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HPLC and molecular spectroscopic investigations of the red dye obtained from an ancient Pazyryk textile

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Abstract

The cloth specimens of Pazyryk culture from frozen burials of Altai Mountains (500–200 B.C.) were investigated by molecular spectroscopy and high performance liquid chromatography coupled with diode-array and mass selective detection. The qualitative and quantitative analyses of ancient red dyes were conducted. Natural dyes of plant origin – alizarin and purpurin and of insect origin – carminic acid and kermesic acid were determined.

Keywords: Alizarin; Purpurin; Carminic acid; Kermesic acid; Molecular spectroscopy; HPLC

1. Introduction

An ancient costume is one of the most short-lived and rare compositions of things that come to our days. Usually the clothes of ancient people are re-established from the works of art in which the people were depicted. Sometimes writing evidence complements these conceptions. Rarer the culture of ancient people comes to us in colour. Colour preferences of ancient cultures can characterize the accessibility of one or more dyestuffs, methods of dyeing that were used in antiquity. The type of the dyestuff can say more about the ways of trading that existed at that time. All these facts allow to re-establish the way of life of ancient man to some extent.

The Pazyryk culture of Altai Mountains falls into few ancient cultures of Siberia and Central Asia the clothes of which kept its original form and colour. Because of this the analysis of dyestuffs with which the clothes of Pazyryk people were dyed may shed light on the appearance and disappearance of these ancient denizens of Altai Mountains.

2. Experimental

2.1. Materials

The objects of investigation were Pazyryk belt and skirt from the Ak-Alakha – 3 burial (500–200 B.C.), which were given by the Institute of Archaeology and Ethnography of the Siberian Branch of the Russian Academy of Science [1]. The skirt was needled of three stripes of wool. The belt trusses and top and bottom stripes of skirt of different shades of red were studied.

For identification of dyes on textiles the standards are needful. As standards in our work we used dyed wool standards, namely pieces of wool those were coloured with the given dyestuff under different dyeing conditions.

Alizarin, purpurin (synthetic); madder (The State Hermitage Museum, Saint-Petersburg, Russia); kermes (*Kermes vermilio* Planchon, Institute of Archaeology

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and Ethnography of the Siberian Branch, Russian Academy of Science, Novosibirsk, Russia); cochineal (*Dactylopius coccus* Costa, Kremer-pigmente, Germany) were used as dyestuffs. Also a piece of wool coloured with *Porphyrophora hamelii* Brandt (The State Hermitage Museum, Saint-Petersburg, Russia) was used as dyed wool standard.

2.2. Dyeing procedure

The standard coloured pieces of wool were prepared according to the following receipts that are based on Ref. [2].

1. Dyeing without mordant: wool fibre (~70 mg), dyestuff (20 mg), sodium hydrocarbonate (30 mg), distilled water (50 ml) were placed into the Erlenmeyer flask and boiled for 60 min. The fibre was washed with soap in distilled water and dried.
2. Dyeing together with mordant: wool fibre (~70 mg), dyestuff (20 mg), sodium hydrocarbonate (30 mg), distilled water (50 ml) and mordant were placed into the Erlenmeyer flask and boiled for 60 min. The fibre was washed with soap in distilled water and dried.
3. Dyeing after treatment with a mordant: wool fibre (~70 mg) was boiled with mordant and sodium hydrocarbonate (30 mg) in distilled water (50 ml) for 20 min. Then dyestuff (20 mg) was added into the solution and this mixture was boiled for 60 min. The fibre was washed with soap in distilled water and dried.
4. Dyeing before treatment with a mordant: wool fibre (~70 mg) was boiled with dyestuff in 50 ml of distilled water for 60 min. Then sodium hydrocarbonate (30 mg) and mordant were added into the solution and mixture was boiled for 20 min. The fibre was washed with soap in distilled water and dried.

As a mordant, we used common alum (80 mg), calcium chloride (15 mg). Addition of $CaCl_2$ into mordant leads to obtaining alum–calcium-lake on the fibre while dyeing with only alum gives alum–alizarin-complex that has low light-resistance and intensity. Sodium hydrocarbonate is used for neutralization of hydrochloride acid, which is formed by the interaction of dye and calcium chloride [2].

Treatment order and mordant have a great influence on the wool colour.

