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ORIGINAL PAPER

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Electrochemical analysis of natural solid organic dyes and pigments

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Abstract Square-wave voltammetry of solid naphthoquinone, anthraquinone, and flavone dyes, carmine, cochineal red, indigo, and Prussian blue, was compared to microanalysis (sample consumption < 1 mg) of traditional painting pigments and dyes without their preliminary dissolution. Electrochemical analysis was also performed after the samples' hydrolysis simultaneously with thin-layer chromatography. Anthraquinone-based pigments and Prussian blue are reversibly reduced, cochineal red and lac dyes are irreversibly reduced, flavones are mostly reversibly oxidized, dragon's blood is irreversibly oxidized and reduced, and indigo yields both reversible oxidation and reduction. The potential window of these reactions is about 1.4 V wide. This variability permits identification of the kind of pigment or dye, and directly distinguishes, for example, alizarin and purpurin; luteolin and quercetin; or indigo and Prussian blue.

Keywords Voltammetry · Microparticles · Identification · Natural dyes

Introduction

Identification of the colouring agents in the paint layers of art works is a complex task due to the simultaneous presence of dyes, pigments, other minerals, polysaccharides, proteins, oils, and/or resins. Pigment identification in paint layers has been relatively well developed for inorganic substances: in restoration practice it is mainly done by optical microscopy in normal and UV light, and by elemental analysis (EDX or XRF). In research laboratories, these methods are accompanied by a wide variety of spectroscopic [1, 2, 3] and separation methods [3, 4], voltammetry [2, 3, 5], and other techniques. However, the complexity of organic binders combined with the small amount of samples available for analysis limits the applicability of the spectroscopic and separation methods.

The scale of organic dyes and pigments commonly used in traditional painting was limited to selected natural materials [6] available before the boom of the modern chemical industry in the 19th century. In traditional fine art, namely in easel painting, polychrome art, and manuscript illumination, derivatives of 9,10-anthraquinone (alizarin, carmine, laccaic acids, and related red dyes), 1,2-naphthoquinone (lawson), flavonols (quercetin, luteolin, and further yellow dyes), and heterocyclic aromatic compounds (indigo) were used. Those compounds are theoretically very suitable for electrochemical detection: the electrochemistry of alizarin [7, 8], natural anthraquinone compounds [9], flavones [10, 11], indigo [12, 13], and many other organic dyes has already been studied. However, the voltammetry of microparticles has only recently been proposed for identification of the anthraquinone and naphthoquinone dyes and pigments used in fine art [5]. As for the sample consumption, the voltammetry of microparticles has already been shown to be suitable for distinguishing some mineral pigments [2, 3]. The voltammetric microanalysis of organic dyes is hence a challenging task. The aim of this report is the study of selected organic dyes and pigments, the general characterization of their voltammetric behaviour, extending the study by Doménech-Carbó et al. [5], and testing the sample handling methods required in analysis of organic lakes (composites of organic dyes in/on an inorganic matrix). Two lakes were

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prepared by traditional procedures and compared to the commercially available specimens: red madder lake and yellow reseda lake. The lakes were hydrolysed and characterized by thin layer chromatography.

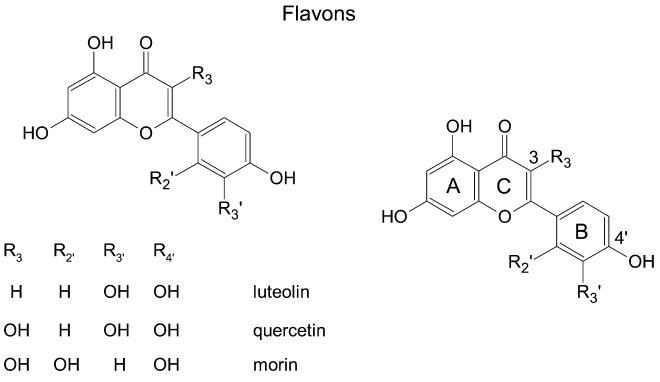
Experimental

The structures of the dyes analysed are shown in Figs. 1, 2, 3. Two kinds of dyes (pure, soluble organic compounds) and pigments (insoluble powders) were used. Chemically pure dyes, obtained from Riedel-de Haën, Sigma-Aldrich, and Merck, are denoted by their systematic chemical names. Natural dyes supplied by a producer of traditional materials for artists and restorers (Kremer, Aichstetten/Allgäu, Germany) are denoted by their traditional names and the provider's catalogue number. The following dyes and pigments supplied by Kremer were used: lac dye (36020, from *Coccus lacca*, contains a mixture of laccaic acids); cochineal red (36040, from *Coccus cacti*); reseda lemon yellow lake (36260, *Reseda luteola*, a flavonol dye); dragon's blood (37000, a resinous product from *Calamus draco*); madder lake (37200B, from *Rubia* sp., an alizarin-like dye); buckthorn berries lake – Still de Grain (37390A, from *Rhamus* sp., a flavonol dye); and cochineal carmine (42100, from *Coccus cacti*, contains carminic and kermesic acids). The term *lake* stands for a pigment made from an organic dye on/in an inorganic substrate (commonly hydrated aluminium oxides or $CaCO_3$). Prussian blue was obtained separately (Paris blue 1225, Deffner and Johann, Röthlein, Germany).

