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Electrochemical identification of flavonoid dyes in solid work of art samples by abrasive voltammetry at paraffin-impregnated graphite electrodes

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Abstract

An electrochemical method for identifying flavonoid-type dyes in microsamples from works of art is reported. Square wave voltammograms of natural insoluble dyestuffs based on flavonoid structure dragoon's blood, weld, old fustic, gamboge, Brazilwood and logwood (Campeche wood) attached to paraffin-impregnated graphite electrodes in contact with 0.25 M HAc + 0.25 M NaAc aqueous buffer display characteristic peaks in the potential region between +0.85 and −0.85 V versus AgCl/Ag. Sequential experiments in contact with 0.05 M $AlCl_3$ and 0.05 M Na_2MoO_4 plus HAc/NaAc and 0.05 M H_3BO_3 + 0.10 M NaOH solutions also provide dye-characteristic signals allowing for an unambiguous identification of each one of the pigments. Individual pigments were satisfactorily identified in mixtures of pigments and real samples from textile pieces found in Castellfort (Valencian Region, Spain) attributed to the 15th century production of local textile workshops.

Keywords: Abrasive voltammetry; Flavonoids; Dye identification; Archaeometry

1. Introduction

Pigmenting materials enter into the composition of painting layers in a variety of works of art and as dyestuffs in textiles. The identification of such dyes is an obvious target in the scientific examination of works of art which in turn plays an essential role if the fields of archaeometry, conservation and restoration. Available methods are conditioned by the fact that the amount of sample is usually restricted to the microgram level or less.

The interest in analyzing dyes is reinforced by the genotoxic or ecotoxic properties of a number of these compounds. Accordingly, the analysis of dyestuffs has claimed considerable attention in the last years. Among them, flavonoids form a group of polyphenol compounds widely distributed in vegetables used as pigmenting agents in textiles and paints [1,2]. Their analysis in art and archaeological materials has claimed attention for studying mediaeval textile collections [3]. Thus, Quye et al. [4] have studied the effect of light-ageing of natural flavonoid-dyed wools using photodiode array high performance liquid chromatography (HPLC) and direct temperature mass spectrometry (DTMS). In this context, the identification of flavonoid dyes in archaeological Coptic textiles by reversed-phase HPLC [5,6] has been recently described.

In addition of such techniques, electrochemical methods in solution phase have been applied for identifying dyes having different chromophores [7]. These included anthraquinone [8–10], azo [11–13] and reactive dyes [14,15].

In this context, the voltammetry of microparticles, developed by Scholz et al. [16,17], has extended the scope of methods for analyzing solid micro- or submicrosamples. Following the seminal work of Scholz et al. on

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the identification of organic solids [18], this methodology has been previously applied for the identification of anthraquinone-type dyes in solid microsamples [19]. A similar scheme has been recently used by Grygar et al. for analyzing anthraquinone-based dyes, Prussian blue and other red and lac dyes, including some flavones [20].

The voltammetry of organic microparticles has been recently studied in detail [21–23]. Theoretical treatments due to Lovric el. [24–26] indicate that in the case of insoluble organic compounds able to undergo proton-exchange reactions with the electrolyte, the surface reactions initially occurs on the particle/electrode/electrolyte interface. From there the redox reaction expands over the surface and into the body of the particle depending on its conductivity. For the common case of organic insulator compounds, electron propagation takes place by means of a series of faradaic reactions accompanied by the exchange of ions or protons with the electrolyte as a consequence of the gradient of the electrochemical potential in the particle. Remarkably, redox conductivity may appear even if electrolyte ions cannot diffuse through the particle [22,23,26].

It is reported here an electrochemical procedure for identifying the most frequent flavonoid dyes used in archaeological artifacts, textiles and/or works of art, namely: dragoon's blood, weld, old fustic, gamboge, Brazilwood and logwood. Flavonoids have in common two benzene units lied by a chain containing three carbon atoms. Depending on the structure of the intermediate three-carbon chain one can distinguish between different groups: chalcones, flavones, flavonols, etc. Most of the coloured flavonids have a chroman (benzopyrone) unit as can be seen in Scheme 1.

The most used variety of the red dye dragoon's blood consists on a mixture of dracorubin and dracohordin, two flavonoids whose structure is depicted in Scheme 1. The red colouring agent of weld (often called arzica) and the yellow colouring agent of old fustic (often called yellow wood) are, respectively, luteolin (3′,4′,5,7-tetrahydroxyflavone) and morin (2′,3,4′,5,7-pentahydroxyflavone), whose structures are illustrated in Scheme 1. Moritamnic acid and maclurin have been also reported as other compounds responsible of the yellow colour of old fustic [27].

The colouring agents of gamboge, Brazilwood and logwood exhibit a flavonoid-like structure. The main component of gamboge is gambogic acid, whose structure is shown in Scheme 1.

Brazilwood is mainly composed of brazilin, which is frequently accompanied by its oxidized form, brazilein. The structures of brazilin and brazilein, both depicted in Scheme 1, are tetracyclic with two aromatic rings, one pyrone, and one a five-membered unsaturated ring [4,5]. Such structures are similar to those of hematoxylin and haematein, the reduced and oxidized forms, respectively, of the main colouring agent of logwood (or litmus Campeche). The structures of such compounds that only differ from brazilin and brazilein by the presence of one OH group are also shown in Scheme 1.

The existence of subtle structural differences between all these compounds makes uneasy their identification in routine analysis. Attempting to provide a rapid but sensitive method for identifying flavonoid-type dyes in microsamples, the electrochemical response of such materials immobilized in the surface of paraffin-impregnated graphite electrodes (PIGES) has been studied upon immersion of sample-modified electrodes in different aqueous media.

Square wave voltammetry (SQWV) was used as a detecting technique by its high sensitivity and immunity to capacitive effects [28]. This technique, whose theory for immobilized reactants was developed by Lovric et al. [29,30], is of interest with regard the electrochemistry of solids [31].

In this study, in addition to the methodology previously described for anthraquinone-type dyes [19], it has been applied a new procedure consisting on the sequential use of electrolytes containing selective complexing agents, namely H_3BO_3, $AlCl_3$, and Na_2MoO_4, and the record of the response after an adsorptive step performed via repetitive cycling the potential scan. The reported procedure has been used for identifying flavonoid-type dyes in solid samples formed by individual dyes, binary mixtures of the dyestuffs, and real samples providing from textile pieces found in Castellfort (Valencian Region, Spain), attributed to the 15th century production of local textile workshops.

