

Characterization of Colouring Matters by HPLC-DAD and Colour Measurements, Preparation of Lake Pigments with Ararat kermes (*Porphyrophora hameli* Brand)

Ozan Deveoglu^{*a}, Emine Torgan^b, Recep Karadag^c

a Department of Chemistry, Faculty of Science and Letters, Marmara University, 34722, Goztepe, Kadıkoy, Istanbul, Turkey.

b Turkish Cultural Foundation, Research and Development Laboratory for Natural Dyes, 34775, Yukarı Dudullu, Umraniye, Istanbul, Turkey.

c Laboratory of Natural Dyes, Faculty of Fine Arts, Marmara University, 34718, Acıbadem, Kadıkoy, Istanbul, Turkey.

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Abstract

The lake pigments aluminium, tin, iron, aluminium-calcium, tin-calcium and iron-calcium ararat kermes were obtained by means of $KAl(SO_4)_2 \cdot 12H_2O$ (alum), $SnCl_2 \cdot 2H_2O$, $FeSO_4 \cdot 7H_2O$ and $Ca(NO_3)_2 \cdot 4H_2O$ solutions from Ararat kermes (*Porphyrophora hameli* Brand) dye insects. A reversed phase high performance liquid chromatography (HPLC) with diode-array detection (DAD) method was utilized for the identification of red anthraquinone dyes present in the lake pigments. The extraction of dyes from the lake pigments was carried out with HCl / MeOH / H_2O (2:1:1; v/v/v) solution. CIELAB values of the lake pigments were measured.

Keywords: Ararat kermes; Carminic acid; Flavokermesic acid; Dye analysis; Lake pigment; HPLC; CIELAB.

Introduction

Ararat kermes (*Porphyrophora hameli* Brand) insect is native to the area of Mount Ararat in Turkish eastern Anatolia and in Armenia. They grow in the salt marshes on both sides of the river Aras (Araxas), which flows past the north side of Mount Ararat and forms the border between Turkey and Armenia. Armenian sources cited their usage in dyeing silk and for colouring at miniature painting in the fifth century AD^[1]. The main dye compound present in ararat kermes dye insect is carminic acid which is an anthraquinone compound^[2,3]. Many articles based on the analysis of lake pigments and the dyes have been indicated by various authors. For the identification of carminic acid that present in the lake pigment of artistic paintings method has been proved by Maguregui et al^[4]. Blanck et al. have used a liquid chromatography with diode - array UV-vis spectrophotometric detection for the identification of dyes in the historical maps^[5]. Karadag and Dolen have investigated a group of historical textiles in the Topkapi Museum by TLC and derivative spectrophotometry^[6]. Maier et al. have achieved the isolation of carminic acid from cochineal (*Dactylopius coccus* Costa)^[7]. Sanyova and Reisse have shown in their

* Corresponding author: Tel.: +902163384474, ext. 17; Fax: +902163367468; E-mail: ozan.deveoglu@marmara.edu.tr (O.Deveoglu).

HPLC-DAD studies on the extraction procedures of red anthraquinone dyes from their aluminum complexes in madder lakes that red dyes may determine^[8]. The natural pigments have been prepared from buckthorn (*Rhamnus petiolaris* Boiss) dye plant and qualitatively identified by using HPLC-DAD measurements by Deveoglu et al^[9]. Balakina et al. have prepared the red natural dyes from an ancient Pazyryk textile by HPLC and molecular spectroscopy^[10]. Schweppe has published a work that including the thin-layer chromatography and the preparation of lakes for the identification of natural dyes on old textiles^[11]. A work based on X-ray fluorescence, Fourier transform infrared and UV–visible absorption and emission spectroscopies of organic pigments derived from different natural sources has been achieved by Clementi et al^[12]. Novotná et al. developed a HPLC method for qualitative and quantitative analysis of dyes in historical textiles^[13]. A spectrophotometric method has been developed for the determination of red dyes in cochineal pigments by González et al^[14]. Orska-Gawryś et al. used reversed-phase HPLC with diode-array UV–Vis spectrophotometric detection for identification of natural dyes in extracts from wool and silk fibres from archeological textiles^[15]. Liquid chromatography combined with ultraviolet–visible and mass spectrometric detection was performed to determine the dye components present in extracts of natural dyes originating from fiber samples obtained from Coptic textiles from Early Christian Art Collection of National Museum in Warsaw by Szostek et al.^[16].