2.3. Procedure

2.3.1. Molecular spectroscopy

In this work the analysis of dyes by spectrophotometric method was based on the identification test for the anthraquinone dyes of madder [3] – boiling of the textile coloured with madder in solution of aluminium sulphate leads to extraction of the dyestuff into the solution possibly as an aluminium lake [4].

Samples of coloured wool (1–4 mg) were boiled in 12% solution of aluminium sulphate (1.5–2 ml) for 10 min. The solutions obtained after filtration were used for registration of electronic absorption spectra. The spectra were registered in UV- and visible ranges (Spectrometer HP 8453, equipped with 1.0 cm cell). The spectra were smoothed and normalized, λ_{max} were determined from the second derivatives of spectra [5]. Peak position determination error was 0.5 nm.

2.3.2. HPLC analysis

Fragments of ancient textile material or dyed wool standards (0.5–3 mg) were boiled in 300 μl of the solution of 37% HCl/H_2O/methanol (2/1/1, v/v/v). Then the solution was quickly cooled and cleared from textile fibres in a centrifuge [6].

The chromatograph Agilent 1100 Series LC/MSD (Agilent Technology) was used. The chromatographic conditions were as follows: column Zorbax RX-C18, 150 × 4.6 mm; methanol/HCl extract injected without dilution, flow rate: 1.0 ml/min; detection wavelengths 255 nm (band width 16 nm), 440 nm (band width 80 nm), reference wavelength 850 nm (band width 100 nm); flow scheme (A = methanol, B = 2.3% water solution of formic acid) 40A/60B, 6 min, linear gradient to 90A/10B, 7 min.

For mass-selective detector (G1946C model) the atmospheric pressure chemical ionisation with positive ion scanning with m/z ratio of 100–1000 (accuracy 0.1) was chosen. Working parameters were: vaporizer temperature 450 °C, nebulizing gas (N_2) pressure 1.8 bar, drying gas (N_2) flow rate 7 l/min, its temperature 340 °C.

Anthraquinones in the extracts were identified according to electronic and mass-spectra. Quantitative analyses were conducted on the basis of electronic spectra.

In the paper [7] the correction factor for the calculation of relative amounts of the anthraquinone dyestuffs from integration data obtained under the same conditions were represented (for the 255 nm line). Control chromatographic analyses with additional detection 255 nm line (the line width 4 nm) and following chromatogram integration allow estimating values of correction factors for peaks registered in the 440/80 nm line. Obtained factors (CF) are given in the Table 1.

Due to the small values of obtained correction factors we didn't consider them, and the composition of investigated mixtures of red dyestuff was estimated from the areas of peaks in chromatograms registered on 440/80 nm line without correction factors. Determined error values were about 5%.

Table 1
HPLC of individual dyestuffs

Dyestuff	R_t (min)	λ_{max} (nm)	Molecular weight	m/z^+ of major ions	CF_{440}
Alizarin	14.4	279, 330, 430	240	241	1.00
Purpurin	16.1	255, 294, 454sh, 482, 516sh	256	256.9	1.05
Carminic acid	3.0–3.2	274, 312, 495	492	493.0	0.94
Kermesic acid	13.0	272, 310, 490	330	331	1.04
Flavokermesic acid	12.85	286, 344, 430	314	315	~1.00[a]

[a] Correction factor for flavokermesic acid was estimated on comparison of the chromatograms registered on 440/80 nm line and extract ion current chromatograms for ions 315 and 331 (m/z^+).

3. Results and discussion

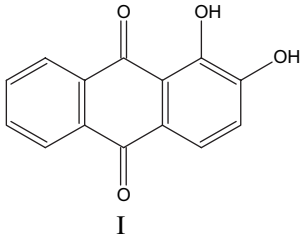
3.1. Analysis of dyed wool standard

In ancient times only natural organic dyes of plant and animal origin were used for fibre dyeing. For obtaining red shades the anthraquinone dyestuffs – madder, cochineal, kermes – were generally used [8].

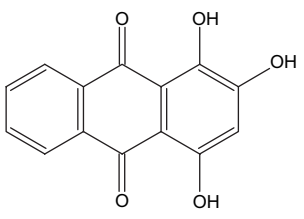
Using of the coloured pieces of wool as standards is conditioned by the fact that electronic absorption spectra of pure dyes in solution of aluminium sulphate differ from spectra of dyes desorbed from coloured fibres. The treatment of the dyed wool with methanol solution in HCl under high temperatures, which is used for obtaining the solutions for HPLC, is rather a hard method (and leads to the destruction of for example laccaic acids, haematein and brazilein). Because of this for spectrophotometric analyses we used solution of aluminium sulphate as a solvent. Aluminium hydroxide sulphate, which is formed as a result of hydrolyses, has strong desorbent properties and transfer the dye (probably, as a complex dye – mordant cation) into the solution. Electronic spectra of such solutions characterize not a dye but this "complex".

