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Methods used in forensic examination to identify cotton fibres dyed with reactive dyes

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Abstract

The large scale of production of cotton products, their wide range of applications, as well as specific physicochemical properties mean that natural cotton fibres are almost always found among physicochemical microtraces secured at crime scenes.

Proper selection of methods for detecting and securing microtraces in the form of fibres, followed by appropriate research methodology and correct interpretation of the obtained result provide important and reliable information about the criminal event that has occurred (Houck et al., 2009; Śmigiel-Kamińska et al., 2019; Śmigiel-Kamińska et al., 2020; Wąs-Gubała, 2000; Wąs-Gubała, 2009; Zięba-Palus et al., 2015).

Research analytics is a tool for identifying and comparing fibres. It is first based on the results obtained using various optical microscopy techniques, followed by microspectrophotometry in the UV-Vis range. The determination of the chemical composition of textile materials is carried out mainly using infrared spectroscopy and Raman spectroscopy, but also high-performance liquid chromatography, mass spectrometry, gas chromatography and numerous variants of combinations of the above techniques.

This article reviews the results of research published so far in the field of identification and comparison of dyes used to dye cotton, in particular those belonging to the group of reactive dyes, using spectroscopic and chromatographic methods.

Keywords: cotton, reactive dyes, forensic analyses, chromatographic methods, spectroscopic methods

1. Characteristics of cotton as a natural fibre

Cotton fibres, belonging to the type of natural fibres, are formed as a result of the development of the cells of the seed shell of cotton (cotton) – a plant from the mallow family. Cotton is a very productive crop, as it loses only 10% of its weight during processing. The basic chemical substance of cotton fibres is cellulose, which accounts for at least 86%. In addition, there are also small amounts of additional substances, such as water, proteins, waxes, or minerals (Houk et al., 2009).

Cellulose is a polysaccharide with an unbranched structure, and its monomer is D-glucose. D-glucose contains a six-membered pyranose ring, similar in structure to a heterocyclic pyranoe with a chair conformation. The cellulose chain consists of 800 to 10,000 glucose residues linked together by a β-1,4-glycosidic bond (Korszak, 1957; Lucia et al., 2018; Morrison, Boyd, 1985; Qiu, Hu, 2013). A fragment of the cellulose chain is shown in Figure 1. The presence of intramolecular hydrogen bonds plays a very important role in the structure of cellulose, as they are difficult to break, which results in the insolubility of cellulose in water. The hydroxyl groups present in the cellulose molecule make it hygroscopic and thus absorb water from the environment (Hatakeyama, 2004; Qiu, Hu, 2013; Ross et al., 1991; Zugenmaier, 2008).

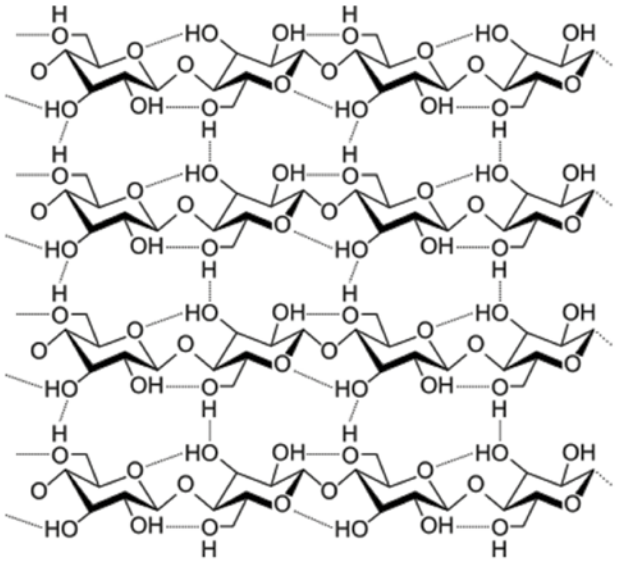


Fig. 1. Fragment of the cellulose chain (Dey, 2021)

Cotton fibre consists of 20-30 layers of cellulose, the structure is conditioned by its maturity. The cotton fibre grows lengthwise for up to 30 days, reaching a length of up to 30 mm, while the fibre wall grows in the second stage, lasting another 35-40 days, reaching a thickness of 6-7 μm. The described characteristics and ripeness of cotton fibres depend to a large extent on external factors, such as temperature, humidity or sunlight (Jeziorny, Lipp-Symonowicz, 1980).

The ripeness of cotton fibres determines their shape. The fibre in the initial stage resembles a flat ribbon, and in cross-section it is shaped like a bean with an empty channel. In the subsequent stages of maturation, the fibre gains an increasing number of twists. These twists disappear when the fibre is too mature. The shape of a mature fibre is cylindrical and cross-section is close to a circle (Jeziorny, Lipp-Symonowicz, 1980).

The physical and chemical properties of cotton fibres are due to the properties of cellulose. As a result, cotton is highly resistant to mechanical factors such as stretching and tearing, although it has low resilience. Cotton treated with alkali does not dissolve, but it does swell. Organic solvents also do not cause the cotton fibre to dissolve. On the other hand, it is sensitive to concentrated acids, such as sulfuric (VI) acid (H2SO4), hydrogen chloride (HCl). Treated with these acids, cotton fibre is destroyed, which is caused by cellulose degradation (Śmigiel-Kamińska et al., 2014).