The aim of this study was two fold. (i) To extract the dyes present in Ararat kermes (*Porphyrophora hameli* Brand) insect by using water. (ii) The preparation of lake pigments by using of $KAl(SO_4)_2 \cdot 12H_2O$, $FeSO_4 \cdot 7H_2O$, $SnCl_2 \cdot 2H_2O$ and $Ca(NO_3)_2 \cdot 4H_2O$ solutions with this extract in order to identify and determine the colour values of the lake pigments. The identification of dyes present in the lake pigments was carried out by a reversed-phase high performance liquid chromatography (HPLC) with diode-array detection (DAD). The red anthraquinone dyes were identified by comparison with the standard reference dyes. The dye extraction was done by using the previously described method^[17-19]. The latter include the investigation of effect of the different amounts of metal solutions on the colouring scale of the lake pigments.

Experimental

Insect and chemicals

Ararat kermes (*Porphyrophora hameli* Brand) insects were obtained from Laboratory for Natural Dyes, Faculty of Fine Arts, Marmara University. $KAl(SO_4)_2 \cdot 12H_2O$ (alum), $FeSO_4 \cdot 7H_2O$, $SnCl_2 \cdot 2H_2O$, CH_3OH , HCl , TFA (trifluoroacetic acid), CH_3CN (acetonitrile) and K_2CO_3 were provided from Merck (Darmstadt, Germany, www.merck.de). $Ca(NO_3)_2 \cdot 4H_2O$ was obtained from Bereket Kimya (Istanbul, Turkey, www.bereketkimya.com) and carminic acid was purchased from Fluka (Sigma-Aldrich, www.sigmaaldrich.com). Kermesic acid and flavokermesic acid were provided by Mohammad S. Mubarak (University of Jordan, Jordan). The HPLC mobile phase was prepared using Milli-Q-water (Millipore, Bedford, MA, USA).

Apparatus

WiseStir MSH-20A Daihan Scientific Co. Stirrer, Precisa XB 220A Gravimetrics AG. (Dietikon, Switzerland), Elga PureLab Option-Q, Elektro-mag M 420 P Hot Air Sterilizer Laboratory Oven and Sigma 2-16P were used in the study.

HPLC equipment

Chromatographic experiments were carried out using an Agilent 1200 series system (Agilent Technologies, Hewlett-Packard, Germany) including a G1329A ALS autosampler, a G1311A gradient quaternary pump with a 50 µl loop and a Rheodyne valve (7725i sample injector), 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 and 276 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 x 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 using the method of Halpine et al.^[20] and Karapanagiotis et al.^[21]. Chromatographic separations of the hydrolysed samples were performed 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 described in table 1.

Table 1: Gradient elution program for HPLC

Time (min)	H ₂ O+0.1% TFA (%)	CH ₃ CN+0.1% TFA (%)
0.0	95.0	5.0
1.0	95.0	5.0
20.0	70.0	30.0
25.0	40.0	60.0
28.0	40.0	60.0
33.0	5.0	95.0
35.0	5.0	95.0
45.0	95.0	5.0

Dye Extraction from Ararat kermes insect

5 g of ground Ararat kermes (*Porphyrophora hameli* Brand) was transferred into a 2000 ml beaker. 1000 ml ultra-pure water was added and the mixture was heated to 100 °C for 30 minutes by using a magnetic stirrer. Finally, the mixture was filtered to obtain the Ararat kermes extract.