Preparation of lakes

Alizarin and purpurin lakes were prepared by adding an aqueous ammonia solution of synthetic 1,2-dihydroxy-9,10-anthraquinone (Riedel-de Haen) or 1,2,4-trihydroxy-9,10-anthraquinone (Sigma) to a warm aqueous solution of $KAl(SO_4)_2.12H_2O$, boiling the mixture until a precipitate was formed, and filtering off the precipitate from the hot mixture. The lake was dried in air at 50 °C.

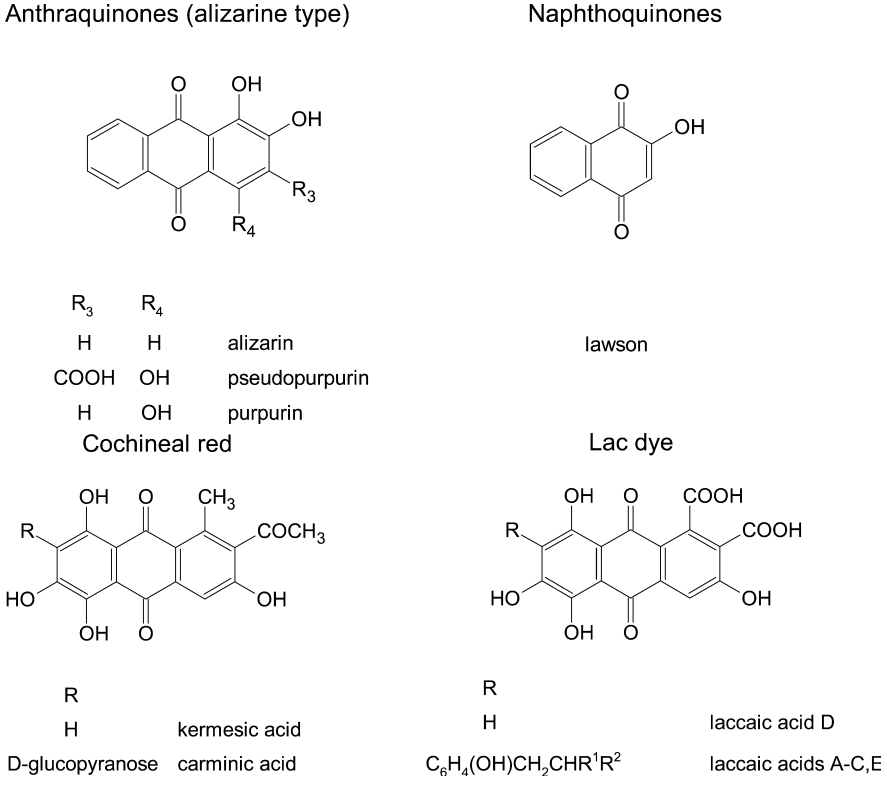
Madder lake was prepared as published [14]: 10 g of madder (Kremer 37200A, dry root of *Rubia* sp.) was left to stand in solution of 10 g sodium sulfate in 120 mL of water for 12 h at room temperature. The residue was pressed on a filter cloth and washed with cold water until no sulfate ions were present in the water, to remove water-soluble impurities. A boiling solution of 10 g of the alum $KAl(SO_4)_2.12H_2O$ in 120 mL of water was added to the solid residue and allowed to stand for 20 min to form a soluble Al^{3+}-dye complex. The solution was filtered and the residue washed with hot water. To the combined filtrates, 10 g of lead acetate was added at 50 °C. The deep-red liquor was then removed from the $PbSO_4$ precipitate and heated to boiling, until the red flakes of the red lake coagulated. Boiling of the solution at this stage must be carefully



R_3	$R_{2'}$	$R_{3'}$	$R_{4'}$	
H	H	OH	OH	luteolin
OH	H	OH	OH	quercetin
OH	OH	H	OH	morin

Fig. 2 Yellow dyes derived from flavone and the numbering of the positions and notation of the rings. The OH groups are responsible for the electrochemical oxidation

Fig. 1 Red dyes derived from 9,10-anthraquinone and 1,4-naphthoquinone. The quinone moiety is responsible for the reversible reduction, the OH groups for oxidation