2. Dyes used to dye cotton

Cellulose fibres, such as cotton fibres, are dyed by several classes of dyes: direct, vat, sulphur and reactive (Broadbent, 2001).

Direct dyes show an affinity for fibres without using a binding agent called mortar (Önder, 2010). Direct dyes completely dissolve in water, which is due to the sulfonate groups (-SO_3-) present in the dye structure, which give them a hydrophilic character (Christie, 2015). The dyeing process takes place at a temperature close to the boiling point of water and in the presence of an electrolyte (e.g. chloride or sodium sulphate). Under such conditions, dyes exhibit affinity for cellulose particles through hydrogen bonds, van der Waals forces, and dipole-dipole interactions (Christie, 2015). Figure 2 shows the structure of an examplary direct dye.

Another group of dyes are water-insoluble vat dyes (Önder, 2010). Carrying out a process commonly called vatting with the addition of sodium dithionine under very high pH conditions allows to obtain the form of dyes that are soluble in water (Shore, 2002). In this form, vat dyes show a very high affinity for cellulose fibres and are absorbed by them. Finally, they are converted back into a water-insoluble form, whereby these dyes are mechanically trapped within the fibre. All stages of dyeing ensure that the textile material dyed in this way is resistant to moisture and does not lose its colour during washing (Önder, 2010). A characteristic element of the chemical structure of vat dyes are two carbonyl groups connected by a conjugated system. Without this groups, the processes that takes place during the dyeing of cotton would not be possible. Carbonyl groups (-C=O) are reduced to enol groups (under alkaline conditions). These groups are responsible for the ability of vat dyes to dissolve in water. Vat dyes include extremely popular blue dyes from the Indigo group (Christie, 2015). The structure of an examplary vat dye is shown in Figure 3.

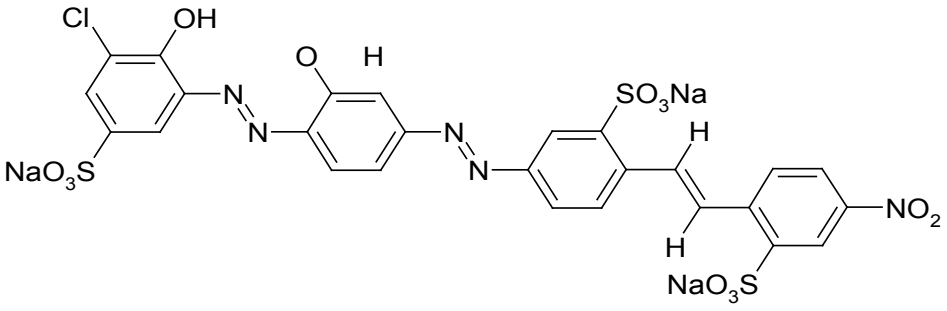


Fig. 2. Structure of Direct Brown Dye 103 [own source]

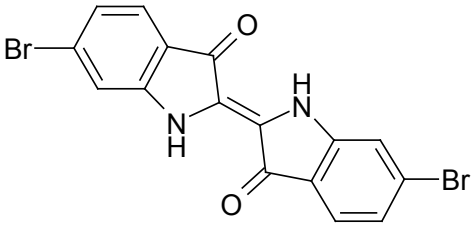


Fig. 3. Structure of vat dye – Dibromoindigo [own source]

Sulfur dyes are a type of vat dyes used to dye cellulose fibres. The chemical structures of sulphur dyes are not fully understood, but it is known that they are mixtures of chemical particles containing various structures with sulphur: sulphide, disulphide, polysulphide and heterocyclic rings. The dyeing process of textile products involves transforming the dye by carrying out a reduction using sodium sulphide. The resulting compound becomes soluble in water and as a result of diffusion enters the interior of the fibre. In the last step, the dye is oxidized to a hydrophobic form (Christie, 2015; Önder, 2010). Sulphur dyes are very cheap, but they have a very negative impact on the natural environment. The structure of an exemplary sulphur dye is shown in Figure 4.

Reactive dyes are the most commonly used, modern group of synthetic dyes for dyeing cotton textiles. They are characterized by excellent durability, a wide range of shades and flexibility of use. Their name comes from the fact that they have reactive groups in the molecule formed during a multi-stage synthesis (Chistie, 2015).