Preparation of Ararat kermes Lake Pigment

Procedure I

15 % KAl(SO₄)₂·12H₂O (alum) solution and 50 ml Ararat kermes extract were heated separately to 90 °C and 60 °C, respectively. 10 ml alum solution at 90 °C was added to 50 ml of Ararat kermes extract at 60 °C. The pH of the mixture was adjusted

to be between 6 and 7 by the addition of 0.1 M K_2CO_3 solution. The mixture was cooled to room temperature to allow the precipitation of the lake pigment. After settling down, the mixture was filtered and the precipitate was washed with ultra-pure water and dried on a filter paper at 100 °C. The dried lake pigment was then powdered. The same procedure was repeated by adding 20, 30, 40 and, 50 ml of alum solution to each part of 50 ml of Ararat kermes extract. These experiments were repeated to precipitate the lake pigments by using 3 % $FeSO_4 \cdot 7H_2O$ and 3 % $SnCl_2 \cdot 2H_2O$ solutions.

Procedure II

10 ml alum solution at 90 °C was added to 50 ml of Ararat kermes extract at 60 °C according to procedure I. The pH of the mixture was adjusted to be between 6 and 7 by the addition of 0.1 M K_2CO_3 solution. Afterwards, 5 ml of 15 % $Ca(NO_3)_2 \cdot 4H_2O$ solution was added to the mixture. After, the mixture was cooled to room temperature and then accomplished the precipitation of lake pigment. Then, the precipitate was filtered and washed with ultra-pure water. The obtained wet precipitate was dried on a filter paper at 100 °C and then powdered.

The experiments were repeated 4 times by adding of different volume of solution (20, 30, 40 and 50 ml of 15 % $KAl(SO_4)_2 \cdot 12H_2O$ (alum) solution) at 90 °C with 50 ml of ararat kermes extract at 60 °C, respectively. After, 15 % $Ca(NO_3)_2 \cdot 4H_2O$ solution (10, 15, 20 and 25 ml) at the room temperature were added to each of the mixture obtained by using alum solution, respectively. These experiments were exactly repeated according to this procedure applied for 10 ml of alum solution at 90 °C and 5 ml of calcium solution at the room temperature. Similarly, the experiment was repeated by using 3 % $FeSO_4 \cdot 7H_2O$ and 3 % $SnCl_2 \cdot 2H_2O$ solutions instead of alum solution.

Extraction Procedure Prior to HPLC Analysis

The dye extractions from the dye insect and the lake pigments were done by using the previously described method^[17-19].

The samples were prepared as follows:

- 1^o) The lake pigment samples (5 mg) were hydrolysed by using H_2O : MeOH: 37 % HCl (1:1:2; v/v/v; 400 μ l) 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 50-65 °C under a gentle stream of nitrogen. The dry residue was dissolved in 400 μ l of a mixture of MeOH: H_2O (2:1; v/v).
- 2^o) The dye insects (5.5 mg) were hydrolysed according to above procedure. Then, the dry insect residue was dissolved in 400 μ l of a mixture of MeOH: H_2O (2:1; v/v). Additionally, the dye insects (9.0 mg) were dissolved in 400 μ l of a mixture of MeOH: H_2O (2:1; v/v) (non-hydrolysed).

The hydrolysates of dry insect and lake pigment were centrifuged at 4000 rpm for 10 min. Then 15 μ l and/or 100 μ l of the supernatant were injected into the HPLC apparatus. Figures 1-4 show the HPLC chromatograms of acid hydrolysed, non

hydrolysed dye insects and acid hydrolysed lake pigments.

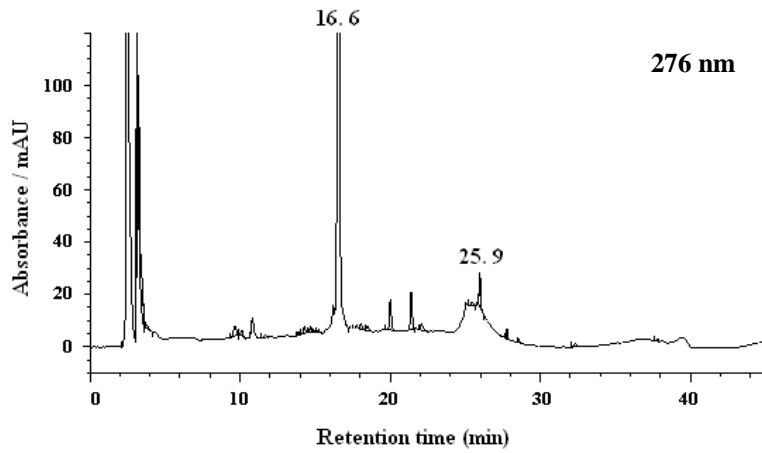


Figure 1: HPLC chromatogram of acid hydrolysed Ararat kermes insect extract. Carminic acid (16.6 min) and flavokermesic acid (25.9 min) are identified.

