



Identification by RP-HPLC-DAD, FTIR, TGA and FESEM-EDAX of natural pigments prepared from *Datisca cannabina* L

Ozan Deveoglu^{a,b,*}, Emrah Cakmakcı^a, Turan Taskopru^{c,d}, Emine Torgan^e, Recep Karadag^{e,f}

^a Department of Chemistry, Faculty of Science and Letters, Marmara University, 34722 Goztepe, Kadikoy, Istanbul, Turkey

^b Department of Chemistry, Faculty of Science, Cankırı Karatekin University, 18100 Cankırı, Turkey

^c Department of Physic, Faculty of Science, Cankırı Karatekin University, 18100 Cankırı, Turkey

^d Department of Physic, Faculty of Science, Anadolu University, Eskisehir, Turkey

^e Turkish Cultural Foundation, Research and Development Laboratory for Natural Dyes, 34775 Umraniye, Istanbul, Turkey

^f Laboratory of Natural Dyes, Faculty of Fine Arts, Marmara University, 34718 Acıbadem, Kadikoy, Istanbul, Turkey

ARTICLE INFO

Article history:

Received 27 May 2011

Received in revised form

26 January 2012

Accepted 2 February 2012

Available online 15 February 2012

Keywords:

Natural pigment

RP-HPLC

FTIR

TGA

FESEM-EDAX

Hemp

ABSTRACT

In this study, natural pigments from the hemp (*Datisca cannabina* L.) dye plant were prepared by using $KAl(SO_4)_2 \cdot 12H_2O$ (alum), $FeSO_4 \cdot 7H_2O$ and $SnCl_2 \cdot 2H_2O$ mordants. A reversed-phase high performance liquid chromatography (RP-HPLC) with diode array detection (DAD) method was utilized for the identification of dyestuffs in the natural pigments. The dyestuff extractions from the pigments were carried out with 37% HCl/MeOH/H₂O (2:1:1 v/v/v) mixture. The pigments were further characterized by ATR-FTIR analysis. It was found that all metals precipitate datiscetin and carbonates. Also results show that the datiscetin–iron complex co-precipitates with glycosides. Thermal degradation of the pigments was determined by thermogravimetric analysis. High char yields were found for all pigments. These char yields are attributed to the high metal chelating capacity of datiscetin. The microstructure and chemical homogeneity of obtained natural pigments were studied by field emission scanning electron microscopy equipped with energy dispersion spectroscopy.

© 2012 Elsevier Ltd. All rights reserved.

1. Introduction

The hemp (*Datisca cannabina* L.) plant is also known as “gence” in Turkey [1]. It is a robust, glabrous perennial plant that grows up to 1–2 m high in the Black Sea region, West and South Anatolia [1–3]. All aerial parts of this plant are used for dyeing. The hemp plant is mainly native to Turkey, North India, Western Asia. In the past, this plant was used by nomads in north-west Turkey as a dye plant. At the present time as in the past, it has been used for carpet and plain weaves.

In addition, it is also used to yellow fibers in Van, Turkey. The aerial parts of hemp are dried and granulated, then the dyeing is accomplished by the mordant dyeing method. Hemp has been used to dye silk, wool and cotton in combination with an alum mordant. It yields a beautiful golden yellow with good fastness to washing

but is not so fast to light. The plant is very rich in flavonols: datiscetin is present in the form of a rutoside, datiscin (amounting to 10% of the weight of the fresh leaves). Kaempferol, quercetin and galangin are also present [2].

The natural pigments are prepared by the reaction of metal salts like aluminum (III) ($KAl(SO_4)_2 \cdot 12H_2O$), tin(II) ($SnCl_2 \cdot 2H_2O$) and iron(II) ($FeSO_4 \cdot 7H_2O$) with the dyestuff compounds (flavonoids, anthraquinones, etc.) present in the dye sources and an alkaline solution (mostly K_2CO_3) is used to adjust the pH. [4–10]. The yellow compounds obtained from plants have been used throughout history, both as textile dyes and to prepare natural pigments used in historical paints [9]. Many historical sources refer to the preparation of yellow pigments from flavonoid-rich plants [10]. The mordant links to specific functional groups of the dye molecule by covalent and coordination bonds and the pigments are precipitated as in-soluble metal–dye complexes in alkaline solution [11]. Flavonoids constitute a group of natural antioxidant substances which have been studied because of their properties such as antibacterial, anti-inflammatory, antiallergic, antimutagenic, antiviral and anticancer [12–15]. Also, interestingly it was found previously that the flavonoid–metal complexes are more effective antioxidants than the free flavonoids [16,17]. The natural dyestuffs

* Corresponding author. Department of Chemistry, Faculty of Science and Letters, Marmara University, 34722 Goztepe, Kadikoy, Istanbul, Turkey. Tel.: +90 216 3384474/124; fax: +90 216 3367468.