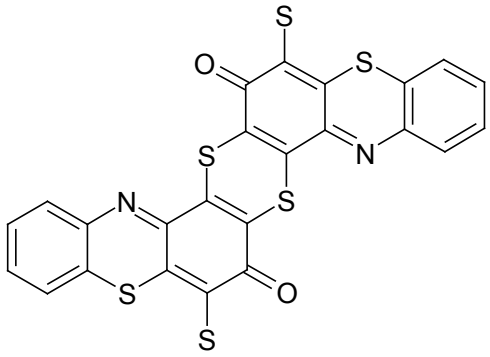
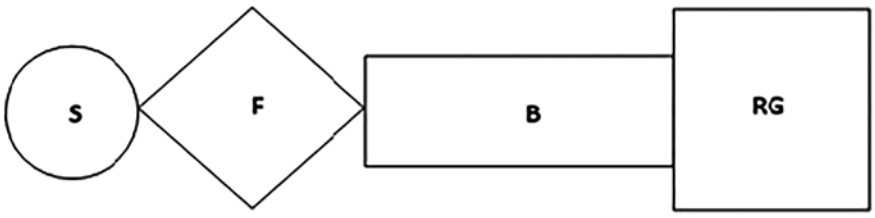


Fig. 4. Example of a sulphur dye - Sulfur Black I [own source]

Reactive dyes are made up of different fragments, as shown in Figure 5. and Figure 6. Each fragment plays a clearly defined role (Blaus, 2014; Chakraborty, 2010; Chattopadhyay, 2011; Hoy, 2013; Lewis, 2011; Mahapatra, 2016; Shang, 2013; Wiggings, 2017; Zollinger, 2003). Characteristic groups of reactive dyes are: chromogen, the group that gives the molecule the properties of water solubility, the bridging group and the group that reacts with fibres.

Chromogen is the part of the molecule that gives it colour and can contribute to giving it various properties. Chromogens typically belong to azoe, carbonyl, or phthalocyane compounds. A bridging group is a group of atoms that is used to combine the chromogenic part of a molecule with a reactive group. In most cases, reactive groups have very simple structures. Reactive dyes must show adequate reactivity towards



S – group giving water solubility properties
F – chromogenic group
B – bridging structure
RG – reactive group

Fig. 5. General scheme of reactive dye (Śmigiel-Kamińska et al., 2020)

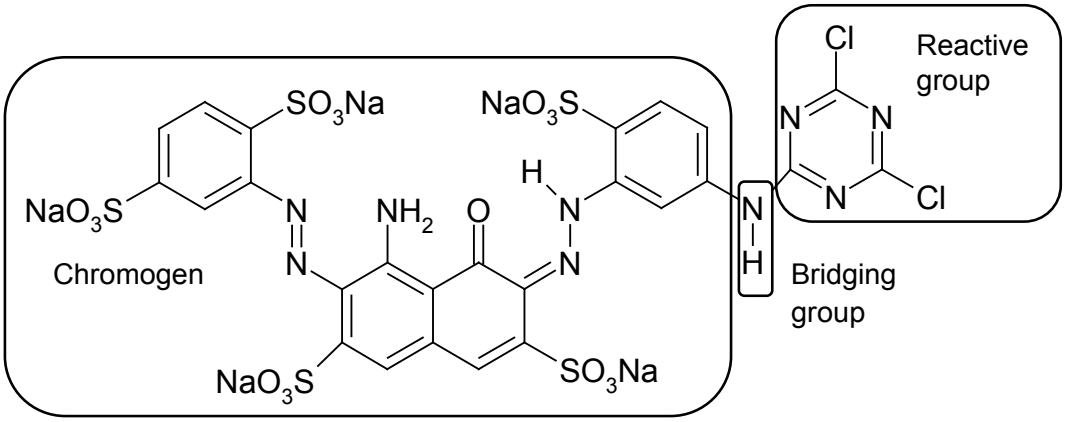


Fig. 6. Structure of REACTIVE BLUE 4 reactive dye (Śmigiel-Kamińska et al., 2020)

cotton, but lower reactivity in relation to water, so that the reactive group of the dye does not hydrolyze.

Chromophore groups show a very high ability to form covalent bonds with nucleophilic groups entering the chemical structure of cotton fibre. This bond remains stable under washing conditions (Lewis, 2014).

A detailed description of reactive dyes is presented in the publication D. Śmigiel-Kamińska et al., 2020.

3. Procedure for dyeing cotton with reactive dyes

The dyeing process of cotton fibres is based on the formation of a strong covalent bond between the reactive groups of the dye and the cellulose fibre. The nucleophilic ability of hydroxyl groups (-OH) present in the cellulose molecule is most commonly used. The reaction between cellulose and reactive dyes takes place in a weakly alkaline environment, in which the deprotonation of hydroxyl groups takes place (Fig. 7). This results in production of strong nucleophilic cellulose anions, which take on the role of active nucleophiles in the reactive dying of cellulose (Fig. 8). (Wiggins, 2017).