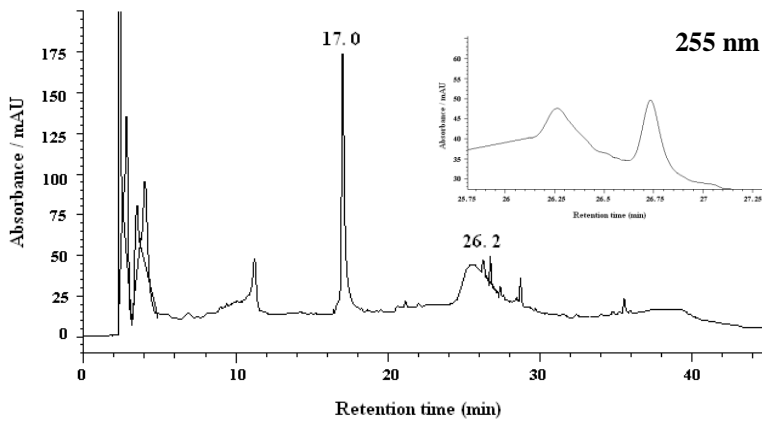


Figure 2: HPLC chromatogram of non-hydrolysed Ararat kermes insect extract. Carminic acid (17.0 min) and flavokermesic acid (26.2 min) are identified.

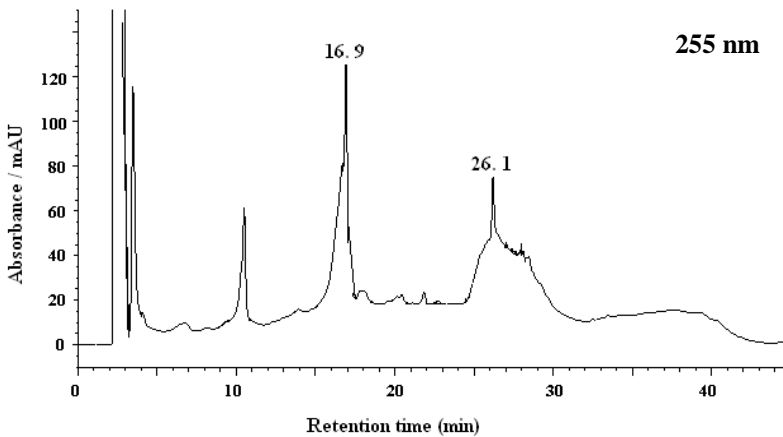


Figure 3: HPLC chromatogram of acid hydrolysed aluminium-ararat kermes lake pigment. Carminic acid (16.6 min) and flavokermesic acid (26.1 min) are identified.

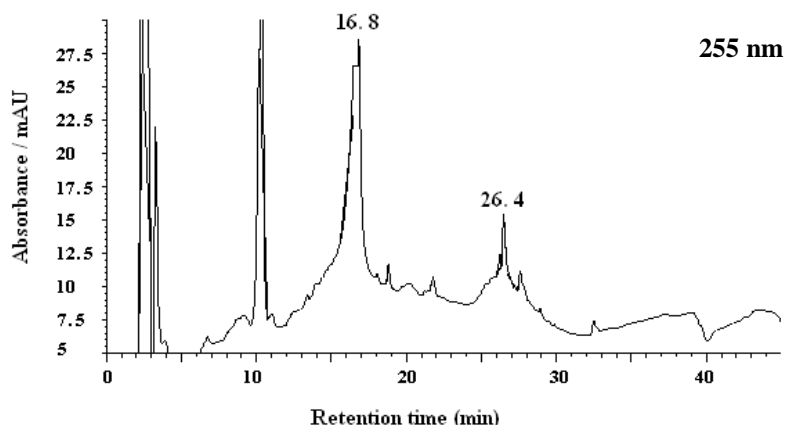


Figure 4: HPLC chromatogram of acid hydrolysed aluminium-calcium-ararat kermes lake pigment. Carminic acid (16.8 min) and flavokermesic acid (26.4 min) are identified.