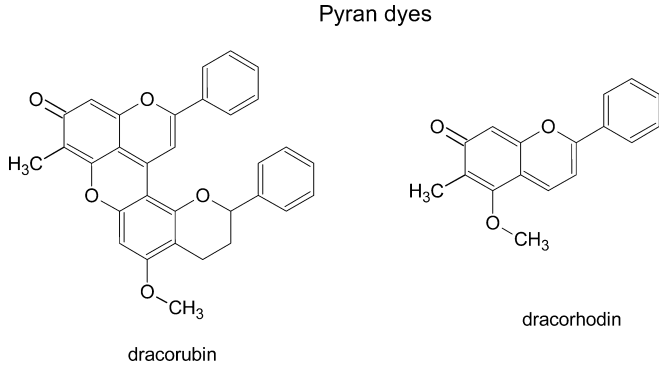


Fig. 3 Some pyran-related dyes from dragon's blood. Dragon's blood is electrochemically irreversibly oxidized

avoided. The hot suspension was centrifuged (8000 rpm for 3 min), because at lower temperatures the lake redissolves. The lake was dried at room temperature in air.

Reseda lake was prepared as published [14] from the dry tops of *Reseda luteola* (Kremer 36250). A sufficient quantity of water was added to cover 40 g of the tops. The mixture was heated and boiled until approximately half of the water had evaporated. Then the mixture was pressed on filter cloth to remove the solid material and the clear solution was heated to boiling point. Then 4 g of $KAl(SO_4)_2.12H_2O$ and 3 g of $CaCO_3$ was added. The mixture was left to cool to room temperature; the lake was then filtered off and dried at room temperature.

Hydrolysis of lakes and their thin layer chromatography [14, 15]

Hydrolysis of madder and reseda lakes was done according to the procedure used for TLC analysis of paint layers to liberate the organic dye from its complexes with Al and from the organic binders. To 2–8 mg of the lakes, 9–10 drops of concentrated sulfuric acid were added. The larger particles were crushed with a glass rod. Then 3–4 mg of boric acid was added. Each solution was diluted with 5 mL of water, allowed to cool, and extracted with 5 mL of 3-methylbutan-1-ol (isoamyl alcohol). The organic phase containing the dyestuffs was repeatedly washed with 1 mL portions of water to remove the acid and the organic phase was then evaporated to dryness. The residues were dissolved in 200 μL of acetone and 2–10 μL of the solutions were used for TLC. For the separation of hydrolysed madder lakes, polyamide K541V plates (Eastman) and toluene/acetic acid (9:1) were used [14]. For reseda lakes, silica gel K301V plates (Eastman) and acetic acid/methanol/water (3:3:4) or cellulose DC on Al foils (Merck) and ethyl acetate/tetrahydrofuran/water (6:35:47) were used [15]. The detection was performed by NH_3 vapour or by UV irradiation; the reference substances were alizarin (Riedel-de Haën) and purpurin (Sigma) for the madder lakes, and luteolin, genistein, and quercetin (Sigma) for the reseda lakes.

Voltammetry of microparticles

In direct electrochemical analysis, solid samples were deposited mechanically by pressing a paraffin-impregnated graphite electrode (PIGE) against about ~0.1 mg of powder on a porcelain plate and removing the excess of powder from the surface by soft tissue. The soluble target compounds (the acetone solutions obtained after hydrolysis of the lakes) were deposited by dropping about 10 μL of the solution on the surface of the PIGE and leaving the solvent to evaporate. Square-wave voltammetric (SWV) measurement [16] was done with a μAutolab (EcoChemie, The Netherlands) with 40 mV square pulses at 10 Hz frequency on a ramp with a 1 mV step potential. A saturated calomel reference (SCE) and Pt-plate auxiliary electrodes and a N_2-deaerated sodium acetate/acetic acid supporting electrolyte (1:1, total acetates 0.2 M) were used. SWV with a similar potential programme and acetate buffer were found by Doménech-Carbó et al. [5] to be most suitable for voltammogram clarity, peak resolution, and reproducibility of the peak potentials. Potential scanning was started at open-circuit potential (about 0.1 V vs. SCE), unless otherwise specified.

Results and discussion

TLC analysis of lakes

For the hydrolysis of lakes, an extraction procedure with cold H_2SO_4, originally proposed for alizarin and carmine reds [14], was used. We considered the technique less drastic than refluxing in $HCl/H_2O/MeOH$ used in the analysis of dyed textiles [1, 4], but further testing of the stability of the dyes in concentrated solution should be performed before using the technique for dyes other than relatively stable anthraquinone derivatives. For example, visual examination showed that dragon's blood is destroyed on contact with the acid, yielding pale yellow products. On the other hand, the principal flavone dye from reseda lakes was not harmed by the hydrolysis procedure.