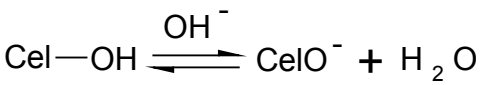


Fig. 7. Formation of the cellulose anion (Soleimani-Gorgani, Karami, 2016)

The most common method of dyeing cotton with the use of reactive dyes is the so-called pull-off method. The following phases occur in it (Benkhaya et al., 2020):

1) depletion phase – the immersion of the fabric in a dye bath where fibres absorb the dye. This process takes place in an inert environment. To increase the saturation of the fibres magnesium chloride or magnesium sulfate are added. Also the gradual increase of temperature increases the migration of dye to the inside of the fibres;
2) fixation phase – actual dyeing in an environment of increased pH (adding the right amount of alkali). During this phase, the hydroxyl groups of the cellulose dissociate, and a reaction with the dye occurs;
3) washing phase – this process is aimed at removing excess dye that has been absorbed but has not bound to the cellulose. Also salt and alkali residues are removed. Washing is divided into several stages. The first stage is pre-washing, in which some of the unfixed dyes, salts and alkalis are removed. Cold and warm water is used in this stage. The main washing process eliminates dyes that are not bound but deeply absorbed into the cellulose fibre. The removal process includes the formation of complexes of dye with cationic compounds that are part of the boiling detergent solutions used. This process should be repeated depending on the depth of the staining, and the detergent solution used should be changed frequently.

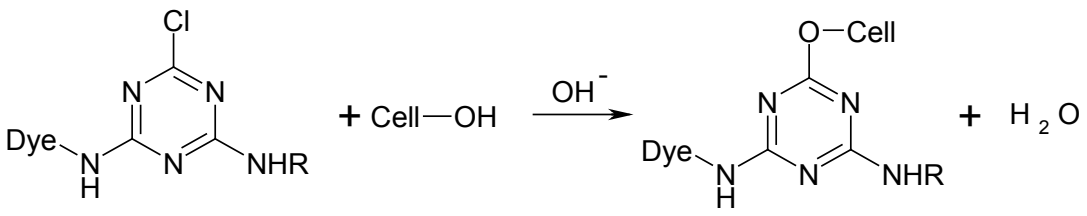


Fig. 8. Reaction of reactive dye with cellulose (Soleimani-Gorgani, Karami, 2016)

4. Spectroscopic methods used in forensic research to identify cotton fibres dyed with reactive dyes

In forensic laboratories, spectroscopic methods are used for identification and comparative testing of textile fibres. Information about the colour and the dyes used is provided primarily by studies using ultraviolet and visible light microspectrophotometry (MSP UV-Vis) and Raman spectroscopy (Chalmers et al., 2012; Wiggins, 2017).

MSP UV-Vis is used for impartial observation of coloured fibres, as it is a non-destructive, repeatable method and does not require the extraction of dye from fibres. However, this method is primarily used for comparative research, i.e. comparison of spectra obtained for individual fibre samples, but not for the identification of dyes (Goodpaster, Liszewski, 2009).

The research of J. Wąs-Gubała and R. Starczak (2015) confirmed the effectiveness of differentiation of single cotton fibres dyed with reactive dyes from the same manufacturer (trade name Cibacron@) and the possibility of assessing the concentration of dyes using MSP UV-Vis. The limit of detection of the MSP UV-Vis method was 0.18% of the dye concentration in the textile sample. However, intra-object and inter-subject variability and the effect of dichroism were observed during the research.

In other studies, J. Wąs-Gubała and R. Starczak (2015) presented an evaluation of the usefulness of MSP UV-Vis and Raman spectroscopy in the analysis of textile fibres dyed with mixtures of synthetic dyes, including reactive dyes. The test samples consisted of cotton fibres dyed with two- and three-component mixtures of reactive dyes. The MSP UV-Vis studies showed limited possibilities for analyzing dyed cotton fibres when the ratio of the concentration of the primary to the secondary dye was higher than four. The obtained results showed that both spectroscopic methods used have a similar ability to distinguish mixtures of dyes used to stain the fibre.

P. Buzzini and G. Massonnet in their research (2013) evaluated the potential and limitations of Raman spectroscopy in the course of studying fibres of various types and colours, including those stained with reactive dyes. Fibre samples collected from 180 textiles were examined using Raman spectroscopy, bright-field optical microscopy, double polarity and fluorescence microscopy, and compared using MSP UV-Vis and thin-film chromatography (TLC). The study showed that Raman spectroscopy can play a complementary role in commonly used forensic fibre studies leading to the detection and comparison or identification of dye. Collective analysis of spectra obtained using lasers of different wavelengths used in Raman spectroscopy allowed to distinguish pairs of fibres that had not previously been differentiated using optical microscopy and MSP UV-Vis (Buzzini, Massonnet, 2015).

J. Wąs-Gubała and W. Machnowski (2014) confirmed the differences between cotton and fibres made of regenerated cellulose (viscose) resulting from changes in the degree of polarization and supramolecular structure using Raman spectroscopy. In more than 80% of the spectra obtained, the presence of bands derived from dyes was confirmed. In fibres with lower dye concentrations, bands originating from cotton and viscose dominated. For higher dye concentrations in fibres, bands characteristic of these dyes were observed.

Techniques that have significantly increased the sensitivity of Raman spectroscopy are: surface-enhanced Raman scattering (SERS) and surface-enhanced resonant Raman scattering (SERRS). However, these techniques are more complex and require experience in sample preparation compared to classical Raman spectroscopy. Therefore, there are not many reports of using these techniques in forensic studies for example to determine reactive dyes in cotton fibres. Nevertheless, in scientific research conducted in other fields, SERS and SERRS techniques are used for the analysis of dyes and textiles (Casadio et al., 2010; Degano et al., 2009; Pozzi et al., 2013; Puchowicz et al., 2019; Sciutto et al., 2017; Zaffiono et al., 2014).

