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Received July 20, 2006
Revised October 16, 2006
Accepted October 16, 2006

Research Article

Simultaneous identification of natural dyes in the collection of drawings and maps from The Royal Chancellery Archives in Granada (Spain) by CE

A simple and rapid capillary electrophoretic method with UV detection (CE–UV) has been developed for the identification of five natural dyes namely, carmine, indigo, saffron, gamboge and *Rubia tinctoria* root. The separation was performed in a fused-silica capillary of 64.5 cm length and 50 μm id. The running buffer was 40 mM sodium tetraborate buffer solution (pH 9.25). The applied potential was 30 kV, the temperature was 25°C and detections were performed at 196, 232, 252, 300 and 356 nm. The injections were under pressure of 50 mbar during 13 s. The method was applied to the identification of carminic acid, gambogic acid, crocetin, indigotin, alizarin and purpurin in the collection of drawings and maps at the Royal Chancellery Archives in Granada (Spain). The method was validated by using HPLC as a reference method.

Keywords:
CE / Dyes / Graphic documents DOI 10.1002/elps.200600446

1 Introduction

The main goal in art conservation and restoration is to present the artwork as the artist intended it to be seen. One of the great challenges in the preservation of old artwork is the investigation of the technique and the material composition used by the artists because this knowledge is essential to use the adequate procedure to restore and preserve the artwork. This is an interdisciplinary task between curators and research scientists.

Color has always been an important element in the cultures of people all over the world. The natural coloring agents are of inorganic (pigments) or organic (dyes) origin. Dyes can be found directly in extracts from natural species (plant, animal) or become colored after various chemical reactions, such as complexation with metals, hydrolysis or oxidation.

The identification of the coloring agents in the paint layers of artworks is a complex task due to the simultaneous presence of dyes, pigments, other minerals, polysaccharides, proteins, oils and/or resins. The rich matrix composition mostly creates the necessity for preliminary separation of the components of dyes prior to their identification. Various analytical techniques have been used for the identification of active coloring agents, *e.g.* spectrophotometry in the UV, Vis and IR regions [1–3], Raman spectrometry [4], fluorescence spectrometry [5], HPLC with UV-Vis diode array detection and fluorescence detection [6–9], HPLC with MS detection [10], CE with UV-Vis diode array detection and electrospray MS detection [11], pyrolysis-GC/MS and abrasive voltammetry [12–14] and voltammetry [15].

CE has proven to be a very powerful tool for the characterization of inorganic and organic compounds since its introduction by Jorgeson and Lukacs in the early 1980s. This is a fast technique allowing the combination of short analysis times and high separation efficiency for the analysis. CE has proved to be an excellent alternative to HPLC. The resolution of CE procedures is greater than that of HPLC and the precision is of the same order. CE is less costly than HPLC and requires only modest quantities of samples. The concentration sensitivity of UV-Vis absorbance detection in CE is, however, relatively poor compared with that in HPLC.

The methods of analysis for the identification of dyes must be highly selective and sensitive because of the complexity of the matrices and the limited quantity of sample available in the case of graphic documents and archive materials. Hereby, CE is the technique selected for developing a new method to identify natural dyes in graphics documents.

The sampling process is really a crucial step here as the reliability of an analytical result is often conditioned by the quality of the sample taken. Further, if the object of the anal-

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ysis is an artefact, there are two important steps in the sampling process in order to respect its physical integrity: (i) the technique for taking samples should be nondestructive and (ii) the amount of sample taken should be minimum. A brush imbibed with the proper solvent leads to amounts of sample taken being a minimum and keeps the physical integrity of an artefact almost in their totality [9]. Sample preparation is then easier, the dye extracted with the brush being adequately dissolved, the solution being centrifuged and injected into the CE.

We wished to identify the natural components of dyes present in historical maps and drawings using a new CE-DAD method. The proposed method has been applied to the historical maps belonging to The Royal Chancellery Archives in Granada and the selected natural dyes are carminic acid, indigotin, crocetin, gambogic acid, alizarin and purpurin.

Carminic acid is a β-*C*-glycopyranosyl anthraquinone derivative which is extracted from a cochineal (*Coccus cacti*), a tropical American insect that feeds on certain species of cactus [16]. Carminic acid is the principal component of this insect, which may represent up to 20% of the total insect female body mass. This is the substance that produces the red color named carmine.

Saffron is the dried stigmas of the flowers of *Crocus sativus* L. The saffron-colored compounds are crocins, a family of unusual water-soluble carotenoids named crocetin [17]. Saffron provides an appreciable yellow coloring.