3.1.1. Standard dyed with madder

The roots of madder (*Rubia tinctoria* L.) in their cells contain a yellow liquid that after drying gives a powder which is used as a dye. The main madder dyestuffs are oxyanthraquinones alizarin (**I**) and purpurin (**II**):



I



II

For obtaining the spectral characteristics wool standards dyed with alizarin, purpurin and madder were prepared. The colour of standard dyed with alizarin – deep crimson, with purpurin – light crimson, with madder – brightly red. The values of λ_{max} of solutions obtained by boiling the standards dyed with alizarin, purpurin and madder in the solution of aluminium sulphate are shown in Table 2.

Spectra of solutions are obtained by boiling alizarin and purpurin in aluminium sulphate solution: λ_{max} – 479, 501 nm for alizarin, λ_{max} – 464, 496, 531 nm for purpurin.

The characteristic absorption lines in UV spectral range (Table 2) are the lines between 253 and 260 nm for alizarin and 263 and 268 nm for purpurin. In the visible range of spectra the major differences occur between 465 and 490 nm. The second derivative of the madder solution spectra obtained in the visible range has a form characteristic of purpurin (possibly absorption coefficient of alizarin derivatives from standards in the visible region is low). However, in UV-region $\lambda_{max} = 260$ nm – the main dyestuff of dyed wool standard is alizarin. After addition of calcium chloride into mordant there is redistribution of line intensities in the regions 502–504 and 543–551 nm.

In the work [7] the water/methanol (1/1) extracts of the different *Rubia* sp. roots were analyzed. For *R. tinctoria* L. the relative contents of main dyestuffs – alizarin and purpurin – are 38 and 23%, accordingly, also there is 15% of pseudopurpurin that is decarboxylated in the acid media with purpurin formation. Thus, their ratio is ~1:1. In the present work we used

Table 2
λ_{max} of UV- and visible spectra of aluminium sulphate solutions obtained from the standards dyed with alizarin, purpurin and madder under different conditions

Dyestuff and dyeing method	λ_{max} (nm) (determined from second derivatives of spectra)				
Alizarin (dyeing together with alum mordant)	258	294	483	504	551
Purpurin (dyeing together with alum mordant)	268	323	469	503	544
Madder (dyeing together with alum mordant)	261	295	469	502	543
Madder (dyeing together with alum mordant with addition of $CaCl_2$)	260	291	469	502	543

Table 3
Relative dyestuffs content in the standards dyed with madder (HPLC)

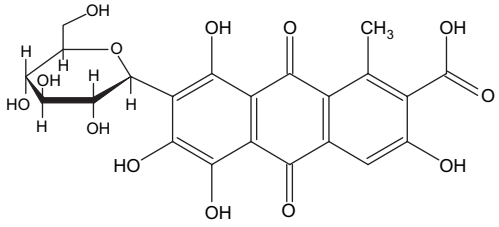
Dyeing method	Alizarin (%)	Purpurin (%)
Dyeing together with alum mordant	84	16
Dyeing together with alum mordant with addition of $CaCl_2$	90	10

solutions containing water/methanol/HCl (1/1/2) and determined relative initial madder composition under such conditions – 75% of alizarin and 25% of purpurin (disregard other minor substances). Chromatographic data of relative content of alizarin and purpurin on the wool dyed standards are given in Table 3.

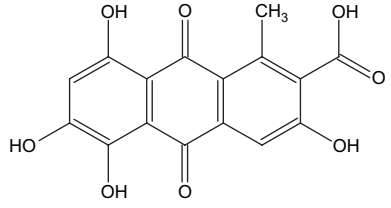
Obtained data show that under given dyeing conditions alizarin mainly fixed on the fibre. Addition of calcium chloride leads to increase in the relative alizarin content on the wool dyed standard.