E-mail addresses: ozan.deveoglu@marmara.edu.tr, odeveoglu@yahoo.co.uk (O. Deveoglu).

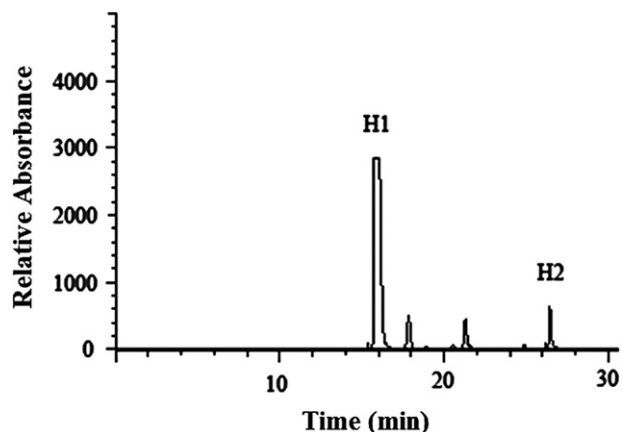


Fig. 1. Chromatogram of dihydrofisetin and datiscetin standard samples. Retention times and UV–Vis absorbance maximas of the detected coloring components are presented in Table 1.

(flavonoids for yellow colour and anthraquinones for red colour) present in the natural pigments and the dye plants are mostly identified by a reversed-phase high performance liquid chromatography (RP-HPLC) with diode array detection (DAD) at the present time [5]. Also, the Fourier-transform infrared (FTIR) technique is a rapid, reliable and an efficient method for the structural determination of natural pigments [11,18,19]. Moreover in this study TGA was performed in order to determine the thermal degradation of the natural pigments and further to characterize impurities such as carbonates. It is well-known that scanning electron microscopy (SEM) and energy dispersive X-ray microanalysis (EDX) can provide information about the surface morphology and the elemental compositions of the pigments. In this work, pigments were analyzed by FESEM/EDAX which can provide the data related to the morphology and elemental composition [20].

2. Experimental

2.1. Dye plant and chemicals

Hemp (*D. cannabina* L.) dye plant was obtained from the Turkish Cultural Foundation, Research and Development Laboratory for

Table 1
Chromatographic and spectral characteristics of the investigated standard dyestuff.

Dyestuff	Coloring component	R_t (min)	Absorbance maxima (nm)	Corresponding peak in Fig. 1
Hemp	Dihydrofisetin	15.7	231, 279, 313	H1
Hemp	Datiscetin	26.4	257, 305, 347	H2

Table 2
Chromatographic and spectral characteristics of the investigated sample extracts.

Sample number	Sample extract	Coloring components detected (min)	Characteristics of the detected coloring components		
			R_t (min)	Peak	Absorbance maxima (nm)
1	Acid hydrolyzed hemp	Datiscetin	25.2	Fig. 2	255, 305, 347
2	Non-hydrolyzed hemp	Datiscetin-3-O-[rhamnosyl(1-6)glucoside]	18.0	Fig. 3	257, 305, 329
		Datiscetin	26.4	Fig. 3	257, 305, 347
3	Al-hemp pigment	Datiscetin	25.3	Fig. 4	253, 305, 347
4	Sn-hemp pigment	Datiscetin	25.4	Fig. 5	253, 305, 347
5	Fe-hemp pigment	Not identified	10.6	Fig. 6	257
		Dihydrofisetin	15.7	Fig. 6	227, 259, 295
		Not identified	20.4	Fig. 6	257

Natural Dyes, Istanbul, Turkey. The following standard dyestuffs were used as references: apigenin from Carl Roth (Karlsruhe, Germany), gallic acid and ellagic acid from Merck (Darmstadt, Germany). Potassium-aluminum sulphate (alum), ferrous sulphate, stannous chloride, hydrochloric acid (37% fuming HCl), acetonitrile (MeCN, HPLC gradient grade), trifluoroacetic acid (TFA, HPLC gradient grade) and methanol (MeOH, HPLC gradient grade) were obtained from Merck (Darmstadt, Germany).

2.2. Extraction of dyestuffs from hemp

Hemp extract was prepared as in previously-described methods [5–8]. Dried and grounded hemp plant (65 g) was transferred into a 5000 mL beaker. 5000 mL of ultra pure water was then added and the mixture was heated to 100 °C while continuously stirring. Subsequently, the mixture was allowed to cool to 65–70 °C, and was maintained at this temperature for 1 h. The mixture was then removed from the heater, was filtered through a filter paper (Whatman No. 1) to collect the precipitated natural pigments.