2. Experimental

2.1. Materials and reagents

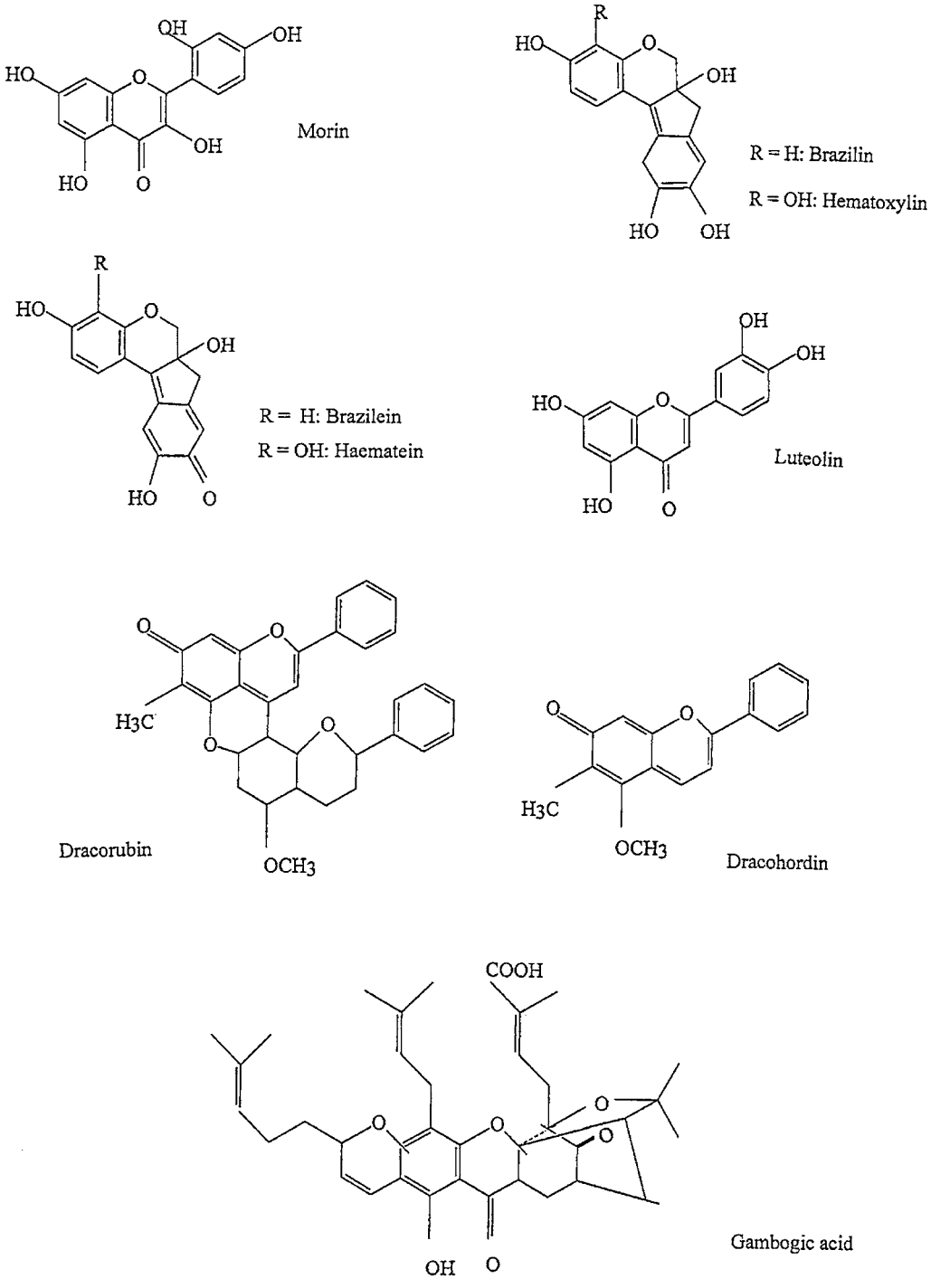
H_3BO_3 (Panreac), Na_2MoO_4 (Merck), $AlCl_3{\cdot}nH_2O$ (Panreac), $NaClO_4$ (Merck), $HClO_4$ (Panreac), acetic acid (HAc, Panreac) and sodium acetate (NaAc, Merck) and NaOH (Panreac) were used for electrolyte preparation. Routine analyses were performed in 0.25 M HAc + 0.25 M NaAc, 0.15 M $NaClO_4$ or 0.10 M NaOH solutions in doubly distilled water.

Dragoon's blood and gamboge were supplied by AP Fitzpatrick (Bethnal Green, London, UK). Weld, old fustic, gamboge, logwood and Brazilwood were supplied by Kremer (Aichstetten/Allgäu, Germany). Luteolin (3′,4′,5,7-tetrahydroxyflavone) and morin (2′,3′,4′,5,7-pentahydroxyflavone) (Aldrich) were used as reference compounds.

In order to assess the presence of carmine, an anthraquinone-type dye, in textile samples (vide infra), blank experiments were performed with samples of that dye. Carmine (cochineal type from *Coccus Cacti* insect) was supplied from AP Fitzpatrick.

2.2. Samples

Three microsamples of coloured fibres from two mediaeval brocade pieces have been analyzed. These pieces belong



Scheme 1.

to a cope found in the Church of Castellfort (Valencian Region, Spain) attributed to the 15th century production of textile workshops in Morella (Northern Valencian Region, Spain), dated ca. 1420. The cope was a sacerdotal garment, also called pluvial, furnished with a hood or a scapulary. Two of the samples analyzed were taken from the back of a fragment of the scapulary whereas the third sample was taken from the back of the capillo, a small decorative piece of the cope. The samples correspond to the following coloured zones:

S.1. Yellow in the background. Scapulary.
S.2. Dark red in the floor tile. Scapulary.
S.3. Red in the mantle of apostle. Capillo.

Microscopic analysis of samples has shown yellow and red stained silk fibres with average width in the range of 10–12 μm.

2.3. Modified electrode preparation

Paraffin-impregnated graphite electrodes (PIGEs) consist on cylindrical rods of 5 mm diameter of graphite impregnated under vacuum by paraffin. Preparation details are described in refs. [16,17].

To prepare dye-modified PIGEs, 0.1–1 mg of the material was powdered in an agate mortar and pestle, and placed on a glazed porcelain tile forming a spot of finely distributed material and then abrasively transferred to the surface of a PIGE by rubbing the electrode over that spot of sample.

For preparing sample-modified PIGEs, a fibre (weight lesser than 1 μg) was immersed into a drop of MeOH + HCl during 10 min on a porcelain tile and then dried in air. Then, the lower end of the electrode was repeatedly rubbed over the residual and the fibre, being further transferred to the cell for electrochemical measurements.

2.4. Instrumentation and procedures

Electrochemical experiments were performed at 298 K in a three-electrode cell under argon atmosphere using a AgCl (3 M NaCl)/Ag reference electrode and a platinum-wire auxiliary electrode. Linear potential scan, cyclic and square wave voltammograms (LSVs, CVs and SQWVs, respectively) were obtained with a BAS CV 50 W equipment. Dye-modified PIGEs were dipped into the electrochemical cell so that only the lower end of the electrode was in contact with the electrolyte solution. This procedure provides an almost constant electrode area and reproducible background currents.

Aqueous 0.25 M HAc + 0.25 M NaAc (pH 4.70), eventually plus 0.05 M Na_2MoO_4 or 0.05 M $AlCl_3$, and 0.05 M H_3BO_3 + 0.10 M NaOH solutions were used as electrolytes. The pH-dependence of the electrochemical response was studied in 0.15 M $NaClO_4$, the pH being adjusted between 3 and 7 upon addition of $HClO_4$ and/or NaOH.

SQWVs at dye-modified PIGEs were performed on initiating the potential scan either at +1.0 and −1.0 V using a potential step increment of 4 mV and a square wave amplitude of 25 mV. The frequency was varied from 2 to 200 Hz. LSVs and CVs were performed at potential scan rates ranging between 20 and 500 mV/s. The following conditions: potential step increment 4 mV, square wave amplitude 25 mV, square wave frequency 15 Hz, were selected for routine analysis for a compromise between sensitivity and repeatability.

In the case of experiments involving prior polarization steps, selected conditions for electrode conditioning were: starting potential 0.0 V, extreme potentials +1.25 and −1.25 V, potential scan rate 100 mV/s and number of cycles 8. After this polarization step, detection scans were performed using the conditions described in the above paragraph.