3.1.2. Standards dyed with American and Armenian cochineal

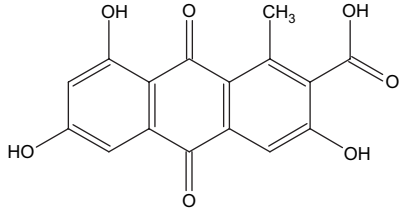
Dyestuff obtained from cochineal is carminic acid (**III**) [9]. It is isolated from specimen of two genus of insects: *Porphirophora* and *Dactylopius* [10]. One of the most known sources of carminic acid is American cochineal (*D. coccus* Costa). Other sources of carminic acid are Armenian cochineal (*P. hamelii* Brandt) and Polish cochineal (*Porphirophora polonica* L.).



III



IV



V

The carminic acid content in American and Armenian cochineal achieves 94–99%, in Polish cochineal – 62–88%. The latter consists of a great amount of kermesic (**IV**) [11] and flavokermesic (**V**) acids – 12–38% [12]. The colour of standards dyed with American and Armenian cochineal is cherry of different shades. Changing the dyeing procedure leads to a redistribution of line intensities in the second derivatives of electronic spectra obtained from dyed standards by boiling in solution of aluminium sulphate. The spectral characteristics of these solutions are shown in Table 4.

Data in Table 4 show that the location of absorption peaks isn't depending on dyeing procedure and is practically constant.

In the data of chromatographic analysis the content of carminic acid on the wool standards dyed with Armenian cochineal and American cochineal with mordant used after dyeing is 97%. Three percent remained is the summary content of kermesic and flavokermesic acids. In other dyed wool standards the acids ratio is 99:1, carminic acid:sum of kermesic and flavokermesic acids. The distributions of dyestuffs occurring in the water/methanol/HCl extracts of American and Armenian cochineal are 93:7 and 92:8, accordingly.

3.1.3. Standards dyed with kermes

The source of this dyestuff is genus *K. vermilio* Planchon known from Mediterranean [8]. The main dyestuff of kermes is kermesic acid. In methanol/water/HCl extracts there were determined 58% of kermesic acid and 29% of flavokermesic acid. The chromatographic data showed that during wool dyeing with kermes without mordant only kermesic acid fixed on the fibre. The colour of wool dyed with kermes without mordant is pink. Dyeing with mordant (in any order) leads to fixation of both kermesic and flavokermesic acids in ratio 1:1. Because of great amount of flavokermesic acid wool standards dyed with mordant have orange shades. Characteristic λ_{max}, determined from the spectra second derivatives of the solutions with aluminium sulphate are presented in Table 5.

Depending on the dyeing method the redistribution of line intensities in the region 280–320 nm occurs. Lines in another part of spectra have the same positions but different intensities.

3.2. Analysis of ancient textile

On the basis of the obtained spectroscopic and chromatographic data for dyed wool standards the samples of ancient textile, coloured stripes of Pazyryk skirt and belt were investigated.

Electronic absorption spectra of the solutions obtained by boiling the samples in aluminium sulphate solution and their second derivatives are shown in Figs. 1 and 2. Characteristic λ_{max} are presented in Table 6.

Spectral properties of the solution obtained from the sample of belt trusses well coincided with the data obtained for standards dyed with madder. Line (267 nm)

Table 4
λ_{max} of spectra in UV/visible region of solutions obtained from standards dyed with American and Armenian cochineal

Dyestuff and dyeing method (mordant − alum + $CaCl_2$)	λ_{max} (nm) (determined from the second derivatives of the spectra)							
Armenian cochineal (dyeing together with mordant)	247	288	305	347	483	520	549	583
American cochineal (dyeing together with mordant)	245	288	305	348	484		544	582
American cochineal (dyeing after treatment with mordant)	244	288	305	346	481		547	584
American cochineal (dyeing before treatment with mordant)	244	288	305	347	484	520	550	582
American cochineal (dyeing without mordant)	245	288	305	346	484	520	550	588

Table 5
λ_{max} of spectra in UV/visible region of solutions obtained from the standards dyed with kermes under different conditions (mordant − alum + $CaCl_2$)

Dyestuff and dyeing method	λ_{max} (nm) (determined from the second derivatives of the spectra)					
Dyeing together with mordant	268	288	309	487	528	576
Dyeing after treatment with mordant	271	287	307	484	525	574
Dyeing before treatment with mordant	270	287	308	484	526	575
Dyeing without mordant	270	285	306	483	526	574

indicates a high amount of purpurin in the sample, so probably the dyeing method was the method of dyeing with *Rubia* sp. with the use of alum as mordant (see Table 2).