2.3. Method for the preparation of natural pigments

15% $KAl(SO_4)_2 \cdot 12H_2O$ (alum) solution and hemp extract were heated separately to 90 °C and 60 °C respectively. 10 mL of alum solution at 90 °C was added to 150 mL of hemp extract at 60 °C. The mixture was left to cool. When the temperature reached 35 °C, K_2CO_3 solution (0.1 M) was added to adjust the pH of the mixture to between 6.5 and 7. Then the mixture was cooled to room temperature to precipitate the natural pigment. After settling-down, the mixture was filtered and the precipitate washed with ultra pure water and dried on a filter paper at 100 °C for 0.5 h. The dried aluminum natural pigment precipitate was powdered. The same process was repeated using 20, 30, 40, 50 and 60 mL of alum solution to each part of 150 mL of hemp extract. These experiments were repeated to precipitate the natural pigments by using 5% $FeSO_4 \cdot 7H_2O$ and 3% $SnCl_2 \cdot 2H_2O$ solutions.

2.4. Dyestuff extraction procedure for HPLC analysis

The dyestuff extraction from the dye plant and the natural pigments carried out using the previously described method [21–23].

The samples were prepared as follows:

For the dyestuff extractions from hemp (*D. cannabina* L.) plant, two procedures were performed.

In the first procedure, the dyestuff extraction from hemp dye plant was achieved by mixing 10.2 mg hemp in 400 μ L of the mixture of MeOH/ H_2O (2/1 v/v) in a conical glass tube without heating.

For the second procedure, hemp dye plant (7.3 mg) was hydrolyzed in 400 μ L of a mixture of 37% HCl/MeOH/ H_2O (2/1/1 v/v/v)

v) until evaporating in conical glass tubes for precisely 8 min in a water bath at 100 °C to extract the organic dyes. After rapid cooling under running cold water, the solution was evaporated just to dryness in a water-bath at 55–65 °C under a gentle stream of nitrogen. The dry residues were dissolved in 400 µL of a mixture of MeOH/H₂O (2/1 v/v).

For the acid hydrolysis of aluminium (4.2 mg), tin (5.8 mg) and iron – hemp (7.3 mg) natural pigments was utilized according to procedure presented in the second step. Then 25 µL and/or 60 µL of the supernatant were injected into the HPLC apparatus.

2.5. HPLC equipment

Chromatographic experiments were carried out using an Agilent 1200 series system (Agilent Technologies, Hewlett–Packard, Germany) including a G1329A ALS autosampler, a G1315A diode-array detector. Chromatograms were obtained by scanning the sample from 191 to 799 nm with a resolution of 2 nm and the chromatographic peaks were monitored at 255 nm. A G1322A vacuum degasser and a G1316A thermostatted column compartment were used. The data were analyzed using Agilent Chemstation. A Nova-Pak C₁₈ analytical column (3.9 × 150 mm, 4 µm, Part No WAT 086344, Waters) protected by a guard column filled with the same material was used. Analytical and guard columns were maintained at 30 °C. The HPLC gradient elution was performed by using the previously described method [24,25]. Chromatographic separations of the hydrolyzed samples were performed by using a gradient elution program that utilizes two solvents: solvent A: H₂O – 0.1%

TFA (trifluoroacetic acid) and solvent B: CH₃CN (acetonitrile) – 0.1% TFA. The flow rate was 0.5 mL/min and the applied elution program is same of the previously performed program [5–8].

3. Results and discussion

3.1. HPLC analysis

In the present study, natural pigments were obtained as the complexes formed by adding aluminum(III), iron(II), and tin(II) solutions to hemp (*D. cannabina* L.) extract. The identification of dyestuffs present in the natural pigments was analyzed qualitatively by reversed phase high performance liquid chromatography