3. Results and discussion

3.1. Cyclic voltammetric response

The CV response of luteolin (3′,4′,5,7-tetrahydroxyflavone), the reference compound for weld, attached to PIGEs in contact with 0.25 M HAc + 0.25 M NaAc is illustrated in Fig. 1. On initiating the potential scan at 0.0 V in the positive direction (Fig. 1a), a well-defined anodic peak at +0.30 V versus AgCl/Ag (A_1) appears, followed by cathodic peaks at +0.15 V (C1), and −0.32 V (C_2). On initiating the potential scan at 0.0 V in the negative direction (Fig. 1b), and prolonging it until −0.85 V, overlapping cathodic peaks at −0.50 (C_3) and −0.75 V (C_4) are recorded while peak C_2 is absent. In the reverse scan, an additional anodic peak at +0.10 V (A_2) appears preceding the more prominent peak A_1.

This voltammetric profile suggests that the electrochemical process C_2 is produced by a species resulting from any chemical reaction following the initial oxidation process A_1. Consistently, on increasing the potential scan rate, the peak C_1 increases at the expense of peak C_2. Upon repetitive cycling the potential scan between +0.50 and 0.0 V, the peaks A_1, C_1 couple approaches a quasi-reversible couple while cycling the potential between +0.70 and −0.40 V, peaks A_1, C_1 and C_2 are recorded with no additional anodic peaks.

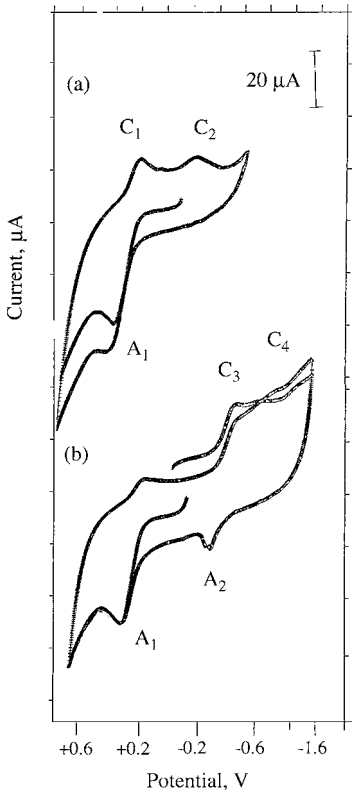


Fig. 1. CVs of luteolin-modified PIGEs immersed into 0.25 M HAc + 0.25 M NaAc. (a) Potential initiated at 0.0 V in the positive direction; (b) I_d at 0.0 V in the negative direction and prolonged to potentials ca. −0.85 V. Potential scan rate 20 mV/s.

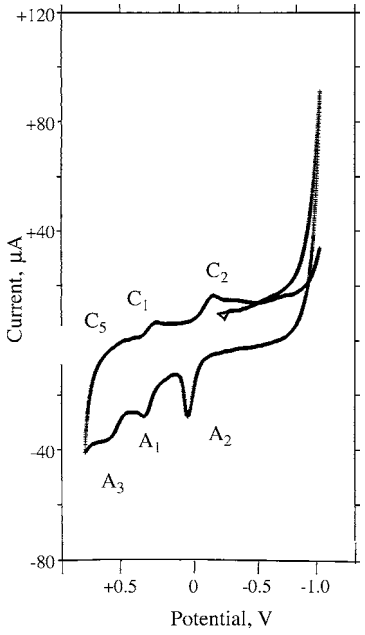


Fig. 2. CV of a weld-modified PIGEs immersed into 0.25 M HAc + 0.25 M NaAc. Potential initiated at −0.25 V in the negative direction and prolonged to potentials ca. −1.05 V. Potential scan rate 50 mV/s.

This suggests that the reduction process C_1 leads to the parent compound responsible of peak A_1, while the peak C_2 is attributable to the apparently irreversible reduction of a secondary species.

If the potential scan is prolonged to values more negative than −1.0 V, the peak A_2 increases whereas an additional anodic peak at +0.60 V (A_3) appears, followed, in the subsequent scan, by an ill-defined peak at +0.50 V (C_5), as illustrated in Fig. 2. The voltammetric response of weld was found to be identical to that of luteolin.

The voltammetric response in 0.15 M $NaClO_4$ was similar to that previously described in the pH range between 4 and 7, the peak potentials of all being shifted toward more negative values on increasing pH. Linear variations of E_p on the pH were obtained for all peaks. The slope of such linear graphs were of 27 (C_1), 23 (C_2), and 45 (A_1) mV/decade for pH values between 4 and 7.

The voltammetric response is particularly sensitive to the application of negative polarization potentials, a feature previously noted [19] and also observed by Grygar et al. [20] and Dai and Shin [32] for anthraquninone-type compounds. As shown in Fig. 2, if the potential is prolonged to values ca. −1.0 V, a prominent anodic peak is recorded at +0.10 V (A_3). This peak, that exhibits a tall profile, was recorded in well-degasified solutions. Accordingly, the peak A_2 cannot be attributed to electrochemical processes involving dissolved oxygen. The peak A_2 increases after applying prolonged constant potential steps of −1.0 V or in repetitive voltammetry. These features suggest that reduction steps at potentials ca. −1.0 V are irreversible, producing a new solid [20] or adsorbates.

The voltammetric profiles changed slightly in successive scans performed on the same dye-modified electrode, suggesting that in these cases a slow dissolution of the microparticles occur at the time scale of voltammetric experiments [33]. This suggests that first scan voltammograms must be used for identifying dyestuffs.

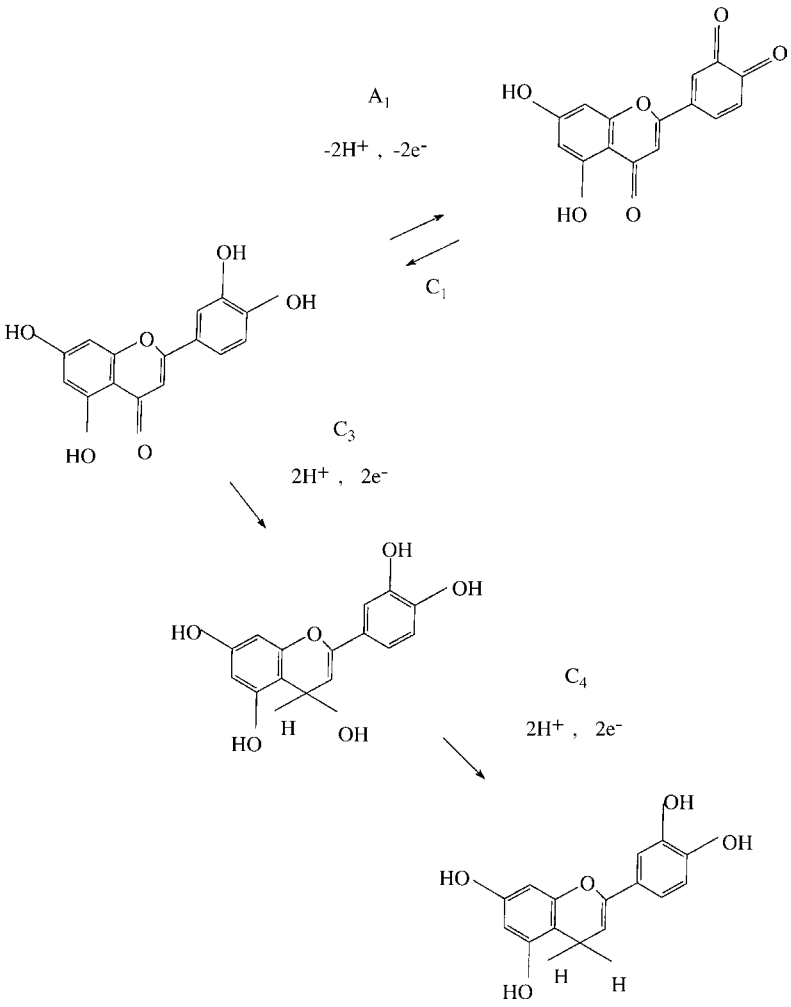
The CV response of morin (2′,3′,4′,5,7-pentahydroxyflavone) and old fustic was entirely similar to that previously described for luteolin/weld. Similar results where obtained also for Brazilwood and logwood (Campechewood). However, for gamboge, only the A_1/C_1 couple remains well-defined. For dragoon's blood, no well-defined initial anodic processes were obtained.