Alizarin and purpurin were identified by TLC as the main components in both madder lakes. Admixtures of munjistin, pseudopurpurin, and xanthopurpurin were identified in madder lake obtained from *Rubia* roots 37200A by comparison of their experimental R_f values and by fluorescence with the published characteristics [14]. Besides the difference in the R_f values, alizarin and purpurin differ by their appearance in UV light: purpurin has a vivid salmon fluorescence. In the lake 37200B supplied by Kremer, only alizarin and purpurin were identified, with purpurin being present in an amount comparable to alizarin.

Luteolin was identified as the main colouring agent in the lake obtained from *Reseda* tops 36250, as well as in reseda lake 36260 supplied by Kremer.

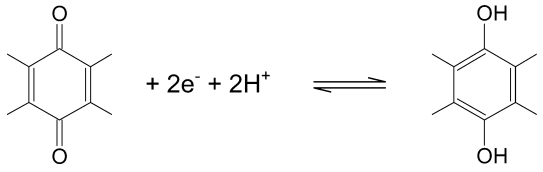
Voltammetry

Compounds yielding cathodic peaks

Anthraquinone and naphthoquinone dyes (Fig. 1) yielded well-developed reversible reduction peaks in linear-sweep voltammetry. The peaks appear in several subsequent scans, indicating a high chemical reversibility of the process and a low solubility of the compounds in the buffer used. This feature permits starting the scanning either at open-circuit potential or at about −1 V vs. SCE.

SWV yielded sharper voltammetric peaks than the linear-sweep mode, with easier reading of the peak potentials. Selected SWV cathodic peak potentials are listed in Table 1; examples are shown in Fig. 4. The

electrochemically active moiety of anthraquinone dyes is the quinone group, reducible to a diphenol. The reaction is known to proceed in a single step in aqueous medium:



The peak potential of polyhydroxyanthraquinones depends linearly on the number of OH groups (N_{OH}) in the anthraquinone skeleton:

$$E_p = -0.40 - 0.053 \times N_{OH}, \quad r^2 = 0.9594 \quad (n = 7,\ \text{SWV cathodic peaks, 2nd scan}) \qquad (1)$$

The equation was valid for the following derivatives: 1-hydroxy-, 2-hydroxy-, 1,2-dihydroxy- (alizarin), 1,4-dihydroxy-, 1,8-dihydroxy-, 1,2,4-trihydroxy- (purpurin), and 1,2,5,8-tetrahydroxy-9,10-anthraquionone (major peak). Surprisingly, the influence position of the OH groups with respect to the electroactive anthraquinone moiety was not observed, probably due to the fact that no theoretically existing isomers were available for the comparison. The dependence clearly indicates a sensitivity of the actual position of E_P to the skeleton substitution that is important, e.g. to distinguish purpurin and alizarin. A decrease of E_P with additional OH groups was also reported by Pournaghi-Azar et al. [9]. The experimental values of the peak potentials for the anthraquinones agree very satisfactorily with the results obtained by Doménech-Carbó et al. [5] under similar conditions: the E_P of alizarin, purpurin, and cochineal red peaks were −0.53, −0.59, and −0.64 V vs. Ag/AgCl (3 M KCl), respectively.

The comparison of the peak potentials of madder lake 37200B and the lake prepared from *Rubia* sp. (Kremer 37520A) indicates that the former pigment contains much more pseudopurpurin than alizarin. Recently also Miliani et al. [17] observed excess of purpurin over alizarin in a madder lake supplied as an artist's pigment. That contradicts the typical composition of traditional lakes from the most common *Rubia* species, *R. tinctorum*; however, the composition of madder lakes depends on the aging (fermentation) of raw *Rubia* and the actual technology of the dye extraction and lake precipitation [14].

The peak potentials of carminic acid, cochineal red, and lac dye occur at about −0.6 V vs. SCE, in accordance with the higher number of OH groups in their anthraquinone skeleton. Contrary to the alizarine-like

Table 1 SWV peak potentials of dyes and pigments yielding well-defined cathodic peaks (V vs. SCE). The empirical dye names of synthetic substances are given in parentheses