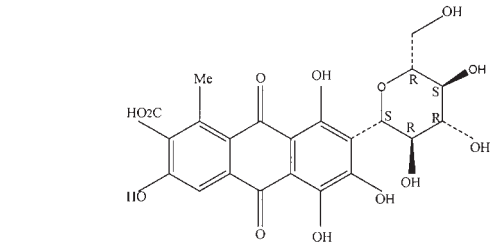
Rubia tinctorum is a plant of the Rubiacea family, which grows in South America and Europe. Madder is the usual name for this dye from the plant. Alizarin and purpurin are coloring agents and are found in the roots of *R. tinctorum*. Both of them are anthraquinones [11].

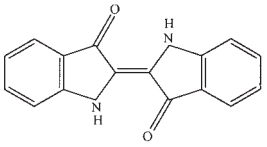
Indigo is obtained from some plants of the Indigofera family. Plants of this family have been found in Africa, Asia, East India, South America, China, Korea and Europe. In this work indigo from *Insatis tinctoria* has been studied. The process for obtaining this dye is very complex. The identification of indigotin (coloring agent of indigo dye) is difficult owing to the low stability and solubility of the compounds [18].

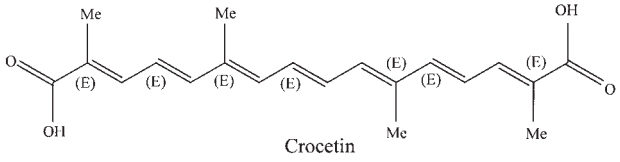
Gamboge is a resin from *Garcinia hamburü,* of the Gutiferae family, grown in Southeast Asia. The plant contains 70–80% of gambigic acid. This substance produces the yellow color [19].

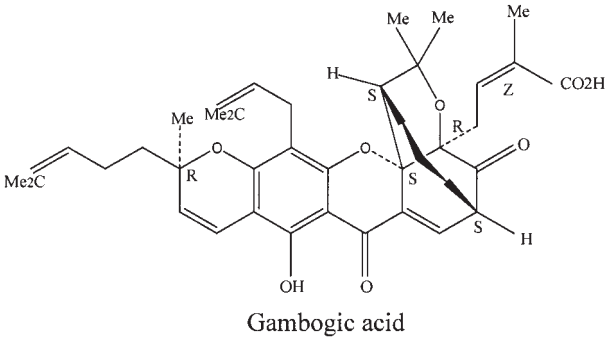
The structures of the coloring agents studied are presented in Fig. 1.

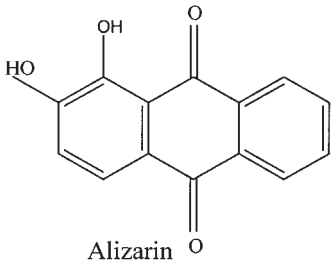
The Royal Chancellery Archives in Granada hold the collections of the institution known as the '*Real Audiencia y Chancillería de Granada*' (1494–1835). The Royal Chancellery was constituted by Isabel I de Castilla and her husband Fernanado II de Aragón, the 'Reyes Catolicos' as the High Court of Justice, some part of high court of appeal, for cases previously heard in the local courts of justice. The collections of the Chancellery are then of paramount value for the history of law and jurisprudence, besides representing a vast











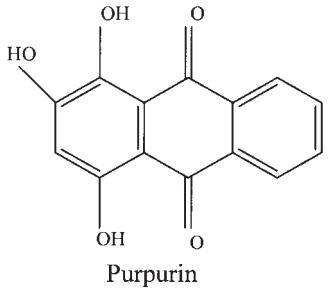


Figure 1. Chemical structures of the natural dyes studied.

source of data and information relevant to historical research on economic, social and religious ideas as well as local history. The maps and drawings which form the existing collection constitute a part of the legal proceedings, as part of the proofs, or testified evidence, the obtaining of which was entrusted to the recipient. Expert draughtsmen were chosen for their skills in their knowledge of a determined subject: masters in the art of architecture or building, alamines, stonemasons, surveyors, masters in the art of painting, along with an extensive network of professional experts. These maps and drawings helped to reach qualified judgements. An elaborate system of numbers and letters was used to codify possessions, property, geographical features, roads, *etc*. with details of the elevation or ground plans of a building. They were incorporated into the lawsuits by virtue of a Royal Recipient Provision at the request of one of the parties for the preparation of the maps. They were then an element of inestimable reference value for attorneys, public prosecutors, and judges to pass sentence. Consequently, their study and analysis is considered essential for their preservation and restoration.

The maps and drawings analyzed come from different places of Andalucia (Spain) from the 16th to the 19th century and can be dated accurately.