Infrared spectroscopy, as a single fibre test method, is mainly used to identify the fibre-forming polymer. It is also possible to use for the identification of dyes, but only for strongly coloured fibres (Kirkbride, 2018). This is due to the low sensitivity of infrared absorbance in relation to those components, which are less than 5% in dyed fibres, which is the concentration for textile dyes, including reactive dyes. Research results of M. C. Grieve and his colleagues (1998) pointed to the possibility of identifying dyes used in textiles using infrared spectroscopy, but for a special type of acrylic fibres. The technique of spectroscopic scattered reflection in infrared (DRIFTS) turned out to be more popular in the identification of dyed fibres, including cotton fibres (Kokot et al., 1997).

A detailed description of the possibilities of spectroscopic research of dyed cotton fibres is presented in the mentioned publication by D. Śmigiel-Kamińska (2020).

5. Chromatographic methods used to identify cotton fibres dyed with reactive dyes

Chromatographic methods of testing cotton dyed with reactive dyes consist of two main stages: extraction of reactive dyes from the fibre and chromatographic analysis of the extracts with interpretation of the results.

5.1. Extraction of reactive dyes from cotton

The extraction of reactive dyes from cotton requires special conditions. Conventional extraction methods are too weak to break the strong covalent bond of the reactive dye – cellulose. The most commonly used

reagent for cleavage of this bond, described in the available literature, is a 1.5% solution of sodium hydroxide (NaOH) (Dockery et al., 2009; Home, Dudley, 1981; Hoy, 2013; Siren, Sulkava, 1995; Sultana et al., 2019; Xu et al., 2001). For this purpose, alkaline hydrolysis is used as an aqueous solution of a strong base. In an alkaline environment, the alcohol groups of the cellulose skeleton of cotton behave like a weak acid and undergo ionization (Dockery et al., 2009). During hydrolysis, other chemical bonds present in the dye molecule can also be cleaved, resulting in multiple products. The mechanism of alkaline hydrolysis is presented in Figure 9.

The duration of alkaline hydrolysis was 25 min (Sirén, Sulkava, 1995) or 60 min (Hoy, 2013; Feng et al., 2020; Sultana et al., 2019), and the extraction temperature is 80°C (Feng et al., 2020; Sultana et al., 2019) or 100°C (Hoy, 2013; Sirén , Sulkava, 1995; Xu et al., 2001). Depending on the size of the test samples, the following volumes of NaOH solution were used: 5 µl (Xu et al., 2001), 50 µl (Hoy, 2013), 500 µl (Dockery et al., 2009) and 1 mL (Feng et al., 2020; Sultana et al., 2019).

The covalent bond reactive dye – cotton has also been tried to be cleaved using: a mixture of sodium sulfide – water – poly(vinylopyrrolidone) (PVP) (Home, Dudley, 1981), hydrogen bromide (Home, Dudley, 1981), 60% H2SO4 (Home, Dudley, 1981), 29.7% ammonium hydroxide (Dockery et al., 2009) and barium hydroxide (Dockery, 2009). Data on the volumes of other extraction media used were not presented.

The above-described studies were carried out on research material in the form of: fragments of cotton clothing with an area of 0.25-5.5 cm^2 (Sirén, Sulkava, 1995), 3 mg of cotton (Feng et al., 2020; Sultana et al., 2019), 10 cm of cotton thread (Dockery et al., 2009) and single fibres 1-15 mm long (Hoy, 2013).

Another method used to extract reactive dyes from cotton fibres is enzymatic extraction (Carey et al., 2013; Feng et al., 2020; Góra, Wąs-Gubała, 2019; Schotman et al., 2017). The first step of this extraction is similar to the alkaline hydrolysis presented above except for the NaOH solution volumes used. They were as follows: 10 µl (Carey et al., 2013; Schotman et al., 2017), 50 µl (Góra, Wąs-Gubała, 2019) and 100 µl (Feng et