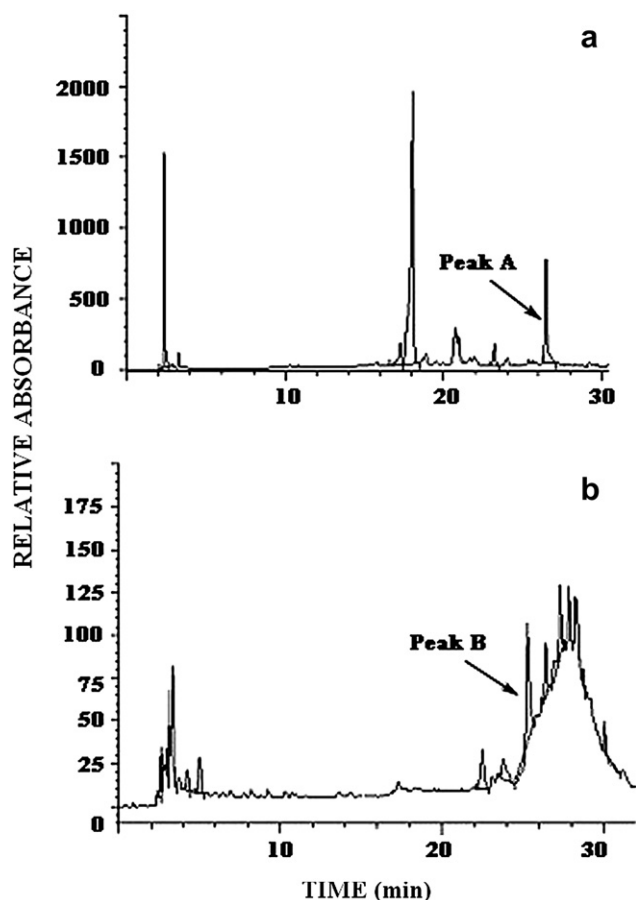


Fig. 2. Chromatograms of non-hydrolyzed (a) and acid hydrolyzed (b) Hemp extracts.

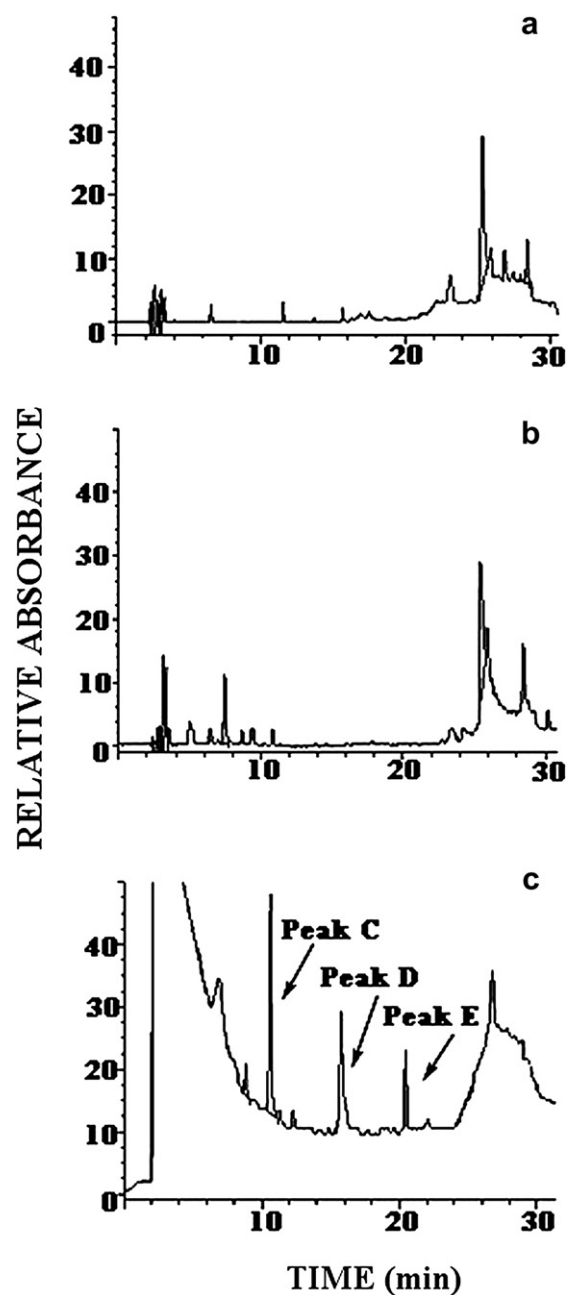


Fig. 3. Chromatograms of aluminum (a), tin (b), and iron (c)-Natural pigments (monitored at 255 nm).

(RP-HPLC). The dyestuff extractions for HPLC analysis were carried out using the previously described method [21–23].

In Table 1, the detected retention times (R_t) and corresponding spectral characteristics of the main coloring components of hemp are presented. The main coloring component of hemp which is datiscetin, can be fully separated, detected, and identified by its UV–Vis spectrum. Generally detection of characteristic absorptions of natural dyestuffs allows identification of pigments and plant components. Fig. 1 shows the chromatogram of standart samples.

The standard dyestuff used in the present study, datiscetin, was also chromatographically and spectrophotometrically (UV–Vis) characterized. Absorbance maxima (nm) and retention time (min) related to datiscetin-3-*O*-[rhamnosyl(1-6)glucoside] dyestuff were evaluated according to the present literature [26] because the corresponding standard dyestuffs were not available. Absorbance maxima in Table 2, which correspond to the one hemp component, appear to be similar and in good agreement with the spectral characteristics of datiscetin, the main coloring component of the hemp, that can be found in the literature [2,26]. Table 2 provides the results of HPLC-DAD analysis of the sample extracts, including retention times and corresponding absorbance maxima. The detection wavelength was selected according to the chemical nature of peaks present. In general, animal dyes were best analyzed at 275 nm, whereas 255 nm was the optimal detection wavelength for vegetable mordant dyes and 288 nm for indigoids [27]. In this study, we analyzed dyestuffs present in the natural pigments and the plant extracts at 255 nm.