3.2. Description of the electrochemical processes

This electrochemistry can in principle be rationalized on the basis of the model of Lovric and Scholz [24–26] for the solid state voltammetry of immobilized microcrystals. Accordingly, there is a transition from the initial three-phase reaction occurring in the electrode/particle/electrolyte junction, to a pure two-phase reaction in which the surface reaction is localized in the particle/electrolyte interface. Here, the progress of the electrochemical reaction can be described in terms of the transfer of electrons across the electrode-microparticle interface, coupled with the ingress/issue of protons into/from the solid microparticle, crossing the particle/electrolyte interface. This scheme results in a solid-state reaction that can be accompanied with reductive/oxidative dissolution processes in which the electron/proton transfer process yields to species in solution phase (vide infra).

In agreement with literature concerning the electrochemistry of phenols, catechols and flavonoids in solution [34–47], the voltammetric response of luteolin/weld is dominated by the oxidation of the orthophenol moiety. It is known that catechols are electrochemically oxidized at potentials ca. +0.5 V, through a two-proton, two-electron process, the resulting quinones being reduced at potentials between +0.4 to −0.2 V [34,35], depending on the kinetics of the proton/electron-transfer processes [36–39]. This scheme applies for flavonoids in solution, that are oxidized at potentials between +0.13 and +0.34 V versus SCE [40].

For luetolin/weld in solid phase, the initial oxidation A_1 must yield the corresponding *o*-quinone. The oxidation process involves to any extent a post-electron transfer reaction producing a secondary product. This scheme is in agreement with that recently reported for luteolin in solution by Hendrickson et al. [41]. Here, two post-electron transfer reactions yield two unstable products through zero- and first-order kinetics, respectively [41]. A similar electrochemical pathway is operative for the oxidation of dopamine with formation of aminochrome and leucoaminochrome [42]. For luteolin/weld, the *o*-quinone is reduced through the process C_1, while the secondary product is reduced at more negative potentials (process C_2). A simplified representation is depicted in Scheme 2.



Scheme 2.

It should be noted, however, that the presence of additional phenol groups can complicate the electrochemical response of flavonoids. Thus, Brett and Ghica have recently reported [43] that the electrochemical oxidation of quercetin (3,3′,4′,5,7-pentahydroxyflavone) at glassy carbon electrodes occur in a cascade mechanism, related with the two catechol hydroxy groups and the other three hydroxyl groups, all being electroactive. Adsorption complicates the voltammetric response, the final oxidation product being not electroactive and blocks the electrode surface [43].

This oxidative pathway can be applied to Brazilwood and logwood (Campeche), both incorporation orthophenol groups as can be seen in Scheme 1. For gamboge and morin/old fustic, phenol oxidation must produce, following literatures [34–37], quinones and polymer products. Under our experimental conditions, however, in which solid-phase processes are involved, it appears that no polymerization reactions occur.

Reduction of the parent products must occur through the ketone group of the benzopyrone moiety present in all tested materials (see Scheme 1). As recently described by Nagarajan et al. [44], the reduction of the keto moiety of 7-hydroxy flavons in solution is irreversible. Reduction occurs via two one-proton, one-electron consecutive steps yielding hydroxyl derivatives at potentials ca. −1 V [36,45,46]. A possible reaction scheme is shown in Scheme 2 for luteolin. Among other possible products, such reduction process can produce chalcones. These species are oxidized to flavonoids at potentials above +0.5 V [47], a process that would correspond to the electrode process A_3.

A possible reaction scheme is shown in Scheme 2 for luteolin. The electrochemical processes recorded for solid products can in principle be regarded as solid state transformations following the aforementioned model [23–26]. Consistently with the structures depicted in Scheme 1, all tested products exhibit reduction waves near to −1.0 V. On initiating the scan at open circuit in the positive direction, oxidation peaks were obtained for luteolin/weld, morin/old fustic, Brazilwood, logwood and gamboge, all having phenol and/or catechol groups. Dragoon's blood, however, in which no hydroxyl groups exist, exhibits no oxidation peaks.

In repetitive voltammetry at potentials ca. −1.0 V, however, the above voltammeric pattern vanishes and a prominent stripping oxidation (peak A_2) appears ca. +0.10 V. The

value of the peak potential of A_2 suggests that an adsorbed hydroquinone-type product is oxidized to a quinone derivative in solution phase. Accordingly, ill-defined reduction processes occurring ca. −1.0 V must lead to polyhydroxy species that remain adsorbed on the surface of the graphite electrode [43].

3.3. Square wave voltammetric response

Identification of dyes in real samples requires the use of low amounts of material. Accordingly, and in view of the rich electrochemistry of the dyestuffs, it is desirable (i) the use of a sensitive techniques, and (ii) to select conditions in which all possible electrochemical processes appear.

For this purpose, SQWVs initiated at relatively high (+0.85 V) or relatively low (−0.85 V) potentials were recorded. The use of SQWV provides a simultaneous examination of both cathodic and anodic processes [34,40].

In Fig. 3, the semi-derivative convolution of SQWVs of (a) dragoon's blood, (b) gamboge, (c) weld, (d) old fustic, (e) logwood, and (f) Brazilwood are shown conjointly with (g) the blank voltamogram recorded at an unmodified PIGE. Elimination of the influence of the supporting electrolyte can in principle be obtained by subtracting the blank voltammogram. This manipulation is, however, problematic in the case of modified electrodes because the electrode modification involves an abrasive procedure that alters the effective surface of the graphite electrode. Then, the background current of the modified electrode differs from that of the bare graphite electrode.

For this reason, semi-derivative convolution was used to increase peak resolution, but no background subtraction has been performed. Thus, for dragon's blood (Fig. 3a), a main peak at +0.12 V appear, preceded by overlapped peaks at +0.52 and +0.26 V. This response is very similar to that of gamboge, depicted in Fig. 3b. For weld (Fig. 3c), two main peaks at +0.46 and +0.05 V appear, followed by a weaker wave at −0.55 V. Old fustic (Fig. 3d) produced overlapping peaks with a profile similar to that of dragoon's blood and gamboge, but slightly displaced towards more negative potentials. Finally, Brazilwood and Campechewood produced very similar SQWVs, with well-separated peaks at +0.45 and +0.10 V. Since SQWV provides simultaneous examination of oxidation and reduction processes, signals for the reduction processes C_1, C_2, C_5, are superimposed with those corresponding to the anodic process A_1 (and eventually A_2 and A_3), in the same region of potentials. Additionally, some peaks may be resolved into two superimposed signals, consistently with their assignment to successive electron-transfer proton-transfer electrochemical processes.

