get full information with DAD, FLD and MS detectors, from one sample actually multiple injections must be done. The most used system consisted of HPLC-DAD-MS, where detectors were used consecutively. The second system was HPLC-FLD-MS, because used software did not allow using DAD and FLD simultaneously. For FLD analysis, excitation wavelengths were firstly found from components DAD spectra – several absorbances that were the most intense. For better sensitivity in MS, two different regions were used for detection – the first injection covering the *m/z* area of 100-600 and second injection *m/z* area of 550-1200.

3.3.4. FT-ICR-MS with the MALDI and ESI/ nESI sources

FT-ICR-MS was used for the determination of different red dye components. Used dual FT-ICR-MS was Varian 930 on MALDI-ICR, ESI-ICR on Varian 910 with a 7 Tesla superconducting magnet for the generation and detection of ions. With FT-ICR-MS, two different ionization sources were used – MALDI and ESI.

MALDI

For MALDI analysis, 2,5-dihydroxybenzoic acid (DHB) was used as a matrix substance. Dye solutions from natural sources were mixed in Eppendorf tubes with a DHB (3:7, DHB/sample) and internal standards solution for sample analyses. For positive mode, phosphazenium cations and DHB peaks were used for internal standards and for negative mode fluorine-rich sulpho-compounds, both previously tested in our work group[97]. Then, 1 μL of each mixture was spotted on the MALDI plate and dried under vacuum. FT-ICR-MS has an intermittent pressure (10–3 mbar) MALDI ion source with a New Wave Orion 50083 Nd:YAG laser (355 nm, 4 mJ energy output, and pulse length of 4–5 ns). Measurements were done both in negative and positive mode in the *m/z* region of 90-1100.

ESI / nESI

For negative-ion ESI-FT-ICR-MS analyses, dye solutions from natural sources were diluted 10–100 times with an 80:20 methanol:Milli-Q water mixture and internal standards were added to this solution. To increase the pH and facilitate ionization of acidic groups, 5 μL of a 25% ammonium hydroxide solution were added to 1 mL of the dilutions. The regular ESI source was used for the preliminary analysis of model samples and standard solutions. Additionally, for historical samples with limited available solution volume, an in-house modified nano-ESI source was used. The ESI needle was cut shorter and the tip was polished and moved closer to the inlet of the MS. This proved to be useful for obtaining better spray and overall ionization efficiency, notably reducing the suppression of analyte signals and being able to use much lower flow-rates, which are needed for very small sample amounts (in the case of historical samples).

All the parameters for both MALDI- and ESI-FT-ICR-MS are more discussed in article III and its supplementary information.

4. RESULTS AND DISCUSSION

Before analysing any object – either a historically valuable textile, archaeological finding, forensic evidence or factory item – we need to ask ourselves a sequence of questions: what do we want to know, what information do we need to obtain the knowledge and how can we actually gain the information. In this work, the main focus is on the analysis of cultural heritage objects, and although many of the developed methods can be used for different applications, we establish the structure of our work around historical textiles.

The results section of this thesis concentrates on the last (rightmost) part of Figure 5 – method development for analysing the main components in textiles, fibres and dyes. Method development in this work was done by finding suitable parameters and measurement conditions for each instrument by analysing different standard samples, by that creating the collection of FT-IR spectra for standard fibres[II,IV] and collection of different chromatograms and spectra for textile dyes[III]. For these spectra and chromatograms, extensive data analysis with interpretation was then performed[II-IV]. All the developed methods were applied for different case-study samples[III-V] from museums and private collections in Estonia. In article V, developed ATR-FT-IR method combined with microscopic analysis[II] was used to analyse textiles found near more than 2000 years old Egyptian mummy. Selection of analysed case-study objects are described in the case-study section (4.3). Results for developed methods and their applications are more thoroughly described in the following sections.

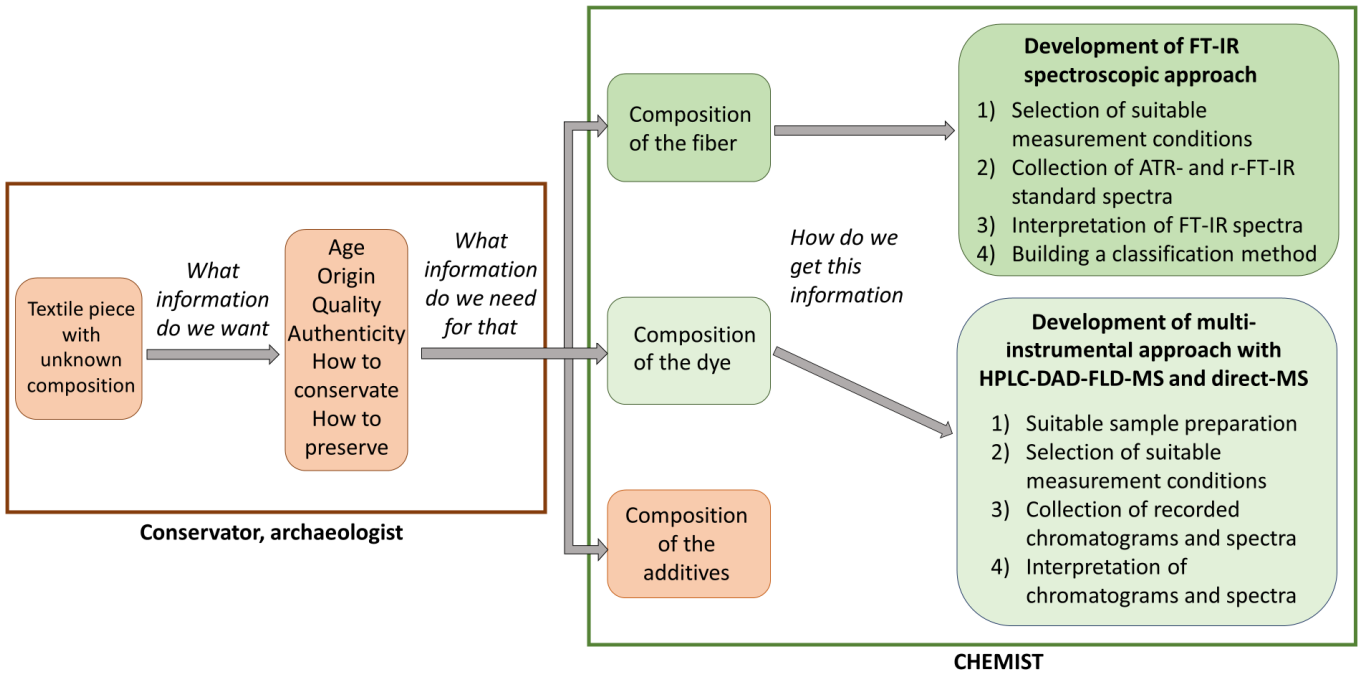


Figure 5. Structure of the work when analysing historical or archaeological textile samples. This thesis focuses on the activities denoted by green backgound

4.1. ATR-FT-IR and reflectance-FT-IR for the analysis of different textile fibres

In this work two analytically different FT-IR sampling techniques (ATR and reflectance), but instrumentally three different approaches – ATR-FT-IR spectrometer (ATR-FT-IR), FT-IR-microspectrometer with ATR (mATR-FT-IR) and reflectance (r-FT-IR) modes – were used and measurement methods were developed. In large scale, ATR-FT-IR spectra recorded with regular ATR-FT-IR spectrometer and ATR-FT-IR microspectrometer are same, but some of the differences still come across due to different optical properties of the ATR crystal (refractive index affect), used detector (wavenumber area, sensitivity) and measured area size (homogeneity of the sample). So, for the best results, all three available approaches were used and compared throughout this research. All approaches combined, from single-component standard textile fibres, thousands of spectra were recorded and in published works, around 4000 of them were used, belonging to 16 different textile fibre classes. Additionally, 316 spectra from 52 multi-component textile pieces were recorded with ATR-FT-IR spectroscopy for qualitative analysis and 175 spectra from 7 different two-component textiles for semi-quantitative analysis. These results are described in article II.