Dye name	Chemical name	1st scan	2nd scan
	1-Hydroxy-9,10-anthraquionone	−0.46	−0.45
	2-Hydroxy-9,10-anthraquionone	−0.60 (double peak)	−0.45
Alizarin	1,2-Dihydroxy-9,10-anthraquinone	−0.53	−0.51
	1,4-Dihydroxy-9,10-anthraquinone	−0.53	−0.52
	1,8-Dihydroxy-9,10-anthraquinone	−0.52	−0.50
Purpurin	1,2,4-Trihydroxy-9,10-anthraquinone	−0.59	−0.58
	1,2,5,8-Tetrahydroxy-9,10-anthraquinone	−0.61, −0.78 (shoulder)	−0.60, −0.77 (shoulder)
Rhein	1,8-Dihydroxy-3-carboxy-9,10-anthraquinone	−0.43, −0.70	−0.43
	Carminic acid	−0.60	−0.61
Lawson	2-Hydroxy-1,4-naphthoquionone	−0.22, ca. −0.3 (shoulder)	−0.21, −0.51 (shoulder)
Alizarin lake		−0.50	−0.50
Purpurin lake		−0.64	−0.64
Madder lake from *Rubia*37200A		−0.52	−0.51
Madder lake from *Rubia*37200A, hydrolyzed		−0.53 (double peak)	
Madder lake, Kremer 37200B		−0.59	−0.57
Madder lake, Kremer 37200B, hydrolyzed		−0.57	
Lac dye 36020		−0.59	-0.59
Cochineal red 36040		−0.61	−0.61
Dragon's blood 37000		−0.56 (double peak)	−0.56 (double peak)
Cochineal carmine 42100		−0.61, −0.76	−0.60

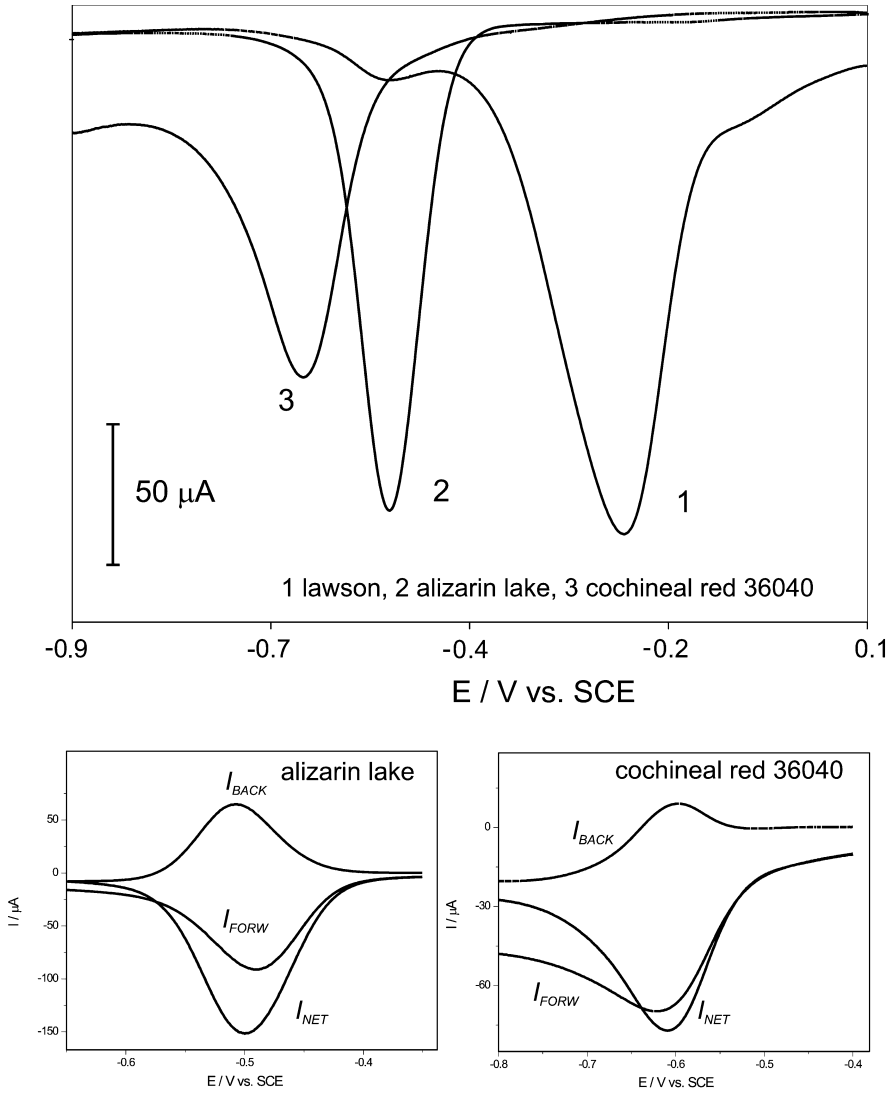


Fig. 4 Cathodic SWV curves for three quinone dyes and pigments: lawson (*1*, a quasi-reversible process), alizarin lake (*2*, a reversible process), and cochineal red (*3*, a quasi-reversible process). Scans from open-circuit potential toward negative potentials. *Insets*: the net, forward, and backward current components are shown for alizarin lake and cochineal red

dyes with highly electrochemically reversible reduction, dragon's blood, cochineal red, and lac dyes yield electrochemically irreversible peaks that could be used to differentiate between those groups of dyes in spite of the similarity of their peak potentials. Electrochemical reversibility here means the negative forward and positive backward currents in the vicinity of E_P [18] (see inset in Fig. 4, alizarin lake).