SQWVs recorded on scanning the potential in the anodic direction provide a more simple profile. Here, a prominent peak near +0.28 V (A_1) appears for all dyes, as can be seen in Fig. 4a–f. The peak potential values are summarized in Table 1. Interestingly, on comparing pairs of polyhydroxy compounds differing in one hydroxy group, the peak potential of A_1 is negatively shifted on increasing the number of OH groups. Thus, the value of E_p for peak A_1 decreases from +0.42 V for Brazilwood to +0.18 V for logwood and from +0.32 V for weld to +0.25 V for old fustic. This result is in agreement with literature data for polyhydroxyanthraquinone compounds [20,47]. Weld (Fig. 4c) can easily be discerned from the other pigments by the presence of a

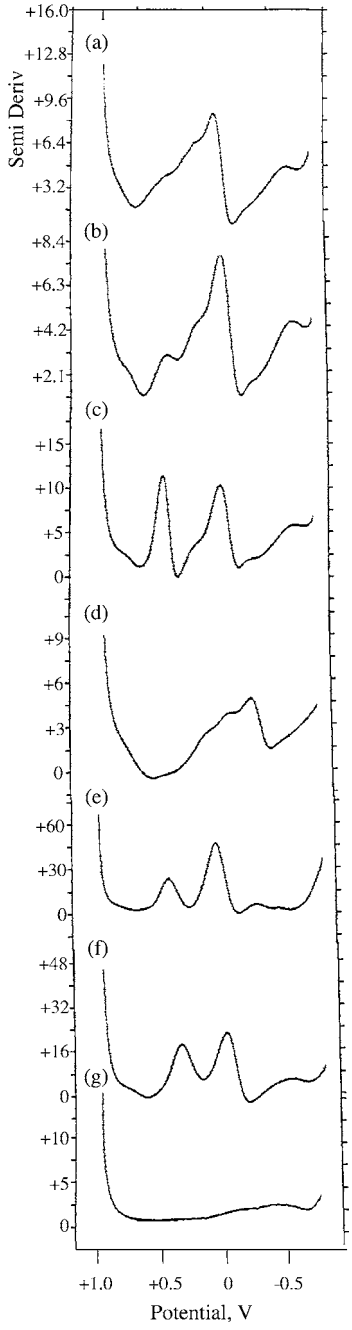


Fig. 3. First scan SQWVs, after semi-derivative convolution, of dye-modified PIGEs immersed into 0.25 M HAc + 0.25 M NaAc. Potential scan initiated at +0.85 V in the negative direction. (a) Dragon's blood, (b) gamboge, (c) weld, (d) old fustic, (e) Campeche wood, and (f) Brazilwood. (g) SQWV at an unmodified PIGE. Potential step increment 4 mV; square wave amplitude 25 mV; frequency 15 Hz.

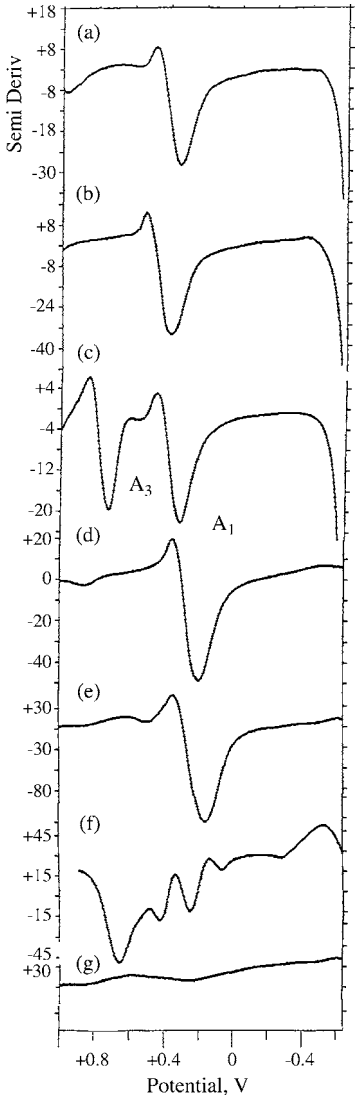


Fig. 4. First scan SQWVs, after semi-derivative convolution, of dye-modified PIGEs immersed into 0.25 M HAc + 0.25 M NaAc. Potential scan initiated at −0.85 V in the positive direction. (a) Dragon's blood, (b) gamboge, (c) weld, (d) old fustic, (e) logwood, and (f) Brazilwood. Potential step increment 4 mV; square wave amplitude 25 mV; frequency 15 Hz.

Table 1
Peak potential data recorded in the initial potential scan of freshly prepared dye-modified PIGEs immersed into 00.25 M HAc + 0.25 M NaAc

Modifier	E_p (mV vs. AgCl (3 M NaCl)/Ag)						
	C_5[a]	C_1[a]	C_2[a]	C_2[a]	C_3[a]	A_3[b]	A_1[b]
Dragon's blood	+515	+260	+120		−435		+340
Gamboge	+465	+235	−105	−180	−530		+360
Weld	+465	+210	+50	−225	−550	+715	+315
Old fustic			+75	−275			+250
Logwood	+445		+75	−250	−435		+185
Brazilwood	+350		+5		−565	+720	+420

[a] Potential scan initiated at +0.85 V in the negative direction.
[b] Potential scan initiated at −0.85 V in the positive direction. Potential step increment 4 mV; square wave amplitude 25 mV; frequency 15 Hz.

prominent peak at +0.73 V while Brazilwood exhibits a relatively complicated profile.

Repeatability tests were performed using series of five freshly prepared modified electrodes for each one of the pigments. In all series peak potentials were almost identical (maximum deviations of ±10 mV from one voltammogram to another). Since the amount of modifier transferred to the electrode was not constant, peak currents varied from one experiment to another.

With exception of peaks A_2 and A_3, well-developed cathodic and anodic components of the peak current (measured at the end of forward and backward pulses) were recorded for all peaks, thus denoting the reversible character of such electron transfer steps. In contrast, for peaks A_2 both forward and backward components of the square wave signal are anodic, denoting that these are electrochemically irreversible processes [48]. This irreversible character is in agreement with the results reported by Grygar et al. [20] and Jovanovich et al. [49]. Following data reported by these last authors concerning the solution phase electrochemistry of 3-hydroxyflavones, the flavone skeleton can be irreversibly reformed at potentials ca. +0.7 V [49].

The voltammetric profile in first scan SQWVs varies slowly on increasing the square wave frequency, *f*. In the range $2 < f < 50$ Hz the peak potentials of peaks C_3 and C_1 are positively shifted on increasing *f*. In this frequency range, the peak C_2 is negatively shifted on increasing frequency. However, for frequencies larger than 50–75 Hz, all peaks become abruptly displaced toward more negative values. This response can be interpreted in terms of the model reported by Schröder et al. [26] for the solid state voltammetry of immobilized microcrystals considering diffusion and charge-compensating cations in the solid microparticle. Following this scheme, there is a transition from the initial three-phase reaction occurring in the electrode/particle/electrolyte junction, to a pure two-phase reaction in which the surface reaction is localized in the particle–electrolyte interface. Here, the electrochemical response is close to those describing planar diffusion of electroactive species in solution, being characteristic of redox reactions coupled with the exchange of protons between insoluble organic compounds [27–29].

Accordingly, the response of solid dyes at low frequencies can be ascribed to a two-phase behaviour conditioned by the rate of preceding or succeeding protonation reactions. Their response at high frequencies should correspond to the initial three-phase step or to a two-phase step with a twin-layer diffusive response, as described theoretically [26]. In agreement with that scheme, plots of the peak current, i_p, versus *f* show a dual regime. Using peak current values measured from the minimum plateau current in each SQWV, i_p appears to vary linearly with *f* at low frequencies, as can be seen in Fig. 5. This behaviour is just that expected for a thin-layer behaviour occurring when the extent of the redox reaction along the surface particle is large. Above 75 Hz, the peak current increases dramatically and becomes proportional to $f^{1/2}$, as expected for an 'ordinary' diffusion-controlled process in solution phase.