For characterization of materials of textile fibres, FT-IR spectra of standard samples were recorded using different sampling techniques (mATR, ATR, reflectance), processed and interpreted. Chemometric approaches were used for data analysis. To characterize each fibre type, thorough interpretation tables were compiled for each standard's ATR-FT-IR spectrum. From each spectrum, the most characteristic absorbance maxima were found. All these tables are presented in the supplementary information of article II. Besides interpretations of absorbance bands, each FT-IR spectrum acts as a finger-print comparison spectrum, which can be used to identify unknown textile fibres. The collection of spectra of standard fibres is the most useful tool for further analysis. While articles I and II focus on ATR-FT-IR usage, article IV brings extra value with a non-contact, thus totally non-invasive reflectance-FT-IR approach. Similarly to ATR-FT-IR spectra, r-FT-IR spectra of standard fibres can also be used as comparison reference spectra for the identification of unknown textile fibres. But unlike ATR-FT-IR spectra, r-FT-IR spectra at present cannot be interpreted to the same level of detail as ATR-FT-IR spectra, due to lack of reference spectra (r-FT-IR approach is not yet widely used for that kind of samples) and unambiguous measurement technique (diffuse and specular reflectance typically get combined when recording spectra from uneven surfaces).

4.1.1. Standard reference spectra of single-component textile fibres

FT-IR spectra of the most common textile fibres, recorded in all used sampling techniques, are presented in Figure 6. Some of the prominent absorbance bands of the fibres in the IR spectra with the interpretation are presented in Table 5. As natural cellulose-based fibres have very similar spectra, only cotton is presented in Figure 6. Spectra of other cellulose-based fibres (linen, jute and sisal) and Tencel™ (lyocell), fibreglass, elastane, and polypropylene are shown in the Additional file of article IV. Complications with Tencel™, elastane and polypropylene are discussed later in this section and in article IV.

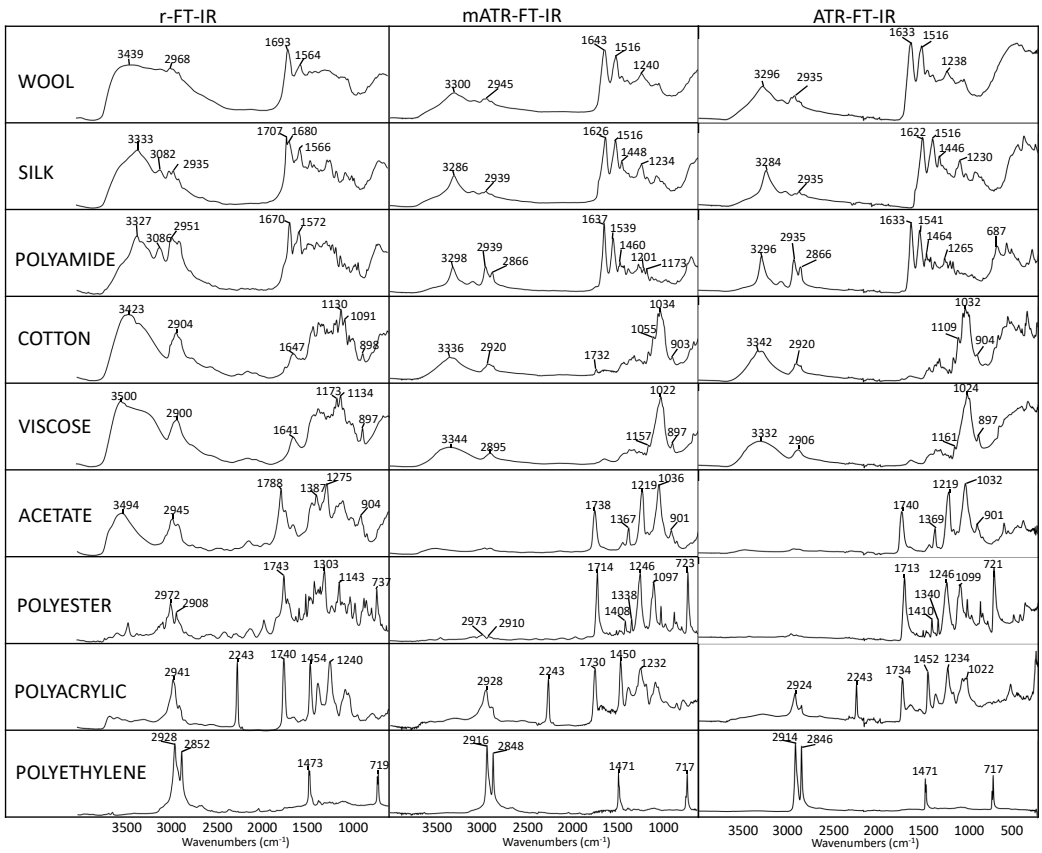


Figure 6. Comparison of r-FT-IR, mATR-FT-IR and ATR-FT-IR spectra of the most common textile fibres. Spectra are normalised and averaged. For FT-IR microspectrometer (r- and mATR-FT-IR) wavenumber region 4000-600 cm^{-1} and for ATR-FT-IR spectrometer 4000-225 cm^{-1} were used.

Table 5. The most prominent absorbance bands in different analysed fibres spectra.