2 Materials and methods

2.1 Instrumentation and software

Electrophoresis was performed with an HP^{3D} CE instrument (Agilent Technologies, Waldbronn, Germany) equipped with a DAD, a thermostated column cartridge, a high-voltage built-in power supply and an autosampler. The software package HP ChemStation version A.0901 was used for acquisition and subsequent processing of eletropherograms.

Separations were carried out in fused-silica capillaries (64.5 cm × 50 μm id) with a capillary inlet to detector distance of 56 cm. An Agilent 8453E (Waldbronn, Germany) UV-Vis spectrophotometer was used to record the absorbance spectra of natural dyes.

The chromatographic system consisted of an Agilent 1100 series HPLC (Agilent Technologies, Palo Alto, CA, USA) equipped with a quaternary pump, an online degasser, an autosampler, an automatic injector, a thermostated column compartment and a DAD connected online. The chromatographic system was controlled by ChemStation for LC 3D (Agilent) software package.

Chromatographic separations of studied compounds were achieved on a Luna 5 μm NH_2 100[a] column (250 mm × 4.6 mm id, 5 μm particle) from Phenomenex (Torrance, CA, USA). The column was protected with a NH_2 amine-aminopropyl (Phenomenex) precolumn (4 mm × 3 mm id).

All pH measurements were made with a Crison (Crison Instruments SA, Barcelona, Spain) combined glass-saturated calomel electrode using a Crison 2000 digital pH-meter earlier calibrated.

An ultrasonic bath from Selecta (Barcelona, Spain) was used for dissolved dyes, nylon filters with 0.20 μm pore size (Supelco, Bellefonte, PA, USA).

2.2 Reagents

All reagents were of analytical grade, unless stated otherwise. Water was purified by means of a Milli-Q plus system (Millipore, Bedford, MA, USA).

The natural dyes used as reference substances were obtained from different natural sources: carminic acid from *Coccus cacti* insect, indigotin from *Isatis tinctoria* pressed leaf, crocetin from *Crocus sativus* L. stigmas, gambogic acid from *Garcinia hanburü* resin and alizarin and purpurin from *Rubia tinctorum* root. *C. cacti* insects, *I. tinctoria* pressed leaf, *C. sativus* L. stigmas, *G. hanburü* resin and *R. tinctorum* root were supplied by Kremer-Pigmente (Krakow, Poland). Alizarin from Merck (Darmstadt, Germany) and purpurin from Fluka (Buchs, Switzerland) were used as a standard to obtain the spectra and identify electropherograms of *R. tinctorum* root.

The solvents methanol, ethanol, ACN, 1-propanol, 2-propanol and acetic acid (all were of HPLC-hipergradient grade), hydrochloric acid 37% (ANALPUR grade) and orthophosphoric acid were acquired from Panreac (Barcelona, Spain) and TFA (99%) from Merck.

Sodium tetraborate decahydrate and ammonium acetate were obtained from Sigma Aldrich (St. Louis, MO, USA), sodium dihydrogen phosphate 1-hydrate, ammonium chloride and sodium hydroxide from Panreac and SDS from Fluka.

Buffer solutions of the required pH value used for HPLC reference method were made from 10 mM sodium phosphate (Panreac) solution and 10 mM *o*-phosphoric acid (Panreac) solution.

2.3 Extraction procedure

2.3.1 Reference substance

The reference substances were obtained by extraction from different sources described above using the adequate solvent. Carminic acid, alizarin, purpurin and crocetin were extracted with deionized water at 30°C for 15 min. Then, the colored solution was filtered with filter paper. The extract was evaporated to dryness and dissolved in 0.1 M SDS. Although alizarin and purpurin are extracted from *Rubia tinctoria* root quickly with higher temperature, purpurin could not be extracted, and the colored solution could turn dark. Gambogic acid was extracted with methanol in an ultrasonic bath in 20 min. The extracts were evaporated to dryness and also dissolved in 0.1 M SDS. Indigotin was extracted using acetic

acid 17.5 M in an ultrasonic bath for 45 min (the solution was blue). Then, the extracts, as the other dyes, were filtered, evaporated to dryness and dissolved in 0.1 M SDS. The final solution was purple and the polarity and solubility changed.

2.3.2 Extraction of sample from maps

Extractions of dyes from samples was carried out using the method proposed by Blanc *et al.* [9]. The extraction was performed using a brush imbibed with 0.1 M SDS and applying directly onto the map. The extract transferred on the brush from the paper-contained particles and SDS-soluble compounds coming from the coloring film. The extracts were transferred to 0.1 M SDS and sonicated for 45 min. The solutions were centrifuged at 2000 rpm for 5 min before being injected into the capillary.

The samples were always evaporated to dryness in order to preconcentrate. Then, they were dissolved with a little drop of 0.1 M SDS solution.