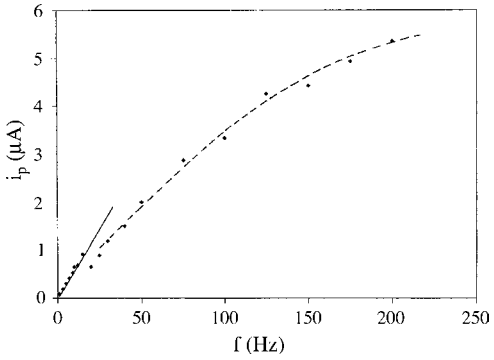


Fig. 5. Dependence of the peak current on the square wave frequency for process C_1 recorded in SQWVs of freshly prepared logwood-modified PIGEs immersed into 0.25 M HAc + 0.25 M NaAc. Potential step increment 4 mV; square wave amplitude 25 mV. Continuous line: i_p and f graph; dotted line: i_p and $f^{1/2}$ graph.

This corresponds to that theoretically expected at short times; i.e., when the extent of the redox reaction is small [26].

For our purposes, it is interesting to consider a compromise between resolution and repeatability. Within the $2 < f < 50$ Hz range, the peak resolution increases in general as the frequency decreases, whereas the repeatability decreases on decreasing frequency. Similar results were obtained with regard to variations in the potential step increment and the square wave amplitude.

3.4. Adsorption processes

As previously noted, in some cases (logwood, Brazilwood, dragoon's blood), repetitive voltammetry after application of potentials close to −1.0 V (or +1.0 V) produces the appearance of prominent peaks ca. 0 V. These peaks adopt a tall shape and increase in successive scans, suggesting that adsorption processes occur. Consistently, peak currents become proportional to the square wave frequency, as expected for surface-confined reactants [29–31].

With regard to pigment identification the relevant point to emphasize is that, although polarization effects obscure the voltammetric response, adsorption peaks can be used in order to enhance sensitivity for logwood, Brazilwood and dragoon's blood. Pertinent data are summarized in Table 2.

In fact, adsorption peaks can be used for the resolution of pigment mixtures. For this purpose, different cycles of potential were tested by varying the potential range from +1.5/−1.5 V to +0.85/−0.85 V, the potential scan rate (between 10 and 1000 mV/s), the number of cycles and the starting potential and the direction of the scan. The selected conditions for electrode conditioning are summarized in Section 2.

3.5. Identification through electrolyte-selective processes

The foregoing procedures are able to distinguish flavonoid-type pigments in solid samples. However, in several cases the distinction between some pigments can be made uncertain by matrix effects. These can be attributed mainly to: (a) presence of spurious peaks associated to other dyes and/or priming and protective organic compounds coexisting in the sample; (b) occurrence of cross-reactions between the intermediate products of electron-transfer processes; (c) modification of the electrochemical response of the dyes by effect of complexation reactions with metal ions from inorganic pigments; (d) appearance of unexpected peaks associated to alteration products.

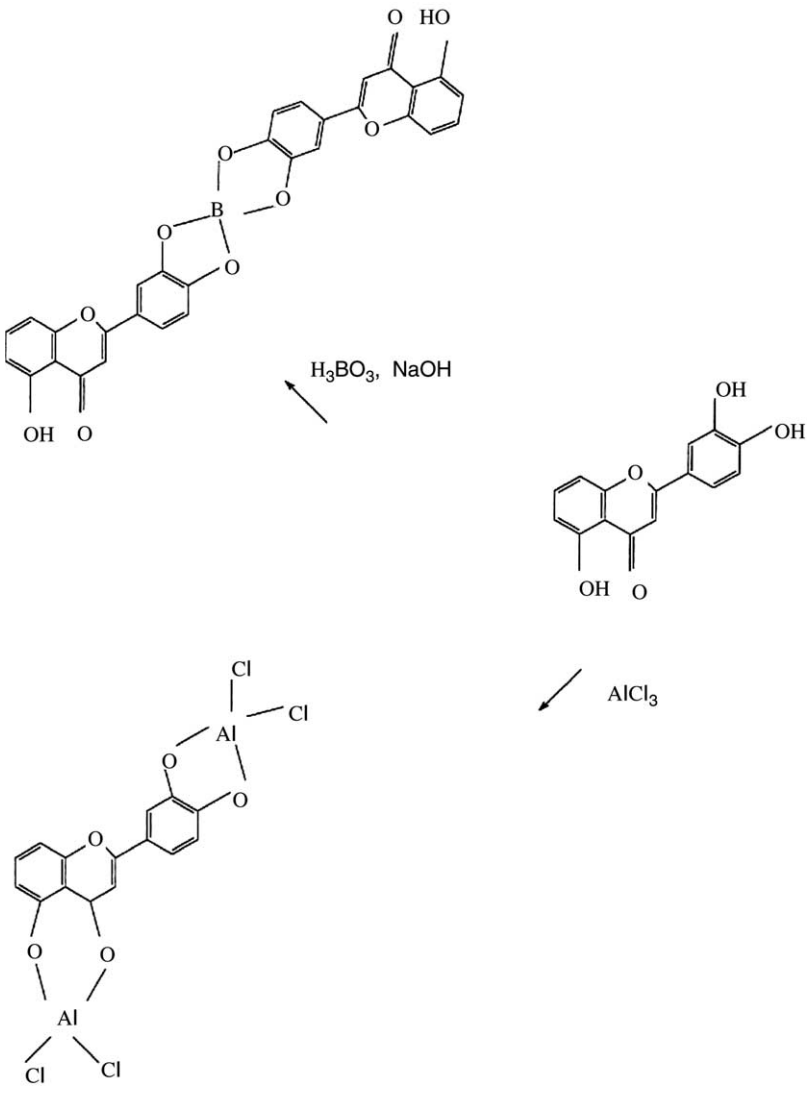
Table 2
Peak potential data recorded for dye-modified PIGEs immersed into 0.25 M HAc + 0.25 M NaAc after adsorptive electrode preconditioning by cycling the potential scan eight times between +1.25 and −1.25 V at 100 mV/s

Modifier	E_p (mV vs. AgCl (3 M NaCl)/Ag)				
	a	a	a	a	b
Dragoon's blood			−375	−575	
Gamboge		−210	−380		−1010
Weld	+10	−40	−380		−940
Old fustic	0	−215	−385		−1050
Logwood	+20	−250	−380		−935
Brazilwood	+55	−150			−820

(a) Potential scan initiated at +0.85 V in the negative direction. (b) Potential scan initiated at −0.85 V in the positive direction. Potential step increment 4 mV; square wave amplitude 25 mV; frequency 15 Hz.

In order to improve the discrimination between the different pigments in real samples, different electrolyte solutions can be used sequentially. The idea is that in the presence of a specific reagent, the voltammetric response of each one of the pigments must change with respect to that previously obtained in the absence of the reagent. Thus, identification of the pigments can be confirmed by comparing the SQWV responses in different electrolytes. Thus, it is known that $AlCl_3$ forms stable chelates in acidic media with 3- and 5-hydroxy derivatives [50] (see Scheme 3). Accordingly, the response of weld, old fustic and "woods" must change from HAc/NaAc to HAc/NaAc + $AlCl_3$ electrolytes.

As expected, the SQWVs of all three dyes change from 0.25 M HAc + 0.25 M NaAc to that plus 0.05 M $AlCl_3$. The obtained results are illustrated in Fig. 6. First, the SQWVs of weld-modified PIGEs in the absence (Fig. 6a) and in the presence (Fig. 6b) of Al^{3+} are shown. Coordination with aluminium apparently blocks electron-transfer processes, and the peaks are significantly decreased. For logwood, the contrast between the SQWVs in the absence and in the presence of Al^{3+}, illustrated in Fig. 6c and d, is also remarkable. In this case, voltammetric peaks are all decreased except the peak at +0.03 V, which is abruptly enhanced. This results probably from the formation of an adsorbate of the aluminium complex on the electrode surface, a situation similar to that existing in alizarin-type aluminium complexes [51]. Since quinone groups are involved in aluminium coordination (see Scheme 3), the peak C_1 is particularly sensitive to that coordination, in agreement with the reaction scheme depicted in Scheme 2.