Wavenumber (cm^{-1})	Assignments
3500-3000	O-H stretching / N-H stretching
3000-2800	C-H symmetric and asymmetric stretchings
2243	C≡N stretching
1790-1710	C=O stretching
1700-1600	amide (-CONH) C=O stretching
1600-1500	C-N-H bending (combined C-N and N-H) and O-H bending in this region, aromatic ring C=C stretching
1500-1300	C-H bending and O-H *in plane* bending
1300-800	C-O, C-O-C, C-O-H, C-N stretchings and aromatic ring C-H bending in this region
800-500	O-H, C-O, N-H *out-of-plane* bendings, C-H bending in this region

The ability of r-FT-IR and ATR-FT-IR techniques to identify textile fibres was evaluated by comparison of standard reference spectra (see Fig. 6). As it can be seen, most of the spectra from different textile fibre types have quite unique absorbance pattern, thus making the differentiation of the fibres with FT-IR spectroscopy a very suitable approach. While different ATR crystal materials (germanium in mATR-FT-IR and diamond in ATR-FT-IR) have only a very small influence on the recorded spectra, reflectance-FT-IR spectra are rather different from the corresponding ATR-FT-IR spectra. As it can be seen in Fig. 6 some of the absorbance bands of the fibres in r-FT-IR spectra are broader, wavenumbers shifted towards higher values, and intensity ratios of bands are different from the corresponding ATR-FT-IR spectra. This finding is rather not surprising since the measuring mechanism is very different. In reflectance-FT-IR actually two quite different mechanisms are in operation: specular reflectance and diffuse reflectance (DRIFTS). In general, specular reflectance is only used to analyse samples with a mirror-like surface, while DRIFTS is mostly used to analyse samples with an uneven surface to enhance the diffuse reflectance. In this work, specular reflectance-FT-IR instrument is used, but the recorded spectra do not follow any Kramers-Kronig equations, neither DRIFTS rules, thus most probably the two reflection mechanisms are combined in our spectra. This hypothesis is relevant since the surface of textile pieces is rarely smooth and even. Nevertheless, this reflectance method gives very characteristic spectra on each class of textile fibre, thus making this approach very useful for identification of polymeric fibre materials.

In this work, in some cases, it was found that with reflectance-FT-IR it can be obtained some more characteristic spectra than with ATR-FT-IR. One of the examples is protein-based fibres silk and wool. Chemical composition of both of these fibres is protein. Wool is composed of keratin, while silk consists mostly of fibroin polymer. These compositions are different enough for identification in ATR-FT-IR when quite pure textile fibres are used. With ATR-FT-IR,

N-H stretching bands of silk are narrower in the region of 3600-3000 cm^{-1} and amides C=O stretching bands ~1626 cm^{-1} is shifted compared to wool. Problems with the differentiation of the fibres may occur when analysing partially degraded or very impure textiles. In these cases, additives from the textiles can disguise the fibre absorbances, making the existing fibre absorbance bands wider. In these cases, ATR-FT-IR spectra of wool and silk are getting quite similar, making the correct identification rather complicated. With reflectance-FT-IR, silk spectrum has very distinct split absorbance bands near 1707 and 1680 cm^{-1} that can be used for the determination of silk in the unknown textiles.

Another problem that occurred when using ATR-FT-IR, but did not affect reflectance mode, was obtaining good quality FT-IR spectrum from the polyacrylic fibre. Measurements of pure polyacrylic and also mixed fibres containing polyacrylic with ATR-FT-IR tended to give poor-quality spectra compared to any other analysed standard[II]. While maximum absorbances in ATR-FT-IR spectra for other fibres were over 0.25, in many cases over 0.50, the highest absorbance in the case of polyacrylic reached 0.085. Due to very low absorbance, the signal-to-noise ratio for these spectra was also very low. In reflectance mode, low spectral quality was not a concern when analysing polyacrylic fibres. However, in reflectance mode, problems occurred when analysing the elastane standard. In this case, the problem did not seem to be in the fibre itself, but in the form of that specific sample. As elastane is rarely used on its own, but rather as an additive in textiles to make the fabric more elastic, in this work it was possible to analyse only elastane thread. While both ATR-FT-IR and FT-IR-microspectrometer with ATR mode enabled to get adequate spectra, with r-FT-IR method only strongly distorted spectra were obtained, even after reducing the regularly measured area 150x150 μm to 25x25 μm. Spectra and more discussion about this problem can be found in article IV. Concerns with purity and inhomogeneous distribution of fibre components in Tencel and polypropylene standards are also described thoroughly in article IV.

Analysing textile fibres simultaneously with all three approaches, enabled to compare the methods, their flaws and advantages. This helps in deciding which of them to use when analysing case-study samples. While ATR-FT-IR with diamond crystal allows analysing samples in broader measuring range (4000-225 cm^{-1}), it is more destructive than mATR-FT-IR, which has a smaller crystal tip. When non-destructivity is critical, then only reflectance mode with a non-invasive approach is suitable. But it has to be kept in mind, that when analysing very small sample pieces, r-FT-IR spectra might be distorted, and unambiguous identification could be impossible. The problem with the extreme similarity of the cellulose-based fibres remains for all the used FT-IR approaches and in this case, microscopic analysis must be included.

4.1.2. Development of classification methods to identify single-component textile fibres

Data analysis of the massive volume of ATR-FT-IR, mATR-FT-IR and reflectance-FT-IR spectra needs easier and automatized approaches. In this research, a lot of work was done with chemometric grouping and classification methods. For the visual differentiation of textile classes, graphs of principal component analysis (PCA) were used in three-dimensions (see Figure 7). Besides that, classification methods were developed for identification of case-study samples. For classification two approaches were used – PCA based discriminant analyse (DA) using Thermo Scientific TQ Analyst Pro software and random forest (RF) using in-house built Python script. In article II only DA-based classification was used. In article IV both DA and Python script based RF method were used for comparison of different chemometric approaches.

In Figure 7, PCA graphs of three different FT-IR data sets are presented – spectra recorded using FT-IR microspectrometer with reflectance (r-FT-IR) and micro-ATR (mATR-FT-IR) modes and ATR-FT-IR spectrometer (ATR-FT-IR). As seen in the previous chapter, r-FT-IR and ATR-FT-IR spectra can be quite different, thus it is not surprising that PCA clustering graphs are also different. The difference in PCA graphs using mATR-FT-IR and ATR-FT-IR spectra can come from (1) the fact that data amounts are very different (612 spectra for ATR-FT-IR and 2022 spectra for mATR-FT-IR) and that consequently the range (spread, scatter) of the spectral features in the two collections of spectra are different, leading to different combinations of initial dimensions into the principal components and (2) possibly from different crystal materials (with different refractive indexes) leading to some differences between spectra of the same material.

Generally, it can be seen that most of the fibre classes are grouped well. Interestingly, the amide-based fibres – silk, wool and polyamide – are clustered in both ATR modes, but are well separated with reflectance mode – better differentiation between amide-based fibres was also done with the interpretation of r-FT-IR spectra (see chapter 4.1.1). This observation means that the ability of differentiation using classification of the different IR sampling modes can occasionally be remarkably different. Problems with distinguishing between cellulose-based fibres (cotton, linen, viscose etc), however, remain and are well visualised in each of these PCA graphs. Classification with more than three principal components have shown better separation of viscose from natural cellulose-based fibres, but differentiation of natural cellulose-based fibres has not been possible with FT-IR spectroscopy. In this case, after placing the sample into the cellulose-based class, optical microscopic analysis is done with the fibre sample. Most of the cellulose-based fibres are distinguished very well with an optical microscope.