Colour measurements of lake pigments

L^* , a^* and b^* values of the lake pigments were measured with Konica Minolta CM-2300d Software SpectraMagic NX (6500 K, 45°). CIELAB graphs of lake pigments were drawn using measured values.

Results and discussion

In the present study, the complexes of red anthraquinone dyes present in Ararat kermes (*Porphyrophora hameli* Brand) insect extract with aluminium (III), iron(II), tin(II) and calcium(II) were obtained and analyzed qualitatively by a reversed phase high performance liquid chromatography (RP-HPLC). The dye extractions for HPLC analysis were done by using the previously described method^[17-19,22,23]. The composition was identified based on the absorption spectra acquired with standard reference dye compounds (figures 5-6). Carminic acid and flavokermesic acid are metal chelates. HPLC analysis shows that carminic acid and flavokermesic acid were identified in the acid hydrolysed Ararat kermes extract, the non - hydrolysed Ararat kermes extract, aluminium - ararat kermes lake pigment, aluminium - calcium - ararat kermes lake pigment. Kermesic acid anthraquinone compound was not identified in the acid hydrolysed Ararat kermes extract and the non - hydrolysed Ararat kermes extract. According to procedures the preparation of lake pigments in the study, the dye could not be identified in the iron-ararat kermes lake pigment, the iron-calcium-ararat kermes lake pigment, the tin-ararat kermes lake pigment and the tin-calcium ararat kermes lake pigment.

The brightness and colour values of ararat kermes lake pigments prepared by using aluminium(III), iron(II), tin(II) and calcium(II) were determined by CIELAB colour space system. The best brightness for aluminium, iron, tin, aluminium-calcium, iron-calcium and tin-calcium lake pigments were observed in samples that have been prepared with 30, 30, 20, (40:20), (50:25) and (50:25) ml of the metal solution,

respectively. When red and green colour values were investigated, it was observed that the best red and green colours were obtained from the pigments that prepared by using 20, 30, 10, (10:5) ml of the metal solution. According to the results of the colour measurement of the prepared lake pigments, the similarity between L*, a* and b* values for the iron-ararat kermes lake pigments (figure 7) and for the pigment prepared by using 30 ml of iron solution were maximum values. This is a very practical desirable. The best colour values of the lake pigments were given with the consumed metal solution volumes in table 2.

Table 2: The best colour values of the lake pigments

Lake pigment	Metal solution volume (ml)	L*	Metal solution volume (ml)	a*
Al-Ararat kermes	30	69.11	20	21.35
Fe-Ararat kermes	30	29.23	30	14.03
Sn-Ararat kermes	20	68.40	10	24.95
Al-Ca-Ararat kermes	40:20	66.38	10:5	34.61
Fe-Ca-Ararat kermes	50:25	39.45	40:20	13.10
Sn-Ca-Ararat kermes	50:25	78.34	10:5	19.63

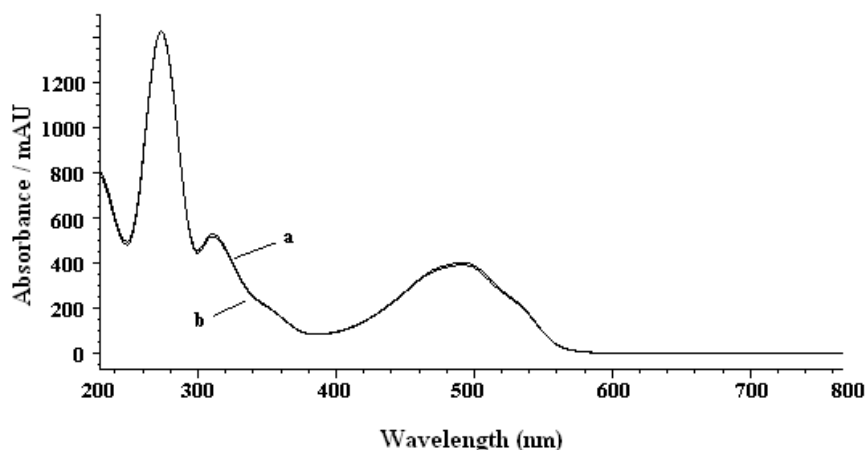


Figure 5: Photodiode array spectra of (a) carminic acid standard compound and (b) peak at 16.6 min from chromatogram in figure 1.