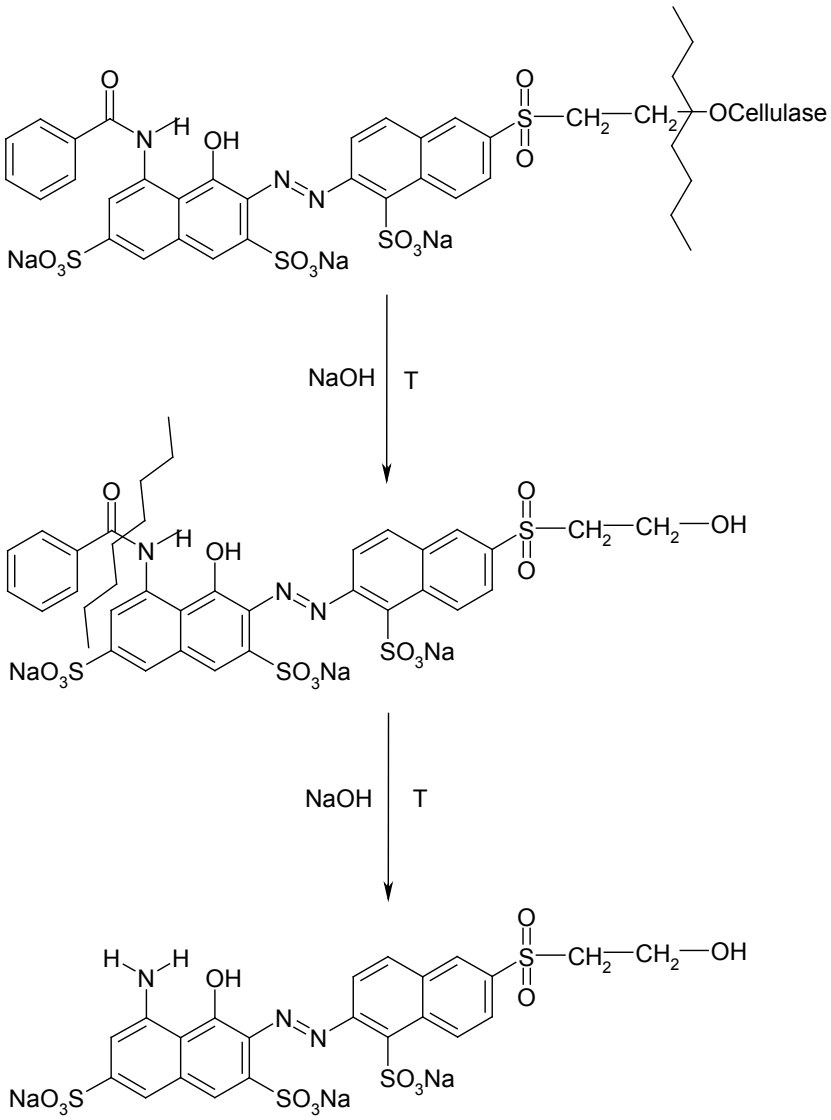


Fig. 9. Mechanism of alkaline hydrolysis of reactive dye (Śmigiel-Kamińska et al., 2020)

al., 2020). After this step, the test material was usually flushed in acetic acid (CH_3COOH) and cellulase solution (Carey et al., 2013), cellulase solution in CH_3COOH with pH 5 (Schotman et al., 2017), or cellulase solution in a vinegar buffer with pH 5 (Feng et al., 2020; Góra, Wąs-Gubała, 2019). Then, a cellulase solution was added to the samples and enzymatic extraction was carried out according to various procedures: 20 h with stirring (500 rpm) 50°C (Carey et al., 2013; Schotman et al., 2017), 20 h in a water bath or with the use of ultrasound at 50, 55 and 60°C (Góra, Wąs-Gubała, 2019) and 24 h in a bath with shaking at 50°C (Feng et al., 2020). The following were used as samples: 3 mg of cotton fabric (Feng et al., 2020), 1 cm of cotton thread (Góra, Wąs-Gubała, 2019) and cotton fibre 10 mm long (Carey et al., 2013; Schotman et al., 2017). Depending on the size of the cotton sample, the volume of the cellulase solution was: 10 µl (Carey et al., 2013; Schotman et al., 2017), 150 µl (Góra, Wąs-Gubała, 2019) and 1 ml (Feng et al., 2020).

In summary, the best results of the extraction of reactive dyes from cotton were obtained for a 1.5% NaOH solution (alkaline hydrolysis) and a cellulase solution (enzymatic extraction). However, the alkaline hydrolysis time is shorter than the enzymatic extraction procedure.

A detailed description of the conditions for the extraction of reactive dyes from cotton presented in the publication D. Śmigiel-Kamińska et al. (2020).

5.2 Chromatographic analysis of reactive dyes extracted from dyed cotton

The fibres as forensic traces are usually no more than a few millimeters long and contain from 2 to 200 ng of dye (Dorrien, 2006). Therefore, the chromatographic methods used to identify reactive dyes isolated from cotton fibres must be highly sensitive. For this reason, the most commonly used identification techniques are high-performance liquid chromatography (HPLC) coupled with the following detectors: UV-Vis spectrophotometric (Zotou et al., 2002), diode array (DAD) (Carey et al., 2013; Chemchame et al., 2010; Chemchame et al., 2012; Feng et al., 2020; Schotman et al., 2017; Sultana et al., 2019), high-resolution mass spectrometer (HRMS) (Carey et al., 2013; Feng et al., 2020; Schotman et al., 2017; Sultana et al., 2019) or tandem mass spectrometer (MS/MS) (Hu et al., 2018). Literature data show that HPLC-MS was typically equipped with two detection systems (Carey et al., 2013; Feng et al., 2020; Schotman et al., 2017; Sultana et al., 2019).

Chromatographic separations are most often performed using silica phases modified with C18 groups (inverted phase) (Chemchame et al., 2010; Chemchame et al., 2012; Feng et al., 2020; Hu et al., 2018; Sultana et al., 2019; Zotou et al., 2002) and column lengths from 50 to 150 mm. In the case of the research conducted by A. Carey and colleagues (2013) and T. G. Schotman and colleagues (2017), the Grom-sil 120 ODS-5 ST chromatographic column with a length of 150 mm was used.