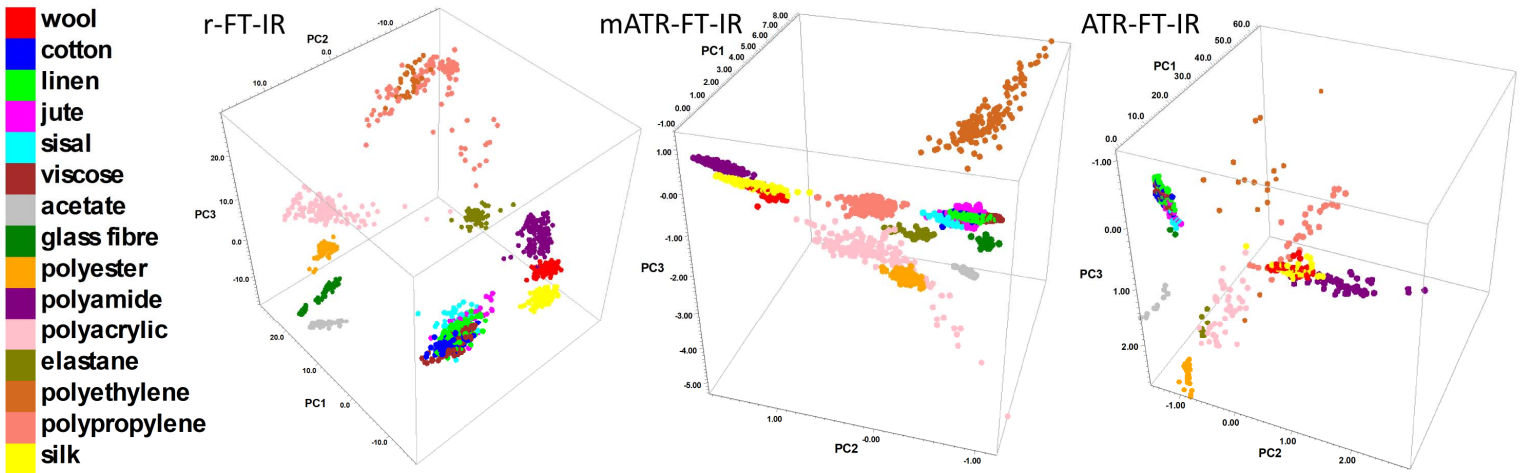


Figure 7. PCA graphs of textile fibre FT-IR spectra recorded with FT-IR microspectrometer with reflectance and mATR modes and ATR–FT-IR spectrometer (PCA graphs composed with TQ Analyst software).

As it was shown in the case of clustering, the different fibre classes group apart from each other. Thus, it can be predicted that classification should work well for distinguishing between the fibre classes. The reliability of developed classification methods is best shown with the analysis of actual textile samples. In article IV two case-study objects were examined with both DA and RF classification. In some cases, it was shown that RF did better work assigning more fibres into the correct classes. One example being scarf samples nr 1 and 2 (see article IV Table 2), where DA with mATR-FT-IR suggested for both samples polyacrylic content, however, spectra from neither of the samples contained C≡N absorbance near 2242 cm^{-1}. Also, for sample nr 5 from the same scarf, only RF with mATR-FT-IR was able to identify the correct class (viscose) for all the recorded spectra.

Clustering of the spectra in this work helped to gather and analyse massive data amounts and visualise the differences and similarities within and between different classes of fibres. Classifications methods developed in this work help to identify unknown fibres in the case of a large number of spectra by automatizing the process.

4.2. Multi-instrumental analysis of natural red textile dyes

Chemical composition of textile dyes is very different from textile fibres, thus a completely different set of instruments was used. Besides the broad and complex composition, dyes' concentration in fibre sample is often very low, especially in the case of degraded museum artefacts and archaeological textile findings. For these reasons, many different instrumental approaches were used to gain as much valuable information as needed and possible. Five instrumental setups were used: HPLC-DAD, HPLC-FLD, HPLC-MS, ESI-FT-ICR-MS and MALDI-FT-ICR-MS. Some of these techniques (HPLC-DAD, HPLC-MS) are widely used for the analysis of textile dyes. Nevertheless, for obtaining an applicable method, many parameters and conditions had to be tuned for each instrument and dye. In this work, the method development was done for seven red dye sources, but all the conditions were developed as universal as possible so that this approach could later be applicable to unknown real-life samples and other dye sources as well. For that reason, in HPLC analysis the gradient was chosen longer and in direct-MS wider *m/z* area (90-1100) was used to be able to identify additional ions besides the known dye components. Besides these well-used detectors mentioned before, also limited-used fluorescence detector was included for HPLC in this work as a novelty. This HPLC-FLD approach proved its usefulness in some specific cases, e.g. for the analysis of logwood and its main component hematein, where MS and DAD detector gave controversial results. Chromatograms and discussion of the data obtained with the logwood standard are found in article III, section 3.1.

Besides widely used HPLC-DAD and HPLC-MS limited-used high-resolution FT-ICR-MS with ESI and MALDI sources was included for the analysis of textile dyes. An advantage of FT-ICR-MS is that besides its ability to identify *m/z* values very accurately (error down to less than 1 part per million (ppm), routinely below 2 ppm) the technique also has very good resolution, enabling separate ions with very close *m/z* values. With this approach, it was possible to confirm the presence of dye components and also identify unknown dyes for which physical references were absent. Thanks to nESI-FT-ICR-MS, synthetic dyes from one of the case-studies were detected without using any standard (see section 4.3.1).

Another novel approach for FT-ICR-MS analysis in this work was the usage on in-house made nano-ESI needle which enabled to analyse real-life samples that had very low dye concentrations and very small sample amounts. While with regular ESI needle, interfering fatty acid ions were dominant in spectra, nESI suppressed the ionisation of these components, enabling to see dye components better. For red anthraquinone dyes, negative ESI mode was especially useful, since many of these dyes have acidic groups, helping them to be well ionised in negative mode.

While natural dye's sources typically consist of multiple different dyes and additional components, the concentrations of the components vary a lot and thus not all components have the same importance in the process of identification of the dye source. In Table 6 the most prominent components for each analysed dye source are presented. In this table the components are listed according to their importance in the multi-instrumental analysis – how intensive is the signal, is it detectable with all used methods.

Combination of different components and their relative concentrations/ intensities are essential information to correctly identify the dye source. For example, the presence of only one carminic acid peak in the chromatogram, might not mean that fibre was dyed with cochineal, but can also indicate the usage of synthetic carminic acid dye. But the presence of very small amounts of isomers dc IV and dc VII as well as other components like flavokermesic and kermesic acid, indicates cochineal usage more reliably. Likewise, the presence of flavokermesic and kermesic acid does not automatically mean that the kermes insect was used unless these two components are clearly dominant. For identifying dyer's madder, the ratio of alizarin, purpurin and other components can be important, since there are other similar dyeing plants, e.g northern bedstraw, common in Estonia, with the same components in different proportions.

Detailed information (retention time, Vis absorbance, exact *m/z* etc) about each component and dye source is presented in article III, Table 1. In the supplementary information of article III, an extended table is given with several other chromatographic peaks that are not fully identified but can still be characteristic to specific dye sources. Bloodred webcap is a less studied dye and that comprehensive instrumental data of the components of dye source is new in this field.

Although five different instrumental techniques were tested for standard dyes, not all of them have to be used together for the case-study samples. A multi-instrumental approach is important in method development and component characterisation, but due to very small amount of sample often available in cultural heritage studies and limited resources, only selected techniques are typically used for real-life samples analysis. In Table *7* all the tested methods are compared according to their capability of analysing seven natural red dye sources. Similar table with discussion is presented in article III (Table 2). In this thesis, the table has been supplemented with additional information about the practical aspects of measurements.