Scheme 3.

The effect of additions of boric acid in alkaline medium was also examined. It is known that borate ions form strong complexes with compounds having *ortho*-diphenols in the benzopyrone group [50] as illustrated in Scheme 3. Since this subunit is present in luteolin, hematoxylin, Brazilin and haematein, one can expect that no significant changes occur in the voltammetry of dragoon's blood, gamboge and old fustic upon addition of boric acid. Experimental data agree well with that expectance. SQWVs of dragon's blood, gamboge, old fustic, in contact with 0.10 M NaOH are indistinguishable of those recorded in 0.10 M NaOH + 0.05 M H_3BO_3. Interestingly, the voltammetric profiles remain identical in both electrolyte media, confirming the high repeatability of the experiments. Confirming the mentioned expectance, SQWV of weld in 0.10 M NaOH (Fig. 7a) differs significantly from that in 0.10 M NaOH + 0.05 M H_3BO_3 (Fig. 7b). However, minor differences were detected in SQWVs of "woods" immersed into 0.10 M NaOH and 0.10 M NaOH + 0.05 M H_3BO_3.

Finally, the effect of molybdate ions on the electrochemical response of dye-modified electrodes was investigated. It is known that molybdate ions form stable complexes in weakly acidic media with polyhydroxy compounds. Complexes with distorted octahedral geometries having *cis*-dioxo or *fac*-trioxo MoO_2 or MoO_3 cores are usually formed conjointly with oxo-bridged dimeric complexes [52]. Although the studied dyes do not form very stable complexes with molybdenum, one can expect that an efficient discrimination between them might be obtained. Since the colouring agents of weld, old fustic, logwood and Brazilwood can act as bidentate ligands, two molecules of such compounds are needed for coordinating each molybdenum core. Accordingly, gamboge and dragoon's blood do not interact with molybdate ions.

Experimental data are in agreement with the foregoing expectances: SQWVs "woods", weld and old fustic experiences significant alterations from HAc/NAc from HAc/NaAc

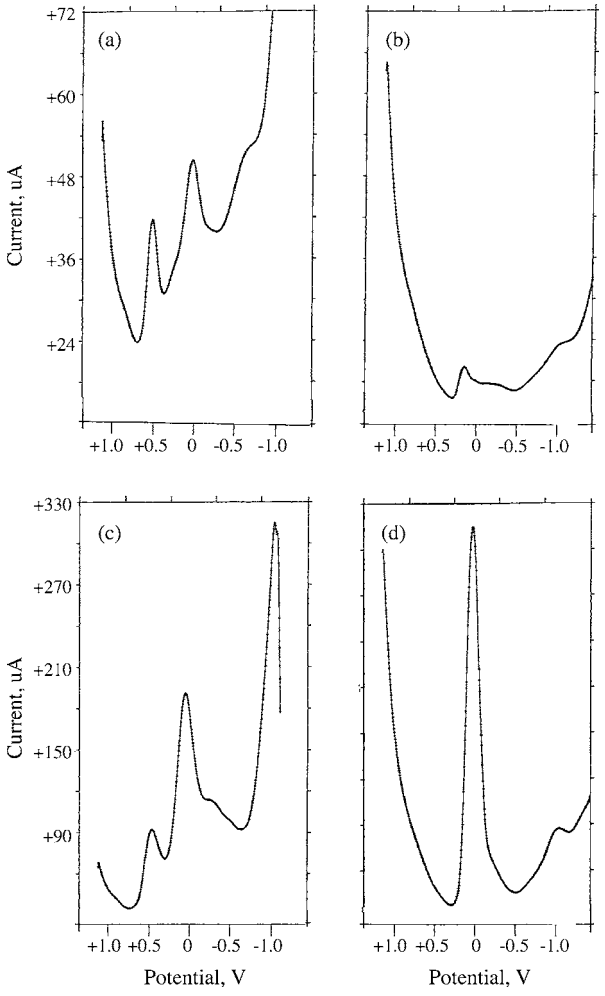


Fig. 6. SQWVs (first scan) of weld (a, b) and logwood (c, d) immersed into 0.25 M HAc + 0.25 M NaAc (a, c) and that electrolyte plus 0.05 M $AlCl_3$ (b, d). Potential step increment 4 mV; square wave amplitude 25 mV; frequency 15 Hz.

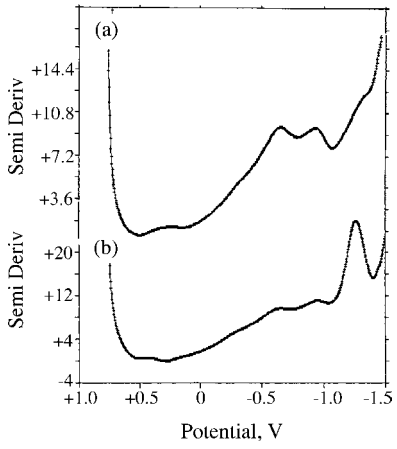


Fig. 7. Semi-derivative convolution of SQWVs for weld-modified PIGEs in contact with (a) 0.10 M NaOH, and (b) 0.10 M NaOH + 0.05 M H_3BO_3. Potential step increment 4 mV; square wave amplitude 25 mV; frequency 15 Hz.