In most cases, chromatographic separation of reactive dyes is carried out in gradient programs using mobile phases such as: aqueous solution of ammonium formate and HCOOH (pH 4) and MeOH/ACN 70/30 (Sultana et al., 2019); ammonium acetate in a mixture of MeOH/H2O (95/5) and ammonium acetate in a mixture of ACN/MeOH (50/50) (Carey et al., 2013; Schotman et al., 2017), an aqueous solution of ammonium formate and HCOOH (pH 4) and MeOH/ACN (70/30) (Feng et al., 2020), ammonium acetate in a mixture of H_2O/ACN (90/10) (pH 6) and ammonium acetate in a mixture of H2O/ACN (10/90) (Chemchame et al., 2012; Hu et al., 2018), a mixture of CH3COOH with H2O and acidified ACN (Hu et al., 2018). In one case, isocratic conditions were used with a mixture of ACN and ammonium acetate (47:53, v/v) as a mobile phase. A buffer containing trimethylammonium bromide (CTAB) was used as an ion-pairing agent (Zotou et al., 2002).

The flow rate of the moving phases ranged from 0.3 mL/min to 0.8 mL/min; analysis time from 9.5 min (Sultana et al., 2019) to 78 min (Schotman et al., 2017); and injection volume of 10 to 20 µl.

Another chromatographic technique used to identify reactive dyes for forensic purposes is ultra-efficient liquid chromatography (UPLC) coupled with DAD and MS/MS detectors (Hoy, 2013). The separation of the analytes was carried out using a 50 mm long C18 column. The following were used as mobile phases: an aqueous solution of 10 mM ammonium acetate (pH 9.3) and ACN. The analysis was carried out in gradient conditions. The phase flow rate was 0.4 mL/min, the analysis time was 5 min, and the injection volume was 10 µL (Hoy, 2013).

As mentioned above, the breaking of the covalent bond formed between the reactive dye and the cotton fibre (reaction with alkaline hydrolysis or enzymatic extraction) can form multiple reaction products from a single molecule of that dye. For this reason, the synthesis of partially and completely hydrolyzed forms of individual reactive dyes has proven to be very useful before analyses (Feng et al., 2020; Nayar, Freeman, 2008; Sultana et al., 2019) and/or obtaining enzymatic extraction products containing cellobiose units (Wąs-Gubała, Machnowski 2014).

A detailed description of the conditions for chromatographic analysis of reactive dyes extracted from cotton is presented in the publication D. Śmigiel-Kamińska et al. (2020).

5.3. Identification of dyes present in cotton dyed with different types of dyes

The above-mentioned analysis conditions refer to cotton fibres dyed with reactive dyes. However, fibres can also be dyed with mixtures of different types of dyes. Therefore, in the available literature, we can find very helpful algorithms to the identification of dyes or their mixtures using characteristic reactions or TLC (Laing et al., 1991; Lewis, 2009; Wiggins, 2017).

One of the first to present a scheme for identifying reactive dyes was D. K. Laing and colleagues (1991). In this procedure, the dyes were not extracted with organic solvents. The fibres were treated with a reducing agent such as sodium dithionine in sodium hydroxide. If the fibres were dyed by azo-dyes, they were discoloured. This irreversible reaction made it possible to distinguish azo reactive dyes from other classes of dyes.

Experts from the former Forensic Science Service in England have developed schemes for the extraction of dyed fibres, including cotton, to isolate and identify different classes of dyes (Laing et al., 1991). Such a scheme is also presented by D. M. Lewis (2009) in one of the chapters of the book entitled "Identification of Textile Fibres" (Fig. 10).

6. Comparison of the potential of chromatographic and spectroscopic methods for the identification of cotton fibres for forensic purposes

Forensic examinations must provide as much information as possible about the evidence being examined. Identification and comparative studies of microtraces in the form of fibres are based on microscopic, spectroscopic, and chromatographic methods.

The first stage of research on dyed textile fibres in forensic laboratories is testing using optical microscopy. The microscopic image obtained in this way provides important information about the structure and