As shown in Fig. 2(a), the main peak in the chromatogram of the non-hydrolyzed hemp extract, was determined as datiscetin-3-*O*-[rhamnosyl(1-6) glucoside] [26]. In the chromatogram related to the same extract, peak A was identified as datiscetin component. In the chromatogram of the acid hydrolyzed hemp extract related to Fig. 2(b), peak B was identified as datiscetin component.

Chromatographic peaks are presented in Fig. 3(a,b) for the samples extracted from natural pigments 3 and 4, respectively. These peaks showed that datiscetin was a main component. The natural dyestuffs (such as flavonoids) are effective metal ion chelators. As shown in Fig. 3(c), peak D was identified as dihydrofisetin. In the chromatogram related to same natural pigment, peaks C and E could not be determined. Chromatographic and spectral characteristic of the investigated sample extracts are displayed in Table 2. R_t and absorbance values are included in this table.

3.2. FTIR analysis

The ATR-FTIR spectra of datiscetin and the yellow pigments precipitated with Al, Fe and Sn salts can be seen in Fig. 4. In the spectra of datiscetin the peak at 3389 cm^{-1} is attributed to the hydroxyl stretching of absorbed water and the absorption band at 3157 cm^{-1} is due to the phenolic –OH groups. Chromone carbonyl absorption and aromatic C=C double bonds are seen at 1657 cm^{-1} and 1598 cm^{-1} . The bands in the $1650\text{--}1050\text{ cm}^{-1}$ range are characteristic flavone skeleton [28].

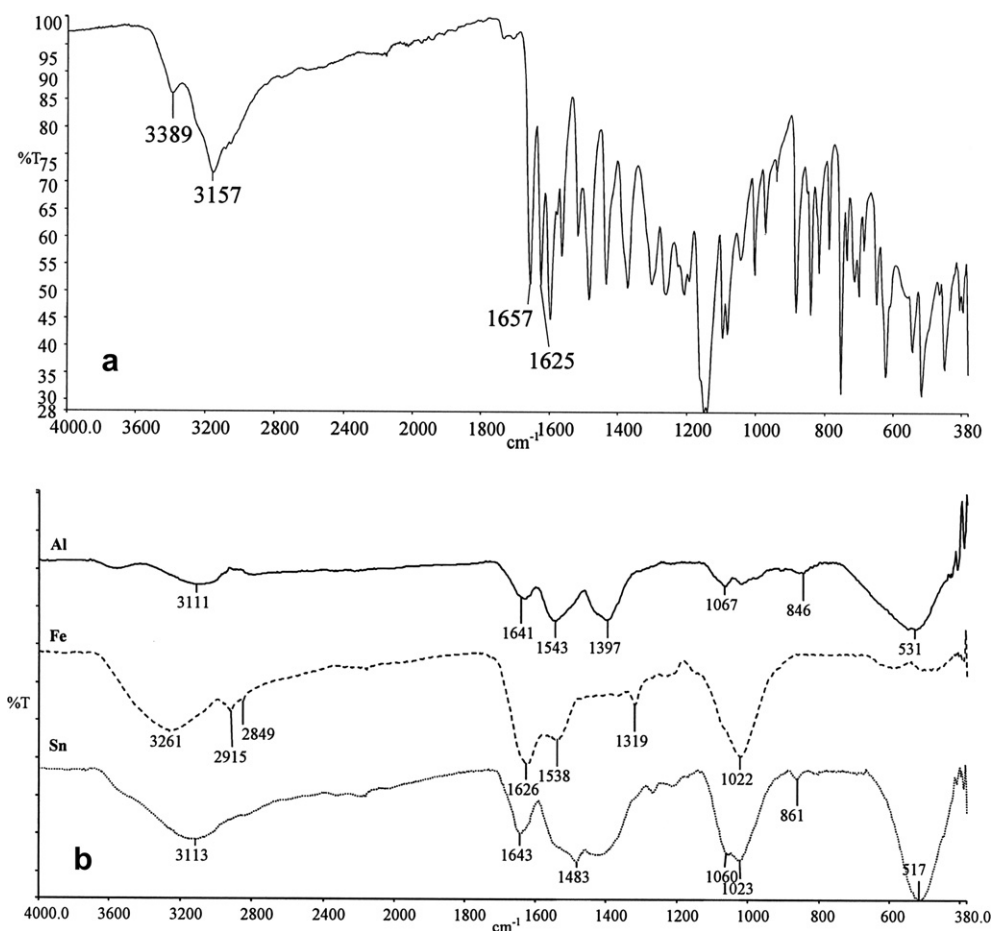


Fig. 4. ATR-FTIR spectra of (a) datiscetin and (b) Al, Fe and Sn yellow pigments respectively.