Compounds yielding oxidation peaks

Some of the dyes and lakes studied yielded anodic peaks (Table 2, Figs. 5 and 6). Electrochemically and chemically reversible anodic peaks were obtained at 0.3–0.4 V vs. SCE with all three available synthetic flavones and also with the reseda lakes, in which the main colouring agent is luteolin according to TLC. Flavonols (3-hydroxyflavones) are known to be oxidized at relatively low potentials (about 0.3 V vs. SCE) [10, 11].

Morin and quercetin, with five hydroxyl groups, have more negative E_P values than luteolin with four hydroxyl groups. According to an empirical rule, the larger the number of OH groups, the lower the oxidation peak potential of a polyphenolic compound. At more positive potentials (about 0.7 V vs. SCE at pH 7.5) the flavone skeleton can be irreversibly reformed at the C3 carbon of ring C [10, 11]. This process can possibly be responsible for the irreversible SWV peaks (shoulder) of morin at about 0.5 V vs. SCE. Obviously very positive potentials could destroy the flavone skeleton, and so the anodic pre-electrolysis of flavones should be avoided.

At about 0.5 V vs. SCE some anthraquinones are also oxidized, namely those having OH groups in the 1,2-positions (alizarin [7, 8] and purpurin), while neither 1,4- nor 1,8-dihydroxy-9,10-anthraquinone is electrochemically active in this region. The Al^{3+}-alizarin complex in both alizarin and madder lakes yields a double peak or an electrochemically reversible peak at ~0.5 V followed by an irreversible peak or shoulder at ~0.7 V. This phenomenon can be related to the different

Table 2 SWV peak potentials for dyes yielding well-defined anodic peaks (V vs. SCE, 1st scan)

Dye name	Chemical name	Reversible	Irreversible
Quercetin	3,3',4',5,7-Pentahydroxyflavone	0.28	
Morin	2',3,4',5,7-Pentahydroxyflavone	0.31	–(shoulder)
Luteolin	3',4',5,5',7-Pentahydroxyflavone	0.37	
Reseda lake from 36250		0.37	0.53 (shoulder)
Reseda lake 36260		0.38	0.54
	Carminic acid	0.41	
Lac dye 36020		0.42	0.67
Cochineal red 36040		0.40	0.68 (shoulder)
Dragon's blood 37000			0.60
Cochineal carmine 42100			~0.6 (shoulder)
Alizarin	1,2-Dihydroxy-9,10-anthraquinone	0.53	
Purpurin	1,2,4-Trihydroxy-9,10-anthraquinone	0.49	
Alizarin lake		0.51	0.67
Madder lake from *Rubia*37200A		0.51	0.69
Madder lake from *Rubia*37200A, hydrolyzed		0.49	
Madder lake 37200B		0.50	0.69 (shoulder)
Madder lake 37200B, hydrolyzed		0.48	

reactivities of the free dye and the Al^{3+}-complexed dye [7]. A similar phenomenon was observed in reseda lakes (double anodic peak) and unsupported luteolin (single peak), indicating that interaction of dyes with the substrates is not merely physical sorption.

At about 0.7 V also, further red dyes are oxidized, but the processes are both chemically and electrochemically irreversible and lead to a complete destruction of the dye, indicated by the absence of SWV peaks in subsequent scans in the case of lac dye and dragon's blood and by the absence of a cathodic counterpart of the anodic peaks in the CVs (not shown here).

Danger of inappropriate starting potential

For a SWV analysis of an unknown sample, a general procedure should be proposed that would permit dye or pigment identification without a priori knowledge of its composition. Anthraquinone and naphthoquinone dyes, flavones, indigo, and Prussian blue yield very stable cyclic and square-wave voltammetric curves in potential ranges from −1 to +1 V vs. SCE after short pre-electrolysis at −1 V (Figs. 6 and 7). However, the prolonged or repeated anodic polarization affects the shape of the voltammetric curves of alizarin and purpurin, causing new, broad voltammetric peaks arising at about 0 V vs. SCE at the expense of the original peaks that obscure the otherwise clear voltammograms of these dyes. Such peaks have already been observed to appear in voltammograms of alizarin after anodic pre-electrolysis in aqueous weakly acidic solution [5, 8]. Additionally, the anodic pre-electrolysis (scanning from positive potentials) should especially be avoided in the case of dyes that are irreversibly oxidized (lac dye, dragon's blood, carminic acid). Probably such an anodic pre-electrolysis of the anthraquinone dyes at +1 V vs. Ag/AgCl (3 M KCl) causes the rather complex spectra of peaks and shoulders, partly unassigned, that were obtained [5].