2.4 Electrophoretic procedure

CE separation was carried out on a fused-silica capillary (50 μm id, total length 64.5 cm, a detection window was created at 56 cm from the capillary inlet). When a new capillary was used, it was preconditioned by rinsing with 1 M NaOH for 20 min at 60°C, followed by a 5-min rinse with deionized water and 20 min with buffer. For the following analyses the capillary was rinsed with 0.1 M NaOH for 5 min at 25°C, followed by a 3-min flush with deionized water to ensure good repeatability. The capillary was equilibrated with the running buffer (40 mM tetraborate of pH 9.25) for 15 min before each sample injection.

Samples were injected hydrodynamically in the anodic end of the capillary with a pressure mode (50 mbar) for 13 s. Electrophoretic separation was performed at 30 kV for 15 min, resulting in a current of about 60 μA. The temperature of the capillary was constant at 25°C. After each analysis, the capillary was rinsed for 3 min with deionized water and dried with air for 2 min.

All solutions, buffers and samples were filtered through a 0.20 μm membrane filter. The running buffer was changed after three runs. UV detection was carried out monitoring at 232, 252, 300, 356 and 196 nm. The wavelengths were selected from the spectrum of each dye. DAD was used over the range of 190–600 nm to achieve spectral data.

3 Results and discussion

To determine the optimum conditions for CE method, experimental and instrumental variables were investigated.

3.1 Effect of experimental variables in the CE method

In the first step, several electrophoretic media were investigated varying the buffer running at different pH in the range of 3–11 in steps of 1.5 U. The alkaline buffer was found to be the unique medium effective for resolving a mixture of the studied dyes. Each dye was examined individually by CE and with the spectrum obtained of each electrophoretic peak.

The effect of pH was tested by adjusting the buffer (40 mM tetraborate) pH between 8 and 10 (in steps of 0.4) by adding a proper amount of 1.0 M HCl or 1.0 M NaOH. We observed (Fig. 2) that at pH values lower than 9.2, the analysis times were shorter, but the resolution for the compounds studied was worse. At pH values higher than 9.2, the analysis time was longer. At pH value 9.2, the resolution for the peaks was good and the analysis time was shorter. Therefore, we selected a pH value 9.2 as the optimum pH value.

Three different buffers were tested: sodium tetraborate decahydrate, ammonium chloride and ammonium acetate. All of them were prepared at pH 9.2. With ammonium chloride and ammonium acetate the current was extremely high. Sodium tetraborate was therefore selected as the buffer because it gave the best resolution in a satisfactory time.

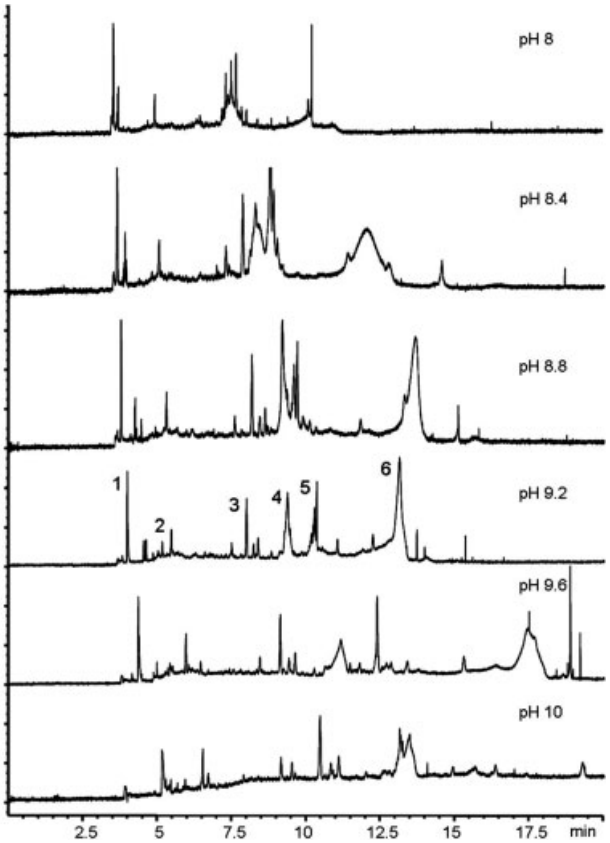


Figure 2. Electropherograms of a mixture of natural dyes studied at different pH values. Peak 1: alizarin; peak 2: purpurin; peak 3: indigotin; peak 4: carminic acid; peak 5: crocetin; peak 6: gambogic acid.