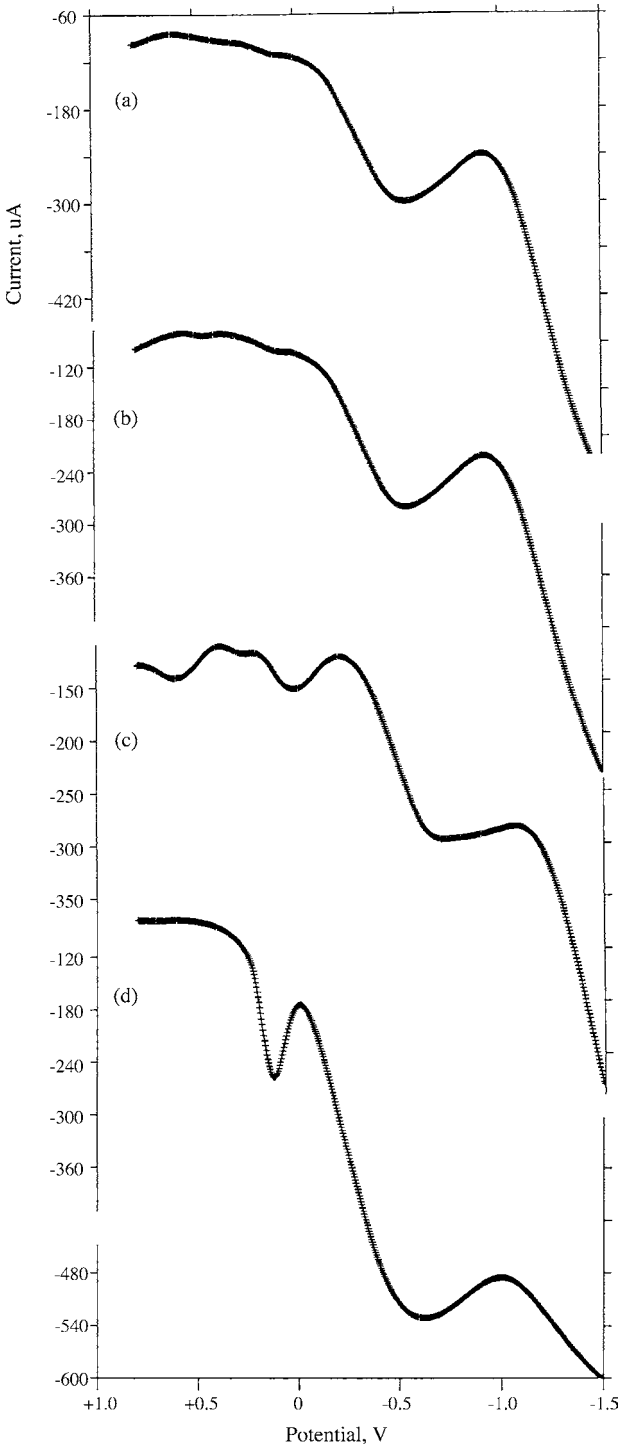


Fig. 8. First scan SQWVs of (a) Brazilwood, (b) dragon's blood-modified electrodes immersed into 0.25 M HAc + 0.25 M NaAc in the presence of 1.5 mM Na_2MoO_4. Potential step increment 4 mV; square wave amplitude 25 mV; frequency 15 Hz.

plus Na_2MoO_4 electrolytes. This can be seen in Fig. 8, corresponding to anodic scan SQWVs of (a) old fustic, (b) logwood, (c) Brazilwood, and (d) dragoon's blood. A prominent wave near to −0.75 V appear in all cases, corresponding to molybdenum-localized processes. In the case of old fustic and

logwood, no additional peaks appear, suggesting that a significant molybdate–dye interaction occurs. For Brazilwood and especially for dragon's blood, however, tall pigment-centred peaks appear, as expected for a weak molybdate–dye interaction. This interaction increases on increasing the concentration of molybdate ions.

In fact, plots of the peak potential versus the concentration of molybdate give typical s-shaped curves, denoting that a complexation process occurs. Our data indicate that Brazilwood and in particular logwood are by far the most sensitive dyes to molybdate ions. This effect is particularly intense if adsorptive voltammetry is used.

3.6. Analysis of real samples

SQWVs of PIGEs modified by samples S.1–S.3 immersed into acetic/acetate buffer were systematically used for pigment identification. Thus, in Fig. 9 are compared the SQWVs of (a) sample S.1, and (b) old fustic. A unique well-defined peak at +0.36 V is recorded in HAc/NaAc in both cases. In agreement with that assignment, this peak disappears upon immersion in HAc/NaAc + Na_2MoO_4 and HAc/NaAc + $AlCl_3$ electrolytes but remains unchanged in HAc/NaAc + H_3BO_3 medium.

For samples S.2 and S.3 the voltammetric response was similar, but does not fit with that of any of the flavonoid-based dyes. However, there was an excellent agreement between sample SQWVs and those recorded in blank experiments with electrodes modified with pristine carmine, an anthraquinone-type pigment. This is illustrated in Fig. 10, in which the SQWVs of (a) carmine and (b) sample S.2 are shown. For sample S.3, the voltammetric response looks like the superposition of peaks corresponding to carmine and Brazilwood. This is clearly suggested by SQWVs corresponding to pristine carmine (Fig. 10a), Brazilwood (Fig. 10c) and sample S.1 (Fig. 10d). Consistently with such ascription, the SQWV response changes in HAc/NaAc + $AlCl_3$ but not in HAc/NaAc + Na_2MoO_4 and HAc/NaAc + H_3BO_3 electrolytes. This last result is in agreement with literature concerning mediaeval dyestuffs used in Spain. In particular, Hofenk de Graaff and Roelofs [3], who have reported the use of a similar mixture of carmine and Brazilwood as a dyestuff of silk after analyzing a red silk velvet wrap of a Spanish illuminated manuscript from the 15th century.

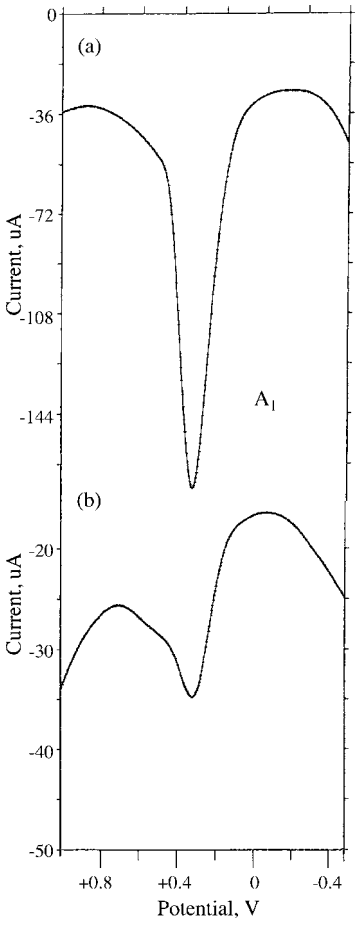


Fig. 9. First scan SQWVs of (a) old fustic; (b) sample S.1, immersed into 0.25 M HAc + 0.25 M NaAc. Potential step increment 4 mV; square wave amplitude 25 mV; frequency 15 Hz.

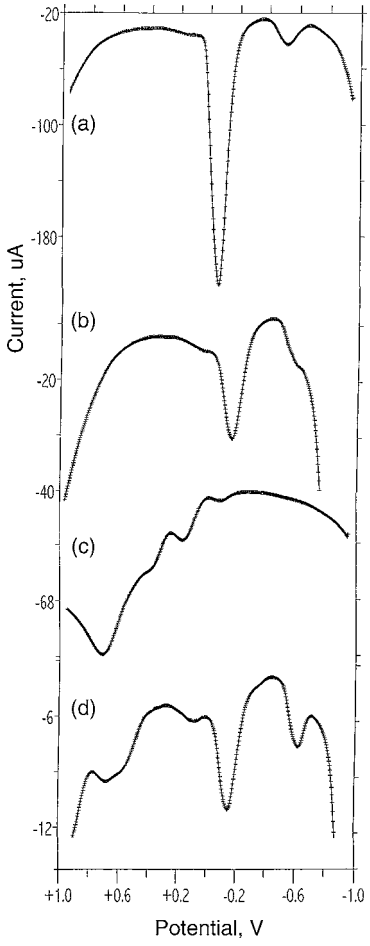


Fig. 10. First scan SQWVs of (a) carmine; (b) sample S.3; (c) Brazilwood; (d) sample S.1 immersed into 0.25 M HAc + 0.25 M NaAc. Potential step increment 4 mV; square wave amplitude 25 mV; frequency 15 Hz.

4. Conclusions

Upon sample attachment to PIGEs, flavonoid pigments produce a characteristic SQWV response in contact with acetic/acetate electrolytes that can be interpreted in terms of current models in solid state voltammetry of immobilized microparticles. The use of repetitive voltammetry and/or the addition of complexing agents such as $AlCl_3$ or Na_2MoO_4 to the electrolyte solution facilitate the identification of individual pigments and mixtures of dyes.

Apart from the presence of possible interfering compounds, analysis of real samples from archaeological fibres is made difficult by the strong fixation of the pigments to the substrate and their eventual ageing. Comparison of SQWVs of real samples with those of pristine and aged flavonoid dyes, however, provide a method for identifying the existing pigments with minimal possibility of sample contamination.

In spite of the wide variety of difficulties for analyzing real samples, it appears that the described methodology, based on the voltammetry of microparticles approach, can be considered as a promising analytical tool in the fields of archaeometry, conservation and restoration.

Acknowledgements

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