The strong point of techniques involving LC is their ability to separate different components, which is very useful when analysing complex systems like extracts from plants and insects. Besides dyes, these mixtures can contain a lot of other compounds like sugars, tannins, oils etc. UV-Vis detector in the visible range (400–640 nm was used in this work) is the first choice for detecting colourful compounds as it enables to conveniently eliminate interfering compounds. In this work, LC-UV-Vis was very helpful in method development in finding colourful components from standard mixtures, but since the sensitivity of this method can be low, this method alone might not be sufficient for case-study samples where concentrations are much lower. Thanks to the possibility to use DAD and MS detectors simultaneously within one run, this combination of LC-DAD-MS is preferred as the first step of the analysis if enough textile sample is present for extraction. LC-DAD part of the approach helps to detect dominant colourful compounds in the solution, while with additional information from MS, it is possible to identify components more reliably. Besides that, since MS detection is much more sensitive, additional dye components that cannot be seen with DAD might be visible in LC-MS chromatogram. In the case of finding unknown colourful compounds with LC-DAD-MS methods, low-resolution MS might still be not enough for component identification, when retention times are not determined with standards. In this case, high-resolution FT-ICR-MS with both ESI and MALDI ionisation are the next important step. Nominal *m/z* values behind chromatographic peaks can be searched from the HRMS spectrum. With *m/z* values accurate to ± 2 ppm (typically meaning 5 digits after comma), the elemental composition of the component can be found with high reliability. In this work, it was found that if enough sample solution was available, nESI-FT-ICR-MS in negative mode tended to give better result in detecting and identifying different dye components. However, in the case of very small amounts of either sample solution or fibre pieces, MALDI is preferred, since this method does not need filtration of the solution and less than 1 µl of sample solution can be used for the analysis.

Table 6. Identified prominent components from seven red dye sources based on the multi-instrumental analysis. Components are listed according to their approximate order of importance.

Dye source	**Dyer's Madder**	**Sandalwood**	**Cochineal**	**Kermes**	**Bloodred webcap**	**Logwood**	**Redwood**
Components	alizarin	santalin A	carminic acid	kermesic acid	dermorubin	hematein	brazilein
	purpurin	santalin B	dc IV	flavokermesic acid	5-chlorodermo-rubin	hematoxylin	brazilin
	rubiadin	santarubin A	dc VII	kermesic acid isomer	emodin	hematein isomers	brazilin isomers
	nordamnacanthal	santal	flavokermesic acid		dermocybin	hematoxylin isomers	brazilein isomers
	munjistin	(iso)liquiri-tegin	kermesic acid		dermolutein	brazilein	
	lucidin	maackiain			endocrocin		
		pterocarpin			5,7-dichloroemodin		
	xantopurpurin	santalin			7-chloroemodin		
	ruberythric acid				physcion		
	lucidin primeveroside						
	pseudopurpurin						

Table 7. Comparison of the general utility of the instrumental techniques in the identification of different dyes.[a] [III]

Dye source	LC-UV-Vis	LC-FLD	LC-ESI(-)-MS	ESI(-)-HRMS	MALDI(-)-HRMS	MALDI(+)-HRMS
Dyer's madder	++	+	++	+	+	+
Sandalwood	++	+	+	++	–	+++
Cochineal	++	+	++	+++	+++	++
Kermes	++	+	++	+	+	–
Bloodred webcap	++	+	++	+++	–	–
Logwood	++	++	++	+	+	++
Redwood	++	+	++	++	+	++
Practical aspects of techniques	* components must be fully dissolved				* possible to analyse insoluble substances and dyes directly from the textile fibre * suitable matrix substance must be chosen * matrix substance peaks in the spectrum can be dominant and interfere with the analysis	
	* possible to differentiate between isomers			* can be definitive if a component exists that is not present in other dyes (even if there is no standard substance) * for precise *m/z* suitable internal calibrants must be used		
	* low sensitivity * low characteristicity	* only for certain components	* not all components ionize well, suitable parameters must be found			

[a] Legend: "+++" Very useful, possibly definitive; "++" useful if other evidence exists; "+" useful as supporting evidence; "–" not useful. All of these assessments are based on our perception and refer to the experimental conditions used in this work.

As a result of using these different instruments, seven red dyes sources were characterised with a collection of chromatograms and spectra (altogether 113) and 23 dye components were identified at a molecular level and provided with corresponding chromatographic retention time, absorption maxima, in some cases fluorescence emission maxima and high-resolution mass spectra. All that data is presented in article III, Table 1. In supplementary information of III, an extended table is given with several other chromatographic peaks that are not fully identified but can still be characteristic to specific dye sources. The obtained collection of chromatograms and mass spectra are a useful set of reference materials for latter identification of unknown dyes. Comparison of these used techniques proved, that in many cases they complement each other and for case-study sample analysis, a combination of techniques is the best option for reliable identification.

4.3. Case-studies

All the previously described developed methods were applied on several case-study samples from the Estonian National Museum, Conservation and Digitization Centre KANUT and private collections. This chapter presents case-studies that have been described in published articles III–V. The results of all of these case studies are collectively presented in Table 8. First three samples are dye analysis case-studies, but prior to dye analysis, fibre identification was also done. From other samples, only fibre identification was done. Two case-studies (carriage blanket and textile painting, see Table 8), that present the capability of developed methods are discussed more thoroughly below (see chapters 4.3.1 and 4.3.2).

Table 8. Analysed case-study samples and their results.[a]

Object	Textile fibre		Dye		Article	Comments
	Identified fibres	Used methods	Identified dyes	Used methods		
Carpet from Estonian National Museum (19th c.)	Wool	ATR-FT-IR, microscopy	Cochineal	LC-DAD-MS, nESI-FT-ICR-MS	III	Two samples from the same carpet were analysed. Carminic acid and isomers, flavokermesic and kermesic acid were found. Unknown components with *m/z* 503.08308 and 533.09322 (corresponding $C_{23}H_{20}O_{13}$ and $C_{24}H_{22}O_{14}$, accordingly) were detected.
Carriage blanket from Estonian National Museum (19–20th c.)	Wool	ATR-FT-IR, microscopy	Acid orange 7, acid red 27[a]	LC-DAD-MS, nESI-FT-ICR-MS	III	Usage of synthetic dyes was found. More information below in section 4.3.1.
Tapestry from KANUT (18th c.)	Wool	ATR-FT-IR, microscopy	Northern bedstraw	LC-DAD-MS, nESI-FT-ICR-MS	III	Purpurin, alizarin, rubiadin and other anthraquinones were found. Ratio of purpurin and alizarin, indicated rather local northern bedstraw instead of dyer's madder.
Scarf from KANUT (20th c.)	Silk, viscose, cotton, unknown mixture	r-FT-IR, ATR-FT-IR, microscopy, classification	-	-	IV	Altogether seven sample pieces were analysed. Four of them were fully identified. Three unknown fibre mixtures were detected.
Textile painting from a private collection (20th c.)	Silk	ATR-FT-IR, microscopy, classification	-	-	IV	Very small sample pieces. Despite that it was possible to detect silk.
Mummy from University of Tartu Art Museum (100–500 BC.)	Linen	ATR-FT-IR, microscopy, classification	-	-	V	Several sample pieces, all of them linen. Many of the fibres were contaminated and degraded.