The buffer concentration was investigated in the range between 20 and 80 mM (in steps of 20). The results of this study are shown in Fig. 3. When the buffer concentration was increased, the analysis times became longer. We selected a 40 mM concentration of the tetraborate buffer as the best compromise solution between the resolution of the mixture of the dyes studied and a reasonable analysis time.

Different organic solvents (methanol, ethanol, ACN, 1-propanol and 2-propanol) were tested as organic modifiers to the buffer at 5 vol%. In all the cases, the electrophoretic peak of gambogic acid (peak 6) disappears or shows an alteration in the migration time. For this reason, the use of organic modifier was not considered (Fig. 4).

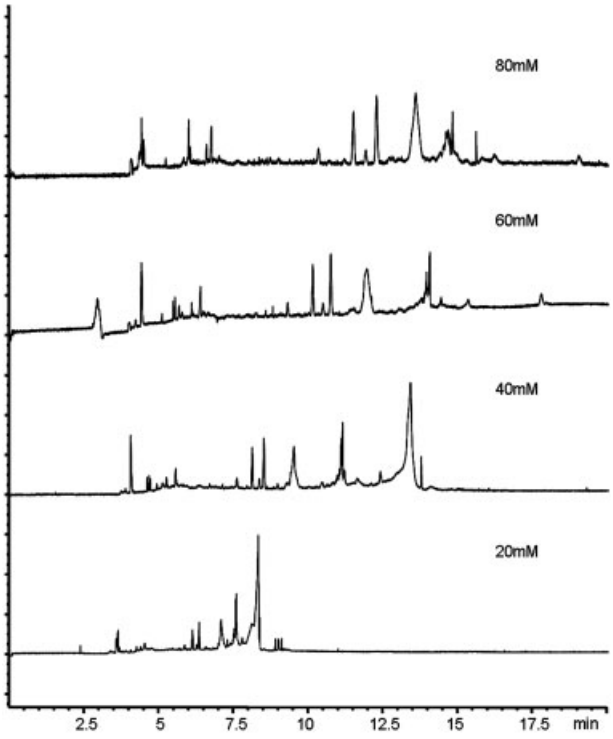


Figure 3. Optimization of the separation of a mixture of natural dyes studied using different concentrations of sodium tetraborate buffer. For identification of the peaks see Fig. 2.

3.2 Effect of instrumental variables in the CE method

The temperature was investigated in the range of 20–30°C. When the temperature was increased, the analysis time was shorter, but the resolution for the compounds studied was worse. The experimental work was carried out at the room temperature of 25°C.

The applied voltage was studied in the range of 20–30 kV. The voltage used to obtain a shorter analysis time and maintain a good resolution was 30 kV (Fig. 5).

The sample was injected into a 0.1 M SDS solution. In order to improve the detection, the injection was examined using a 50 mbar hydrodynamic injection and the injection

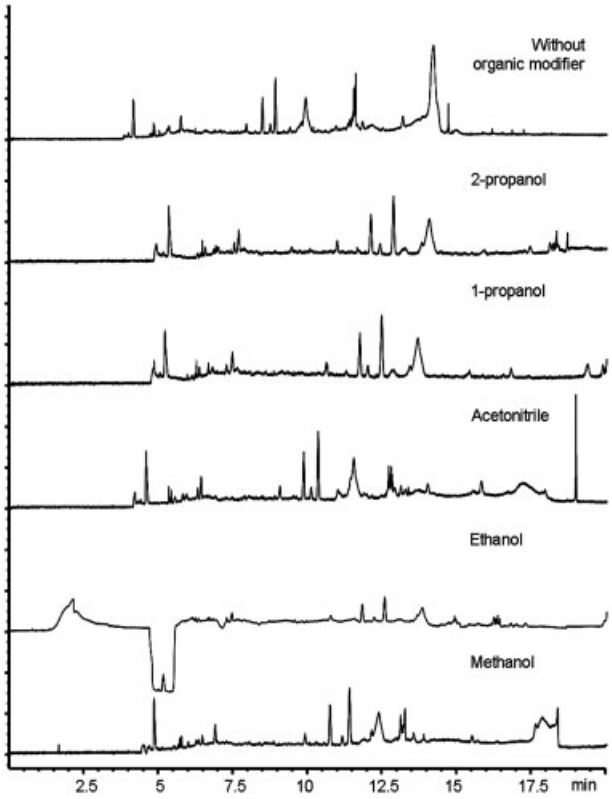


Figure 4. Effect of the use of different organic modifiers in the separation of a mixture of natural dyes studied. For identification of the peaks see Fig. 2.

