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Discrimination of reactively-dyed cotton fibres with thin layer chromatography and UV microspectrophotometry

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ABSTRACT

Reactively-dyed black, navy blue and medium red cotton samples showing metamerism under fluorescent tube illumination were examined. Optical microscopy (bright field, polarization and fluorescence microscopy) was used, followed by microspectrometry in the visible range (MSP Vis), to differentiate the samples in each block of colours. Additionally, the non-discriminated samples were subjected both to microspectrophotometry in the UV-range (MSP UV) and to enzymatic digestion followed by high performance thin layer chromatography (HPTLC) on the digests. While it was found that both methods may potentially result in higher discrimination, preparation of reactively-dyed cotton for HPTLC was found to be a very tedious and time-consuming step and HPTLC only led to a better discrimination than MSP UV for the red cotton samples. The results suggest that in order to increase the discrimination for reactively-dyed cotton fibres, measurement of the UV absorption spectrum (MSP UV) is preferred over HPTLC.

1. Introduction

In forensic fibre examination, transferred fibre traces collected at the crime scene or in the laboratory are compared to control samples coming from a known garment or textile. A sequence of examination methods is chosen in order to obtain the best possible discrimination. If no differences are found using this series of methods, the known material could be the source of the fibre traces.

Man-made fibres usually possess a multitude of morphological characteristics and a high discrimination can already be obtained using optical microscopy only (bright field, polarization and fluorescence microscopy). On dyed fibres the absorption spectrum can be measured using microspectrophotometry in the visible range (MSP Vis). Moreover, the chemical composition of man-made fibres (fibre class and subclass) can be determined using FT-IR or Raman spectroscopy.

Natural fibres are already identified by their morphological appearance in bright field microscopy. Other techniques include polarization and fluorescence microscopy and dyed fibres can be measured using MSP Vis. Because of the absence of supplementary morphological features, further discrimination with other techniques is highly recommended.

Cotton fibres are one of the most commonly encountered fibre classes. Reactive dyes are one of the dye classes that are applied on cotton. These dyes were first developed and commercialized by ICI in 1956 and are gaining in popularity since the '80s due to their improved wash fastness over direct dyes and their improved brightness in all shades of colour as compared to vat dyes [1,2]. One of the disadvantages of reactive dyes is that under the alkaline conditions some of the dye is hydrolyzed and becomes unreactive. Therefore higher quantities of dye have to be used. Also, to promote dye uptake fairly large amount of electrolytes are added to the dye bath, which lead to higher costs. To overcome these drawbacks much research has been performed. More than 40 different reactive groups have been developed that are based on a higher reactivity or better substantivity for the substrate [3,4].

For the fibre practitioner, reactively-dyed cotton can cause some analytical difficulties. As these dyes react with the cellulose hydroxyl groups to form covalent bonds, they cannot be readily extracted using solvents in order to perform chromatography on the dye mixture. According to the extraction–classification scheme of Laing *et al.* [5] reactively-dyed cotton:

- cannot be extracted in glacial acetic acid at 100 °C after 20 min, indicating that it is not an azoic dye;
- cannot be extracted in pyridine/water 4:3 (*v*/*v*) at 100 °C after 20 min, indicating that it is not a direct dye;

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- undergoes a change in colour when treated with a dithionite/polyvinylpyrolidone solution (80 mg sodium dithionite, 30 mg PVP, 450 μL 10% NaOH in 9 mL water). This is observed as both a fibre colour change and as the extract being not coloured or having not the original fibre colour when spot on a TLC plate

Another method to verify if a sample is reactively-dyed, consists in treating the cotton sample with dimethyl formamide at boiling temperature. No colouration of the extraction solvent should occur for reactive dyes. Some sulphur or vat dyes may not extract as well but these can be easily distinguished when observing their reduction–oxidation behaviour [6].

According to Home and Dudley, a 1.5% (*v*/*v*) aqueous NaOH solution at 100 °C will extract most of the reactive dyes on cellulose. The extracts are suitable for spotting on TLC plates [7]. However, often only partial extraction is obtained and bleaching occurs if the fibres are treated longer than 3–4 min. Instead, an enzymatic digestion procedure using cellulase has been proposed [8].