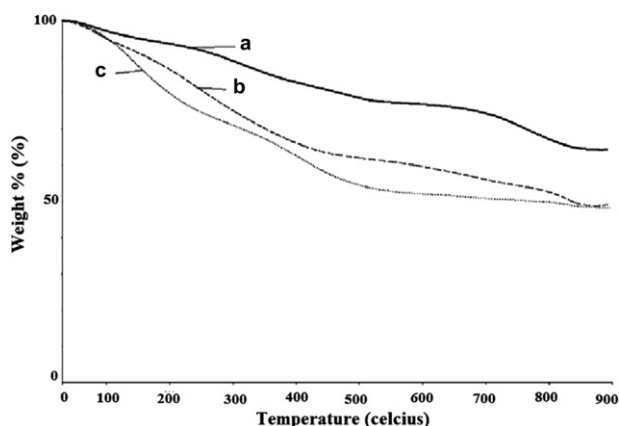


Fig. 5. TGA thermograms of yellow pigments (a) Sn, (b) Fe and (c) Al.

In the ATR-FTIR spectrum of alum precipitated yellow pigment, the band at $\sim 3500\text{ cm}^{-1}$ is assigned to the $-\text{OH}$ stretching. The absorption band at 1420 cm^{-1} is attributed to the CO_3^{2-} group. Thus it can be concluded that carbonate co-precipitates with hydrated alumina [11]. Also the absorption band seen in the region between 750 and 400 cm^{-1} can be related with $\text{Al}-\text{O}$ vibrations.

In the FTIR spectra of yellow pigments, the peaks at $\sim 1020\text{ cm}^{-1}$ and 1060 cm^{-1} correspond to $\text{C}-\text{O}$ stretchings. Also the shifts in the carbonyl and the hydroxyl absorptions in the spectra of yellow pigments can be related to the strong chelation [29].

Fe precipitated yellow pigment shows a broad peak at 598 cm^{-1} which can be related to $\text{Fe}-\text{O}$ bond. The peaks between 1640 and 1400 cm^{-1} belong to the aromatic vibrations of datisctetin. Also in this spectrum, the peaks detected at 2915 and 2850 cm^{-1} are due to asymmetric $\text{C}-\text{H}$ stretching in alkyl hydrocarbons. This result shows that datisctetin–iron complex co-precipitates with glycosides. Also in this spectrum a small peak corresponding to CO_3^{2-} can be seen.

Same observations were also made for Sn precipitated yellow pigments (glycosides were not detected). $\text{Sn}-\text{C}$ and $\text{Sn}-\text{O}$ peaks can be seen in the range between 410 and 600 cm^{-1} [29].

The peaks related to presence of carbonates can also be seen in this spectrum. So it can be said that all metals precipitate datisctetin with carbonates. However no peaks were detected due to SO_4^{2-} for any of the three samples.

3.3. TGA analysis

TGA thermograms of the yellow pigments are shown in Fig. 5. In all three thermograms, high inorganic content was observed. This situation was attributed to strong complexation between the metals and ligand species. The weight loss between 30 and $200\text{ }^\circ\text{C}$ is due to the absorbed water. After $200\text{ }^\circ\text{C}$ a slow degradation is observed. The weight loss during the period between 200 and $500\text{ }^\circ\text{C}$ is attributed to the degradation of the organic moiety. A third weight loss was also observed for Sn and Fe. This weight loss can be related to the release of carbon dioxide due to the presence of precipitated CO_3^{2-} [30]. However, no weight loss was observed with the Al samples. However har yields were found as 48, 49 and