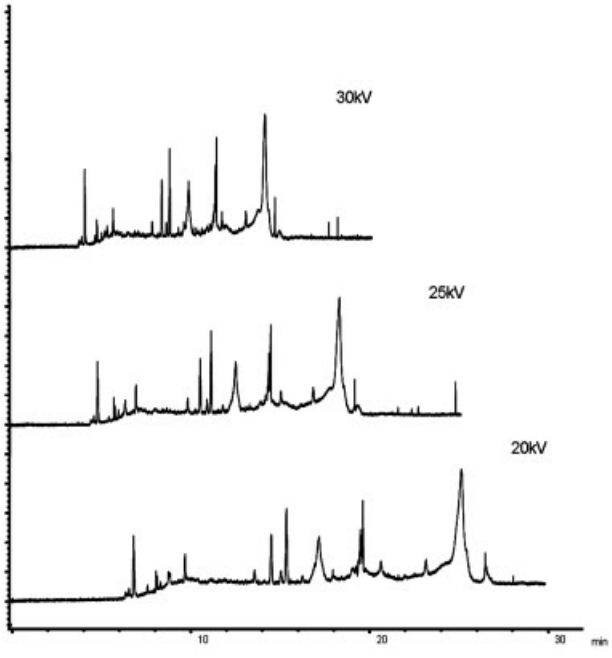


Figure 5. Effect of the applied voltage in the separation of a mixture of natural dyes studied. For identification of the peaks see Fig. 2.

time was varied between 5 and 15 s. When the injection time was increased, the peak height increased, but the peak shape was not adequate. Therefore, the injection time of 13 s is a compromise between the peak height and the peak shape.

3.3 Application and validation of the method

The reported investigations were carried out in two stages. Firstly, electrophoretic measurements were carried out for natural dyes collected from various sources and the electrophoretic procedure was applied. Then CE data were recorded for extracts of dyes from historical maps. Identification of dyes was based on migration time and their comparison with reference substances as well as on UV-Vis spectra recorded for sample extracts and reference substances. Identity of UV-Vis spectra was confirmed when agreement between their maxima was obtained within ±1 nm.

3.3.1 EC of natural dyes

Electrophoretic identification of natural dyes was carried out with the use of these substances purchased from different natural sources previously indicated. Electropherograms obtained for solutions of natural dyes are shown in Fig. 6 together with the UV-Vis spectra corresponding to identified chemical species.

The UV-Vis spectra were obtained at electropherogram peaks and are similar to those spectra obtained for reference substances. The complete list of examined natural dyes together with their migration times in the electrophoretic conditions used and spectrophotometric data are shown in Table 1.

The optimum electropherogram obtained for a mixture of the dyes studied under optimized conditions is presented in Fig. 7. It shows that the electrophoretic procedure selected is adequate for the separation of the analytes involved. The time for one analysis was approximately 14 min. This method is quicker than the HPLC method, which for the same mixture standard analysis runs at around 45 min [9].

Table 1. Electropherographic migration time and absorption maxima for the studied dyes

Dye	Migration time (min)	Peak no.	Absorption (nm)	Natural source
Alizarin	4.0	1	201, 252, 280, 430	*R. tinctorum*
Purpurin	5.4	2	205, 257, 489, 521	*R. tinctorum*
Indigotin	8.0	3	227, 294, 608	*I. tinctoria*
Carminic acid	9.4	4	206, 234, 284, 334, 521, 563	*C. cacti*
Crocetin	10.3	5	259, 431	*C. sativus* L.
Gambogic acid	13.1	6	205, 236, 282, 290, 364	*Garcinia hanbüru*

3.3.2 CE of extracts from maps

Samples taken from the collection of drawings and maps from the *Royal Chancellery Archives in* Granada dated from the 16th to the 19th century have been examined using the proposed analytical procedure.

The CE method was validated by comparison with HPLC as reference method [9]. Analyses were carried out on a Luna NH_2 100[a] column (250 mm × 4.60 mm id, 5 μm particle) from Phenomenex. The mobile phase consisted of 40 mM SDS, 10 mM phosphate buffer solution (pH 2.3), 0.1% TFA (eluent A) and ACN (eluent B) using a programmed gradient (5–95% B). The column temperature was set at 35°C and the injection volume was 20 μL.

The results obtained, summarized in Figs. 8 and 9 and Table 2, showed that both methods are optimal for analysis, separation and identification of dyes present in this study.

Table 3 shows the obtained results in the study of the collection of drawings and maps in the *Royal Chancellery Archives in* Granada (Spain).

Table 2. Electrophoretic migration time and chromatographic[1] retention time for the studied colors

Map No.	Color sample	Identified dye	Migration time (min)	Retention time (min)
13	Yellow	Crocetin	11.2	33.8
		Gambogid acid	14.6	42.2
19	Red	Carminic acid	9.6	13.7
42	Yellow	Gambogic acid	14.3	42.9

Table 3 Identification of the dyes in the maps.