In this work, reactively-dyed cotton samples were selected that showed metamerism under fluorescent tube illumination. Black, blue and red samples were chosen as these are the most frequently used colour shades. Each block of colours was subjected to the examination sequence:

- Optical light microscopy (LM) using bright field, polarization and fluorescence
- Microspectrophotometry in the visible range (MSP Vis)
- Microspectrophotometry in the UV-range (MSP UV) and high performance thin layer chromatography (HPTLC)

The samples that could not be discriminated by LM were subjected to MSP Vis. The remaining non-discriminated samples were then subjected to both MSP UV and HPTLC. After each step in the comparison process the total discriminating power (DP) was calculated according to Smalldon and Moffat [9].

Several papers already dealt with the discrimination within black [10–12], blue and red cotton [10,11,13]. These studies mainly used LM and MSP UV–Vis and concerned all dye classes applied on cotton, including reactive dyes.

The focus of this work was to determine an optimal examination sequence for the analysis of reactively-dyed cotton fibres in each of the chosen colour shades. In particular, the study was designed to determine whether MSP UV or HPTLC would provide the best discrimination after optical microscopy and MSP Vis. The results of this work will be compared to those of Wiggins *et al.* who also used MSP UV and TLC in order to discriminate reactively-dyed black and blue cotton samples [14].

Other instrumental methods have not been considered in this work, as this was beyond the scope of the research performed for the master thesis by one of the authors (KVD). Complementary methods may result in even better discrimination. Non-destructive methods, such as Raman spectroscopy [15–18] and the measurement of dichroism using MSP-PPL [19,20], are recommended. The use of HPLC is also very promising; although destructive, the high sensitivity of this method allows for the analysis of single fibres or fibre fragments [21].

2. Experimental

2.1. Material

17 black, 11 navy blue and 12 medium red cotton fabric swatches were selected by a panel of four individuals. The samples within these three groups could not be discriminated by visual inspection under fluorescent tube illumination. A photograph of all samples is shown in Fig. 1 in order to illustrate the choice and the depth of shade of the samples. All swatches are obtained from manufacturer sample books for reactive dyes on cotton. For all samples the commercial dye names (and often also the Colour Index generic names) are known. For some of the dyes the chemical composition (Colour Index number) is also known. A list of the samples is given in Table 1.

2.2. Methods

The analytical sequence involved the use of optical light microscopy (LM), visible microspectrophotometry (MSP Vis) and finally UV microspectrophotometry (MSP UV) or enzymatic fibre digestion followed by high performance thin layer chromatography (HPTLC).

2.2.1. LM

A tuft of fibres from each sample was mounted on glass microscope slides and cover slips using Histomount (National Diagnostics) mounting resin. High power microscopy was performed using a DMRXP research microscope (Leica) at a total magnification of 400×. A first discrimination was obtained based on colour and morphology (bright field), dichroism (polarization microscopy) and fluorescence colours (fluorescence microscopy). All microscopic observations were performed using a Plan Fluotar 40×/NA 0.70 objective and HC Plan 10× oculars and a universal condenser (NA 0.90). The dichroic behaviour was checked using a polarizer with the procedure described in [22]. Fluorescence was checked using episcopic irradiation with a HBO 50 W mercury vapour source and two different fluorescence filter blocks: excitation with blue–violet light (H3: BP 420-490|RKP 510|LP 515) and with green light (N2.1: BP 515-560|RKP 580|LP 590).

2.2.2. MSP Vis

Absorption spectra have been collected in the visible range between 380 and 800 nm using a Tidas 800 microspectrophotometer (J&M) coupled to a Zeiss microscope (Axioplan 2) and a diode array detector (type MCS-TSPEX 1024, range 190–1020 nm). The absorption spectra

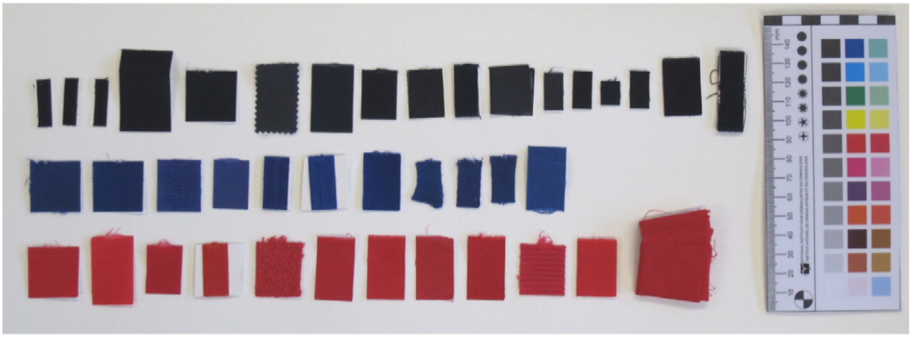

Fig. 1. Reactively-dyed cotton samples used in this work.