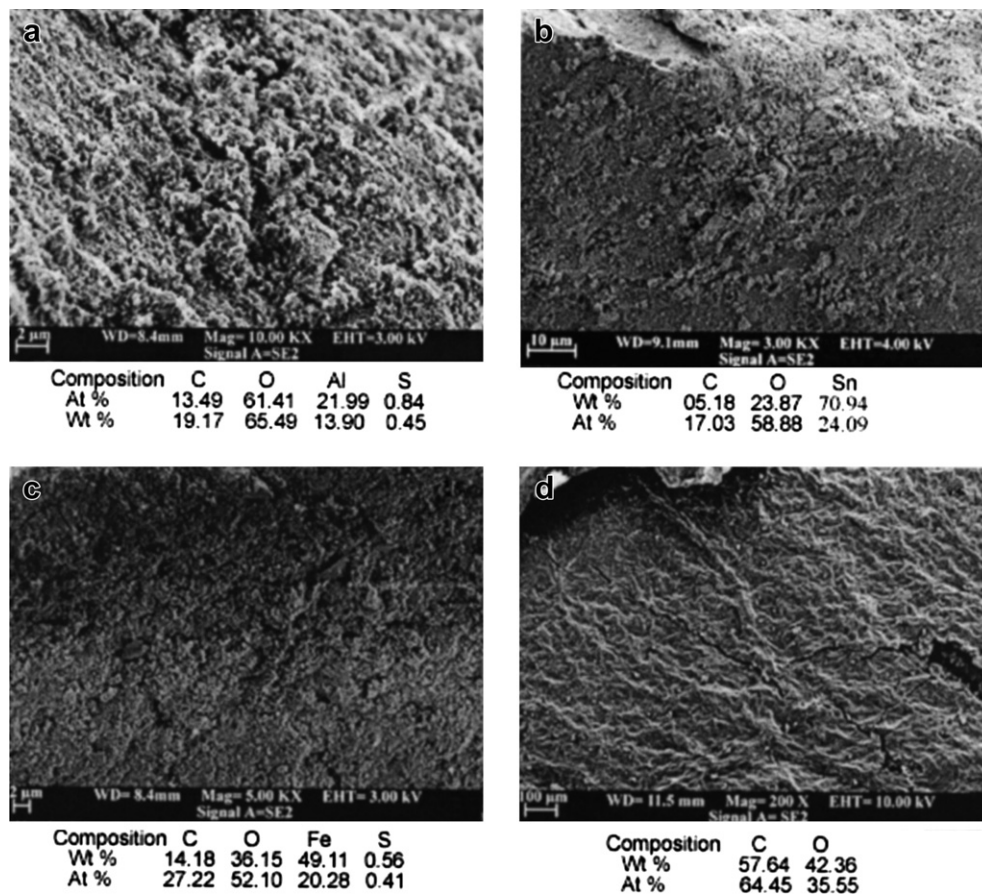


Fig. 6. FESEM-EDAX images and elemental compositions of (a) aluminum pigment (b) tin pigment (c) iron pigment and (d) crude plant.

65% for Al, Fe and Sn respectively. These char yields also indicate a high metal chelating capacity for datiscetin.

3.4. FESEM-EDAX analysis

The microstructure of obtained natural pigments were studied by field emission scanning electron microscopy (FESEM Zeiss Ultra Plus) equipped with energy dispersion spectroscopy (EDS–EDAX with detector Bruker AXS, software: Genesis).

Samples were analyzed as loose powders on carbon stubs. Secondary and back-scattered electron modes were employed to examine the nature, homogeneity and microstructure of the samples. The micrograph of hemp could be only taken after coating with Au–Pd by means of an Agar sputter coater to dismiss the charging effect. EDAX was applied to achieve the elemental composition of pigments with accelerating voltage at 10 kV and the working distance at 8.5 mm without coating. As seen in Fig. 6, natural yellow pigments successfully deposited with the reaction of related metals. Deposited natural pigments show good dispersion.

4. Conclusions

In this study, natural pigments from the hemp (*D. cannabina* L.) dye plant were prepared by using KAl(SO₄)₂·12H₂O (alum), FeSO₄·7H₂O and SnCl₂·2H₂O mordants. The HPLC analyses of the natural pigments revealed that the main organic component of the precipitated pigments was datiscetin. The ATR-FTIR spectra of the dye stuff also provided important data on the structure of the compounds. Moreover, it was found that the iron-precipitated pigments contained glycosides. TGA thermograms showed high char yields for all pigments and these high char yields were attributed to the strong metal-chelating capacity of datiscetin. Using FESEM and EDAX analysis, deposited natural yellow pigments were analyzed. The obtained experimental data showed that the pigments were obtained successfully as a result of reaction with the desired metals.

Acknowledgement

The support by Turkish Cultural Foundation is gratefully acknowledged (www.turkishculturalfoundation.org).