Map No.	Color sample	Identified dye
2	Brown	Gamboge
	Red	Carmine
11	Green	Saffron + gamboge
	Brown	Saffron + gamboge
13	Yellow	Gamboge
	Green	Gamboge
	Brown	Gamboge
37	Red	Carmine
41	Red	Carmine + gamboge
	Yellow	Gamboge
69	Green	Indigo
74	Green	Gamboge
83	Blue	Indigo
87	Blue	Indigo
	Brown	Indigo
94	Yellow	Gamboge
	Red	Carmine
	Green	Gamboge
121	Red	Carmine
136	Green	Indigo

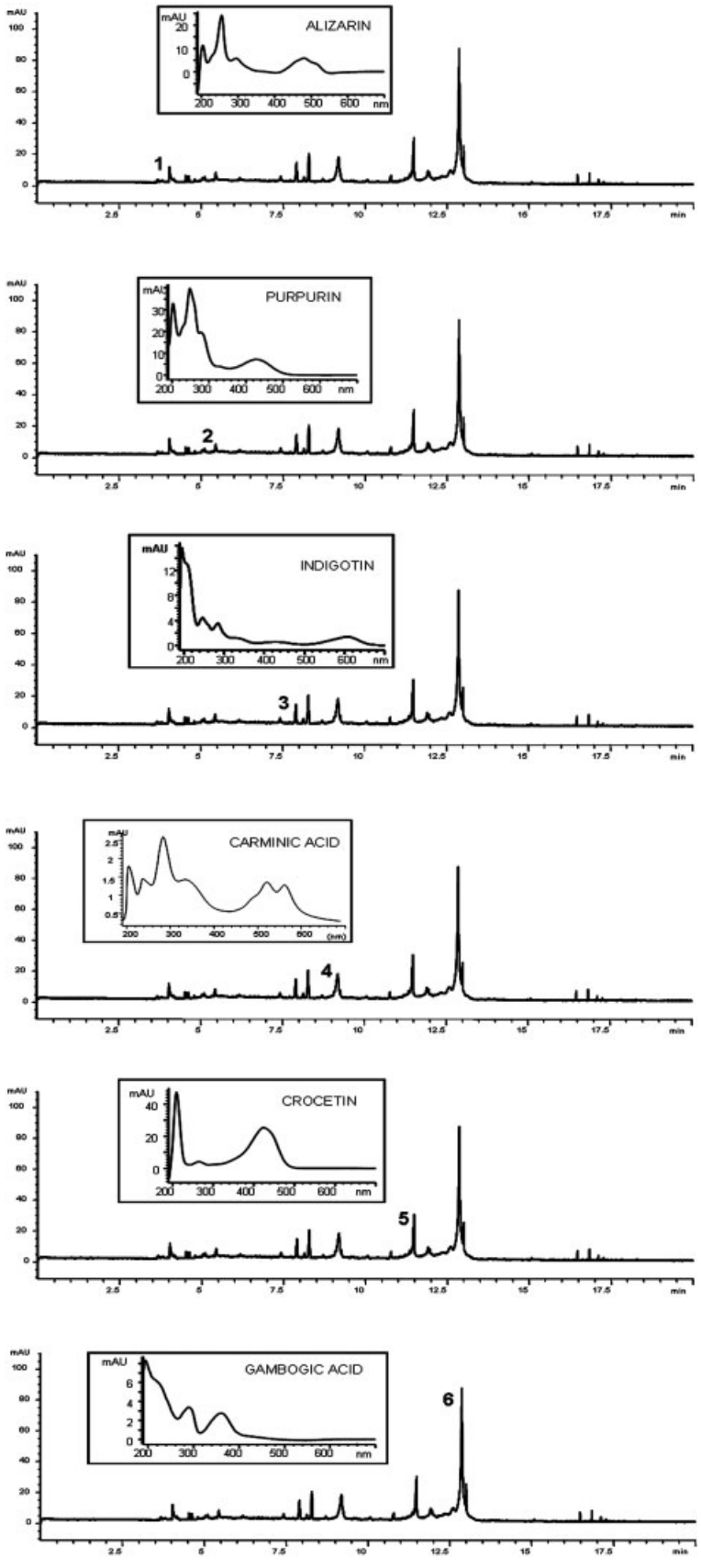


Figure 6. Electropherograms obtained for solutions of natural dyes extracted from natural sources.