Absorption maxima of the solution of the bottom stripe of a skirt indicate high content of kermesic acid (dyeing without mordant, lines 247, 306 nm, Table 5), and carminic acid (line 348 nm, Table 4).

High absorption intensity of the solution obtained from the top stripes of the skirt in the region 579–583 nm points out the high content of carminic and kermesic acids. The remaining part of the spectrum in the visible

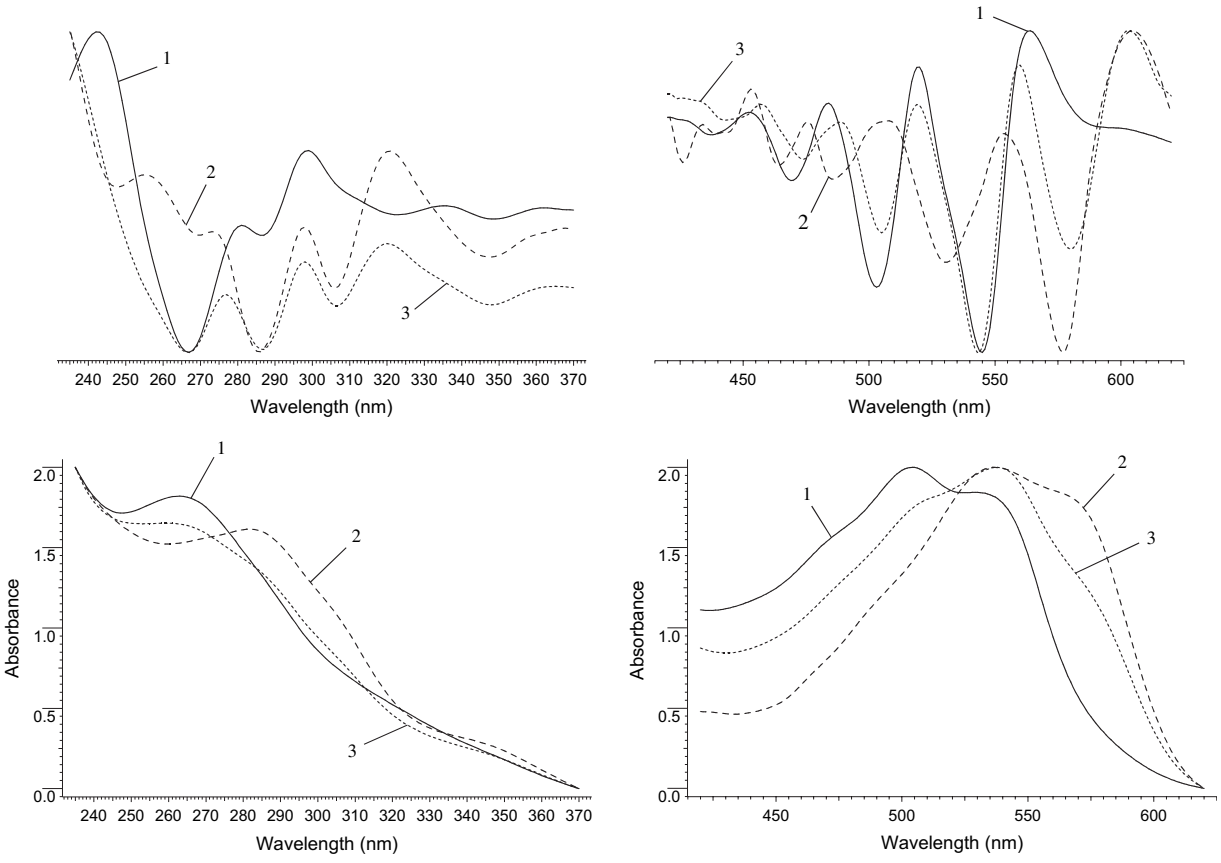


Fig. 1. Absorption spectra in UV- and visible regions of the solutions obtained by boiling ancient textile samples in aluminium sulphate solution. Top − the second derivatives of spectra. 1 − Trusses of skirt belt, 2 and 3 − bottom and top stripes of skirt accordingly.