References

- [1] Karadag R. Doğal boyamacilik. Ankara: Geleneksel El Sanatları ve Magazalar Müdürlüğü Yayınları; 2007.
- [2] Cardon D. Natural dyes. Paris: Archetype Publications; 2007.
- [3] Böhmer H, Enez N, Karadag R, Kwon C. Koekboya. Germany: Remhüb-Verlag; 2002.
- [4] Schweppe H. Identification of dyes on old textiles. J Am Inst Conserv 1979; 19(1):14–23.
- [5] Deveoglu O, Karadag R, Yurdun T. Preparation and HPLC analysis of the natural pigments obtained from buckthorn (*Rhamnus petiolaris* Boiss) dye plants. Jordan J Chem 2009;4(4):377–85.
- [6] Deveoglu O, Torgan E, Taskopru T, Karadag R. SEM-EDX/HPLC analysis and production of natural pigments from *Quercus ithaburensis* with Al³⁺, Fe²⁺ and Sn²⁺ metals. In: Proceeding 6th Conference on medicinal and aromatic plants of Southeast European Countries; Turkey; 2010.
- [7] Deveoglu O, Torgan E, Karadag R. Characterization of colouring matters by HPLC-DAD and colour measurements, preparation of lake pigments with ararat kermes (*Porphyrophora hameli* Brand). Jordan J Chem 2010;5(3): 307–15.
- [8] Deveoglu O, Torgan E, Karadag R. Identification of dyestuffs in the natural pigments produced with Al³⁺, Fe²⁺ and Sn²⁺ mordant metals from cochineal (*Dactylopius coccus* Costa) and wallon oak (*Quercus ithaburensis* Decaisne) by HPLC-DAD. Asian J Chem 2010;22(9):7021–30.
- [9] Singer BW, Perry JJ, Brown L, Jurneczko E, Ludkin E. Identifying the plant origin of artists' yellow lake pigments by electrospray mass spectrometry. Archaeometry 2011;53(1):164–77.
- [10] McNab H, Ferreira ESB, Hulme AN, Quye A. Negative ion ESI–MS analysis of natural yellow dye flavonoids—an isotopic labelling study. Int J Mass Spectrom 2009;284:57–65.
- [11] Miliani C, Clementi C, Doherty B, Gentili PL, Romani A, Brunetti BG, et al. Vibrational and electronic properties of painting lakes. Appl Phys A Mater Sci Process 2008;92:25–33.
- [12] De Souza RFV, Sussuchi EM, De Giovanni WF. Synthesis, electrochemical, spectral, and antioxidant properties of complexes of flavonoids with metal ions. Synth React Inorg Met Org Chem 2003;33(7):1125–44.
- [13] Cushnie TPT, Lamb AJ. Antimicrobial activity of flavonoids. Int J Antimicrob Agents 2005;26:343–56.
- [14] Harborne JB, Williams CA. Advances in flavonoid research since 1992. Phytochem 2000;55:481–504.
- [15] Havsteen BH. The biochemistry and medical significance of the flavonoids. Pharmacol Ther 2002;96:67–202.
- [16] De Souza RFV, De Giovanni WF. Synthesis, spectral and electrochemical properties of Al(III) and Zn(II) complexes with flavonoids. Spectrochim Acta Part A 2005;61:1985–90.
- [17] Malešev D, Kuntić V. Investigation of metal–flavonoid chelates and the determination of flavonoids via metal–flavonoid complexing reactions. J Serb Chem Soc 2007;72(10):921–39.
- [18] Silva CE, Silva LP, Edwards HGM, De Oliveria LFC. Diffuse reflection FTIR spectral database of dyes and pigments. Anal Bioanal Chem 2006;386:2183.
- [19] Konvar M, Baruah GD. On the nature of vibrational bands in the FTIR spectra of medicinal plant leaves. Arch Appl Sci Res 2011;3(1):214–21.
- [20] Zenga QG, Zhang GX, Leung CW, Zuod J. Raman identification of natural red to yellow pigments: ochre and iron-containing ores. Microchem J 2010;96: 330–6.
- [21] Wouters J. High performance liquid chromatography of anthraquinones: analysis of plant and insect extracts and dyed textiles. Stud Conserv 1985; 30:119.
- [22] Wouters J, Verhecken A. The coccid insect dyes: HPLC and computerized diode-array analysis of dyed yarns. Stud Conserv 1989;34:189.
- [23] Wouters J, Verhecken A. The scale insect dyes (Homoptera: Coccoidea): species recognition by HPLC and diode-array analysis of the dyestuffs. Ann Soc Entomol Fr 1989;25:393.
- [24] Halpine SM. An improved dye and lake pigment analysis method for high-performance liquid chromatography and diode-array detector. Stud Conserv 1996;41:76.
- [25] Karapanagiotis I, Daniilia S, Tsakalof A, Chrysosoulakis Y. Identification of red natural dyes in post-byzantine icons by HPLC. J Liq Chromatogr Relat Technol 2005;28:739.
- [26] Campos MG, Markham KR. Structure information from HPLC and on-line measured absorption spectra: flavones, flavonols and phenolic acids. Coimbra University Press; 2007.
- [27] Wouters J, Rosario-Chirinos N. Dye analysis of Pre-Columbian Peruvian textiles with high-performance liquid chromatography and diode-array detection. J Am Inst Conserv 1992;31(2):237–55.
- [28] Mishra BB, Yadav SB, Singh RK, Tripathi V. A novel flavonoid C-glycosid from *Sphaeranthus indicus* L. (Family Compositae). Molecules 2007;12:2288–91.
- [29] Nagy L, Mehner H, Christy AA, Sletten E, Edelmann FT, Andersen QM. Preparation and structural studies on organotin (IV) complexes with flavonoids. J Radioanal Nucl Chem 1998;227(1–2):89–99.
- [30] Serifaki K, Boke H, Yalcin S, Ipekoglu B. Characterization of materials used in the execution of historic oil paintings by XRD, SEM-EDS, TGA and LIBS analysis. Mater Charact 2009;60:303–11.