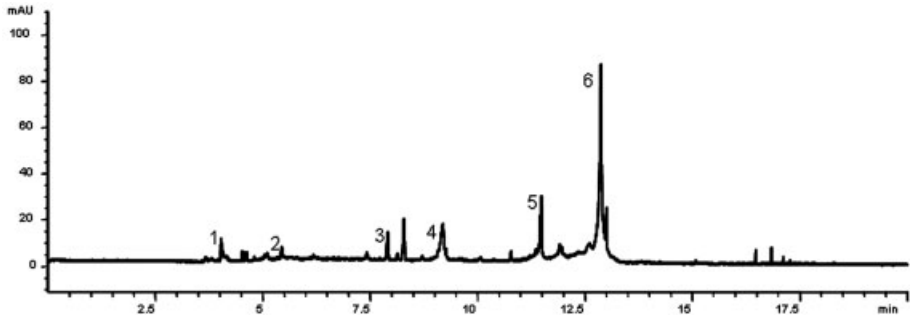


Figure 7. Electropherogram of a mixture of the different natural dyes studied obtained under the optimized conditions. Peak 1: alizarin; peak 2: purpurin; peak 3: indigotin; peak 4: carminic acid; peak 5: crocetin; peak 6: gambogic acid.

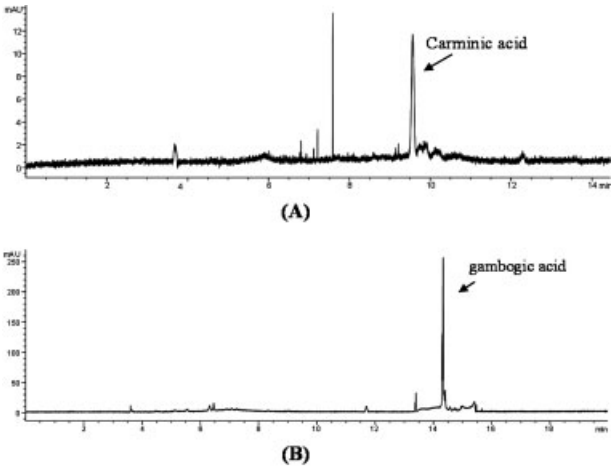


Figure 8. Electropherograms of map 19, sample red (A) and map 42, sample yellow (B).

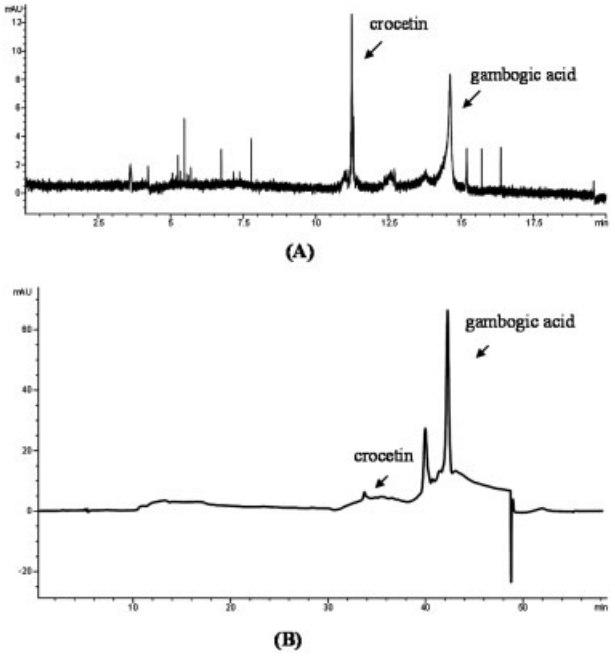


Figure 9. Electropherograms and chromatograms of map 13, sample yellow. (A) CE, (B) HPLC.

4 Concluding remarks

CE with diode-array UV-Vis spectrophotometric detection has been proved to be an optimal method of analysis for separation and identification of dyes present in this study. The results presented in this work demonstrate that CE is a very fast and easy alternative to identification of colorants by HPLC-DAD. Even the sample needed for the analysis by CE is minor. The methodology proposed does not affect negatively to the artefact. The main advantages of the method are that only small amounts of sample are required, sample treatment is simple and analysis times are short.

The identification of natural dyes of the Real Chancillería de Granada (Spain) has been successful. Identification of dye mixtures is possible and effective with only one sample, therefore sampling is considerably reduced.

The characterization of these materials has a double use. On one side, it allows us to put them in their historical context, and on the other it can be used to gain a deep knowledge that could be useful for a possible restoration or conservation process. This analytical methodology is likewise apt for the study of a wide variety of other dyes and artefacts.

This work was partly supported by the Spanish Ministry of Science and Technology (project no. MAT2002-01903). The authors are grateful to David Torres, Director of Archivo de la Real Chancillería de Granada, for kindly providing the real samples, and the authors also thank F. Lara-Vargas, Ph.D. student from University of Granada, for his help.

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