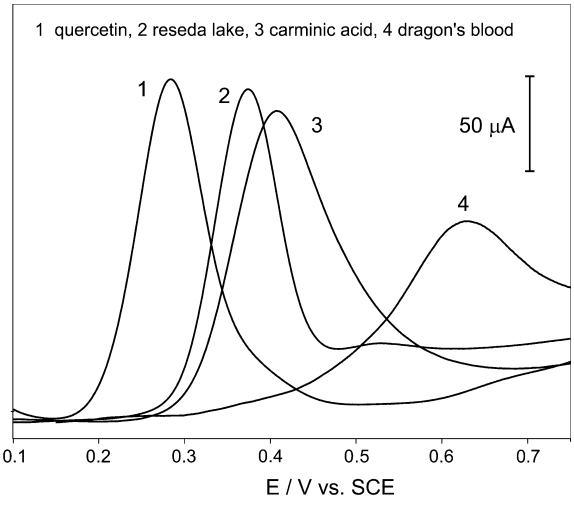


Fig. 5 Anodic SWV curves of flavones (*1* and *2*, reversible processes), an anthraquinone-related dye (*3*, a quasi-reversible process), and a pyran dye (*4*, a totally irreversible process). Scans from open-circuit potential toward positive potentials

For the analysis of an unknown sample containing some of the compounds mentioned above, the cathodic scanning from the open-circuit potential to −0.9 V or −1 V vs. SCE followed by scanning toward positive potentials could be proposed. Flavones are not harmed by this procedure, as is obvious from the voltammetric curve of reseda lake in Fig. 6. Although dragon's blood yields an electrochemically irreversible reduction peak (Table 1), the dye is not destroyed and yields a normal oxidation peak (see Table 2) in the subsequent anodic scan (Fig. 6).

Indigo and Prussian blue

The electrochemical reactions responsible for the voltammetric curves of indigo and Prussian blue have

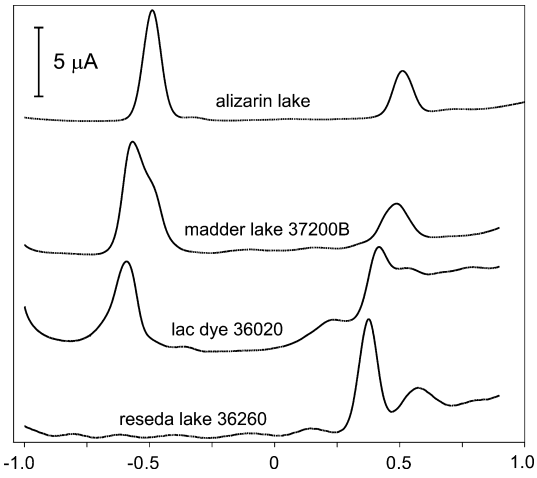


Fig. 6 SWV curves of four lakes, with 10 s pre-electrolysis at −1 V vs. SCE, scanning toward positive potentials. The first anodic process of madder lake is composed of overlapping peaks of purpurin and alizarin. The shoulder after the major anodic peak for reseda lake is due to luteolin bound to the substrate

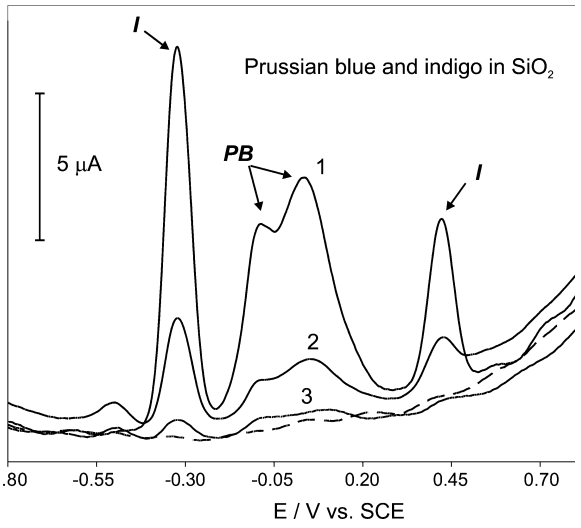


Fig. 7 A mixture of Prussian blue and indigo diluted with SiO_2 (by grinding). Concentration of the colouring components: 3.6% and 1.9% (*1*), 0.7% and 0.4% (*2*), 0.07% and 0.04% (*3*); the background is plotted as a *dashed line*. Indigo and Prussian blue peaks are denoted by *I* and *PB*, respectively