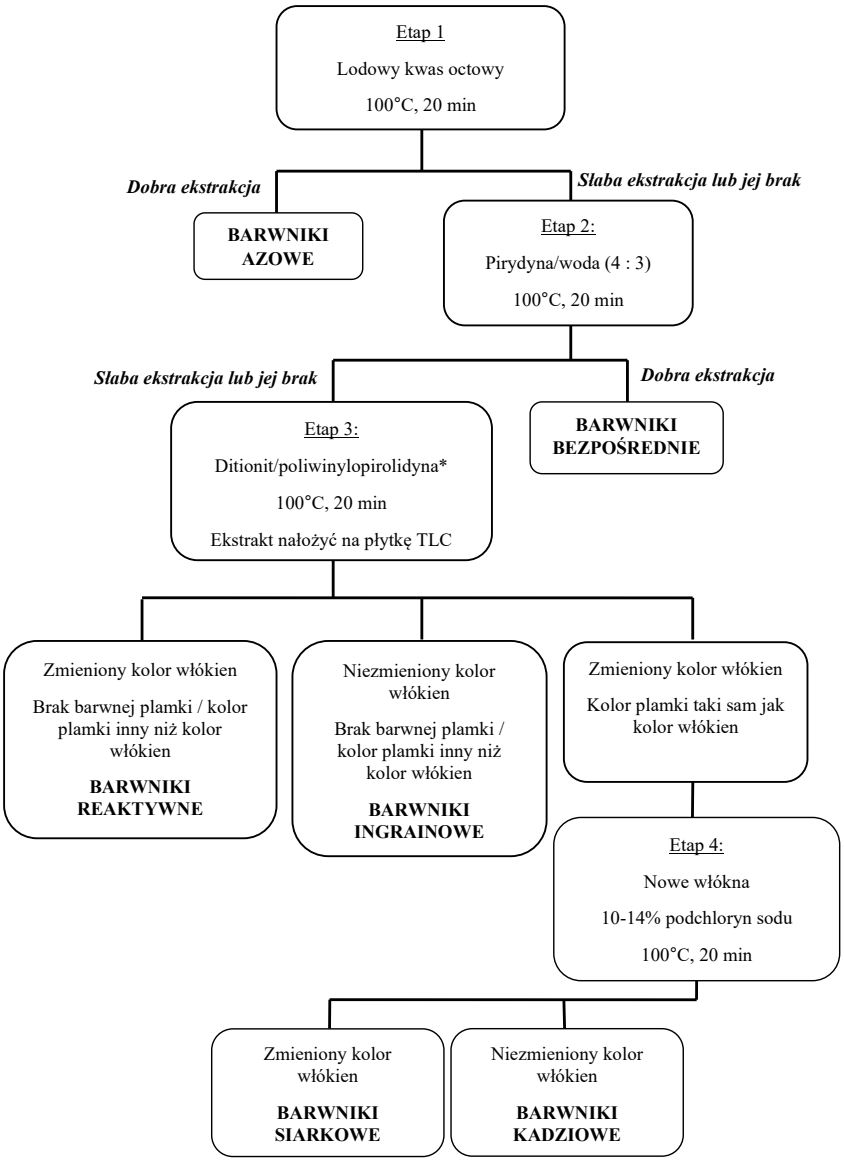


Fig. 10. Diagram of Extraction of Dyes from Cotton and Cellulose Fibres (Lewis, 2009)

physicochemical properties of the fibres, including their colour. In this case, stereoscopic (with reflected light), research microscopy (with transmitted white, polarized and ultraviolet (UV) light) and fluorescence microscopy are used.

The microscopic stage is followed by the analysis of chemical composition of the fibres. For this purpose, mainly spectroscopic techniques are used. Chromatographic techniques are used much less frequently. The choice of research methodology is determined by the form and amount of both evidence and comparative material, but also by the appropriate equipment available in forensic laboratories.

The spectroscopic techniques are used because they do not have a destructive effect on the examined samples, which is very important in forensic research. The results obtained are spectra with bands characteristic of fibre-forming polymers, dyes or both of these. It is depend on the kind of spectroscopic chosen for the study (MSP UV-Vis, FTIR, Raman spectroscopy) and measurement parameters (e.g. excitation line length).

Fibre tests can also be complemented by scanning electrone microscope examination, especially when one of the aspects of fibre analysis is their damage. However, the resulting image is black and white, so this technique is not suitable for identifying dyes.

Chromatographic techniques are destructive to the research material, but the results of such tests can provide important information about the dyes. Such identification is based not only on retention times, but often also on mass spectra and selected m/z values specific for given dyes. These techniques make it possible to compare dyes extracted from fibres constituting evidence with a similarly prepared sample from comparative material. It is also often possible to determine the chemical structure of the textile dyes which are analyzed.

Other parameters differentiating spectroscopic and chromatographic techniques are: sample preparation and analysis times. Both of these are longer for chromatography.

During spectroscopic studies, the fibre is treated as a finished sample, but in some cases the analysis conditions must be determined individually (Raman spectroscopy).

In chromatographic techniques, the examination can be divided into stages:

1) extraction of dye(s) from a sample of fibre or fibres,
2) analysis of the obtained extract,
3) analysis of the results.

In addition, the preparation process requires the use of additional reagents and special equipment, but the conditions for the analysis of individual dyes are constant. As mentioned, the identification of these dyes is based on the chromatographic parameter (retention time) and the mass spectrum (m/z values).

In summary, the use of spectroscopic and chromatographic methods and techniques in the identification and comparative analysis of evidence such as dyed fibres has both advantages and disadvantages. Therefore, the selection of appropriate test methods depends on many aspects, including the type, form and amount of evidence or equipment of the research/forensic laboratory. We should also not forget about the important role of the body commissioning forensic examinations, which indicates the scope of examinations relevant from the point of view of the analyzed case. (Śmigiel-Kamińska et al., 2019; Śmigiel-Kamińska et al., 2020).

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