[a] *Additional results for article III were found later. See section 4.3.1*

4.3.1. Carriage blanket from Estonian National Museum

Carriage blanket (see Figure 8) from the Estonian National Museum was analysed to determine the main red dyes of the blanket. The blanket was catalogued in the museum in 1940, but the date and origin are unknown.

Preliminary results are presented in article III. In this thesis, the workflow of the case-study sample is discussed more thoroughly, and the importance of multi-instrument approach is demonstrated for such samples. During ongoing work with the sample after the publication of article III, additional components were detected and these results are more discussed in this thesis.

Figure 8. Pictures of a carriage blanket (photo Estonian National Museum), a spot ("proov 1") where the sample was taken (photo Estonian National Museum) and the final sample for dye analysis.

At first, HPLC-DAD-MS was used (after sample preparation as described in section 3.3.2). With the first part – HPLC-DAD – the most important information gained was that in chromatogram recorded in a visible area (400-640 nm) there was a very intensive peak with retention time 34.7 min (see Figure 9) and wide peak with retention time 66 min (see Figure 9), that was in the first round of analysis unnoticed since it lays in the so-called rinsinging area (see section 3.3.3). UV-Vis spectrum from the first peak revealed that that absorbance maximum was at 490 nm and 510 nm for the second peak, indicating red or close to red colour. Since those peaks did not match with any of the results from our natural red dye standards collection (Table 1 in article III), it was impossible to identify the dye component using only the HPLC-DAD results. Since the retention time is highly dependent on exact parameters and equipment used during measurements, it cannot be compared with literature data, also the UV-Vis spectra of components are usually not very characteristic and can be dependent on used solvents, thus the same applies when comparing with literature.

When observing LC-MS results separately, chromatogram with several different peaks is obtained (see Figure 9), representing unknown dyes but also many other components extracted from the analysed fibre. From this chromato-

gram, components of natural red dye standards with known retention time and *m/z* can be searched, but identification of unknown dye components not present in the standard collection can be difficult due to abundance of possibilities. Even when dominant peaks are found and the corresponding mass spectra analysed, the nominal *m/z* behind the peak is not specific enough to tell the structure of the component. Thus, telling which peak belongs to the dye and which peak belongs to the matrix, can be very difficult. However, with collective information from DAD and MS, in this case, it was possible to tell that behind the colourful peak near 34.7 min, dominant *m/z* is 327. Behind the wide peak near 66 min, there was mass spectra with many *m/z* values: 279, 268, 457, 313, 321, 581, 597, 550 etc (*m/z* are listed according to intensities). Although this information narrows down the possibilities, there still can be several different compounds behind these nominal *m/z* values.

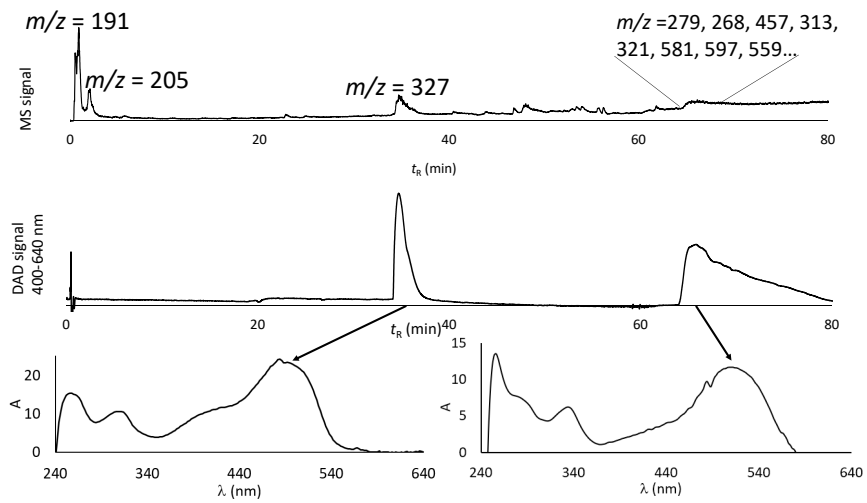


Figure 9. Top: LC-ESI(-)-MS chromatogram of the carriage blanket fibre sample extract. Middle: LC-DAD chromatogram of the carriage blanket fibre sample extract. Bottom left: DAD absorbance spectrum of found dye component with t_R=34.7 min. Bottom right: DAD absorbance spectrum of found dye component with t_R=66 min.

To solve the problem, the third step of the analysis was using of HRMS for getting the exact *m/z* values of the components. As in this case we already had dye in solution, nESI-FT-ICR-MS in negative mode was used (Figure 10). With HRMS the exact *m/z* of 327.04443 was attributed to chemical composition $C_{16}H_{11}N_2O_4S^-$. This composition corresponds to the anion of the synthetic azo dye acid orange 7[III]. Another compound that was identified due to exact *m/z* was azo dye amaranth, also named acid red 27. For this compound six peaks were identified, belonging to different fragments – many of them corresponding to nominal *m/z* behind wide peak t_R=66 min in LC-DAD-MS analysis (see Figure

9). More thorough interpretation of the nESI-FT-ICR-MS spectrum (Figure 10) is in Table 9.

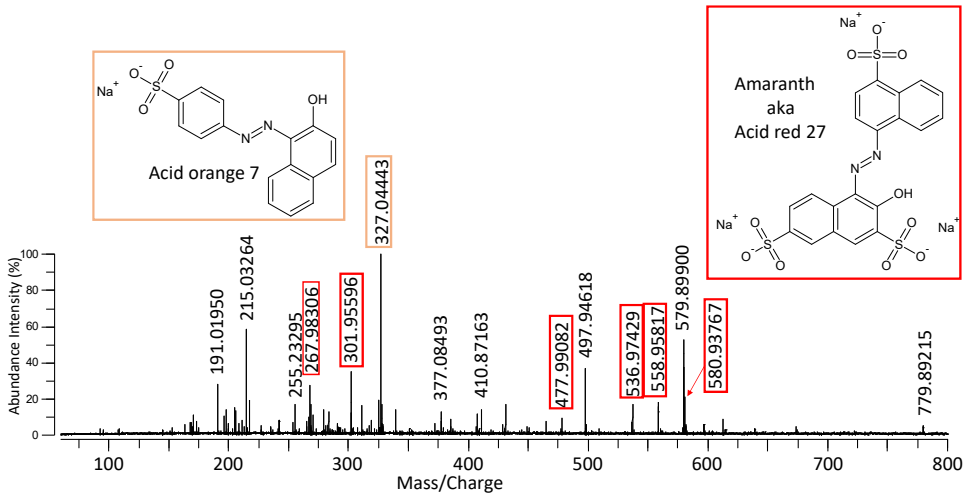


Figure 10. nES(-)I-FT-ICR-MS spectrum of case-study "carriage blanket" sample.

Table 9. Interpretation of nESI(-)-FT-ICR-MS spectrum of case-study "carriage blanket".