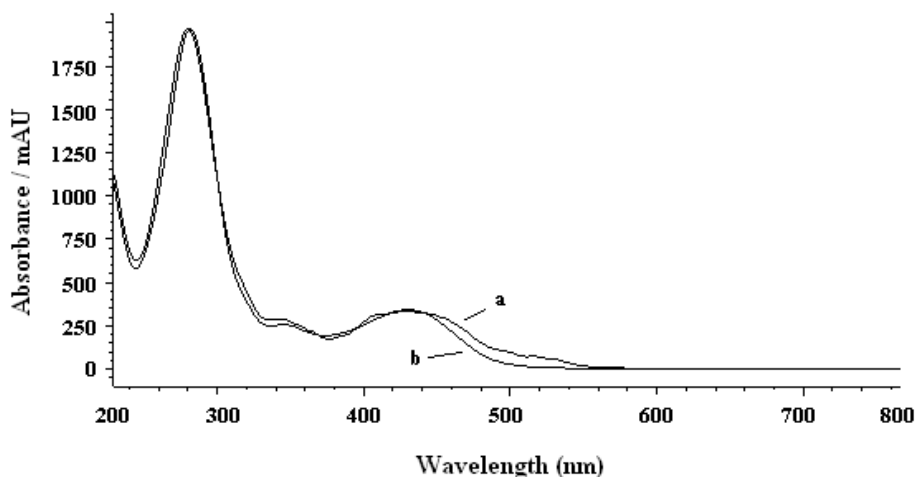


Figure 6: Photodiode array spectra of (b) flavokermesic acid standard compound and (a) peak at 25.9 min from chromatogram in figure 1.

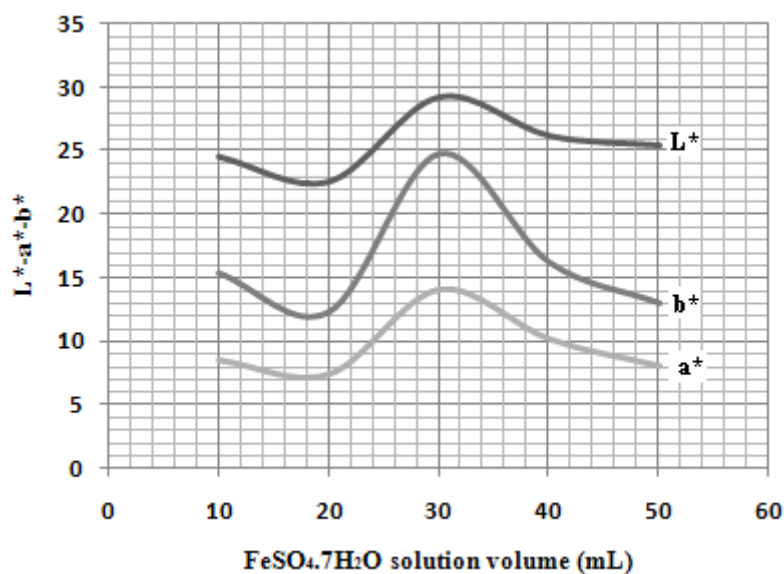


Figure 7: CIELAB graph of iron-ararat kermes lake pigment.

Conclusion

In this study, the reaction of the red anthraquinone dyes present in Ararat kermes (*Porphyrophora hameli* Brand) insect extract with aluminium(III), iron(II), tin(II) and calcium(II) has been used to prepare the lake pigments. Results from the HPLC analysis of the acid hydrolysed Ararat kermes insect extract show that carminic acid and flavokermesic acid are presented in the aluminium-ararat kermes lake pigment, the aluminium-calcium-ararat kermes lake pigment and the non-hydrolysed Ararat kermes insect extract. The effect of different amounts of metals on the colouring scale of the lake pigments were investigated.

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