already been well described [12, 13, 16, 19]. Both compounds yield highly reversible redox processes and both are very sparingly soluble in acetate buffer. The electrochemical reactions of both solids involve ion insertion that is responsible for diffusion waves in linear-sweep voltammetry, but due to a remarkable reversibility of the corresponding electrochemical process, they yield easily recognizable SWV peaks that are much more suitable for detection and identification. Distinguishing between these two blue pigments in colour layers of art works could be worthy in restoration practice because both these compounds are usually diluted by an inert solid to tune their hue and their direct detection by other methods is hence complicated. SWV of the two blues in their mixtures diluted by SiO_2 is presented in Fig. 7. The detection limit is, in order of magnitude, about 0.1 wt% in such a plain physical mixture with an inert substrate. However, the detection limit might be worse or higher in a real paint layer, especially if the dye was bound in a very stable, chemically inert lake.

Conclusions

Voltammetry can be used as a fast method to identify many solid or dissolved organic dyes and solid pigments, including lakes, without the necessity of any sample pre-treatment or preliminary dissolution. The sample consumption (<1 mg) is comparable to microchemical tests or TLC that could alternatively be used for analysis of artists' organic pigments [14]. Voltammetry, contrary to chromatography, does not require the extraction/dissolution of a solid sample that can harm the target compound [4]. The applicability of voltammetry is enhanced by a redox activity of the chromophores of many organic dyes and inactivity of the binders and matrices (substrates) that, on the other hand, worsen the interpretability of UV/Vis, IR, and Raman spectra of paint layers and textiles [1].

In this study, voltammetry was shown to be suitable for powdered samples of dyes and pigments even after their dilution by an inert matrix. A problem to be solved before proposing the method for the analysis of art works is the sample preparation. A paint layer is a much more complex matrix than, for example, textile samples, and in paint layers mainly lakes were used with the dye component present inside the substrate grains. Their hydrolysis with cold H_2SO4 and voltammetry of the residue after evaporation of the dye extract seems to be most promising for the identification of red anthraquinone dyes and pigments (alizarin, cochineal reds, and their lakes) and flavone yellows.

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References

1. Martoglio PA, Bouffard SP, Sommer AJ, Katon JE, Jakes KA (1990) Anal Chem 62:1123A
2. Doménech-Carbó A, Doménech-Carbó MT, Gimeno-Adelantado JV, Bosch-Reig F, Sauri-Peris MC, Sanchez-Ramos S (2001) Analyst 126:1764
3. Doménech-Carbó MT, Casas-Catalan MJ, Doménech-Carbó A, Mateo-Castro R, Gimeno-Adelantado JV, Bosch-Reig F (2001) Fresenius' J Anal Chem 369:571
4. Novotná P, Pacáková V, Bosáková Z, Štulík K (1999) J Chromatogr A 863:235
5. Doménech-Carbó A, Doménech-Carbó MT, Sauri-Peris MC, Gimeno-Adelantado JV, Bosch-Reig F (2003) Anal Bioanal Chem 375:1161

6. Thompson DV (1967) The materials and techniques of medieval painting. Dover, New York
7. Downard AJ, Powell HKJ, Xu S (1991) Anal Chim Acta 251:157
8. Dai H-P, Shin K-K (1998) Electrochim Acta 43:2709
9. Pournaghi-Azar MH, Shemirani F, Pourtork S (1995) Talanta 42:677
10. Jovanovic SV, Steenken S, Hara Y, Simic MG (1996) J Chem Soc Perkin Trans 1 2497
11. Jorgensen LV, Cornett C, Justesen U, Skibsted LH, Dragsted LO (1998) Free Radical Res 29:339
12. Komorsky-Lovrić S, Mirćeski V, Scholz F (1999) Microchim Acta 132:67
13. Bond AM, Marken F, Hill E, Compton RG, Hugel H (1997) J Chem Soc Perkin Trans 2 1735
14. Schweppe H, Winter J (1997) Madder and alizarin. In: Fitz-Hugh EW (ed) Artist's pigments, vol 3. National Gallery of Art, Washington, pp 109–142
15. Schweppe H (1993) Handbuch der Naturfabstoffe. Ecomed, Landsberg
16. Scholz F, Meyer B (1998) Voltammetry of solid microparticles immobilized on electrode surfaces. In: Bard AJ, Rubinstein I (eds) Electroanalytical chemistry, vol 20. Dekker, New York, pp 1–86
17. Miliani C, Romani, Favaro G (1998) Spectrochim Acta A 54:581
18. Lovrić M (2002) Square-wave voltammetry. In: Scholz F (ed) Electroanalytical methods. Springer, Berlin Heidelberg New York
19. Schröder U, Scholz F (2000) Inorg Chem 39:1006