Measured exact *m/z*	*m/z* error (ppm)	Anion formula	Anion	Assigned compound
191.01950	-1.20	$C_6H_7O_7^-$	$[C_6H_8O_7\text{-}H]^-$	Citric acid
205.03512	-1.26	$C_7H_9O_7^-$	$[C_7H_{10}O_7\text{-}H]^-$	Homocitric acid
215.03264	-0.72	$C_6H_{12}O_6Cl^-$	$[C_6H_{12}O_6\text{+}Cl]^-$	Glucose
253.21747	0.66	$C_{16}H_{29}O_2^-$	$[C_{16}H_{30}O_2\text{-}H]^-$	Palmitoleic acid
255.23295	0.00	$C_{16}H_{31}O_2^-$	$[C_{16}H_{32}O_2\text{-}H]^-$	Palmitic acid
265.14766	-0.93	$C_{12}H_{25}O_4S^-$	$[C_{12}H_{25}NaO_4S\text{-}Na]^-$	Lauryl sulfate
267.98306	-0.73	$C_{20}H_{12}N_2O_{10}S_3^{2-}$	$[C_{20}H_{11}N_2Na_3O_{10}S_3\text{-}3Na\text{+}H]^{2-}$	Amaranth
301.95596	-0.28	$C_{10}H_6O_7S_2^-$	$[C_{20}H_{11}N_2Na_3O_{10}S_3\text{-}C_{10}H_6N_2NaO_3S\text{-}2Na\text{+}H]^-$	Amaranth
325.18421	-0.23	$C_{18}H_{29}O_3S^-$	$[C_{18}H_{29}NaO_3S\text{-}Na]^-$	Dodecylbenzenesulfonate
327.04443	-0.20	$C_{16}H_{11}N_2O_4S^-$	$[C_{16}H_{11}N_2NaO_4S\text{-}Na]^-$	Acid orange 7
377.08493	-1.81	$C_{12}H_{22}O_{11}Cl^-$	$[C_{12}H_{22}O_{11}\text{+}Cl]^-$	Sucrose
410.87163	-0.61	$C_4F_9O_6S_3^-$	$[(CF_3SO_2)_3CH\text{-}H]^-$	Calibrant
477.99091	-0.55	$C_{20}H_{11}N_2O_7NaS_2^-$	$[C_{20}H_{11}N_2Na_3O_{10}S_3\text{-}SO_3Na\text{-}Na]^-$	Amaranth
536.97429	0.94	$C_{20}H_{13}N_2O_{10}S_3^-$	$[C_{20}H_{11}N_2Na_3O_{10}S_3\text{-}3Na\text{+}2H]^-$	Amaranth
558.95817	4.36	$C_{20}H_{12}N_2O_{10}NaS_3^-$	$[C_{20}H_{11}N_2Na_3O_{10}S_3\text{-}2Na\text{+}H]^-$	Amaranth
579.89900	0.55	$C_8F_{18}NO_4S_2H^-$	$[CF_3(CF_2)_3SO_2]_2NH\text{-}H]^-$	Calibrant
580.93767	6.75	$C_{20}H_{11}N_2O_{10}Na_2S_3^-$	$[C_{20}H_{11}N_2Na_3O_{10}S_3\text{-}Na]^-$	Amaranth

With a combination of these three different techniques (LC-DAD, LC-MS, nESI-FT-ICR-MS) and comparison of result with information from literature[107–109], the presence of dyes acid orange 7 and acid red 27 were confirmed.

4.3.2. Textile painting

Small fibre from a textile painting (Figure 11) was provided as a case-study sample from a private collection. The fibre piece was taken from a portrait (Figure 11), reportedly from China and the aim was to confirm if the used material is silk.

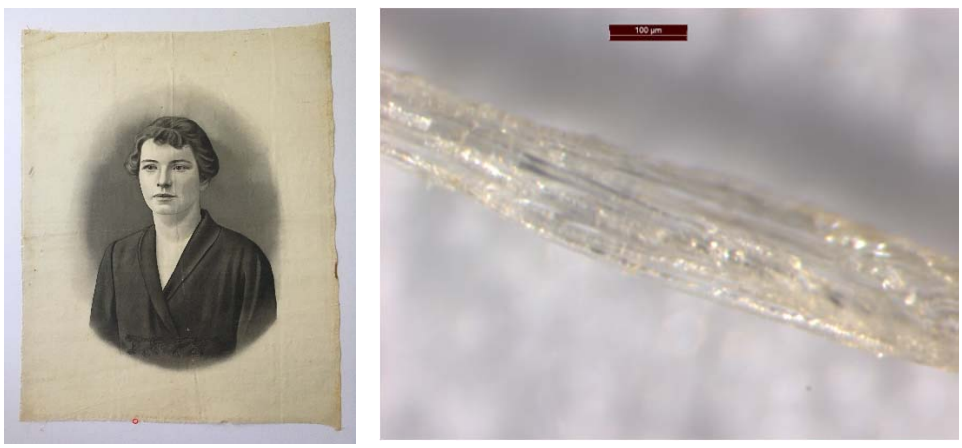


Figure 11. Picture of the analysed textile painting (photo by Nele Ambos) and microscopic image of the sample.

In this work two non-invasive methods, optical microscopy and r-FT-IR, and semi-destructive mATR- and ATR-FT-IR were used. At first, observations with optical microscopy revealed that yarn consist of thin shiny fibres. This could indicate the use of silk, but also some man-made fibre e.g viscose. For reliable identification, FT-IR spectroscopic method was applied, starting with non-invasive FT-IR microspectrometer with reflectance mode (r-FT-IR). This thin sample revealed the deficiency of this approach – samples with too thin cross-section are unsuitable for r-FT-IR. Even when the measured area was reduced from 150x150 to 25x25 µm, it was impossible to get good quality spectra. The r-FT-IR spectra were distorted and uncharacteristic (Figure 12, third spectrum). As with this specific case, non-invasive analysis methods did not give desired results, the next step would be applying semi-destructive methods, to make as little harm as possible. FT-IR microspectrometer with ATR mode (mATR-FT-IR) with measured area diameter of 3 micrometres and applying weak pressure, leaves only very small, hardly noticeable marks on the fibre but gives plenty of information. Analysis with FT-IR microspectrometer with ATR mode (Figure

12) showed all the characteristic absorbances to amide-based fibres like silk, wool and polyamide. Classification results with both DA and RF and comparison with standard spectra all indicated that the analysed fibre is most likely silk.