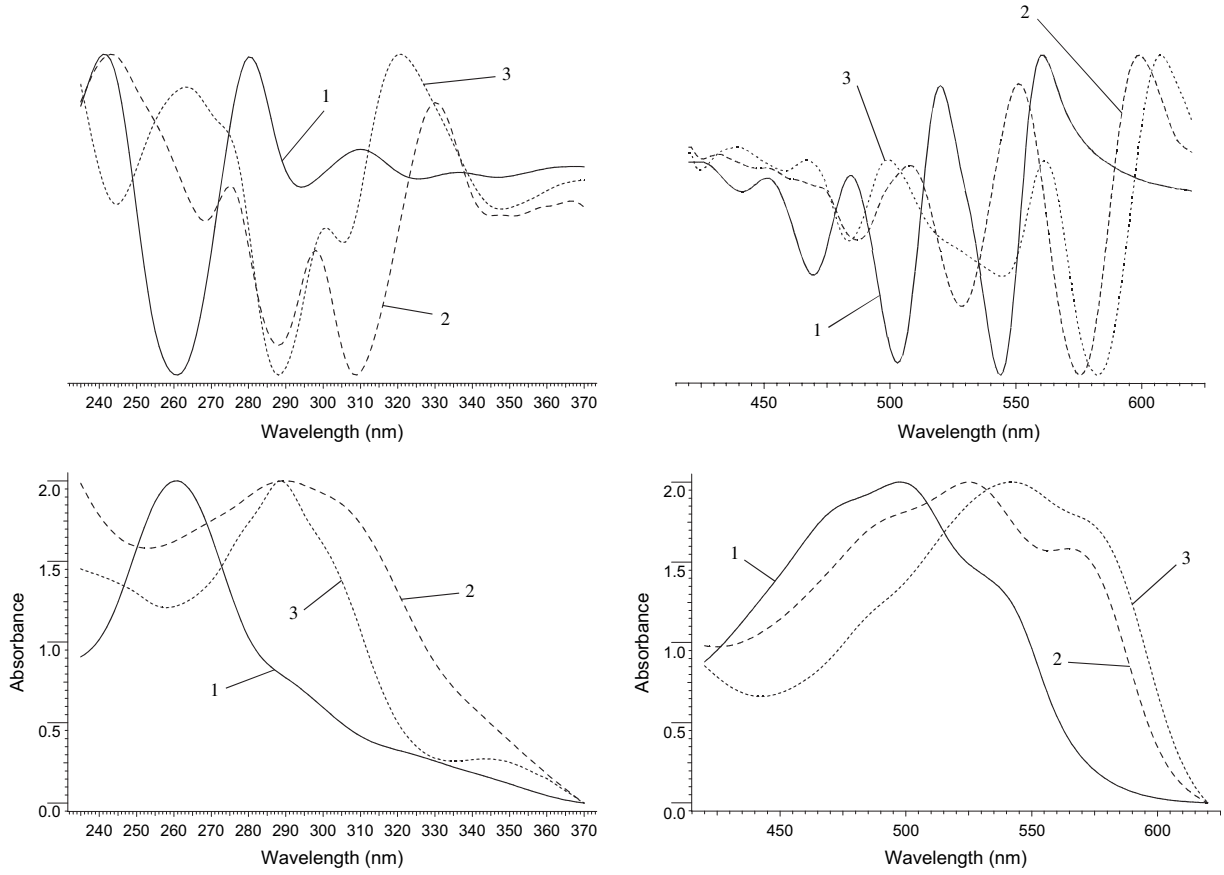


Fig. 2. Absorption spectra in UV- and visible regions of solutions obtained from dyeing standards with madder with alum mordant (1), kermes without mordant (2), and cochineal (3). Top – the second derivatives of the spectra.

Table 6
λ_{max} UV- and visible spectra of the solutions obtained from ancient textile

Sample	λ_{max} (nm) (determined from the second derivatives)								
Belt trusses		267	286			469		503	545	
Top skirt width		267	286	306	348	474		505	544	580
Bottom skirt width	247	270	286	306	348		486		530	577

region coincides with the spectra of the standards dyed with madder (Table 2).

Depending on chromatographic analyses (Table 7) the skirt stripes were dyed with several dyestuffs, for dyeing the belt trusses only the extract of *Rubia* sp. was used. Flavokermesic acid was not detected in the samples. These data are in a good agreement with spectrophotometric analysis data, thus electronic absorption spectra obtained in this work can be used for qualitative and approximate quantitative expression analysis of red dyestuffs of ancient textile.

Table 7
Relative content of red dyestuffs in the samples of ancient textile

Sample	Alizarin (%)	Purpurin (%)	Carminic acid (%)	Kermesic acid (%)
Belt trusses	64	36		
Top stripe of skirt	32	22	20	26
Bottom stripe of skirt	16	9	26	49

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