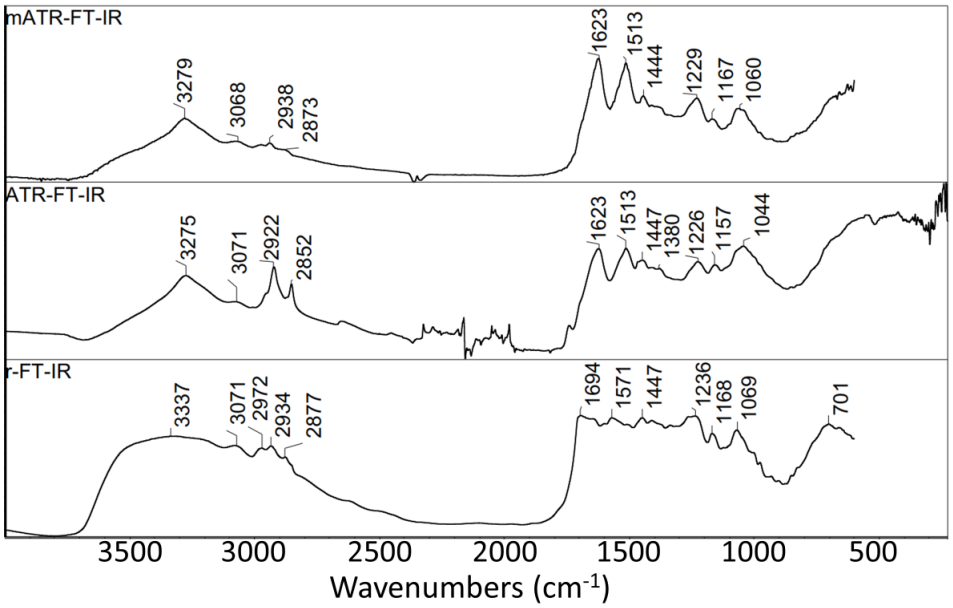


Figure 12. Comparison of spectra recorded from a textile painting with ATR-FT-IR microspectrometer (mATR-FT-IR), ATR-FT-IR spectrometer (ATR-FT-IR) and FT IR microspectrometer with reflectance mode (r-FT-IR).[IV]

Although in the case of cultural heritage samples, non-invasive r-FT-IR is very useful, this specific case-study showed that such non-invasive methods are not always universally suitable for every sample case and thus is good to have the ability to use several different approaches.

SUMMARY

The aim of this work was to develop methods to analyse and identify textile fibres and natural red textile dyes in cultural heritage objects. For determination of textile fibers, 16 different fibre types – wool, silk, cotton, linen, jute, sisal, viscose, cellulose acetate (acetate), Tencel™ (lyocell), fibreglass, polyester, polyamide, polyacrylic, elastane, polyethylene and polypropylene – were used for the development of analytical methods using FT-IR spectroscopy in ATR and reflectance modes combined with chemometric classification methods. For dye analysis, method development was done using seven natural red dyes (dyer's madder, redwood, logwood, sandalwood, kermes, cochineal, bloodred webcap) and five different instrumental sets (HPLC-DAD, HPLC-FLD, HPLC-MS, MALDI-FT-ICR-MS and ESI-FT-ICR-MS).

For textile fibre analysis it was concluded that both ATR and reflectance techniques in FT-IR spectroscopy can be suitable for identifying textile fibres. While the advantage of the reflectance technique is its non-invasive nature, in some cases, like with very small sample pieces, ATR-FT-IR or even mATR-FT-IR should be preferred. While most of the fibre types are well distinguished with FT-IR spectroscopy, problems remain with cellulose-based fibres (cotton, linen etc), in which case optical microscopy must be used as well. For the identification of unknown textile fibres, classification methods were also developed using discriminant analysis and random forest.

For textile dye analysis, it was found that for reliable identification of dyes, using multiple instruments simultaneously is often the best approach. While HPLC-DAD is a good technique for separating and identifying colourful components, MS detector is much more sensitive and universal. Usage on HRMS (FT-ICR-MS in this work) enables to identify components' chemical compositions with high-accuracy (less than 2 ppm error) *m/z* measurements, thus identification can be done without using standard compounds.

In this work, a large collection of standard spectra and chromatograms was created, which is a helpful tool for identifying unknown fibres and dyes in case-study samples. Fibre standard collection consists of more than 4000 FT-IR spectra from 16 different fibre types recorded in ATR, mATR and reflectance mode. Seven natural red dyes are characterised with 113 chromatogram/spectra using five different instrumental sets.

The usefulness of the developed methods was demonstrated by analysing several historical samples from Estonian National Museum, Conservation and Digitization Centre Kanut (Estonia) and private collections.

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SUMMARY IN ESTONIAN

Instrumentaalmeetodite väljatöötamine tekstiilimaterjalide ja -värvainete analüüsimiseks

Käesoleva doktoritöö eesmärgiks oli välja töötada meetodid tekstiilikiudude ja looduslike tekstiilivärvainete analüüsimiseks kultuuriväärtuslikest objektidest. Tekstiilikiudude materjali identifitseerimiseks, kasutades 16 kiuklassi – vill, siid, puuvill, lina, džuut, sisal, viskoos, tselluloosatsetaat (atsetaat), Tencel™ (lyocell), klaaskiud, polüester, polüamiid, polüakrüül, elastaan, polüetüleen ja polüpropüleen – töötati välja analüütiline meetod FT-IR spektroskoopia koos ATR- ja peegeldustehnikate jaoks. Värvainete analüüsimiseks töötati välja meetodid kasutades selleks seitset looduslikku punast värvaineallikat (punavärvik, siiltsesalpiiniapuu, kampetše veripuu, sandlipuu, kermes, košinell, verev vöödik) ja viit erinevat instrumenti (HPLC-DAD, HPLC-FLD, HPLC-MS, MALDI-FT-ICR-MS ja ESI-FT-ICR-MS).

Tekstiilikiudude analüüsil leiti, et nii ATR kui ka peegeldustehnika sobivad kiudude identifitseerimiseks. Kuigi peegeldustehnikal on suur eelis olles mittekontaktne, seega mitte-destruktiivne meetod, on väga väikese läbimõõduga proovide korral eelistatud ATR-FT-IR mikrospektromeeter või ATR-FT-IR spektromeeter. Kuigi enamik kiuklassidest on FT-IR spektroopilisel meetodil hästi üksteisest eristatavad, on tselluloosipõhiste kiudude (puuvill, lina, viskoos jne) eristamine siiani raskendatud ning sel juhul tuleb abimeetodina kasutada optilist mikroskoopi. Tundmatute kiuproovide tuvastamiseks töötati välja ka klassifitseerimismeetodid kasutades diskriminantanalüüsi ja otsustusmetsa (*Random Forest*) algoritmi.

Värvainete analüüsi jaoks leiti, et tihti on usaldusväärse tulemuse saamiseks kõige parem kasutada erinevaid analüüsimeetodeid koos. Kuigi HPLC-DAD on sobiv meetod värviliste ühendite eraldamiseks ja tuvastamiseks, on MS detektor palju tundlikum ja universaalsem. Kõrglahutusega MS (selles töös FT-ICR-MS) võimaldab tuvastada komponentide elementset koostist tänu *m/z* määramise väga kõrgele täpsusele (alla 2 ppm veaga) ning seetõttu on ainete identifitseerimine enamasti võimalik ka ilma standardeid kasutamata.

Käesolevas töös koostati suur standardspektrite ja -kromatogrammide kogum, mis on kasulik tundmatute tekstiilikiudude ja värvainete tuvastamisel reaalsetest proovidest. Tekstiilikiudude kogumis on 16 erineva kiuklassi FT-IR spektrid mõõdetud peegeldus-, ATR ja mATR mõõtmismeetoditel. Seitse punast värvainet on karakteriseeritud 113 kromatogrammi/spektriga, mis on mõõdetud viie erineva instrumendiga.

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PUBLICATIONS

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